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della Società Italiana di Anatomia e Istologia
Genova, 12-14 settembre 2024

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In lasting memory of Carlo Enrico Grossi
and Alessandro Moretta

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From September 12 to 14, 2024, Genoa will host the meeting of the Italian Society of Anatomy and Histology (Società Italiana di Anatomia e Istologia - SIAI). Scientists dedicated to different aspects of morphological sciences will gather to discuss the latest scientific findings on both traditional and forward-looking issues.

The Scientific Committee, in close collaboration with the SIAI Board, has developed a program addressing highly impactful areas within our discipline.

The program is structured into invited lectures, oral communication sessions, and poster sessions.

The invited lectures honor the work of Prof. Carlo Enrico Grossi and Prof. Alessandro Moretta, two eminent scientists from the Genoese school of morphology, who prematurely passed away.

Invited Lectures

Title: Morpho-functional organization of the lymphoid follicle
Prof. Claudio Tripodo
Full Professor of Pathology, Head, Tumor Immunology Unit, Department of Health Sciences, University of Palermo School of Medicine, Istituto di Patologia Generale, Palermo, Italy

Title: Cells of immune system: from molecular mechanisms to clinical applications
Prof. Daniel Olive
Head of the Immunity and Cancer lab, CRCM, Professor of Immunology and Director of the Oncology Research Programs at Aix Marseille University, Scientific founder of ImCheck, Marseille, France

The following selected topics cover various fields where morphological sciences provide fundamental contributions. Strategies for successfully disseminating specific expertise and knowledge related to our discipline have also been considered.

- The immune system and its morpho-functional organization: from molecular mechanisms to therapeutic applications
- Neuroscience and neuromorphology
- Histogenesis, functions and dysfunctions of the musculoskeletal system
- 3D models and organoids, tissue engineering and regenerative medicine
- Ageing and degenerative diseases
- Epithelial tissues: epithelium-mesenchymal transition in organogenesis and carcinogenesis
- Innovation in teaching strategies and multimedia technologies: present and future
- Stem cells, histogenesis and differentiation
- Connective tissues
- Neuro-muscular-skeletal system in motor and sports activities
- Clinical and forensic anatomy, promotion of sectorial activity and its role in education

Oral communication and poster sessions

The immune system and its morpho-functional organization: from molecular mechanisms to therapeutic applications

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Probiotic supplementation improves colonic wall morphology and gut microbiota community in a murine model of colitis

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Inflammatory bowel diseases (IBD) are chronic relapsing-remitting gastrointestinal disorders associated with altered intestinal permeability, which causes a degeneration of the intestinal wall[1]. The treatments for IBD have insufficient therapeutic efficacy; however, studies identified that disease progression appears to be improved by a probiotic- supplemented diet[2]. The potential health-promoting properties of *Pediococcus acidilactici* 46A (*Pa*) were evaluated by an orally 10 days supplementation of the bacterial strain (1×10^8 CFU daily) on a murine model of Dextran-sulfate sodium (DSS)-induced colitis[3], by 7 days administration of DSS (2.5% w/v).

Progression of colitis was monitored after DSS administration. The gut microbiota community was evaluated using Illumina sequencing. Morphological and immunochemical analyses were performed on the proximal and the distal colon to assess disease severity score, neurodegeneration of myenteric plexus, pro-inflammatory cytokines expression, and oxidative stress status.

Pa supplementation reduced the disease activity index score while not affecting weight loss. The group receiving *Pa* exhibited higher microbiota biodiversity than the group receiving only DSS. Furthermore, bacterial co-occurrence network analyses revealed that *Pa* maintained favourable inter-species interactions, indicative of a more resilient and balanced microbial community.

The crypts architecture alteration, goblet cells depletion, and pro-inflammatory microenvironment were ameliorated in the *Pa*-supplemented mice compared to the DSS group. *Pa* ameliorates DSS-induced dysfunction of the colonic barrier by enhancing mucin 2 (MUC2) and zonula occludens-1 (ZO-1) expression. Moreover, in the *Pa*-supplemented mice was appreciated the stability of the neuronal network, evaluated by HuC/D pan-neuronal, nitrergic, and cholinergic markers.

These results showed the protective effects of specific bacterial strain to maintain colonic mucosal integrity, emphasizing the potential applications in the management of inflammation-related diseases.

This work was supported by University of Camerino (FAR-BVI000068).

References

1) Tse, C.S.; Hunt, M.G.; Brown, L.A.; Lewis, J.D. Inflammatory Bowel Diseases-related Disability: Risk Factors, Outcomes, and Interventions. Inflamm Bowel Dis. 2024, 30, 501-507.
2) Haneishi, Y.; Furuya, Y.; Hasegawa, M.; Picarelli, A.; Rossi, M.; Miyamoto, J. Inflammatory Bowel Diseases and Gut Microbiota. Int J Mol Sci. 2023, 24, 3817.
3) Katsandegwaza, B.; Horsnell, W.; Smith, K. Inflammatory Bowel Disease: A Review of Pre-Clinical Murine Models of Human Disease. Int J Mol Sci. 2022, 19, 934.

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Effect of the MoviS lifestyle intervention program on breast cancer survivors' blood immune cells: a preliminary framework

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The antitumorigenic effects of exercise might be related to increased cancer immunosurveillance [1]. Moreover, also nutrition plays an essential role in regulating optimal immunological response [2]. The study aims to evaluate possible phenotypic and functional changes in leukocyte subpopulations of breast cancer survivors (BCS) patients enrolled in the MoviS Trial (ClinicalTrials.gov: NCT04818359), which follow a 3-month of aerobic exercise and nutritional education program.

All tests were performed at baseline, at 3- 6- 12-month post lifestyle intervention. The peripheral blood samples were labelled by monoclonal antibody cocktail by lyse-no-wash flow cytometric technique. Mitochondrial potential and reactive oxygen species (ROS) were also investigated by fluorescent dyes: TMRE and MitoSOX, respectively.

During the time-course evaluation, we found a moderate but significant decrease of CD3+ T-lymphocytes and a significant increase of CD16+ NK at 12-month after the MoviS enrollment. The mitochondrial membrane potential improves over the time. Indeed, since mitochondria are one of the primary sources of ROS, we assessed the level of mitochondrial superoxide anion in all subpopulations. For their involvement in the oxidative burst, granulocytes show the greatest MitoSOX positivity: of note, after an initial ROS production increase, unstimulated granulocytes seem to stabilize their ROS level.

Finally, the presence of the insulin-like growth factor 1 receptor (IGF-1R) on leukocytes was also investigated. IGF-1R, a key member of the IGF axis, is known for its oncogenic role in multiple cancer lineages. The IGF-1R immunophenotyping aims to establish the level of the IGF-1R leukocyte expression and activation [3]. Our preliminary data related to a shorter time course (6 months) suggests a decrease in the IGF-1R percentages and Mean Fluorescence Intensity, detected on granulocytes.

Based on current and literature data, these results showed a beneficial effect induced by MoviS program on the immune system of BCS patients, paving the way for a possible prognostic application.

References

1) Fiuza-Luces, C., Valenzuela, P. L., Gálvez, B. G., Ramírez, M., López-Soto, A., Simpson, R. J., & Lucia, A. (2024). The effect of physical exercise on anticancer immunity. Nature reviews. Immunology, 24(4), 282–293.
2) Munteanu, C., & Schwartz, B. (2022). The relationship between nutrition and the immune system. Frontiers in nutrition, 9, 1082500.
3) Erlandsson, M. C., Lyngfelt, L., Åberg, N. D., Wasén, C., Espino, R. A., Silfverswärd, S. T., Nadali, M., Jood, K., Andersson, K. M. E., Pullerits, R., & Bokarewa, M. I. (2019). Low serum IGF1 is associated with hypertension and predicts early cardiovascular events in women with rheumatoid arthritis. BMC medicine, 17(1), 141.

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Anatomical considerations in lymph node retrieval: a comparative study of laparoscopic and open colorectal cancer surgeries

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Accurate staging and prognosis of colorectal cancer require careful retrieval of lymph nodes following surgery. It is the third most common form of cancer worldwide. This study, conducted at a single institution between 2018 and 2023, analyzed 560 patients who underwent colorectal cancer surgery. The aim was to compare lymph node retrieval in laparoscopic versus open procedures and evaluate the influence of body mass index (BMI). The data we collected indicates that there is no notable difference in the number of lymph nodes removed during laparoscopic surgeries compared to open surgical procedures. Furthermore, our statistical analysis shows that this lack of difference is not statistically significant. It is worth mentioning that the BMI of the patients did not have a significant impact on the retrieval of lymph nodes. The study employed an enhanced fat clearance technique to chemically dissolve mesenteric fat, allowing for improved visualization and retrieval of lymph nodes. This method has the potential to increase lymph node yield when compared to traditional manual dissection methods. The consistency in surgical outcomes across different treatments and patient BMIs can be attributed to the meticulous approach of the surgical team and the precise fat clearance technique utilized. Based on the findings, it appears that both laparoscopic and open surgical methods yield similar results when it comes to retrieving lymph nodes in colorectal cancer patients, regardless of BMI. This emphasizes the significance of using standardized surgical techniques and relying on a skilled surgical team to achieve consistent anatomical outcomes. This study provides significant insights into the anatomical aspects of colorectal cancer surgery, highlighting the comparable effectiveness of minimally invasive and traditional approaches in achieving comprehensive lymph node dissection. This is a crucial factor in patient care and prognosis.

References

1. Betge, J.; Harbaum, L.; Pollheimer, M.J.; Lindtner, R.A.; Kornprat, P.; Ebert, M.P.; Langner, C. Lymph node retrieval in colorectal cancer: determining factors and prognostic significance. Int. J. Colorectal Dis. 2017, 32, 991-998.
2. Ouyang, M.; Liao, T.; Lu, Y.; Deng, L.; Luo, Z.; Wu, J.; Ju, Y. Laparoscopic versus Open Surgery in Lateral Lymph Node Dissection for Advanced Rectal Cancer: A Meta-Analysis. Gastroenterol. Res. Pract. 2019, 2019, 7689082.

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The role of the Pentose Phosphate Pathway in Chronic Lymphocytic Leukemia and its contribution to chemotherapy resistance

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Chronic lymphocytic leukemia (CLL) is a hematological malignancy displaying a highly heterogeneous clinical course.

Several studies highlight the capability of CLL B cells to control the persistent redox stress by triggering antioxidant pathways that are particularly efficient in aggressive CLL cells[1]. Antioxidant pathways and anabolic reactions strictly depend on NADPH, whose intracellular pool is provided by the pentose phosphate pathway (PPP). Recent evidence showed that the level of PPP rate-limiting enzyme, glucose-6-phosphate-dehydrogenase (G6PD), is significantly increased in CLL B cells compared to normal B cells and is directly correlated with poor prognosis[2].

We thus evaluated the enzymatic activity of G6PD, the mitochondrial respiratory function, and the glycolytic flux in CLL cells derived from patients with 'indolent' and 'aggressive' disease, classified according to molecular prognostic markers.

The results showed that *in vitro* stimulation selectively increased G6PD activity only in samples with features of higher aggressiveness. Interestingly, in activated CLL cells, the mitochondrial dependence on fatty acid was significantly higher in patients with more aggressive disease, whereas mitochondrial dependence on pyruvate appears to be reduced. Given that levels of glucose consumed by cells and the lactate produced are similar in 'indolent' and 'aggressive' activated samples, these data suggested that part of the cell-entering glucose might be processed through PPP, thus configuring the limitation of this enzyme as a possible therapeutic target. In line with these data, we observed a possible involvement of G6PD in the resistance to the Bcl-2 inhibitor Venetoclax. Indeed, the anti-apoptotic protein Mcl-1 increment might be involved in resistance to Venetoclax[3]. In line with these observations, we observed a possible direct correlation between G6PD and Mcl-1 expression.

These data highlight a novel role of G6PD function in the aggressiveness of CLL patients and anti-Bcl-2 therapy resistance, suggesting the PPP-limiting enzyme may represent a new therapeutic target in CLL.

References

1) Cavallini, C.; Chignola, R.; Dando, I.; Perbellini, O.; Mimiola, E,; Lovato, O.; Laudanna, C.; Pizzolo, G.; Donadelli, M.; Scupoli, MT. Low catalase expression confers redox hypersensitivity and identifies an indolent clinical behavior in CLL. Blood. 201, 131(17):1942-1954.
2) Xiao, G.; Chan, LN.; Klemm, L.; Braas, D.; Chen, Z.; Geng, H.; Zhang, QC.; Aghajanirefah, A.; Cosgun, KN.; Sadras, T.; Lee, J.; Mirzapoiazova, T.; Salgia, R.; Ernst, T.; Hochhaus, A.; Jumaa, H.; Jiang, X.; Weinstock, DM.; Graeber, TG.; Müschen, M. B-Cell-Specific Diversion of Glucose Carbon Utilization Reveals a Unique Vulnerability in B Cell Malignancies. Cell. 2018,173(2):470-484.e18.
3) Bojarczuk, K.; Sasi, BK.; Gobessi, S.; Innocenti, I.; Pozzato, G.; Laurenti, L.; Efremov, DG. BCR signaling inhibitors differ in their ability to overcome Mcl-1-mediated resistance of CLL B cells to ABT-199. Blood. 2016, 127(25):3192-201.

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Altered miRNome impacts on immune responses, inflammatory processes and tissue repair in children affected by Long Covid

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The emerging research on the involvement of microRNAs (miRNAs) in paediatric Long Covid, drawing insights from studies on adults. Various miRNAs (are implicated in COVID-19 pathology, including antiviral responses and interaction with the SARS-CoV-2 genome. In adult Long Covid patients, distinct miRNAs involved in immune responses and inflammation are dysregulated and the ones especially regulating ACE2 expression, could be responsible for organ-specific complications (1). In our study we intended to dissect the potential

implications of miRNAs in paediatric Long Covid, to better understand their role and potential clinical applications (2-3).

Children were evaluated at least 8 weeks after their initial infection and classified as "fully recovered" (no persisting symptoms and return to pre-Covid activity levels) or as having "Long Covid" (persistent symptoms impacting daily life for at least eight weeks, with other diagnoses excluded). Through epigenetic/transcriptomic analysis we compared miRNome of different patients (n=34) e once revealed potential microRNA, we studied their pathways. We found miRNAs able to characterize the disease when compared to healthy children; moreover, we found 4 miRNAs (hsa-miR-26a-5p, hsa-miR-423-3p, hsa-miR-29a-3p, hsa-miR-424-5p) able to significantly distinguish Long-Covid and recovered cohorts. We found that these miRNAs are all involved in the regulation of similar gene pathways. Among them we predicted that the 4 miRNAs characterizing the disease state were able to modulate AKT expression and consequently different functions of many immune cells. In particular, AKT represents one of the main triggers of immune response and is responsible for cell activation and proliferation and antibody production. These data are in line with the long-lasting persistency of Long-Covid.

The identification of specific miRNA signatures could help in diagnosing, prognosing, and monitoring paediatric Long Covid, and miRNAs may serve as potential therapeutic targets. Moreover, targeting the gene(s) modulated by dysregulated miRNAs could represent al alternative strategy to counteract this disease.

Keywords: epigenetic, autoinflammation, host pathogen interactions

References

1 Widiasta, A.; Sribudiani, Y.; Nugrahapraja, H.; Hilmanto, D.; Sekarwana, N.; Rachmadi, D. Potential Role of ACE2-Related microRNAs in COVID-19-Associated Nephropathy. Non-coding RNA Research 2020, 5, 153–166, doi:10.1016/j.ncrna.2020.09.001.

2 Di Sante, G.; Buonsenso, D.; De Rose, C.; Tredicine, M.; Palucci, I.; De Maio, F.; Camponeschi, C.; Bonadia, N.; Biasucci, D.; Pata, D.; et al. Immunopathology of SARS-CoV-2 Infection: A Focus on T Regulatory and B Cell Responses in Children Compared with Adults. Children 2022, 9, 681, doi:10.3390/children9050681.

3 Di Sante, G.; Buonsenso, D.; De Rose, C.; Valentini, P.; Ria, F.; Sanguinetti, M.; Sali, M. Immune Profile of Children with Post-Acute Sequelae of SARS-CoV-2 Infection (Long Covid); Pediatrics, 2021

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Integrated stress response shapes the immune and metabolic landscape of Triple-negative Breast Cancer

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Breast Cancer (BC) is the most lethal disease for women worldwide. Among all BCs, about 15% are classified as Triple-Negative Breast Cancer (TNBC) and considered the most aggressive subtype for the high metastatic rate and lack of targeted therapy.

Metastasis, the process by which cancer cells leave the primary tumor and colonize the surrounding tissue and/or distant organs, is considered the deadliest aspect of cancer. Here we focus on Integrated Stress Response (ISR), an evolutionarily conserved pathway that fuels cancer metastatic potential regulating cancer cells and Tumour MicroEnvironment (TME) interplay. TME-derived stress such as hypoxia, starvation, immune attack and dissemination-associated stresses activate ISR in cancer cells, increasing their metastatic potential and the capability, in turns, of remodelling the surrounding milieu.

Interrogating The Cancer Genome Atlas (TCGA) gene expression dataset of breast cancer patients' samples we revealed that the highest activation of ISR is found in TNBC, is in line with its the aggressiveness. Since the ISR signature also showed a strong correlation with macrophages signature in METABRIC (Molecular Taxonomy of Breast Cancer International Consortium) analysis, we investigated the role of ISR in TNBC cells and macrophages crosstalk.

Our results show a paradoxical role of the pro-inflammatory macrophages (M1-like phenotype), conventionally considered anti-tumor. Indeed M1-like macrophages' secretome leads to the activation of ISR in TNBC. Beyond the well-established anti-tumour activity, we demonstrated for the first time that M1-like secretome is able to promote TNBC invasiveness. Strikingly, inhibiting ISR is sufficient to impair the invasive capability of M1-like secretome treated TNBC cells, confirming a causal relationship between M1-driven invasiveness and ISR activation.

Highlighting the mechanisms by which activation of ISR in BC cells reshapes their phenotype, in search for a targetable vulnerability, could represent a tremendous opportunity to counteract the metastatic spread.

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Human Cytomegalovirus infection induces adaptive reconfiguration of NK cells resulting in increased atherosclerotic plaque instability

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Human Cytomegalovirus (HCMV) infects a broad range of host cells and remains latent upon primary infection. Recent works detected HCMV in Atherosclerotic Plaque (AP), suggesting that the virus may contribute to atherosclerotic processes (1). HCMV-seropositive individuals exhibit "memory-like or adaptive" NK cells characterized by a distinct phenotype and enhanced functional capabilities, including antibody-dependent cell-mediated cytotoxicity, and cytokines production, such as Interferon- γ (IFN-γ) (2,3). Although NK cells were detected in AP, the involvement of these cells and of HCMV in promoting AP destabilization remains unclear. Aim of this study was to investigate the role of HCMV in the induction of adaptive NK cells pool that could be implicated in plaque instability.

A total of 64 patients (pts) were enrolled and classified according to conventional criteria as bearing high-risk plaques (High-risk pts- HR pts) or low-risk plaques (Low-risk pts- LR pts). HR pts underwent carotid endarterectomy according to the European Society for Vascular Surgery guidelines. Carotid Atherosclerotic Plaque (CAP) and Peripheral Blood (PB) samples were examined by histological and flow cytometric analyses.

Immunohistochemical analysis revealed that NK cells (NKp46$^+$ cells) and macrophagic cells (CD68$^+$ cells) infiltrate the CAP of HR HCMV-seropositive pts, and that HCMV is localized within CD68$^+$cells. Remarkably, in seropositive pts, adaptive FcεR1γ$^-$ NK cells are enriched in PB of HR pts compared to LR, and accumulate in CAP of HR pts. Moreover, NK cells from seropositive HR pts show enhanced antibody-dependent IFN- γ production that correlate with the frequency of FCεR1γ$^-$ NK cells. Interestingly, the presence of FcεR1γ$^-$ NK cells in CAP of HR pts is associated with the expression of matrix metalloproteinase 9, endopeptidases produced by CD68$^+$ cells.

In conclusion, our data demonstrate the potential impact of HCMV and adaptive NK cells on atherosclerotic process, suggesting their use as a possible marker of AP destabilization.

References

1) Beyaz, M.O.; Ugurlucan, M.; Oztas, D.M.; Meric M.; Conkbayir, C.; Agacfidan A.; One M, Alpagut U. Evaluation of the relationship between plaque formation leading to symptomatic carotid artery stenosis and cytomegalovirus by investigating the virus DNA. Arch. Med. Sci. Atheroscler. Dis, 2019, 4, 19-24.
2) Schlums, H.; Cichocki, F.; Tesi, B.; Theorell, J.; Beziat, V.; Holmes, T.D.; Han, Hongya.; Chiang, S. C.C.; Foley, B.; Mattsson, K.; Larsson S.; Schaffer M.; Malmberg K.J.; Ljunggren, H.G.; Miller, J. S.; Bryceson, Y.T. Cytomegalovirus infection drives adaptive epigenetic diversification of NK cells with altered signaling and effector function. Immunity, 2015, 42, 443-56.
3) Capuano, C.; Pighi, C.; Battella, S.; Pulcinelli F.; Santoro, C.; Ferretti, A.; Turriziani, O.; De Federicis D.; Fionda, C.; Sciumè G.; Galandrini, R.; Palmieri, G. (Auto) Antibody Responses Shape Memory NK Cell Pool Size and Composition. Biomedicines 2022, 10, 625.

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Phylogenetic and tissue expression pattern conservation of Trop-2 across species support preclinical modeling of anti-Trop-2 antibody therapy

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The Trop-2 calcium signal transducer is a driver of tumor growth and progression and a target for anticancer therapy (1). The anti-Trop-2 Sacituzumab govitecan (TRODELVY) antibody-drug conjugate has received FDA approval for therapy of patients with metastatic estrogen receptor-positive and triple-negative breast cancer and urothelial carcinomas. However, Sacituzumab govitecan limited half-life in plasma (11–14 hours) and dose-limiting toxicities indicate an urgent need for next-generation anti-Trop-2-targeted therapy (2). We recently showed that Trop-2 undergoes cancer-specific proteolytic activation, and we generated humanized monoclonal antibodies (Hu2G10, Hu2EF) to specifically exploit this cancer vulnerability (3).

With the aim to identify reliable models for in vivo toxicity and pharmacokinetic (PK) profiling of next-generation anti-Trop-2 therapeutics we analysed phylogenetic, structural and expression pattern conservation of Trop-2 across vertebrate species. Sequence divergence and incomplete conservation of expression patterns were observed in mouse and rat. On the other hand, non-human-primate Trop-2 sequences were found to be 95%-100% identical to the human sequence, with high structure conservation. Immunohistochemistry analysis of rhesus monkey tissues showed Trop-2 expression patterns that closely followed those in human tissues. Flow-cytometry analyses showed that Hu2G10 and Hu2EF efficiently recognized primate Trop-2. This led us to test Trop-2 targeting in vivo in *Macaca fascicularis*. Intravenous (IV)-injected Hu2G10 and Hu2EF at 5 to 10 mg/kg were well tolerated, with no signs of adverse symptoms or biochemical or hematological toxicities over 28 days. PK profiling showed that the Hu2G10 and Hu2EF were stable in plasma ($t_{1/2}$=6.5 days and $t_{1/2}$=5.5 days respectively). Our findings validate non-human primate for reliable in vivo modeling of anti-Trop-2 anticancer therapy and candidate the Hu2G10 and Hu2EF antibodies as first-in-class anti-Trop-2 immunotherapeutic agents.

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References

1) Guerra, E.; Trerotola, M.; Alberti, S. Targeting Trop-2 as a Cancer Driver. J. Clin Onc. 2023, 41, 4688.
2) Guerra, E.; Alberti, S. The anti-Trop-2 antibody-drug conjugate Sacituzumab Govitecan-effectiveness, pitfalls and promises. Ann. Transl Med 2022, 10, 501.
3) Guerra, E.; Trerotola, M.; Relli, V.; Lattanzio, R.; Tripaldi, R.; Ceci, M.; Boujnah K.; Pantalone, L.; Sacchetti, A.; Havas K.M.; Simeone, P.; Travali, N.; Querzoli, P.; Pedriali, M.; Roversi, P.; Iezzi, M.; Tinari, N.; Antolini, L.; Alberti, S. 3D-Informed Targeting of the Trop-2 Signal-Activation Site Drives Selective Cancer Vulnerability. Mol Cancer Ther 2023, 22, 790.

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Expression of inflammatory molecules in rat liver infected with Lipopolysaccharides (LPS)

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The general histological structure of rat liver is comparable to that of other mammalian species; indeed, it is characterized by a lobular organization based on the distributions of portal areas and central venules. The presence of canaliculi and a bile duct system also appear to be analogous to that reported for other species. The evidence that rat and mammalian livers are similar indicates that rats serve as valuable animal models for investigating the anatomy and physiology of the liver. Since innate immunity is considered the first line of defense against microbial invasion, this study aims to characterize rat liver hepatic immune cells involved in inflammation caused by lipopolysaccharide (LPS). The bacterial endotoxin lipopolysaccharide (LPS) affects levels of inflammatory mediators, playing an important role in the development of systemic inflammatory response which may trigger sepsis and be linked to liver injury. In addition, LPS activates hepatic macrophages, including Kupffer cells, to secrete inflammatory cytokines and induces hepatocyte necrosis or apoptosis, ultimately resulting in liver injury. In this study immune hepatic cells have been characterized with the following antibodies: TLR4, TLR2 (both belonging to Toll-like receptors family), α-SMA (Alpha smooth muscle actin), MHCII (Major Histocompatibility Complex) and Langerin/CD207(c-type lectin expressed by different types of DCs). Our results showed a significant difference between control and LPS-infected samples. Several immune cells were found to be positive for these antibodies in the samples obtained from inflamed rat liver, especially in the region between the portal space and the central vein. In control samples, instead, there is an extremely small number of antibody-positive cells. In conclusion, this study demonstrates that bacterial LPS can bridge the activation of various cellular populations of the innate immune system such as dendritic cells, neutrophils and Kupffer cells.

References

1) Baratta, J.L.; Ngo, A.; Lopez, B.; Kasabwalla, N.; Longmuir, K.J.; Robertson, R.T. Cellular Organization of Normal Mouse Liver: A Histological, Quantitative Immunocytochemical, and Fine Structural Analysis. Histochem Cell Biol 2009, 131, 713–726.
2) Pergolizzi, S.; Fumia, A.; D'Angelo, R.; Mangano, A.; Lombardo, G.P.; Giliberti, A.; Messina, E.; Alesci, A.; Lauriano, E.R. Expression and Function of Toll-like Receptor 2 in Vertebrate. Acta Histochem. 2023, 125, 152028.
3) Miller, A.; Cutroneo, G.; Lombardo, G.P.; D'Angelo, R.; Pallio, S.; Migliorato, A.; Fumia, A.; Favaloro, A.; Lauriano, E.R.; Pergolizzi, S. Association between Neuropeptides and Mucins in Crohn's Disease Mucous Cells. Acta Histochem. 2023, 125, 152115.

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PD1-1+ NK cell subset: a phenotypic and molecular characterization in healthy and pathological conditions

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Recently several studies have attempted to uncover the role of PD-1+ NK cells (NKs) in multiple physiopathological contexts, including cancer. However, the precise contribution of this NK subset is still unclear due to the lack of a well-defined characterization. In this context, firstly we identified PD-1 on NKs derived from the peripheral blood healthy adult donors (HAD). Recently, we showed that also NKs of newborn cord blood (CB) may express PD-1. In addition, we observed that in ovarian cancer patients the level and frequency of PD-1 on NKs is higher compared to HAD and CB. The increase, detectable in the peripheral blood (PB), is more evident in the tumor microenvironment such as peritoneal fluid (PF). Importantly, a higher expression of PD-1 correlates with a higher severity of disease. PD-1+ HAD-NKs are consistently NKG2A-KIR/LILRB1+NKp46lowCD57high, showing a fully mature phenotype, whereas PD-1+ CB-NKs and PF-NKs show more immature features, characterized by the NKG2A+KIR+/-NKp46+CD57low/- phenotype. This different maturation state is confirmed by a transcriptomic analysis on sorted PD-1+ and PD-1- HAD-NKs and PF-NKs. In addition, the transcriptomic profile reveals that PD-1+ NKs is characterized by a diminished presence of mRNA for, EOMES, and IL2RB both in HAD and PF, while FceRg transcript is downregulated in PD-1+HAD NKs and upregulated in PD-1+PF NKs. Interestingly, we observed a markedly higher number of upregulated genes in PD-1+ PF-NKs, compared to both PD-1-NKs of the same patient and PD-1+ HAD-NKs, suggesting that the PD-1+ NK subset is a key subset on which to bet on in cancer. The research leading to these results has received funding from Fondazione AIRC under IG 2021 – ID. 26037 project – P.I. EM. Additional grants from University of Genova: PRIN-MIUR 2022, Grant N° 2022YCKH7K-P.I. EM; PRIN-MIUR 2022, Grant N° P2022PKFNB P.I. SP; AIRC 5x1000 Id. 21147-G.L. SS. MG was supported by a Post-Doctoral Fellowships from Fondazione Veronesi (Post-Doctoral Fellowships - Anno 2024).

References

1) Pesce, S.; Greppi, M.; Tabellini, G.; Rampinelli, F.; Parolini, S.; Olive, D.; Moretta, L.; Moretta, A.; Marcenaro, E. Identification of a Subset of Human NK Cells Expressing High Levels of PD-1 Receptor: A Phenotypic and Functional Characterization. The Journal of Allergy and Clinical Immunology, 2017.
2) Greppi, M.; Obino, V.; Goda, R.; Rebaudi, F.; Carlomagno, S.; Della Chiesa, M.; Sivori, S.; Ubezio, G.; Agostini, V.; Bo, A.; Pesce, S*.; Marcenaro, E*. Identification of a novel cord blood NK cell subpopulation expressing functional programmed death receptor-1. Front Immunol. 2023 * These authors share senior authorship.

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Temporal and spatial morphofunctional shifts in CLL B cells during transition between lymphoid organs and peripheral blood

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Chronic lymphocytic leukemia (CLL) cells can be divided into subsets based on time since last cell division. This can be determined by patients drinking deuterated water (^2H_2O) and then using flow cytometry to identify intraclonal subpopulations with reciprocal surface levels of CXCR4 and CD5.

We redefined the kinetics of CLL fractions and provide novel insights about their functional dynamics. *Ex vivo* CLL cells from patients who drank ^2H_2O were sorted by CXCR4/CD5 relative densities, 5 fractions. Using ^{2}H-DNA as determinant of age, a unidirectional path of phenotypic change could be defined, with the bigger and complex active cells transitioning to the small, quiescent status.

Since BCR signaling is fundamental for CLL proliferation, we analyzed the densities of smIgM, smIgD (smIGs) and smCD19, finding higher smIGs densities on fractions with higher ^{2}H-DNA incorporation. Notably, these findings were not consistent with cell division being uniquely initiated by BCR engagement. Chronological combinations of stimuli via TLR9 and smIGs showed that increased IG density required TLR9 stimulation before or concurrently with the latter. Thus, recently-divided cells might have experienced multifactorial stimulation.

The fractions sorted before and during ibrutinib treatment in vivo, displayed diverse intraclonal changes in smIG densities, cell size and complexity, and metabolic activation, with ^{2}H-enriched and higher smIG density cells being more affected.

These data defined additional CXCR4/CD5 subpopulations of divergent ages, phenotypes, and sensitivities to treatment, suggesting that CLL B-cell kinetics are more complex than the current model describes. This complexity originates in secondary lymphoid organs, where stimulation by the BCR and other pathways generates the young CLLs. In the blood, CLLs age to the quiescent fraction. Since each cell within a clone appears to traverse these stages, the unique biologic features at each phase represent novel processes for therapeutic targeting.

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Involvement of rat intestinal mucosal cells in inflammation caused by Lipopolysaccharides (LPS)

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Rat and human are quite similar in physiology and anatomical structures, particularly, the intestinal tracts in both species are composed of anatomically similar organs. Indeed, rat intestinal mucosa is characterized by three distinct portions: a layer of epithelial cells, a connective tissue and the muscularis mucosae. In addition, the presence of villi and intestinal glands makes rat models widely used in biomedical studies. The different cell types of the intestinal mucosa cooperate, forming a physical, chemical and immune barrier in the defense against microbial invasion.

This study aims to characterize rat intestinal mucosal cells in inflammation caused by Lipopolysaccharides (LPS). Lipopolysaccharide (LPS) is a component of the outer membrane of Gram-negative bacteria, which works as a strong activator of the intestinal inflammatory system. LPS functions as the typical endotoxin because it binds the receptor complex in various cell types, such as enterocytes, macrophages and B cells, stimulating the release of pro-inflammatory cytokines.

In this study, intestinal mucosal cells have been characterized by immunostaining for: Alpha smooth muscle actin (α-SMA), Toll-like receptor 2 (TLR2), and Serotonin (5-HT).

Our results showed in the samples obtained from inflamed rat intestinal mucosa, TLR2-positive intestinal epithelial cells (IECs), demonstrating that these cells can be considered immunologically active working as the first line of defense. Numerous α-SMA-positive cells (subepithelial myofibroblasts) were present in *lamina propria* of the villus, around glands forming a pericryptal fibroblast sheath (PCFS) and in the *muscularis mucosae*, highlighting an inflammatory state. Conversely, there are very few antibodies-positive cells in control samples.

Furthermore, it has been shown an increase of neuroendocrine cells 5-HT positive compared to normal, which could be associated with intestinal inflammation. This study concludes by showing that bacterial LPS can mediate the activation of different intestinal mucosal cells, including enterocytes, myofibroblasts, macrophages and neuroendocrine cells.

References

1) Cario, E.; Rosenberg, I.M.; Brandwein, S. L.; Beck, P.L.; Reinecker, H.C.; Podolsky, D.K. Lipopolysaccharide activates distinct signaling pathways in intestinal epithelial cell lines expressing Toll-like receptors. J. Immunol., 2000, 164(2), 966-972.
2) Marino, A.; Santoro, G.; Spataro, F.; Lauriano, E.R.; Pergolizzi, S.; Cimino, F.; Dugo, G. Resveratrol role in Staphylococcus aureus-induced corneal inflammation. Path. and Dis., 2013, 68(2), 61-64.
3) Miller, A.; Cutroneo, G.; Lombardo, G.P.; D'Angelo, R.; Pallio, S.; Migliorato, A.; Pergolizzi, S. Association between neuropeptides and mucins in Crohn's disease mucous cells. Acta Histochemica., 2023, 125(8), 152115.

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Immunohistochemical characterization of the mononuclear phagocyte system in murine lymphoid tissues

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The mononuclear phagocyte system (MPS), which includes circulating monocytes, macrophages, and dendritic cells, consists of highly heterogeneous cell populations characterized by intrinsic plasticity. These cells play crucial roles in various physiological and pathological processes. While the role of MPS cells in different experimental settings has been extensively investigated through various techniques, immunohistochemistry stands out for its ability to identify MPS cells within spatial contexts. However, existing studies often focus on specific tissues and/or pathological conditions. This study aims to broadly characterize MPS cells in non-pathological murine lymphoid tissues, using an extensive panel of macrophage and dendritic cell markers. Spleen, lymph node, and thymus tissues from 25 C57BL/6 control mice, confirmed to be free of significant background lesions, were included in the study. Formalin-fixed and paraffin-embedded sections were immunostained for Iba1, F4/80, MARCO, CD206, Ym1, HO1, Arginase-1, iNOS, and MHC-II. For each marker, the number of positive cells was semiquantitatively scored in each microanatomical subcompartment. Additionally, cell shape and staining intensity were recorded. The applied panel, which included pan-macrophagic markers and population-specific ones, highlighted the heterogeneous phenotype of murine MPS cells in lymphoid organs and, by combining morphological features, microanatomical localization, and marker expression allowed the definition of the immunophenotype of the different MPS subsets known to populate each organ. The obtained results expand the knowledge regarding the immunophenotype of MPS cells in murine lymphoid tissues under steady-state conditions, providing a valuable reference for future experimental research. Furthermore, they highlight the importance of considering cell morphological features and spatial distribution for a comprehensive characterization of these populations.

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Morphological study of bone marrow microenvironment in Multiple myeloma patients

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Multiple myeloma (MM) is a clonal plasma cell proliferative disorder characterized by the abnormal increase of monoclonal immunoglobulins (Ig). The increased monoclonal Ig cause hyper viscosity, platelet dysfunction, and renal tubular damage, leading to derangement syndrome, bleeding, and renal failure. The expanding plasma cell clone observed in the Bone marrow are the consequence of anemia, thrombocytopenia, and leukopenia.

In addition, myeloma cells which are located in the bone microenvironment stimulate the osteoclasts activity and inhibit the osteoblast bone matrix deposition, resulting in bone loss.

The Plasma cells adhesion to hematopoietic cells is a consequence of Several intra- or extra-cellular signaling cascades and the secretion of many cytokines and growth factors such as IL-6, VEGF and IGF-1, by supporting the autocrine and paracrine plasma cell growth.

The Bone marrow mesenchymal cells (BMSC) are involved myeloma malignant proliferation, promoting the BMSC-myeloma malignant cell interaction.

It's has been recently demonstrated that in *in vitro* culture the CD155/TIGIT signaling plays a pivotal role in the regulation of BMSCs inducing the Natural Killer (NK) cell exhaustion in MM.

Considering these previous observations, we investigated by the TIGIT expression in the bone marrow smear biopsies cultured in cytomatrix from MM patients.

According to TIGIT expression we divide our experimental group in $TGIT^+$ and $TGIT^-$ patients.

We investigate the morphological microenvironment for both groups, in $TIGIT^-$ patients plasma cells are increased in dimensions, with vacuolized cytoplasm and clustered. By contrast in the bone marrow microenvironment of $TIGIT^+$ patients the plasma cells are smaller, not clustered surrounded by neutrophils, witch are increased in this experimental group.

Given the complexity and heterogeneity of MM, with this study we firstly identified the main morphological alteration that occur in MM patients and the TGIT involvement in this complex disease.

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Characterization of immune cells in human placenta in pathological conditions

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The placenta is a transient organ that allows metabolic exchanges between maternal and fetal blood, also acting as a protective barrier to prevent the passage of harmful substances to the fetus. The placenta is also considered a metabolic mirror reflecting the health status of the mother and the fetus. Hofbauer cells are the typical placental antigen-presenting cells, they are macrophages involved in a wide range of functions crucial for successful pregnancy; these cells express on their surface MHCII molecules, TLR4 recognizing PAMPs, and CD14 protein binding LPS and detecting bacteria. Langerhans cells are dendritic cells that present antigens to T lymphocytes, activating immune responses. They are among the first lines of defense in various tissues and organs in which they are highlighted with Langerin/CD207, but there is little evidence regarding the presence of Langerhans cells in the human placenta. Therefore, this study aimed to investigate, with immunohistochemical methods, the antigen-presenting cells in human term placenta in pathological conditions. The tissues were obtained from spontaneous birth of healthy women and women affected by obesity and related diseases. Paraffin sections were incubated with primary antibodies directed against TLR4, CD14, and CD207, and processed for light and confocal microscopy observations. In the control placenta, Hofbauer cells were identified through localization of anti-CD14 and anti-TLR4 antibodies, no positivity for CD207 was detected. In the pathological placenta, Hofbauer cells are more numerous and have been identified near the blood vessels of the villi and in the peripheral region, while CD207-positive cells have been observed in the stroma of the villi. In conclusion, in pathological conditions such as diabetes, hypertension and obesity, there is a subset of antigen-presenting cells, confirming the protective role of the placenta, which is important in safeguarding the life of the fetus and allowing its proper development.

References

1) Wong, I.C.; Moller, A.; Giachini, F.R.; Lima, V.V.; Toledo, F.; Stojanova, J.; Sobrevia, L.; San Martin, S. Placental structure in gestational diabetes mellitus. Biochim. Biophys. Acta Mol. Basis Dis. 2020, 1866(2), 165535.
2) Reyes, L.; Golos, T.G. Hofbauer Cells: Their Role in Healthy and Complicated Pregnancy. Front. Immunol. 2018, 9: 2628
3) Pergolizzi, S.; Marino, A.; Capillo, G.; Aragona, M.; Marconi, P.; Lauriano, E.R. Expression of Langerin/CD 207 and α-smooth muscle actin in ex vivo rabbit corneal keratitis model. Tissue and cell 2020, 66, 101384.

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Downmodulation of PD-1 receptor in NK cells using siRNA tecnology: a novel approach for immune-checkpoint blockade therapy

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Recently, we explored the potential application of small interfering RNA (siRNA) technology as an adjunct to immunotherapy to enhance Natural Killer (NK) cell antitumor activity. siRNAs, small non-coding RNAs, play a crucial role in gene regulation by silencing or downregulating the expression of specific genes. Our focus was to modulate the expression of immune-checkpoints in NK cells, in particular PD-1, to restore their functionality against tumor cells. To achieve efficient transfection of human NK cells with siRNAs, we developed a new protocol based on a cell transfection, with a smart pool of siRNAs specifically targeting the PD-1 receptor. In our experiments we employed both a human NK cell line called, YT, as they express PD-1 and PD-1+ NK cells from healthy donors. After 72 hours of incubation with siRNAs, we conducted phenotypic, molecular, and functional analyses on the collected cells. Phenotypic analyses demonstrated a significant down-modulation of PD-1 expression compared to the control conditions. This down-modulation was also observed at the PD-1 mRNA level. Preliminary functional experiments provided promising results, indicating that siRNA-mediated PD-1 down-modulation significantly increased NK cell cytotoxicity against tumor cells expressing PD-1 ligands. These findings suggest a potential avenue to enhance the antitumor activity of NK cells through siRNAs technology, specifically targeting immune checkpoints such as PD-1. In conclusion, our study introduces a novel approach involving siRNA technology to modulate immune-checkpoints in NK cells, highlighting its potential in enhancing antitumor responses. Further research and clinical validation are needed to explore the full therapeutic potential of this approach.

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References

1) Identification of a subset of human natural killer cells expressing high levels of programmed death 1: A phenotypic and functional characterization. Pesce S, Greppi M, Tabellini G, Rampinelli F, Parolini S, Olive D, Moretta L, Moretta A, Marcenaro E.J Allergy Clin Immunol. 2017 Jan;139(1):335-346.e3. doi: 10.1016/j.jaci.2016.04.025. Epub 2016 May
2) Targeting the RNA m6A modification for cancer immunotherapy. Li X, Ma S, Deng Y, Yi P, Yu J.Mol Cancer. 2022 Mar 16;21(1):76. doi: 10.1186/s12943-022-01558-0.
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Analysis of natural killer cells phenotype in refractory relapsing Hodgkin lymphoma treated with autologous lymphocyte infusion and immune checkpoint inhibitors

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Hodgkin lymphoma (HL) is a lymphoid malignancy of B-cell origin with a high cure rate. However, a minority of patients exhibit refractory/relapsing disease (R/R) or partial response (PR) despite several cycles of high-dose chemotherapy and autologous stem cell transplantation (ASCT). Several studies have concluded and approved the clinical use of immune checkpoint inhibitors (IC) such as Nivolumab for the treatment of advanced-phase solid tumours. Furthermore, the use of Nivolumab in R/R HL showed promising results after autologous stem cell transplantation. Previously, we observed that autologous lymphocyte infusion (ALI) plus Nivolumab treatment early post-ASCT may accelerate the NK cell development/maturation. This expansion of mature NK cells (CD56dimCD16brightNKG2A-KIR+CD57+) was evident following the last ALI cycle as compared to one month after the 1st cycle where NK cells showed a rather immature phenotype (CD56brightCD16dimNKG2A+KIR- phenotype).

Recently, we conducted an in-depth analysis of the peripheral blood of HL patients which revealed that HL-derived NK cells (HL-NK) may have lower levels of activating receptors (NKG2D and DNAM-1) in samples collected before infusion (pre-ALI) as compared to NK cells derived from Healthy Donors. This compromised activating receptors expression matches with an impaired functional activity against tumour target cells. Interestingly, our data suggest that after ALI and Nivolumab treatment, HL-NK cells may restore the expression of NKG2D and DNAM-1 and accordingly also cytotoxic activity maybe resumed. In conclusion, these data indicate that our protocol based on combination of ALI and Nivolumab is able to potentiate effective NK cell-mediated tumour cell killing in these patients.

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References

1) Guolo, F.; Minetto, P.; Pesce, S.; Ballerini, F.; Clavio, M.; Cea, M.; Frello, M.; Garibotto, M.; Greppi, M.; Bozzo, M.; Miglino, M.; Passannante, M.; Marcolin, R.; Tedone, E.; Colombo, N.; Mangerini, R.; Bo, A.; Ruzzenenti, M.R.; Carlier, P.; Serio, A.; Luchetti, S.; Dominietto, A.; Varaldo, R.; Candiani, S.; Agostini, V.; Ravetti, J.L.; Del Zotto, G.; Marcenaro, E.; Lemoli, R.M.; (2021) Post-Transplant Nivolumab Plus Unselected Autologous Lymphocytes in Refractory Hodgkin Lymphoma: A Feasible and Promising Salvage Therapy Associated With Expansion and Maturation of NK Cells. Front. Immunol. 12:753890.
2) Ansell, S.M.; Hodgkin Lymphoma: 2016 Update on Diagnosis, Risk-Stratification, and Management. Am J Hematol (2016) 91:434–42.
3) Pesce, S.; Greppi, M.; Grossi, F.; Del Zotto, G.; Moretta, L.; Sivori, S.; et al. PD/1-PD-Ls Checkpoint: Insight on the Potential Role of NK Cells. Front Immunol (2019) 10:1242.

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ACE2 up-regulation by MDM2 inhibition reduces inflammation and monocytes adhesion in HUVEC

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Endothelial cells play a pivotal role in vascular homeostasis and innate immunity, modulating inflammation by adapting their activation status and recruiting immune cells. Reduced expression of the cell surface receptor Angiotensin-Converting Enzyme 2 (ACE2) has been identified under inflammatory conditions; therefore, our work aimed to explore a potential treatment to restore ACE2 levels and rescue cells from inflammation. Recent studies have suggested that Murine Double Minute 2 (MDM2), a well-known p53 inhibitor, can control ACE2 ubiquitination. To pursue our goal, we tested nutlin-3a, a known MDM2 inhibitor, to avoid ACE2 degradation and repristinate homeostasis in a model of endothelial inflammation based on Human Umbilical Vein Endothelial Cells (HUVEC) treated with exogenous inflammatory stimuli.

Nutlin-3a at different concentrations was used alone or in the presence of 1ng/mL TNFα or 5ng/mL LPS, and biological events on cell behavior were analyzed at 24 and 48 hours' time points after treatment, with respect to controls. ACE2 protein level was investigated by western blotting and immunofluorescence. In parallel, the impact on cellular proliferation and migration was assessed by xCELLigence real-time assays; cell cycle and apoptosis were evaluated by cytofluorimetric analysis. Protein levels of p53 and MDM2 were assessed by western blotting. For the evaluation of inflammation status, pro-inflammatory cytokines release and monocytes (THP-1) adhesion to cultures were investigated.

Nutlin-3a efficiently upregulated ACE2 protein and induced p53-dependent pathway activation, leading to reduced cell proliferation and cell cycle block without significant apoptosis. These effects were also associated with the inhibition of HUVEC cell migration. Interestingly, nutlin-3a decreased IL-6 release and impaired monocyte adhesion to endothelium.

These findings indicate that MDM2 is an efficient target for anti-inflammatory therapies and nutlin-3a, used as proof-of-concept MDM2 inhibitor molecule, can restore a physiological condition in inflamed endothelium upregulating ACE2 protein level.

Keywords: ACE2, MDM2 inhibitor, Inflammation, Innate immunity

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ER-mitochondria association negatively affects wound healing by regulating NLRP3 activation

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most common causative agent of acute bacterial skin and skin-structure infections (ABSSSI), one of the major challenges to the health system worldwide. Although the use of antibiotics as the first line of intervention for MRSA-infected wounds is recommended, important side effects could occur, including cytotoxicity or immune dysregulation, thus affecting the repair process. Here, we show that the oxazolidinone antibiotic linezolid (LZD) impairs wound healing by aberrantly increasing interleukin 1 β (IL-1β) production in keratinocytes. Mechanistically, LZD triggers a reactive oxygen species (ROS)-independent mitochondrial damage that culminates in increased tethering between the endoplasmic reticulum (ER) and mitochondria, which in turn activates the NLR family pyrin domain-containing 3 (NLRP3) inflammasome complex by promoting its assembly to the mitochondrial surface. Downregulation of ER-mitochondria contact formation is sufficient to inhibit the LZD-driven NLRP3 inflammasome activation and IL-1β production, restoring wound closure. These results identify the ER-mitochondria association as a key factor for NLRP3 activation and reveal a new mechanism in the regulation of the wound healing process that might be clinically relevant.

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NFATc1 regulates cell signaling and mitochondria functionality through the modulation of intracellular cholesterol abundance

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NFAT is a family of five different transcription factors (NFATc1-5), and the first four are activated by the Ca2+/calmodulin-dependent phosphatase calcineurin. Notably, the calcineurin/NFAT signaling pathway, which is fundamental to maintaining normal T-cell physiology, has been found to be deregulated both in B/T-cell lymphomas and leukemias, as well as in solid tumors (i.e prostate, breast and pancreatic cancer). Starting from the evidence of an involvement of NFATc1 in the resistance to glucocorticoid (GC) treatment in T cell acute lymphoblastic leukemia cells, we aimed to unveil the biological processes driven by NFATc1, involved in GC resistance. To achieve this goal, we applied Gene Expression Profile and Nuclear Magnetic Resonance analysis on NFATc1 knockdown cells, and we observed, among the most significant biological processes, the downregulation of the intracellular cholesterol abundance. Additionally, by Chromatin Immune Precipitation we revealed that NFATc1 can directly control the transcription of HMGCS1, EBP and DHCR7, key enzymes of cholesterol biosynthesis process. In addition, since cholesterol is a key component of the plasma membrane lipid raft (LR) elements by immunofluorescence we demonstrated that its downregulation decreases the number of LRs, as well as the anchoring and activation of key proteins and co-receptors such as the lymphocyte-specific protein tyrosine kinase (LCK) and CD4 and CD8, thus impairing the TCR signaling cascade. Finally, 3% of intracellular cholesterol is located into the mitochondria membrane, we wondered whether the decrease in cholesterol levels could impact on mitochondria functionality. Intriguingly, through membrane polarization and mitochondrial ROS production assays, we unveiled a loss of mitochondria functionality as well as an increase in autophagy markers. Transmission Electron Microscopy observations are ongoing to further confirmed these evidences. All together these results reveal a novel role for NFATc1 in which trough the regulation of cholesterol intracellular abundance can modulate the intracellular cell signaling and the mitochondria functionality.

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Dissection of the heterogeneity of peripheral blood and tumor-associated NK cell subsets in Pancreatic ductal adenocarcinoma (PDAC) patients

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Pancreatic ductal adenocarcinoma (PDAC) is the most common and lethal form of pancreatic cancer, known for its rapid progression, early metastasis, and resistance to chemotherapy and radiotherapy, resulting in a dismal five-year survival rate of around 10%. The presence of tertiary lymphoid structures and anti-tumor immune infiltrates correlates with better outcomes in surgically resected PDAC, suggesting the potential benefit of precision immunotherapies based on a deeper understanding of immune cell interactions within the tumor microenvironment.

This study employed multiparametric flow cytometry to analyze conventional NK cells from peripheral blood (PB-NK) and tumor-associated NK cells (TANK) in PDAC patients. Findings revealed a reduction in NKG2A+ and an increase in CD57+ PB-NK cells in early-stage (I-II) PDAC compared to advanced stages (III-IV) and healthy donors (HDs). Advanced PDAC PB-NK cells showed elevated TIM3 receptor expression compared to early-stage PB-NK cells and increased LIR-1 compared to HDs' PB-NK cells.

In 40% of resectable PDAC patients, TANK cells, despite their low abundance, were evaluated. These TANK cells exhibited higher levels of PD1, LAG-3, and CD69, and lower levels of CD57 and CD16 compared to PB-NK cells. Although KIRs were reduced in TANK cells, they were present in both tumor and non-tumor tissues. Further analyses reveled that CD103+/CD49+ tissue resident TANK (trTANK) expressed PD-1, LAG-3 and NKG2A with higher frequency compared to non-tissue resident TANK (ntrTANK).

The study also assessed the expression of ligands for inhibitory NK receptors (including PDL1/2 and HLA-I) and stem cell markers (CD133, CD44, CD24) on PDAC tissue cells.

This analysis, focused on NK cells-PDAC crosstalk, is useful to unveil one of possible PDAC tumor evasion mechanisms from immunosurveillance and for the developing of NK cell-based immunotherapies.

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TGFβ as a novel therapeutic target to restore the Natural Killer antitumor activity in Uveal Melanoma

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Natural Killer (NK) cells are cytotoxic lymphocytes that play a crucial role in controlling tumor growth. However, in cancer patient, NK cells may acquire an immune-tolerant, poorly cytotoxic, and pro-angiogenic decidual-like (dNK-like) phenotype. It has been demonstrated that the polarization of NKs towards a dNK-like phenotype is due to a reprogramming process guided by immunosuppressive factors within the tumor microenvironment, such as transforming growth factor β (TGFβ) [1,2].

Uveal melanoma (UM) is the most common eye adult malignancy. It has been hypothesized that UM exploits strategies to inhibit NK-mediated cytotoxicity, but the mechanisms are not fully elucidated [3].

Here, we conducted a bioinformatic analysis of UM gene expression data obtained from a publicly available dataset and we showed that the overexpression of isoforms of TGFβ is associated with a reduced disease-free survival in patients and may be linked to more aggressive tumors. Accordingly, we confirmed high mRNA and protein levels of TGFβ1 and/or TGFβ2 in UM cell lines 92.1, Mel270 and Mel285 UM.

Our findings demonstrate that the conditioned media (CM) from UM cells induces a decidual-like polarization in NK cells from healthy donors. Indeed, CM-treated NK cells significantly downregulate the expression of the major activating receptors NKG2D, NKp30, NKp46, and DNAM1, whereas CD9 and CD49a decidual markers are upregulated. These phenotypic alterations are matched by a decreased expression of perforin and granzyme-B and by a strong reduction of NK degranulation capabilities against K562 cells. Preliminary results suggest that blocking TGFβ in UM-CM partially rescues the physiological expression of NKG2D and CD9, underscoring the role of TGFβ in UM immunomodulatory effect.

Together, these data indicate that UM modulates NK cell function to foster an immune-tolerant microenvironment and they pave the way for targeting TGFβ as a novel therapeutic strategy.

References

1) Albini, A. and D.M. Noonan, *Decidual-Like NK Cell Polarization: From Cancer Killing to Cancer Nurturing.* Cancer Discov, 2021.
2) Bruno, A., et al., Angiogenin and the MMP9-TIMP2 axis are up-regulated in proangiogenic, decidual NK-like cells from patients with colorectal cancer. FASEB J, 2018.
3) Rossi, E., et al., Uveal Melanoma Metastasis. Cancers (Basel), 2021.

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Trastuzumab-deruxtecan in HER2+ breast cancer cells: complex subcellular mechanisms and immune microenvironment interactions

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Breast cancers (BC) positive for human epidermal growth factor receptor 2 (HER2+) are aggressive with poor survival rates. While therapies targeting ERBB2/HER2, such as Trastuzumab and tyrosine kinase inhibitors, have significantly improved patient outcomes, Trastuzumab-deruxtecan (T-DXd) is now revolutionizing treatment for both ERBB2/HER2+ and HER2-low BC. Despite the remarkable clinical results, the intricate subcellular and molecular mechanisms modulated by T-DXd remain elusive. We investigated the effects of T-DXd on ERBB2/HER2+ BC cells and examined the influence of tumor cell conditioned medium (CM) on macrophage polarization using advanced microscopy, biochemistry, and flow cytometry. We observed a biphasic response in ERBB2 downstream signaling, with transient activation after 2 hours of treatment followed by attenuation at 72 hours. Intriguingly, ERBB2 phosphorylation persisted over time, likely serving an inhibitory role. The extent of ERBB2 internalization was variable among cells but was accompanied by significant DNA damage, highlighting the predominant role of DXd payload in inducing tumor cell death. Unexpectedly, confocal microscopy revealed that T-DXd can also localize directly in the nucleus, within LAMP-1 positive structures. Additionally, electron microscopy showed nuclear autophagosome-like structures and nucleus-cytoplasm connections. T-DXd triggered time-dependent autophagy, modulated energy metabolism and induced extensive mitochondrial network fusion in ERBB2/HER2+ BCa cells, ultimately inducing apoptosis in over 50% of cells. Furthermore, cytokine analysis of CM from treated BCa cells showed enrichment with pro-tumor cytokines (IL-6, IL-8, and TNF-α), potentially contributing to M2 polarization of macrophages. These findings not only demonstrate T-DXd's potent anti-tumor effects but also highlight the complex interplay between tumor cells and the immune microenvironment. The observed M2 macrophage shifting suggests potential challenges in T-DXd therapy, underscoring the necessity for further research to optimize its impact on the tumor microenvironment.

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Intranodular follicle-associated epithelium in rabbit Peyer's Patches

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The specialized follicle-associated epithelium (FAE) of intestinal Peyer's patches (PP) play a pivotal role in the initiation of the mucosal immune responses by providing portals through which luminal antigens interact with the underlying immunological machinery, comprising Antigen-Presenting Cells (APCs) and the various subsets of lymphocytes. To this end, the PP-associated FAE displays complex functional properties, the most notable being the presence of specialized membraneous (M) cells, responsible for the unique morphological and functional features of the FAE compared to the rest of the "conventional" absorptive intestinal epithelium. In this study, we have described the existence of peculiar epithelial structures deeply located within the PP-associated lymphatic nodules in rabbit small intestine PP which we named "intranodular follicle-associated epithelium." Initially, consecutive sections stained with hematoxylin-eosin, allowed the analysis of the spatial development of the follicular epithelium in the formation of intranodular epithelial structures. These structures exhibited two main types of morphology. Indeed, these display either a hollow labyrinth-like or a micropseudocyst-like structure. Their epithelial nature was immunohistochemically confirmed by the expression of cytokeratins, whereas the presence of M cells was ascertained by monitoring the expression of M cell-specific marker vimentin. Further, immunofluorescence allowed the initial characterization of immune cells in relation to these portions of intranodular follicle-associated epithelium. Additional analyses are being carried out, such as the expression of entactin as a basement membrane marker to further characterize these intranodular structures and differentiate them from regular invaginations of the FAE. Currently, the biological relevance of epithelial structure embedded within the lymphoid tissue of the PP remains elusive; however, based on the observations made, hypotheses are formulated regarding the formation of these epithelial arrangements and their functional significance.

Neuroscience and neuromorphology

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Effects of a peripheral nerve injury on the sensorimotor thalamo-cortical synaptic integration of a DYT1 mouse model

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Introduction. Gene-environment interactions may be relevant in the pathogenesis of hereditary forms of dystonia with reduced penetrance, such as DYT1 (DYT-TOR1A), DYT6 (DYT-THAP1), DYT25 (DYT-GNAL). Based on the hypothesis of a "second hit", proposing that the manifestation of dystonia results from the interplay of an intrinsic predisposition and an environmental trigger, a dystonia-like phenotype has been disclosed in genetic rodent models by exposing them to a sensorimotor stressor, the compression of the sciatic nerve. **Materials and Methods.** Here, we examined thalamo-cortical synaptic integration within striatal tissue from Tor1a+/Δgag mice at different time points following sciatic nerve compression. To induce this model of dystonia-like phenotype, we compressed the sciatic nerve of 30-day-old Tor1a+/Δgag mice for either 15 or 30 seconds. The severity of the dystonic phenotype was assessed through the tail suspension test (TST) performed 24 hours before and after surgery, and subsequently on a weekly basis for eight weeks. TST video recordings were analyzed using automated software. **Results.** The analysis of the motor score in the TST for the evaluation of the dystonic phenotype on mice subjected to compression of the sciatic nerve for 15 seconds at the age of 30 postnatal days showed a difference between the genotypes in the recovery of normal postures during the Tail Suspension Test (TST). In fact, we observed a higher frequency and duration of nerve lesion-induced dystonic movements in Tor1a$^{+/\Delta gag}$ mice compared to their wild-type littermates. This difference was particularly evident 4 weeks after the injury. The patch clamp analysis carried out on the animals subjected to compression of the sciatic nerve showed that the thalamo-striatal fibers stimulation, that mimics the signal induced by salient sensory stimuli, evokes a shorter pause and an altered discharge of action potentials in striatal cholinergic interneurons in DYT1 mice compared to wildtype controls. **Discussion.** These results support a relationship between an impaired sensorimotor thalamo-cortical synaptic integration in the striatum and the manifestation of a dystonic-like motor phenotype.

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Unravelling the role of Schwann cell-cancer cell interplay in Hepatocellular carcinoma progression

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In the last two decades, peripheral nerves involvement in cancer growth and metastasis has been well elucidated. More recently, an increasing attention has been dedicated to Schwann cells (SCs), the main glial component of the peripheral nervous system (PNS). These cells are known to make a significant contribution to various types of cancer by actively regulating the tumor microenvironment (TME) and through direct interplay with cancer cells[1]. For example, recent results obtained in our laboratory revealed that SCs-derived paracrine factors enhance malignant traits of intrahepatic cholangiocarcinoma cells in a TGF-β dependent manner[2]. Hepatocellular carcinoma (HCC), the most common primary liver tumor, is one of the leading causes of cancer-related death worldwide. Several studies have highlighted the potential implication of autonomic innervation in HCC onset and progression[3], but the role of SCs has not yet been investigated. Based on these premises, the aim of the current study is to characterize SCs distribution and degree of activation in the TME of HCC, and to uncover molecular mechanisms and biological effects involved in SCs-HCC cells crosstalk. Using an immunohistochemical approach on human healthy liver and HCC tissue sections, we observed a considerable presence of SCs in the tumor capsule and infiltrating cancerous parenchyma. Accordingly, *in vitro* experiments revealed that paracrine signals released by the human hepatoma Hep3B cell line exhibit a chemo attractive action on SC, fostering their migration. Moreover, HCC cells treated with SCs-conditioned medium showed more aggressive features, including activation of the epithelial-mesenchymal transition, increased migratory abilities and morphological alterations, compared to control CM-treated cells. Overall, these results suggest that a better understanding of the PNS participation in HCC, as well as the mechanisms underlying the dialogue between glial cells and tumor cells, will aid in the discovery of new markers and potential therapeutic strategies.

References

1) Deborde S, Wong RJ. The Role of Schwann Cells in Cancer. Adv Biol (Weinh). 2022; 6(9), e2200089.
2) de Franchis, V.; Petrungaro, S.; Pizzichini, E.; Camerini, S.; Casella, M.; Somma, F.; Mandolini, E.; Carpino, G.; Overi, D.; Cardinale, V.; et al. Cholangiocarcinoma Malignant Traits Are Promoted by Schwann Cells through TGFβ Signaling in a Model of Perineural Invasion. Cells 2024, 13, 366.
3) Parent R, Gidron Y, Lebossé F, Decaens T, Zoulim F. The Potential Implication of the Autonomic Nervous System in Hepatocellular Carcinoma. Cell Mol Gastroenterol Hepatol. 2019;8(1):145-148

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Cognitive impairment in is associated with early transcriptome changes in a mouse model of multiple sclerosis

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Cognitive impairment (CI) represents a significant clinical hallmark of multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE), the mouse model of the disease. To investigate the most relevant EAE-associated changes in brain regions relevant for cognition, we sequenced RNA isolated from prefrontal cortex (PFC) samples of control (n=4) and EAE (n=7) mice sacrificed at the peak of the acute phase of the disease. Cytokine-mediated pathways and inflammatory response terms were significantly enriched among the genes differentially regulated (DR) in the PFC of EAE-mice. Heat-map representation of the top 100 DR genes indicated the presence of two EAE-subgroups, one showing more marked differences with the controls and the other displaying lower changes. Thus, we stratified EAE-mice into EAE-L (low immune-reaction/inflammation) and EAE-H (high immune-reaction/inflammation) subgroups. Cell-type-Specific-Expression Analysis (CSEA) of genes upregulated in both EAE subgroups highlighted microglial categories. However, astrocytic genes were significantly enriched only in the EAE-H PFC, consistent with a more advanced stage of inflammation in these mice. Moreover, downregulated genes of the EAE-H group showed highly significant overlaps with genes expressed by cortical neurons, suggesting that high levels of inflammation exert a pronounced impact on neurons. Correlated of gene expression changes with cognitive performances of pre-symptomatic EAE-mice by the Object-in-place test identified a subset of genes that were significantly associated with CI. In particular, the expression of genes belonging to the antigen presentation pathway (*Cd74*, *H2-Ab1*, *H2-Eb1*) and inflammatory genes (*C1qb*, *Saa3*, *Ccl5*) was negatively correlated with cognitive performances. By contrast, no significant correlation between CI and expression of neuronal genes (*Bdnf*, *Homer1*, *Nptx2*, *Pnoc*, *Sst*) was observed. Collectively, these results suggest that increased activation of immune-related and inflammatory pathways in the PFC during EAE leads to progressive functional impairment of this cortical region.

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The chaperone system in glioblastoma multiforme-derived cell lines: Diagnostic and mechanistic implications

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Glioblastoma multiforme (GBM) is the most common and malignant primary brain tumor in adults, for which the development of new treatments capable of counteracting the molecular mechanisms underlying its growth and drug resistance is urgently needed[1]. Members of the chaperone system (CS) are typically cytoprotective but some, named Hsps, can become pathogenic and participate in carcinogenesis.[2] We have studied some Hsps and the VEGF-VEGFR axis[3] in GBM biopsies and derived cell lines with the aim of identifying diagnostic-prognostic biomarkers and gathering information for the development of chaperonotherapy. *Methods*. Primary and secondary cell lines from GBM were stabilized, characterized (morphology, growth characteristics, and specific markers) and preserved. Chaperones (Hsp10, Hsp27, Hsp60, Hsp70, and Hsp90) and angiogenic factors [FLT-1 (VEGFR-1), FLK1 (KDR, VEGFR-2), and FLT-4 (VEGFR-3)] were studied by immunomorphology-immunofluorescence. *Results*. Primary cell lines obtained from four tumor biopsies showed a fusiform or polygonal morphology and high-rate growth. They were positive for astrocyte phenotyping and malignancy criteria, i.e, for astrocyte-specific glial fibrillary acidic protein (GFAP), vimentin (VIM), and proliferating cell nuclear antigen (PCNA); and were negative for neuron-specific enolase (NSE), which characterizes neurons. Primary and secondary cell lines showed high levels of Hsp10, Hsp27, Hsp60, Hsp90, and Flk1; and low levels of Hsp70, Flt1, and Flt4. *Conclusions*. All cell lines showed intense positivity for the chaperones and one angiogenic factor, all at increased levels, making them potential diagnostic-prognostic biomarkers.

References

1) Louis, D.N.; Perry, A.; Wesseling, P.; Brat, D.J.; Cree, I.A.; Figarella-Branger, D.; Hawkins, C.; Ng, H.K.; Pfister, S.M. et al. The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. Neuro Oncol. 2021, 23,1231-1251.
2) Babi, A.; Menlibayeva, K.; Bex, T.; Doskaliev, A.; Akshulakov, S.; Shevtsov, M. Targeting Heat Shock Proteins in Malignant Brain Tumors: From Basic Research to Clinical Trials. Cancers (Basel). 2022, 14, 5435-5457.
3) Rainer, E.; Wang, H.; Traub-Weidinger, T.; Widhalm, G.; Fueger, B.; Chang, J. et al. The prognostic value of [123i]-vascular endothe- lial growth factor ([123i]-VEGF) in glioma. European Journal of Nuclear Medicine and Molecular Imaging. 2018, 45, 2396-2403.

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Acetylated α-Tubulin in *post-mortem* human brain: region-specific changes in Parkinson's disease patients

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Acetylation of α-tubulin is one of the most investigated post-translational modifications of the microtubule cytoskeleton. In neurons, acetylated α-tubulin (Ac-tubulin) has been mainly linked to regulation of morphogenesis, differentiation, and transport; and found along stable, long-lived microtubules in axons. In the last decade Ac-tubulin have been demonstrated to be linked to microtubule physical properties, being responsible for resistance to mechanical stresses and breakages. Interestingly, defective regulation of α-tubulin acetylation emerged to be linked to α-Synuclein pathology in cellular and animal models of neurodegenerative diseases including Parkinson's disease (PD), whose hallmarks are the presence of insoluble α-Synuclein aggregates, called Lewy Bodies (LBs) and the loss of dopaminergic neurons in *Substantia nigra*. With the aim to investigate this interplay in human samples, we investigated Ac-tubulin in *post-mortem* human brains from PD patients (Braak stage 6) and control subjects. First, we analysed different subcortical regions and found that Ac-tubulin accumulates in neuronal soma in PD but not in controls. Then, we analysed α-Synuclein in parallel to Ac-tubulin, to verify if its alteration could be linked to α-Synuclein pathology and LB formation. Taking advantage of immunofluorescence, Proximity Ligation assays and confocal analyses, we found that Ac-tubulin changes correspond to α-Synuclein changes, in terms of aggregation and phosphorylation (Mazzetti et al. 2024). Finally, we moved to Inferior Olivary Nucleus, whose involvement in PD is emerging even if not affected by α-Synuclein pathology. Interestingly, preliminary results indicate an increase of Ac-tubulin positive neurons also in this brainstem region of PD patients.

All together, our data confirm that changes in acetylated tubulin are linked to α-Synuclein pathology and are region-specific in human brain affected by PD neurodegeneration.

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References

Mazzetti, S.*; Giampietro, F.*; Calogero, A.M.*; Isilgan H.B.; Gagliardi, G.; Rolando, C., Cantele, F.; Ascagni, M.; Bramerio, M.; Giaccone, G.; Isaias, I.U.; Pezzoli, G.; Cappelletti, G. Linking acetylated α-Tubulin redistribution to α-Synuclein pathology in brain of Parkinson's disease patients. NPJ Parkinsons Dis. 2024, 10(1), 2

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Locus Coeruleus sexual dimorphism and its impact on cognitive impairment and cortical atrophy in Alzheimer's Disease

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Introduction: Female sex is a significant risk factor for Alzheimer's Disease (AD), following age and the APOE4 allele. Female subjects are more likely to develop AD with age and in a more severe form. Experimental data suggest estrogen may influence the activity and structure of the noradrenergic nucleus Locus Coeruleus (LC) [1], which is notably involved in AD pathogenesis [2]. This study aims to detect sex-related LC morphological dimorphism in cognitively intact (HC) elderlies and AD patients and explore if sex influences the association between LC integrity, cortical volume, and cognitive performance. *Methods:* We included 152 subjects – 53 HC, 70 mild cognitively impaired (MCI) individuals, and 29 AD patients – who underwent High Field Brain MRI with a 3D whole brain morphological sequence and an LC-sensitive sequence. LC images were processed using a standardized template-based approach [3], and cortical volume was extrapolated [3]. Cognitive performance was assessed, exploring memory, visuospatial, and executive functions. *Results:* The diagnostic groups (HC, MCI, and AD) were homogeneous in terms of age, sex, and APOE4 carrier percentage. LC volume and signal intensity were significantly higher in females than males ($p<0.05$) in the HC and MCI groups, but not in AD patients. LC signal correlated with the cortical volume of the inferior, middle, and superior frontal gyri and the superior temporal gyrus in males (rho: 0.40, $p<0.05$), and with the cuneal cortex in females (rho: 0.37, $p<0.05$). No significant sex-related association between LC and cognitive performance was found, although a correlation with cognitive reserve was detected in females (rho: 0.30, $p<0.05$). *Conclusion:* LC volume is larger in females than males, aligning with experimental literature. However, this difference has minor effects on AD pathophysiology, showing only weak associations with cortical atrophy and cognition. Further studies are necessary to fully understand the sex influence on the interplay between LC and AD.

References

1) Bangasser, D. A., Wiersielis, K. R., & Khantsis, S. Sex differences in the locus coeruleus-norepinephrine system and its regulation by stress. Brain research 2016, 1641(Pt B), 177–188.
2) Kelly, S. C., He, B., Perez, S. E., Ginsberg, S. D., Mufson, E. J., & Counts, S. E. Locus coeruleus cellular and molecular pathology during the progression of Alzheimer's disease. Acta neuropathologica communications 2017, 5(1), 8.
3) Galgani, A., Lombardo, F., Frijia, F., Martini, N., Tognoni, G., Pavese, N., & Giorgi, F. S. The degeneration of locus coeruleus occurring during Alzheimer's disease clinical progression: a neuroimaging follow-up investigation. Brain structure & function 2024, 229(5), 1317–1325. https://doi.org/10.1007/s00429-024-02797-1

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Post-mortem study of human glial network in suicide patients affected by psychiatric disorders

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Suicide is a major public health problem today and one of the leading causes of death worldwide. There are numerous risk factors, but psychiatric disorders are certainly one of the most important. In this context, the study of the postmortem brain has attracted considerable attention in recent decades, as alterations in astrocytes have been identified in individuals who died by suicide and suffered from major depressive disorder or schizophrenia. These changes included alterations in astrocyte density, morphology and protein expression. Therefore, a pilot study on human post-mortem brain aimed to investigate astrocyte patterning in the white and gray matter regions of the dorsolateral prefrontal cortex (dlPFC), mostly implicated in regulating motivation and emotion and in mental illness, and of the somatosensory cortex (SS), less involved in behavioral regulation, both in cases of suicide with psychiatric disorders and in control cases (natural deaths in healthy individuals). In addition, a tissue clarification protocol was developed for future studies that would allow 3D analysis of the glial network in the human brain. Cell counting analysis was performed using the protein markers glial fibrillary acidic protein (GFAP) and S100β, as well as protocol setting for aldehyde dehydrogenase-1 L1 (ALDH1-L1), aquaporin 4 (AQP4) and lectin from Lycopersicon esculentum (LEA). GFAP, S100β and ALDH1-L1 enabled the labeling of astrocytes, AQP4 mainly labeled peculiar bushes of astrocytic processes and LEA identified blood vessels. Preliminary results indicated a decreasing trend in the total number of GFAP+ cells in both white and gray matter of the dlPFC, while S100β showed an increasing trend in both regions in suicide cases. These interesting and encouraging results confirm that this is an area of research that should be further investigated to shed light on a topic about which little is known today.

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References

1) O'Leary, L.A.; Belliveau, C.; Davoli, M.A.; Ma, J.C.; Tanti, A.; Turecki, G.; Mechawar, N. Widespread Decrease of Cerebral Vimentin-Immunoreactive Astrocytes in Depressed Suicides. Frontiers in Psychiatry, 2021, 12.
2) Forrest, S. L.; Kim, J. H.; Crockford, D. R.; Huynh, K.; Cheong, R.; Knott, S.; Kane, M. A.; Ittner, L. M.; Halliday, G. M., Kril, J. J. Distribution Patterns of Astrocyte Populations in the Human Cortex. Neurochemical Research, 2023, 48(4), 1222–1232.

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Creation and development of SINEUP to increase the production of RAI1 protein in wild-type fibroblasts and in fibroblasts from patients affected by Smith-Magenis syndrome (SMS): a proof-of-concept study to hypothesize new therapeutic approaches

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Smith-Magenis syndrome (SMS)[1] is a neurodevelopmental disorder caused by a deletion of the 17p11.2 region or a pathogenic variant of the RAI1 gene, which is located within the 17p11.2 region. Various psychiatric and neurological disorders have been reported in patients, including intellectual disability, characteristics related to the autism spectrum, attention deficit and hyperactivity disorder, self-harming and aggressive physical behavior, sleep-wake disorders and convulsions. To date, there is no therapy and treatments are mostly symptomatic, for example the administration of melatonin to regulate the sleep-wake cycle. A possible therapeutic strategy consists in increasing the expression of the target protein, RAI1, to correct haploinsufficiency. However, any excess production of this protein causes a pathological phenotype, Potocki-Lupski syndrome, which is why the increase in RAI1 levels must fall within physiological limits.

SINEUPs[2] are small noncoding antisense RNA molecules made up of two domains: an effector domain capable of recruiting polysomes into the cytosol, increasing translation and a binding domain capable of exclusively binding the mRNA of interest, in our case RAI1. As regards the approach with SINEUPs, the first point of the research has been identifying the most appropriate gene sequences. Then, SINEUPs have been cloned into commercial expression vectors (pcDNA3.1) and delivered into SMS affected fibroblasts. The objective of this phase has been to physiologically increase RAI1 levels. The protein levels of RAI1 have been assessed by western blotting and visualized by immunofluorescence, compared to RAI1 levels in healthy fibroblasts. The second goal will be to evaluate the functional recovery of SMS fibroblasts towards healthy fibroblasts, in relation to lipid metabolism, mitochondrial dysfunctions and alterations of autophagy. All these biological processes are involved in SMS.

If this second objective is achieved, in vivo mouse models will be created, using adenoviral vectors, to hypothesize therapeutic strategies in humans.

This work was supported by Associazione Smith Magenis - ASM17 Italia

References

1) Smith, A.C.; McGavran, L.; Robinson, J.; Waldstein, G.; Macfarlane, J.; Zonona, J.; Reiss, J.; Lahr, M.; Allen, L.; Magenis, E. Interstitial deletion of (17) (p11.2p11.2) in nine patients. Am. J. Med. Genet. 1986, 24(3), 393-414.
2) Espinoza, S.; Bon, C.; Valentini, P.; Pierattini, B.; Matey, A.T.; Damiani, D.; Pulcrano, S.; Sanges, R.; Persichetti, F.; Takahashi, H.; Carninci, P.; Santoro, C.; Cotella, D.; Gustincich, S. SINEUPs: a novel toolbox for RNA therapeutics. Essays Biochem. 2021, 65(4):775-789.

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Treatment with Polyphenols and Probiotics Counteracts LPS-Induced Morphological and Functional Polarization of BV-2 Microglia

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Neuroinflammation is involved in the onset and progression of neurodegenerative diseases and occurs primarily as a result of hyperactivation of glial cells in the central nervous system (CNS). Microglia cells respond to insults through the release of inflammatory mediators such as cytokines and chemokines. Our research focuses on the study of some nutraceutical compounds that promote the morpho-functional switch of microglia toward an anti-inflammatory phenotype. We have recently demonstrated the presence of bioactive compounds in blueberry extracts that can polarize LPS-stimulated BV2 microglial cells toward an anti-inflammatory phenotype. In addition, we considered the role of the gut-brain axis and gut dysbiosis in the induction of pathological processes at the CNS level. Here, we demonstrated that yeast, obtained from *S. cerevisiae* after exposure to electromagnetic millimeter wavelengths, revert LPS-stimulated microglial cells toward an anti-inflammatory phenotype. Microglial polarization was assessed by analysis of cytokine expression, M1 and M2 markers, and antioxidant enzymes by RT-PCR and immunofluorescence assays. Evaluation of cell morphology, analysis of pro- and anti-inflammatory cytokines and key markers of microglial activation (iNOS and ARG-1) demonstrated the ability of the factors analyzed to direct microglial activation toward an anti-inflammatory condition.

Overall, our data suggest that the molecules we have analyzed may drive microglial polarization toward an anti-inflammatory phenotype to counteract the development of chronic inflammatory conditions.

References

1) Armeli, F.; Mengoni, B.; Maggi, E.; Mazzoni, C.; Preziosi, A.; Mancini, P.; Businaro, R.; Lenz, T.; Archer, T. Milmed yeast alters the LPS-induced M1 microglia cells to form M2 anti-inflammatory phenotype. Biomedicines 2022, 2;10(12):3116.
2) De Caris, M.G.; Grieco, M.; Maggi, E.; Francioso, A.; Armeli, F.; Mosca, L.; Pinto, A.; D'Erme, M.; Mancini, P.; Businaro, R. Blueberry counteracts BV-2 microglia morphological and functional switch after LPS challenge. Nutrients 2020, 19;12(6):1830.

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Bio-hybrid scaffolds based on polyvinyl alcohol and decellularized human cartilage for the recovery of articular focal lesions in haemophilic patients

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Haemophilic Arthropathy (HA) is one of the major complications of Haemophilia and is caused by repeated joint bleeding (hemarthrosis) resulting in the intra-articular deposition of iron, which leads to the damage to both the articular cartilage (AC) and subchondral bone [1]. Current therapies can slow down osteochondral damage, but do not stimulate cartilage regeneration. Tissue Engineering can offer valid alternative strategies for the treatment of the AC damage. Therefore, this work investigated the fabrication of bio-hybrid polyvinyl alcohol (PVA) scaffolds, assuring for mechanical support, combined with decellularized human AC to enhance polymer bio-activity. Human AC was harvested from cadaver donors and minced into fragments which underwent decellularization by detergent-enzymatic treatment. The quality of acellular AC was assessed by DNA quantification assay and histological/histomorphometric analyses, confirming that decellularization correctly removed the immunogenic tissue components (i.e., cells, DNA), while preserving the structural biomolecules of the extracellular matrix (i.e., collagen, elastic fibers, glycosaminoglycans). In parallel, PVA hydrogels at two different concentrations (15%, 20%) were investigated by mechanical tests, showing that the almost-instantaneous compressive behavior of PVA varies with the hydrogel concentration. Finally, PVA/AC hybrid supports were fabricated by two methods: a) the mechanical incorporation of the homogenized acellular AC matrix into the polymer or b) the cross-linking of a layer of homogenized and freeze-dried matrix onto the hydrogel to obtain a double-layer support. The ultrastructure of the PVA/AC scaffolds was studied by scanning electron microscopy, revealing different characteristics of roughness and porosity, depending on how the matrix was combined with PVA. The cytocompatibility of the hybrid supports was tested by seeding mesenchymal stem cells on PVA/AC scaffolds and verifying cell growth at 7 and 14 days, with better results obtained on double-layer scaffolds. These data highlight some promising properties of PVA/AC bio-hybrid scaffolds for cartilage regeneration in haemophilic patients with HA.

References

1) Gualtierotti, R.; Solimeno, L. P.; Peyvandi F. Hemophilic arthropathy: Current knowledge and future perspectives. Journal of Thrombosis and Haemostasis, 2021, 19, 2112–2121.

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The replication principle revisited: a shared functional organization between pulvinar-cortical and cortico-cortical connectivity and its structural and molecular imaging correlates

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The pulvinar, the largest nucleus in the human thalamus, is a complex, highly interconnected structure. Through a dense, organized network of cortical and subcortical areas, it provides adequate cooperation between neural systems, which is crucial for multiple high-order functions such as perception, visuospatial attention, and emotional processing. Such a central role is made possible by a precise internal topographical organization, which is mirrored by anatomical connections as well as by the expression of neurochemical markers. While being traditionally subdivided into sub-nuclei, each characterized by distinct connectional and morphological features, recent studies in both primate and human brains have highlighted that this topographical organization only marginally aligns with the conventional histological subdivision. Instead, it has been delineated in the context of continuous gradients of cortical connections along the dorsoventral and mediolateral axes. The characterization of this multi-gradient organization remains relatively underexplored in the human brain. The present work combines high-quality, multi-modal structural and functional imaging data with a recently published whole-brain, large-scale, positron emission tomography (PET) atlas detailing 19 neurotransmitters and receptors distributed across the human brain. By applying diffusion embedding analysis to tractography, functional connectivity, and receptor coexpression data, we identify and characterize multiple topographically organized gradients of structural connections, functional coactivation, and molecular binding patterns. We demonstrate that such gradients converge on a shared representation along the dorsoventral and mediolateral axes of the human pulvinar. This representation aligns with transitions in both structural and functional connectivity, spanning from lower-level to higher-order cortical regions. Moreover, it is paralleled by gradual changes in the expression of molecular markers associated with key neuromodulator systems, including serotoninergic, noradrenergic, dopaminergic, and opioid systems. We contend that our findings mark a significant stride towards a more comprehensive understanding of pulvinar anatomy and function, providing a nuanced characterization of its role in health and disease.

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Ultrastructural evaluation of PC-12 cell line treated in vitro with the pesticide Rotenone

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Rotenone is a commonly used pesticide known for its ability to induce biochemical and histological changes similar to those observed in Parkinson's disease (PD) [1]. These alterations lead to the selective death of dopaminergic neurons in the substantia nigra. The exact mechanism through which rotenone alters the structure and function of neurons remains unclear. The Neuroendocrine Pheochromocytoma Cell Line (PC12) strictly resembles dopamine terminal neurons. They represent an ideal model for analyzing the morphology of central dopamine neurons and predicting neurotoxicity [2]. In this study, we examined the effects of 0.5 μM rotenone for 24-48 h on PC12 cell viability *in vitro*, trying to identify by Transmission Electron Microscopy (TEM) the primary and evident alterations related to neuronal damages similar to that seen in PD animal models. Morphometric evaluation of mitochondria and neuropeptide granules was also performed [3]. Our results showed a reduction of cell viability after 24 h rotenone treatment, with a further decrease after 48 h. Both the control and rotenone-exposed cells did not display major alterations in shape, size, or general organization of the cytoplasm, and only minor changes in subcellular organelles were detected. However, 20-35% of rotenone-treated cells presented ultrastructural changes, mainly evident with the longer time of treatment and strictly associated with increased cell death. Specifically, TEM analysis showed vacuolar degeneration, mitochondrial slight swelling, a decrease in the numerical density of neuropeptide granules, and the loss of cell-to-cell adhesion. These findings align with previous data indicating that rotenone inhibits energy production and increases ROS generation, leading to significant ultrastructural alterations and cell death in PC12. Consequently, our results imply a potential link between rotenone exposure and changes in dopamine metabolism, vesicle formation, and transport suggesting that rotenone may have a significant role in the pathogenesis of PD.

References

1) Betarbet, R.; Sherer, T.B.; MacKenzie, G.; Garcia-Osuna, M.; Panov, A.V.; Greenamyre, J.T. Chronic Systemic Pesticide Exposure Reproduces Features of Parkinson's Disease. Nat. Neurosci. 2000, 3, 1301–1306.

2) Chiaradia, E.; Renzone, G.; Scaloni, A.; Caputo, M.; Costanzi, E.; Gambelunghe, A.; Muzi, G.; Avellini, L.; Emiliani, C.; Buratta, S. Protein Carbonylation in Dopaminergic Cells Exposed to Rotenone. Toxicol. Lett. 2019, 309, 20–32.

3) Belli, M.; Zhang, L.; Liu, X.; Donjacour, A.; Ruggeri, E.; Palmerini, M.G.; Nottola, S.A.; Macchiarelli, G.; Rinaudo, P. Oxygen Concentration Alters Mitochondrial Structure and Function in in Vitro Fertilized Preimplantation Mouse Embryos. Hum. Reprod. 2019, 34, 601–611.

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Drug repositioning as a therapeutic strategy for Spinal Muscular Atrophy treatment

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Spinal Muscular Atrophy (SMA) is caused by the mutation of the survival motor neuron 1 (Smn1) gene. The consequent lack of SMN protein determines motor neuron (MN) impairment, skeletal muscle atrophy, and premature death. Fundamental limitations of current therapies still drive the need for new approaches aimed at increasing functional SMN production. Drug repositioning for SMA treatment represents a reliable tool to address significant unmet therapeutic needs.

A Drosophila-based screening identified GT5 (code name) as a promising therapeutic candidate for SMA. Then, the efficacy of GT5 was tested in vivo (delta7 mice, murine model of severe SMA) and in vitro (patient's iPSCs-derived-MNs and primary SMA fibroblasts and myoblasts). The SMN expression, the neuroprotective and anti-inflammatory effects have been evaluated by immunofluorescence reactions, morphometric analyses and WB assays. Moreover, we assessed the behavioral performance and survival in treated and untreated mice.

We observed that daily subcutaneous administration of GT5 in delta7 mice increased the SMN levels in the spinal cord (≥50%), quadriceps and gastrocnemius (≥1 fold), compared to controls, also leading to improved motor skills. The analysis of the spinal cord ventral horns (lumbar tract) of GT5-treated mice confirmed: i) delayed MN degeneration (≤87%); ii) reduced levels of cleaved-caspase-3 (apoptotic marker) (≤63%), iii) lower neuroinflammation with reduced astrogliosis (GFAP signaling) (≤37%) and different degree of microglia ramification/activation compared with controls. Furthermore, GT5 mice skeletal muscles showed improved trophism and neuromuscular junction phenotypes. In vitro analyses also revealed that GT5 administration significantly prevented iPSC-derived MN degeneration and rescued the impaired formation of myotubes in an MN-myoblast co-culture.

Overall, these results support the GT5 repositioning for the SMA treatment and strengthen the value of this strategy for discovering new therapies for rare diseases.

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References

1) Menduti, G.; Rasà, D.M.; Stanga, S.; Boido, M. Drug Screening and Drug Repositioning as Promising Therapeutic Approaches for Spinal Muscular Atrophy Treatment. Front Pharmacol. 2020, 11, 592234.
2) Januel, C.; Menduti, G.; Mamchaoui, K.; Martinat, C.; Artero, R.; Konieczny. P.; Boido, M. Moxifloxacin rescues SMA phenotypes in patient-derived cells and animal model. Cell Mol Life Sci. 2022, 79, 441.

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Magnetic resonance imaging of neurovascular interactions in Hyperactive Dysfunction Syndromes: a specialist-based inquiry

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Background: Hyperactive dysfunction syndromes (HDS) encompass a spectrum of neuropathies where cranial nerves are pathologically excited due to vascular compressions at their root entry or exit zones. This anatomical anomaly can be pivotal for operative planning and outcome prediction in microvascular decompression (MVD) procedures. Despite the utility of preoperative Magnetic Resonance Imaging (MRI) in visualizing these conflicts, its role in cases with negative MRI findings in symptomatic patients remains under debate.

Materials and Methods: We retrospectively analyzed MRI scans of patients who underwent MVD and their age-matched controls. The study used a blinded and non-blinded review system by three raters, focusing on the identification of neurovascular contact (NVC) involving specific nerves.

Results: Accuracy of NVC identification by specialists varied significantly under different conditions of blinding and awareness of control presence. Initial blind assessments identified 40% of the patients who had NVC confirmed intraoperatively. This recognition improved significantly when the raters were informed about the specific nerve involved, and further increased when no blinding was applied to the presence of neurovascular conflict, suggesting anatomical knowledge significantly influences diagnostic outcomes.

Conclusions: This study underscores the critical role of detailed anatomical imaging in managing symptomatic primary HDS. It highlights the necessity of MVD in managing symptoms effectively, regardless of the MRI findings, and stresses the importance of integrating anatomical insights with clinical strategies to enhance diagnostic accuracy and treatment outcomes.

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Cadmium-induced neuroinflammatory cytokines impairs BBB permeability by altering tight junction morphological localization

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Cadmium (Cd) is a widespread environmental pollutant found in cigarette smoke, traffic and industrial fumes, as well as paints, batteries, and pesticides. It is toxic to many organs including the central nervous system (CNS) by crossing the blood-brain barrier (BBB), leading to inflammation in the brain parenchyma affecting the glial compartment [1]. As previously reported, Cd-induced glial activation leads to cytokines secretion, such as TNF-α, INF-γ, IL-6, and IL-6, thus contributing to CNS homeostasis perturbance [2]. The present research aimed to study the effect of microglial-released cytokines on endothelial cell compartments at different time points in order to better understand the role of pro-inflammatory molecules in BBB permeability. For this purpose, different concentrations of TNF-α (1-100 ng/ml) at different time points (3-48 hours) were used on rat brain endothelial cell line (RBE4). Immunofluorescence staining, western blotting analysis, and Trans-Endothelial Electrical Resistance (TEER, IVTech, Pisa, Italy), were performed. The results clearly demonstrated that TNF-α 10ng/ml induces the NF-κB nuclear translocation after 3h of treatment, thus underlining the transcription factor activation pathway. Furthermore, the cytokine treatment alters the *zonula occludens* 1 (ZO1) distribution on the peripheral edge of RBE4 as early as 10ng/ml after 24-h treatment, showing a more perinuclear localization without a significative decrease of protein expression and no F-actin altered localization. This morphological alteration indicates a putative BBB permeability, confirmed by a TEER decrease with no cell viability alterations in comparison to control, untreated cells.

Our findings highlight the deleterious role of environmental pollutants on the CNS directly affecting BBB permeability and indirectly inducing cytokine release from glial compartment, leading to sustained brain endothelial cells permeabilization.

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References

1) Branca, J.J.V.; Fiorillo, C.; Carrino, D.; Paternostro, F.; Taddei, N.; Gulisano, M.; Pacini, A.; Becatti, M. Cadmium-Induced Oxidative Stress: Focus on the Central Nervous System. Antioxidants 2020, 9(6), 492.
2) Martínez-Hernández, M.I.; Acosta-Saavedra, L.C.; Hernández-Kelly, L.C.; Loaeza-Loaeza, J.; Ortega, A. Microglial Activation in Metal Neurotoxicity: Impact in Neurodegenerative Diseases. Biomed. Res. Int. 2023, 2023, 7389508.

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Diffusion magnetic resonance imaging and connectome of the olfactory system in Parkinson's disease: a systematic review

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Parkinson's disease (PD) is a movement disorder characterized by motor symptoms that manifest clinically, yet neurodegenerative processes likely initiate years earlier. Olfactory dysfunction, affecting 75-90% of PD patients, holds significant predictive value for PD development. Advanced imaging techniques, such as diffusion MRI (dMRI), offer insights into structural changes within olfactory pathways. This systematic review summarizes dMRI findings, elucidating structural correlates of olfactory dysfunction in PD. We systematically searched PubMed and Embase databases and retrieved 37 articles, of which 15 met inclusion criteria and were reviewed in detail. Among the studies investigating diffusion metrics, the most consistent finding was the reduction of fractional anisotropy in the olfactory tract and anterior olfactory structures. The impact of PD on global brain networks has also been highlighted by the reviewed studies, highlighting alterations in the structural integrity and connectivity of white matter networks in PD patients. While some of the reviewed studies might not directly focus on olfaction, the observed reduction in connectivity across various brain regions, such as the olfactory cortex, frontal areas, and the anterior cingulate cortex, point to a generalized network disruption that can potentially affect sensory processing pathways, including olfaction. By providing a comprehensive perspective on the use of dMRI to explore the olfactory connectome in PD, our review might facilitate future research aiming to lead to earlier diagnosis and more targeted therapeutic and neurorehabilitation strategies.

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Dystrophin-glycoprotein complex in epileptic rat brain

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The dystrophyn glycoprotein complex is an important transmembrane proteins system that mediate the interaction with the extracellular environment necessary for a variety of biological functions. The DGC contains protein members such us dystroglycans and sarcoglycans. It is known that this complex plays a key role in nervous tissue even if here the expression and the role of sarcoglycan sub-complex remains unclear. The aim of the present study was to evaluate the expression of sarcoglycan in the hippocampus of rat brain sham and with induced epilepsy to understand if their expression is influenced by this pathological condition. The brains were processed for both histology and immunohistochemistry; anti each sarcoglycan antibody were used. Our results show that all sarcoglycans are expressed at plasmalemma level of neuronal cells of CA1, CA2 and CA3 regions; their fluorescence pattern decreases strongly in CA3 region of epileptic rat's brains. These data suggest a role of these glycoproteins in nervous tissue, probably mediating receptors clustering at post-synaptic membrane level as already suggested [1]. Further investigation to understand the exact function of sarcoglycan in nervous tissue are needed.

References

[1] Cutroneo G., Bramanti P., Ananstasi G., Bruschetta D., Favaloro A., Vermiglio G., Trimarchi F., Di Mauro D., Rizzo G. Sarcoglycans and GABAa receptors in rat central nervous system: an immunohistochemical study. IJAE, 2015, 120 (2): 105-116.

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Structural and functional connectivity predicts MRgFUS thalamotomy outcome in Parkinson's disease

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Magnetic Resonance-guided Focused UltraSound (MRgFUS) thalamotomy has shown efficacy in reducing tremor symptoms in Parkinson's disease (PD). We aimed to determine if the connectivity between the ablation target and other brain areas predicts treatment outcomes better than the target site itself. We analyzed structural and functional connectivity associated with successful MRgFUS ablation of the Ventral intermediate thalamic nucleus (Vim) to predict treatment outcomes We assessed how ablation features (volume- and distance-based) correlated with clinical outcomes and combined our tremor-dominant PD dataset with normative human connectome data. This included diffusion tractography and resting-state functional connectivity to identify connectivity networks linked to clinical improvement. We employed leave-one-patient-out cross-validation to predict clinical outcomes based on these connectivity profiles. Additionally, we analyzed cortical thickness correlation networks before and after treatment and used positron emission topography (PET) imaging data related to dopamine neurotransmission. Standard ablation features did not significantly correlate with clinical outcomes (all $p>0.05$). However, connectivity between the ablation area and a network of brain regions, including structural connectivity to pre-supplementary and supplementary motor areas, superior frontal gyrus, and cerebellum, correlated with clinical improvement. Functional connectivity patterns, including anticorrelation between the ablation area and primary somatosensory cortex and lateral primary motor cortex, also correlated with outcomes. Cross-validation showed that structural ($R^2 = 0.53$, $p = 0.002$) and functional connectivity ($R^2 = 0.23$, $p = 0.007$) profiles predicted clinical improvement. etwork analysis suggested increased network segregation post-treatment, indicating specialized local processing. Furthermore, dopamine uptake showed positive correlations with both functional ($R = 0.59$, $p = 0.01$) and structural networks ($R = 0.35$, $p = 0.04$) in the basal ganglia, but not in the cerebral cortex or cerebellum (all $p>0.05$). Our results suggest that both structural and functional connectivity of the target area are independent predictors of clinical improvement in tremor-dominant MRgFUS-thalamotomized PD patients.

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Diagnostic potential of combined skin morphometric analysis and salivary alpha-synuclein oligomers in Parkinson's Disease

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Oligomeric alpha-synuclein (α-syn) in saliva and phosphorylated α-syn deposits in the skin have emerged as promising diagnostic biomarkers for Parkinson's disease (PD). However, none of the previous studies have evaluated the diagnostic potential of not phosphorylated α-syn and different other cutaneous morphological parameters, including the aggregation of α-syn in melanocytes and the variations in collagen in both derma and epidermis. This study aimed to assess and compare the diagnostic value of these skin morphometric aspects in discriminating between 16 PD patients and 9 healthy subjects (HSs) and to correlate them with the presence of α-syn oligomers in saliva. Salivary oligomeric α-syn levels were quantified using competitive ELISA, while skin biopsies were analysed through immunofluorescence to detect not phosphorylated α-syn. α-syn oligomers were accurate in discriminating PD patients from HSs as previously reported. Skin biopsies have shown a significant reduction of α-syn positive fibres in PD in comparison to HSs (AUC 0.73 – $p<0.05$), while both collagen area of staining and α-syn positive melanocytes were significantly increased in the skin of PD patients in comparison to HSs. In particular, collagen staining area was particularly higher in PD patients in derma and around sweat glands, reaching an optimal diagnostic power in differentiating PD patients from HSs (AUC 0.80 – $p<0.001$). The number of α-syn positive melanocytes was also significantly increased in the skin of PD patients in comparison to HSs, disclosing a sub-optimal diagnostic potential (AUC 0.70 – $p<0.05$). The correlations between concentrations of monomeric and oligomeric salivary α-syn and the parameters obtained from the quantification of skin biopsies were studied. Our study suggests that salivary and skin biomarkers may reflect different aspects of PD pathology, thus providing complementary information when combined. Moreover, it highlight the potential of utilizing a multimodal morphometric analysis of the skin to enhance diagnostic accuracy in PD.

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Radioactive-free staining and automated tissue procedure in Serial Block Face Scanning Electron Microscopy (SBF-SEM)

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Serial block face scanning electron microscopy (SBF-SEM) provides an automated three-dimensional mesoscale acquisition of the specimen with ultrastructural resolution.

However, SBF-SEM usage remains limited due to its labor-intensive processes. In fact, en bloc staining, dehydration and resin embedding are still largely manual procedures.

Here we compared the ultrastructural contrast and the resolution achieved with the gold standard and manually-performed en bloc staining procedure (1) with two en bloc stainings: the first used the radioactive-free lantanides (2), the second the standard molecular weight specimen processed with an automated tissue processor, which increases the throughput and reproducibility of specimen staining.

We analyzed biological structures of two different regions of the peripheral nervous system, caudal nerves and dorsal root ganglia, selected because their histological structure is remarkably different and, therefore, they allow a more extensive comparison providing images with different technical features and compartments for volume representation (Arivis Vision 4D Pro imaging software). Cubic microns of imaging data were achieved with a Zeiss Gemini SBF-SEM 360 equipped with Volutome

The automated tissue processing ensured homogeneous contrast enhancement within each sample and staining reproducibility across the same batches. Good-quality images were obtained with low contrasted specimens by using higher electron doses than the values suggested by manufacturers and other studies and minimizing the resulting charging effects with local discharging with the focal charge compensation tool. Acquired micrographs ensure fine resolution and 3D view of the biological structures of interest for moderate and extremely charge-prone specimens.

We demonstrated a valuable new pathway for ultrastructural analysis of bulk specimens suggesting the advantages of using the automated sample processor technology and uranyl-free staining which can compete with gold standard staining techniques for large structures analysis.

References

(1) Deerinck, T.J. et al. NCMIR methods for 3D EM: A new protocol for preparation of biological specimens for serial block face scanning electron microscopy. Nat Center Microsc Imag Res. 2010, 6-8.
(2) Moscardini A et al. Uranium-free X solution: a new generation contrast agent for biological samples ultrastructure. Sci Rep 2020,10(1):11540. doi: 10.1038/s41598-020-68405-4.

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Long-term effects of SARS-CoV-2 infection in patients with and without chemosensory disorders at disease onset: a psychophysical and magnetic resonance imaging exploratory study

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A preserved sense of smell and taste allows us to understand many environmental "messages", and results in meaningfully improvement to quality of life. With the COVID-19 pandemic it became clear how important these senses are for social and nutritional *status* and catapulted this *niche* chemosensory research area towards widespread interest. In the current exploratory work, several months later the onset, we assessed two groups of post-COVID-19 patients who reported having had (Group 1) or not (Group 2) a smell/taste impairment at the disease onset. The aim was to compare them using validated smell and taste tests (UPSIT smell identification, Taste Strips Test) as well as brain magnetic resonance imaging volumetric analysis. Eight patients *per* group were hospitalized at the COVID-19 onset. All the other participants had an overall mild disease course that did not require hospitalization. All patients underwent detailed clinical neurological examination and none of them showed neurological deficit. Normative data were used for smell scores comparison. A pool of healthy subjects, recruited before the pandemic, served as controls for taste scores. The majority of patients in both groups showed an olfactory impairment, which was more severe in Group 1 (median UPSIT scores: 24.5 Group 1 vs 31.0 Group 2, p= 0.008), particularly amongst women (p=0.014). No significant differences emerged comparing taste scores between Group 1 and Group 2, but regarding qualitative disorders, dysgeusia was only present in Group 1 patients. However, for taste scores, a significant difference was found between Group 1 and controls (p=0.005). No MRI anatomical abnormalities emerged in any patients while brain volumetric analysis suggested a significant difference among groups for the right caudate nucleus (p=0.028), although this was not retained following Benjamini-Hochberg correction. This exploratory study could add new information in COVID-19 chemosensory long-lasting impairment and address future investigations on the post-COVID-19 patients' research.

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Serine metabolism in the post-mortem caudate-putamen of Parkinson disease (PD) patients

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Atypical D-amino acids, D-serine (D-Ser) and D-aspartate (D-Asp), regulate synaptic transmission/plasticity by activation of glutamatergic ionotropic N-methyl-D-aspartate (NMDA) receptors (NMDAR). However, the role and possible alterations in D-amino acid metabolism in aging and neurodegenerative disorders is still debated, producing either harmful or beneficial effects depending on the neuropathological context.

D-ser, an endogenous obligatory co-agonist at the glycine modulatory site on GluN1 subunit of NMDAR, might be involved in the neurotoxic theory of the neurodegeneration based on the evidence of high D-ser levels in Parkinson's disease (PD) and other neurodegenerative disorders (MND and AD).

In contrast to its D-enantiomer, L-Ser regulates a plethora of crucial metabolic processes in the central nervous system (CNS), therefore a deficiency in L-ser levels compromises brain development and functioning. Evidence suggests that increase of both D-Ser and L-Ser (observed in the caudate/putamen (CPu) of PD patients) may represent an adaptive biochemical event aimed at contrasting the ongoing degeneration of midbrain dopaminergic neurons. To understand the role of the CNS cells in the D/L aminoacid homeostasis and its specific role in the neurodegenerative process, we aim to analyze morphological changes of post-mortem CPu of PD patients at different Braak stages and normal healthy control subjects (HCS). Specifically, we will study the expression and the localization of key enzymes of the serine metabolism in the brain (serine racemase - SRR, D-amino acid oxidase - DAAO, 3-phosphoglycerate dehydrogenase – PHGDH) using immunohistochemistry (IHC). Consecutive human CPu sections will be processed for IHC) and stained for morphological analysis of neuronal (GAD/vGAT/DARPP32) and glial markers (GFAP, Iba1). Multiparameter and high-resolution microscopy imaging will be performed for all the markers.

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Cellular and Extracellular plasticity at the spinal Neurovascular Interface following nerve injury

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The peripheral nerve injury (PNI) represents a peculiar model for direct interference with the spinal cord circuitry without violating the integrity of the Central Nervous System (CNS). The lesion of the sciatic nerve with sural sparing (SNI) combines functionally a motor axotomy of the ventral horn and a transsynaptic deafferentation of the dorsal horn in the lumbar spinal cord. The neurovascular interface (NVI) represents our region of interest with morpho-molecular interactions on multiple cellular and extracellular levels. We analyzed the early spinal NVI (sNVI) changes following SNI in a rat model. We considered the sNVI within the afferent and efferent compartments of the lumbar spinal cord at 1, 2, and 7 days. The multiple time points revealed a pleiotropic effect of the coagulation protein thrombin and its associated receptor PAR-1. PAR-1 is firstly localized on neurons and in sparse perivascular networks. Following the damage, the receptor is clustered in rafts close to the astrocytic endfeet. Matrix Metalloproteinase 9 (MMP9) can cleave and activate the same receptor. Furthermore, MMP9 profoundly reshape the spinal extracellular matrix (ECM). In particular, at the basal lamina level, we observe profound protein changes corroborating the involvement of the sNVI in maladaptive plasticity. The timely upregulation of the MMP9 target has been coherently observed through Immunohistochemistry, RNAseq, and RNAscope techniques. Moreover, the early changes of the sNVI include differential expression of tight junctions, and channel proteins observable only using protein-assessment, while their transcription is unaffected. The delocalization of astrocytic Aquaporin4 (AQP4) and Connexin43 (Cx43) hemichannels or gap-junctions is accompanied by an increasing invasion of microglial/macrophagic elements. The dorsal and ventral horns of the spinal cord showed different characteristics in response to the different inflicted damage. Our data widen the field of sNVI involvement in the damage response of the spinal cord. In conclusion pathophysiology of the maladaptive changes is observed in the vascular compartment, while the CNS integrity is not compromised.

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HO-1 targeting to counteract glioblastoma progression

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Glioblastoma multiforme (GBM) is classified as grade IV astrocytoma and represents the deadliest brain cancer, affecting adults with poor prognosis [1]. The uncontrolled cell proliferation characterizing GBM induces the formation of hypoxic niches inside the cancer core. Inducing hypoxia-inducible factors (HIFs) transcription, the hypoxic microenvironment activates different signaling cascades, making the tumor highly aggressive. The specific transcription of HIF-1α regulates many downstream target genes, including vascular endothelial growth factor (VEGF), the main factor responsible for aberrant neovascularization characterizing GBM progression. Besides this, hypoxia also modulates gene expression of many other factors, with consequent upregulation of protective enzymes, such as human heme oxygenase 1 (HO-1). Recent emerging evidence has reported aberrant levels of HO-1 in different human cancers, including GBM, whose overexpression is linked to a poor prognosis [2]. Therefore, targeting HO-1 could be suggested as a novel strategy to improve cancer cell sensitivity to conventional pharmacological approaches.

In the present work, we aimed to investigate the role of HO-1 in hypoxia-driven GBM progression by testing the effect of the new synthesized azole-based HO-1 inhibitor (VP18/58) on the hypoxia-triggered pathway. In the present study, we used two human glioblastoma cell lines, U87MG and A172, characterized by different tumorigenic potential, exposed to a hypoxic mimetic agent, deferoxamine (DFX) for 24h. Results have demonstrated that hypoxic conditions induced upregulation and nuclear expression of HO-1 in U87MG and its faint immunoreactivity was detected exclusively in the cytoplasm and perinuclear area of A172.

Moreover, our data demonstrated that the HO-1 inhibitor counteracted GBM progression by modulating the HIFα/HO-1/VEGF signaling cascade in cancer cells bearing more malignant phenotypes.

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References

1) Wesseling, P.; Capper, D. WHO 2016 Classification of gliomas. Neuropathol. Appl. Neurobiol. 2018, 44, 139–150.
2) 31. Ghosh, D.; Ulasov, I.V.; Chen, L.; Harkins, L.E.; Wallenborg, K.; Hothi, P.; Rostad, S.; Hood, L.; Cobbs, C.S. TGFβ-Responsive HMOX1 Expression Is Associated with Stemness and Invasion in Glioblastoma Multiforme. Stem Cells 2016, 34, 2276–2289.

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Identification of archaic introgression tuning the functionality and connectivity of the human brain

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Phenotypic variations across worldwide populations reflect the widespread impact of human evolutionary trajectories, including processes related to natural selection and demographic history. So far, the introgression from Neanderthals and Denisovans contributes to the human phenotypic spectrum. Signatures of archaic introgression have been reported for hair and skin pigmentation, immunity, neoplasms and metabolic traits, and male sterility. Surprisingly, Neanderthal-derived variation is enriched for associations with neuropsychiatric phenotypes, suggesting that there are traces of this archaic species that shape the present-day human brain.

Our project investigates how archaic introgressions contribute to the human neurobiological traits across different ancestry groups. Specifically, leveraging large-scale cohorts, we are exploring how archaic introgression and other evolutionary processes contributed to the phenome and connectivity evolution of the brain.

We are leveraging association data for 3,935 brain imaging-derived phenotypes (IDPs), and data pertaining to Mental Health (MH), Drug Prescription impacting the Nervous System (FUNCT), International Classification of Diseases 10 (ICD) F and G code clusters from UK Biobank, which are able to characterize more than 3,000 participants.

An integrated multipronged approach involving association data for archaic introgressed and present-day specific variants from pan-ancestry analyses, latent causal variable (LCV) analysis, Mendelian Randomization (MR), and colocalization among IDPs, MH, FUNCT, and ICDs, and brain transcriptomic regulation, will implement a multi-trait colocalization approach assessing the shared liability of brain phenotypes and prioritizing causal variants. After colocalized traits and the shared causal variants were identified, we also plan to extract expression quantitative trait loci (eQTL) statistics from the GTEx v.8 to test their colocalization with brain-specific transcriptomic regulation.

The newly developed analytical approach will permit us to reveal the causal relationships across complex brain traits that are challenging to assess using traditional randomized controlled trial design by investigating systematically complex traits pleiotropy with brain structures and functions.

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The role of primary cilia in human cerebral cortex development and vascularization

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Primary cilia are present in the developing brain, implicated in transduction of several signaling pathways essential during cerebral cortex development. Primary cilia have been described on the apical membrane of ventricular progenitor cells, bathed in the cerebrospinal fluid rich in morphogens and growth factors. At this site, primary cilia sense and transduce a variety of signals which control cell precursor proliferation, as well as migration of differentiating neurons and glial cells. Moreover, during brain vascularization, also endothelial cells (ECs) are characterized by apical cilia specialized in mechanosensing and seem to be involved in stabilization of the neo-formed vessels, which is a critical phase associated to pre-term intraventricular hemorrhage (IVH). Interestingly, previous studies on endothelial cilia in zebrafish and mouse brain have unexpectedly revealed endothelial cilia not only on the apical, but also on the basolateral endothelial surfaces. These results have been confirmed with ciliary markers that co-localize with PDGF-BB ligand in primary human brain microvascular ECs (hBMECs) and the visualization of cilia on both luminal and abluminal side. In the present study we have investigated the presence and subcellular localization of EC primary cilia during brain development in a 22-week-old human fetus, using cell-specific markers of ECs and pericytes and ARL13B as a ciliary marker, by immunofluorescence confocal microscopy. The results show ARL13B-stained primary cilia of radial progenitor cells at the ventricular surface, together with cilia of intermediate progenitors in the ventricular zone (VZ) and subventricular zones (SVZ). The periventricular vascular plexus that lies at the VZ/SVZ border, is revealed by endothelial CD31 and helps in the identification of the endothelial luminal and abluminal sides. On double CD31/ARL13B immunostainings, EC primary cilia are detected on both apical and basal endothelial surfaces. During differentiation of the blood-brain barrier (BBB) endothelial phenotype and assembly of neurovascular unit (NVU) cells, the abluminal EC cilium-associated PDGF-BB ligand fulfills autocrine and juxtacrine (endothelium-pericyte) roles and promotes vascular BBB function and NVU stability.

References

1) Thirugnanam K, Prabhudesai S, Van Why E, Pan A, Gupta A, Foreman K, Zennadi R, Rarick KR, Nauli SM, Palecek SP and Ramchandran R. Ciliogenesis mechanisms mediated by PAK2-ARL13B signaling in brain endothelial cells is responsible for vascular stability. Biochemical Pharmacology. 2022, PMID: 35700757.
2) Zhang H, Chalothorn D. & Faber J.E. Collateral Vessels Have Unique Endothelial and Smooth Muscle Cell Phenotypes. Int J Mol Sci. 2019, 20.

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Schwann cells and melanoma progression: bystanders or promoters?

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In the last few years nerves are gaining attention for their role in cancer, as researchers discover their connection to metastasis and poor prognosis. It is well established that cancer cells rely on the recruitment and interaction with various non-malignant cells to support tumor growth, creating what is referred to as the tumor microenvironment often compared with a nonhealing wound. Schwann cells are glial cells of the peripheral nervous system found in virtually every organ of the body and therefore serving as an early witness of the emerging tumor. After nerve injury, Schwann cells drive and support axonal growth in regenerating nerves. Recent studies suggest that the plasticity of Schwann cells intended to assist with nerve repair is utilized by melanoma to help create conditions favorable to tumor growth and progression. In animal models Schwann cells adjacent to melanoma tumors demonstrate a functional switch to a repair-like immunosuppressive phenotype (Kruglov et al., 2023).

We stained tissue sections from human melanoma samples for glial markers (p75NTR, GAP43 and S100b). While Schwann cells are rare around the margins of benign nevi, they appear in abundance in the peritumoural areas of melanomas. In coculture experiments the proliferation of human melanoma cell lines is only slightly modified by secreted factors produced by human Schwann cells consistently with previous reports (Shurin et al., 2019). In addition, Schwann cells promote the migration of human melanoma cells. These data are in accordance with our group's recent observations obtained in cholangiocarcinoma (de Franchis et al., 2024).

References

1) Kruglov O.; Vats K.; Soman V.; Tyurin V.A.; Tyurina Y.Y.; Wang J.; Williams L.; Zhang J.; Donahue Carey C.; Jaklitsch E.; Chandran U.R.; Bayir H.; Kagan V.E.; Bunimovich Y.L. Melanoma-associated repair-like Schwann cells suppress anti-tumor T-cells via 12/15-LOX/COX2-associated eicosanoid production. Oncoimmunology. 2023, 12, 2192098.
2) de Franchis V.; Petrungaro S.; Pizzichini E.; Camerini S.; Casella M.; Somma F.; Mandolini E.; Carpino G.; Overi D.; Cardinale V.; Facchiano A.; Filippini A.; Gaudio E.; Fabrizi C.; Giampietri C. Cholangiocarcinoma Malignant Traits Are Promoted by Schwann Cells through TGFβ Signaling in a Model of Perineural Invasion. Cells, 2024, 13, 366.

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Poly-ubiquitin and p62 exceed the increase of alpha-synuclein during degeneration of catecholamine cells induced by methamphetamine

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Alpha-synuclein (alpha-syn) is routinely assumed to be the primary component and seeding molecule of intracellular inclusions known as Lewy bodies (LBs), which develop within diseased catecholamine neurons in a number of degenerative disorders including Parkinson's disease (PD). In addition, alpha-syn aggregates are induced by methamphetamine (METH). To date, no study has provided a quantitative assessment of alpha-syn *in situ* nor the amount of alpha-syn compared with other key proteins or non-protein structures, neither following METH exposure nor in LBs.

In this study, we profited from an *in vitro* model, consisting of a catecholamine cell line, to assess within METH-induced cytopathology the amount of alpha-syn and other proteins involved in the pathology of PD. Immunofluorescence at light microscopy and immunogold at transmission electron microscopy (TEM) were used to measure the amount of alpha-syn, p62/sequestosome, ubiquitin and poly-ubiquitin. We found that p62 and poly-ubiquitin are more densely packed compared to alpha-syn within METH-induced pathological cell domains. Specifically, small seed-like areas, where the highest concentrations of p62 and poly-ubiquitin were assessed, correspond to regions rich in tubulo-vesicular structures. These correspond to autophagosome-like vesicles where shuttling of ubiquitinated autophagy substrates occurs via p62. The prevalence of other proteins compared with alpha-syn was unexpected, although it was evident only at TEM where the stoichiometry quantitative amount of these proteins was calculated. In contrast, light microscopy did not provide this evidence. These findings provide new insights about proteins involved in catecholamine cell damage and challenge the routine assumption that alpha-syn prevails and precedes other proteins.

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Calretinin in the human brain. A preliminar report

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Calretinin (CR) is an intracellular calcium binding protein of the EF-hand family, firstly described in the chick retina. CR is composed by 261–271 amino acids. CR and Calbindin D28k present 58% of the same amino acids residues. In the brain of mammals CR is widely expressed in the neuronal cell bodies and processes and is localized in spines, dendrites and somata. In neurons CR contributes to calcium homeostasis and in synaptic plasticity mechanisms in the asymmetrical and symmetrical synapses. Neuropathological studies suggest a role of CR in neurologic, psychiatric and neuropsychiatric disorders (e.g. Alzheimer's disease, epilepsy, Parkinson's disease, schizophrenia, bipolar disorder, attention deficit hyperactivity disorder) and in some rare brain disorders (e.g. Huntington's disease, spinocerebellar ataxias). Currently, non-exist a detailed CR human brain mapping. Therefore, the aim of this study using an immunohistochemical approach, is to carry out a detailed distribution of CR in the adult human brain and in particular, in the areas of the central nervous system (CNS) involved in brain disorders. The study was carried out on postmortem fragments of human brains fixed in neutral buffered formalin, embedded in paraffin, cut into 4-5 μm sections and subjected to light microscopic immunohistochemistry with mouse polyclonal antibody for CR. For positive controls were used fragments of human mesothelioma subjected to the same experimental procedure. CR-immunoreactivity was observed in the gray matter cell bodies and processes of neurons and astrocytes, in the white matter in bodies and processes of oligodendrocytes of different regions of the adult human brain (e.g. midbrain, cerebellum, thalamus, basal ganglia, neocortex gyri). Moreover, CR immunoreactivity was also observed in microgliocytes variously distributed in the human brain nervous tissue. Therefore, the results of this immunohistochemical study indicate a role of CR in neurotransmission and gliotransmission mechanisms. Furthermore, CR functional mechanisms could be damaged in brain disorders.

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NF-kB Regulation and the Chaperone System Mediate Restorative Effects of the Probiotic *Lactobacillus fermentum* LF31 in the Small Intestine and Cerebellum of Mice with Ethanol-Induced Damage

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Probiotics are live microorganisms that yield health benefits when consumed, generally by improving or restoring the intestinal flora (microbiota) as part of the muco-microbiotic layer of the bowel. In this work, mice were fed with ethanol alone or in combination with the probiotic Lactobacillus fermentum (L. fermentum) for 12 weeks. The modulation of the NF-κB signaling pathway with the induction of Hsp60, Hsp90, and IkB-α by the probiotic occurred in the jejunum. L. fermentum inhibited IL-6 expression and downregulated TNF-α transcription. NF-κB inactivation concurred with the restoration of the intestinal barrier, which had been damaged by ethanol, via the production of tight junction proteins, ameliorating the ethanol-induced intestinal permeability. The beneficial effect of the probiotic on the intestine was repeated for the cerebellum, in which downregulation of glial inflammation-related markers was observed in the probiotic-fed mice. The data show that L. fermentum exerted anti-inflammatory and cytoprotective effects in both the small intestine and the cerebellum, by suppressing ethanol-induced increased intestinal permeability and curbing neuroinf lammation. The results also suggest that L. fermentum could be advantageous, along with the other available means, for treating intestinal diseases caused by stressors associated with inflammation and dysbiosis.

Keywords: gut microbiota; gut-brain axis; *Lactobacillus fermentum*; ethanol; chaperone system; heat shock proteins.

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Benzo[a]pyrene impairs the migratory ability of human GnRH neuroblasts through the inhibition of RhoA/ROCK pathway

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There is increasing evidence that environmental pollutants like polycyclic aromatic hydrocarbons (PAHs) and heavy metals may act as endocrine disruptors, negatively affecting various physiological functions, including reproduction. In particular, both pollutants may impact the neuroendocrine circuits of the reproductive axis, with the hypothalamic gonadotropin-releasing hormone (GnRH) system being the crucial target. Our previous research using human fetal GnRH neuroblasts (FNCB4) demonstrated that treating cells with benzo(a) pyrene (BaP, 10μM, 24h), a representative PAH, interferes with their migratory properties and therefore maturation of GnRH neurons [1]. Here, we extended our studies with the main purpose of clarifying the mechanisms through which BaP affects FNCB4 migration. In the gene expression profile analysis, performed with the RNA-seq technique, we identified 2,324 differentially expressed genes (DEGs) in BaP-treated compared to untreated cells, including 1,128 up-regulated and 1,196 down-regulated. Reactome enrichment analysis indicated that BaP exposure induced significant changes in genes involved in cell motility pathways, such as Rho GTPase signaling, semaphorin interactions, ECM proteoglycans, integrin and non-integrin cell surface interactions. Interestingly, we found 31 DEGs that are related to RhoA/ROCK pathway, an important signaling implicated in cell adhesion, cytoskeletal remodeling and migration, especially in neurons. To better investigate the BaP mechanism of action and confirm the implication of RhoA/ROCK pathway, we analyzed the subcellular localization of the small GTPase RhoA in FNCB4. Immunofluorescence analysis showed that BaP exposure inhibited RhoA membrane translocation and, therefore, its activation, thus compromising the downstream signaling. Interestingly, exposing cells to cadmium (Cd, 10μM, 24h), a widespread pollutant belonging to the heavy metal category, also affected FNCB4 cell migration without interfering with RhoA membrane translocation. In conclusion, our findings suggest different molecular mechanisms of action for the two pollutants and identified the alteration of the RhoA/ROCK pathway as a possible mechanism through which BaP affects GnRH neuron development.

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References

1) Guarnieri, G.; Becatti, M.; Comeglio, P.; Vignozzi, L.; Maggi, M.; Vannelli, G.B.; Morelli, A. Benzo[a]pyrene impairs the migratory pattern of human gonadotropin-releasing- hormone-secreting neuroblasts. Eur J Histochem. 2021, 65, 3282.

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BRAFV600E mutation and PTEN deletion in neural stem precursor cells give rise to glioma and neurofibromatosis

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The RAS/BRAF/MEK/ERK pathway is highly mutated in cancer, with mutations of the *BRAF* gene accounting for about 7% of cancers. The most frequently observed BRAF mutation is the V600E, that induces persistent activation of BRAF, inhibiting its inactivation and leading to a continuous pathway stimulation.

In the nervous system, BRAFV600E mutation has been found among low-grade glioma, pediatric[1] oligodendroglioma-like tumors, pediatric glioblastoma, and adult epithelioid glioblastoma[2], as well as in peripheral nervous system tumors[3].

To understand if BRAFV600E mutation can drive nervous system tumor formation, we developed a mouse model in which BRAFV600E along with *Pten* mutations are driven by the Tamoxifen-inducible Sox2-CreER, a deleter specifically active in Neural Stem/Progenitor Cells (NSPC). We observed that Pten deleted and BRafV600E mutated central NSPCs are prone to transform into low grade gliomas while peripheral NSPCs transform into paraspinal plexiform neurofibromas and MPNSTs. To prove that NSCPs were the tumor cells of origin we specifically deleted *Sox2* in these cells by crossing BRaf/Pten mice with conditional $Sox2^{loxP/loxP}$ mice. None of the Sox2-deleted BRaf/Pten mice developed tumors compared to Sox2-wildtype BRaf/Pten mice.

In vitro analysis on BRaf/Pten mutated NSPCs revealed that these cells show increased proliferation and preferentially differentiate towards oligogendroglial-like fate.

Moreover, BRaf mutated NSPCs show increased Sox2 protein levels, compared to wildtype. RNA-seq and transcript analysis revealed that Sox2 mRNA levels, instead, are equal between mutated and wildtype NSPCs, suggesting that Sox2 increased protein levels are due to its stabilization in the BRaf mutated genotype. In support of this observation, in BRaf mutated NSPCs, Sox2 protein levels do not change following proteasome degradation and translation inhibition. In addition, we observed a biochemical interaction between Sox2 and BRafV600E, suggesting that BRafV600E mutation might be responsible for Sox2 protein stabilization, and this could be the mechanism through which BRaf mutated NSPCs drive tumor formation.

References

1 Jones DT, et al. Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. Cancer Res (2008) 68(21):8673–7.
2 Dodgshun AJ, et al.. Disseminated glioneuronal tumors occurring in childhood: Treatment outcomes and BRAF alterations including V600E mutation. *J Neurooncol* (2016) 128(2):293–302.
3 Kaplan H.G. Vemurafenib Treatment of BRAF V600E-Mutated Malignant Peripheral Nerve Sheath Tumor JNCCN (2013) 11 (12): doi.org/10.6004/jnccn.2013.0173

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Exploring Biomarkers in Autosomal Dominant Leukodystrophy: Insights from stem cell differentiation and cerebrospinal fluid metabolomics

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Autosomal Dominant Leukodystrophy (ADLD) is an ultra-rare and fatal late-onset neurodegenerative disorder that affects the central nervous system myelination and lacks effective therapy. The disease is caused by lamin B1 (*LMNB1*) gene alteration that leads to demyelination with the disease mechanisms remaining unknown. Although oligodendrocytes are responsible for myelination, astrocytes and ADLD patients' cells overexpressing *LMNB1* have displayed nuclear alterations with activation of proinflammatory and oxidative stress mechanisms that were absent in oligodendrocytes[1,2]. The present study involved the differentiation of patient human induced pluripotent stem cell (hiPSC)-derived neuronal progenitor cells (NPC) into astrocytes and the first metabolomic evaluation of cerebrospinal fluid (CSF) of ADLD patients. NPC from hiPSC of three ADLD patients with *LMNB1* duplication were differentiated into astrocytes[3]. After 42 days of differentiation, immunocytochemical analysis revealed the presence of aberrant nuclei with nuclear blebbing in ADLD patient cells that were not present in healthy control differentiated astrocytes. Furthermore, High Resolution-Magic Angle Spinning (HR-MAS) Nuclear Magnetic Resonance (NMR) spectroscopy metabolomics of the CSF of two ADLD patients was performed for the first time. The complete absence of glutamate and gamma-aminobutyric acid (GABA) with the presence of glutamine was indicative of astrocyte dysfunction and subsequent dysfunction of glutamatergic and GABAergic neurons, which can also affect oligodendrocyte progenitor cells (OPCs) and their differentiation into mature myelinating oligodendrocytes. Moreover, low levels of alanine, along with the presence of pyruvate and lactate, indicated a possible disruption in the alanine-lactate cycle between astrocytes and neurons. Notably, N-acetylaspartate was absent in the CSF of the ADLD patients, indicating compromised neuronal integrity and function that subsequently negatively affects astrocytes and the myelinating ability of oligodendrocytes. These findings underscore the critical role of astrocyte dysfunction in the pathogenesis of ADLD and highlight the identification of specific biomarkers that could enhance diagnostics and inform future therapeutic strategies.

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References

1. Ratti, S. *et al.* Cell signaling pathways in autosomal-dominant leukodystrophy (ADLD): the intriguing role of the astrocytes. *Cell. Mol. Life Sci.* 78, 2781–2795 (2021).
2. Ratti, S. *et al.* Lamin B1 accumulation's effects on autosomal dominant leukodystrophy (ADLD): Induction of reactivity in the astrocytes. *Cells* 10, 1–14 (2021).
3. Perriot, S., Canales, M., Mathias, A. & Du Pasquier, R. Differentiation of functional astrocytes from human-induced pluripotent stem cells in chemically defined media. *STAR Protoc.* 2, 100902 (2021).

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Effect of 1-methyl, 4-phenylpyridinium (MPP+) on SN56 cholinergic cell line

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The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) affects dopaminergic neurons and induces Parkinsonism in humans and experimental models. The active metabolite of MPTP is 1-methyl-4-phenyl pyridinium (MPP^+), which is selectively taken up by the catecholamine transporters (DAT and NET) into dopaminergic and noradrenergic neurons, where it is sequestered into the mitochondria acting as a potent inhibitor of the electron transport chain complex I. More recently, one study documented that MPTP neurotoxicity may involve cholinergic neurons of the pro-encephalon. In the aim to bcharacterize at subcellular level the cholinergic damage induced by MPP^+, in the present study, we investigated alterations induced by MPP^+ within cholinergic neurons. At this purpose, we profited from the cholinergic cell line SN56, derived from the mouse medial septal nucleus, which was exposed to different doses of MPP^+ (0.1 M, 1 M, 10 M, 100 M and 500 M) for 72h. In the first set of experiments, the effects of MPP^+ on cell viability were evaluated, along with the status of mitochondria. The effects of MPP^+ on cell survival were assessed by combining classic histological staining with Hematoxylin and Eosin, and Trypan Blue. We found that MPP^+ produces a dose-dependent reduction in cell viability, which starts from the dose of 1 M. Such a neurotoxic effect is accompanied, within spared neurons, by mitochondrial damage, assessed both at light microscopy and transmission electron microscopy. These preliminary findings confirm that cholinergic neurons are affected by MPP^+ and they are one hundred-folds more vulnerable than catecholamine cells.

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Characterization of a mouse model of chronic stress-induced neuroinflammation

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Chronic stress, together with ageing, is a major risk factor for the development of many brain disorders and diseases. Many of these disorders are characterized byinflammation activation, which is involved in both the insurgence and progression of neurodegenerative pathologies, such as Alzheimer and Parkinson's disease. To study the relationship between chronic stress, inflammation and related diseases, rodent models are commonly used. Models of stress include a variety of socially and physically stressful events that are carried out for a variable period of timein order to simulate either acute or chronic stress. The complexity and diversity of stress paradigms, i.e. the types and combinations of stressors, as well as the duration of the protocol,challenge the effectiveness and reproducibility of these models. Therefore, it's critical to carry out the characterization of the models before investigating the role of inflammation and their relationship to chronic stress in humans.

In the chronic stress model applied in this work mice were randomly assigned to control or stress group: animals of stress group were exposed every day for 24 weeks to a random chronic mild stress stimulus among Isolation, Social stress, Damp bedding, Removal of bedding, Cage tilting at 45°, Restraint Stress Loading, Alteration of light/dark cycle, Intermittent illumination, Food deprivation, Water deprivation, Tail Suspension Test, Forced swimming test. Inflammation activation was evaluated by GFAP and IBA1 expression; moreover, considering the known impact of stress on the synthesis and release of dopamine within mesocortical, mesoaccumbens, and nigrostriatal dopamine projections, TH and DAT expression were evaluated. Preliminary results showed evidence of microglial cell activation following stress stimulus, as well as a slight decrease of DAT expression in the caudate putamen. Further studies are needed to measure the changes in specific inflammatory mediators, including NF-κB and TLRs, and inflammatory cytokines, such as TNF-α, IL-1β, and IL-6.

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Polylactic acid nanoplastics can be efficiently internalized by CNS cell lines and affect NGF-induced differentiation in PC12 cells

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Microplastics (MPs) and nanoplastics (NPs) pollution is recognized as a critical global environmental issue. One proposed solution to mitigate their accumulation is replacing non-degradable plastics with biodegradable alternatives, such as Polylactic acid (PLA), commonly used in food and medical industries. Previous studies on nonbiodegradable polymers have demonstrated that MPs/NPs can enter the human body, breach the blood-brain barrier (BBB) and accumulate in the brain, with negative effects on CNS functions [1,2]. However, the PLA MPs/NPs impact on organisms is poorly understood [3].

To address this gap, two different in vitro cell models were used: the rat adrenal pheochromocytoma PC-12 cells, as a neuronal cell model, and the rat glioma C6 cell line with astrocyte phenotype. Both cell types were treated with increasing concentrations (10 - 300 μg/ml) of Rhodamine-conjugated PLA-NPs (150 nm size) from 24 to 72 hs and cell uptake and viability were analyzed. Flow cytometric analysis of cells treated with PLA-NPs revealed that both C6 and PC12 cells were able to internalize the PLA-NPs in a dose-dependent manner. The cellular uptake reached 100% at higher concentrations in both cell types starting from 24 hs of treatment. Results were confirmed by immunofluorescence analysis showing perinuclear and cytoplasmic localization of the PLA-NPs in both C6 and PC12 cells. However, cell viability did not show a significant cytotoxic effect compared to the controls. To provide deeper insights into the potential effects of PLA-NPs, we investigated nerve-growth-factor-induced neurite outgrowth in PC12 cells. Preliminary results indicated a decrease in the number and length of cells expressing neurites, suggesting a possible negative effect on neuronal differentiation. These data suggest that PLA-NPs may interfere with CNS cell function and with neuronal development, emphasizing the importance of evaluating the neurological risks associated with PLA-NPs exposure.

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References

[1] Prüst, M., Meijer, J., Westerink, R.H.S., 2020. The plastic brain: neurotoxicity of micro- and nanoplastics. Part Fibre Toxicol 17, 24.
[2] Santoro, A., Marino, M., Vandenberg, L.N., Szychlinska, M.A., Lamparelli, E.P., Scalia, F., Rocca, N.D., D'Auria, R., Pastorino, G.M., Porta, G.D., Operto, F.F., Viggiano, A., Cappello, F., Meccariello, R. PLASTAMINATION: Outcomes on the Central Nervous System and Reproduction. Curr Neuropharmacol. 2024 Epub ahead of print.
[3] Lamparelli, E.P., Marino, M., Szychlinska, M.A., Della Rocca, N., Ciardulli, M.C., Scala, P., D'Auria, R., Testa, A., Viggiano, A., Cappello, F., Meccariello, R., Della Porta, G., Santoro A. The Other Side of Plastics: Bioplastic-Based Nanoparticles for Drug Delivery Systems in the Brain. Pharmaceutics. 2023 28;15(11):2549.

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Targeting glioblastoma stem cells via Prelamin A accumulation and ROS-induced DNA damage

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Glioblastoma is the most aggressive and prevalent tumor of the Central Nervous System (CNS), with a five-year survival rate of only 5%. Conventional treatments, including surgery, radiotherapy, and chemotherapy, often fail primarily due to the recurrence of drug-resistant tumor stem cells. Notably, glioblastoma cells exhibit increased expression of lamin A, a critical component of the nuclear lamina, unlike the healthy CNS tissue (1-2). Lamin A undergoes a complex maturation process from its precursor, prelamin A. In certain genetic disorders, known as laminopathies, impaired maturation of prelamin A leads to premature cellular aging and heightened sensitivity to oxidative DNA damage (3). Building on these insights, our project explores a novel therapeutic approach that exploits the vulnerability of glioblastoma cells to prelamin A accumulation and reactive oxygen species (ROS)-induced DNA damage. We propose a combined treatment using the farnesyltransferase inhibitor Lonafarnib to induce prelamin A accumulation and Menadione to generate ROS. Our study demonstrates that the combined administration of Lonafarnib and Menadione results in significant morphological alterations, nuclear reorganization, reduced migratory and invasive capabilities, and decreased colony formation in glioblastoma cells. After confirming the non-toxicity of the proposed dual treatment on primary human astrocytes, we focused our analysis on evaluating its effects on glioblastoma stem cells, which are responsible for tumor aggressiveness and recurrence. The dual treatment seems to have a significant effect on this particular cell population, resulting in the loss of its stemness. These findings suggest that targeting glioblastoma stem cells through the accumulation of prelamin A and the induction of ROS can significantly impair their survival and aggressiveness, providing a promising new avenue for therapy.

References

1) Gatti, G., Vilardo, L., Musa, C., Di Pietro, C., Bonaventura, F., Scavizzi, F., Torcinaro, A., Bucci, B., Saporito, R., Arisi, I., De Santa, F., Raspa, M., Guglielmi, L., & D'Agnano, I.. Role of Lamin A/C as Candidate Biomarker of Aggressiveness and Tumorigenicity in Glioblastoma Multiforme. Biomedicines. 2021, 9(10), 1343.
2) Jung, H. J., Lee, J. M., Yang, S. H., Young, S. G., & Fong, L. G. Nuclear lamins in the brain - new insights into function and regulation. Molecular neurobiology. 2013, 47(1), 290–301.
3) Cenni, V., Capanni, C., Mattioli, E., Schena, E., Squarzoni, S., Bacalini, M. G., Garagnani, P., Salvioli, S., Franceschi, C., & Lattanzi, G. Lamin A involvement in ageing processes. Ageing research reviews. 2020, 62, 101073.

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Nutraceutical activity of bioactive molecules obtained from vegetal compounds

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The most recent experimental results have identified neuroinflammation as the main causes of the development of chronic degenerative diseases in the central nervous system (CNS), such as Alzheimer's disease and Parkinson's disease. The aim of complementary medicine is to provide support to traditional pharmacology by limiting disease progression through anti-inflammatory as well as anti-oxidant treatments. We have recently demonstrated the presence of wheat-derived bioactive compounds capable of polarizing LPS-stimulated microglial cells toward an anti-inflammatory phenotype. We evaluated the nutraceutical activity of durum wheat extracts obtained from the ancient cultivar "Senatore Cappelli" on microglial polarization by analyzing the mRNA expression of M1 and M2 markers such as iNOS, COX2, ARG-1 and CD206 and pro- and anti-inflammatory cytokines such as IL-1β, TNF-α, IL-6 and IL-10 by real-time PCR. Experiments were carried on BV2 microglia cells pretreated with extracts derived from the ancient cultivar "Senatore Cappelli" at the concentrations chosen from the cytotoxicity curves in the presence or absence of LPS. Through immunofluorescence experiments, we evaluated the ability of durum wheat extracts to reverse the pro-inflammatory M1 phenotype into the anti-inflammatory M2 phenotype. In addition, cytotoxicity and mRNA expression analyses of anti- and pro-inflammatory cytokines of extracts derived from samples of "Tomato variety Pera D'Abruzzo" and "Tomato variety Riccio di Parma." are also underway. Our preclinical studies underscore the possibility of isolating bioactive compounds from waste products of agricultural and industrial processing. The molecules we have analyzed so far have shown to possess phytochemical and nutraceutical properties to counteract inflammation in the CNS.

References

1) De Caris, MG; Grieco, M; Maggi, E, Francioso; Armeli, F; Mosca, L; Pinto, A; D'Erme, M; Mancini, P; Businaro, R. Blueberry Counteracts BV-2 Microglia Morphological and Functional Switch after LPS Challenge. Nutrients 2020, 12(6):1830.
2) Vinci, G; Prencipe, S;, Armeli, F; Businaro, R. A Multimethodological Approach for the Valorization of "Senatore Cappelli" Wheat Milling By-Products as a Source of Bioactive Compounds and Nutraceutical Activity. Int J Environ Res Public Health 2023, 20(6):5057.

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Novel insights into neuroinflammatory changes and immune biomarkers associated with chemotherapy-induced peripheral neurotoxicity

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Chemotherapy treatment negatively affects the nervous and immune systems inducing a peripheral neurotoxicity (CIPN), which results in a dose-limiting side-effect. Despite our increasing understanding of the key mechanisms that drive neuropathic pain, the impact of neuroinflammation in CIPN remains unclear. In particular, the effects of antitumor drugs on glial cells have so far not been described accurately, and few studies have examined the association of immune biomarkers with CIPN.

In our study, the rats were treated with paclitaxel (PTX, 10mg/kg) and vincristine (VCR, 0.2 mg/kg) once a week, for four weeks. Morphological, behavioral and neurophysiologic analyses performed at mid and end treatments, revealed a statistically significant decrease in sensory action potential amplitude for caudal nerve, allodynia and axonal degeneration in caudal nerve of animals treated with PTX, which was more severe respect to the rats injected with VCR. In addition, huge macrophages infiltration was observed in caudal nerve only after PTX treatment and a different time course of satellite glial cells, astrocytes and microglia activations were observed in dorsal root ganglia (DRG) and spinal cord after chemotherapy administrations.

Afterwards, we analyzed the presence of immune marker proteins in serum and tissues of rats treated with PTX, 10mg/kg 1qwx4weeks and oxaliplatin (OHP) 5 mg/kg 2qwx6weeks. RT-PCR was used to quantify gene expression of inflammasome protein NLRP3, interleukin (IL) 1b, IL6 and chemokine CCL2 in nervous tissue, and immunochemistry was used to identify macrophages infiltration after chemotherapy treatments. PTX-treated rats showed a significant neuroinflammation condition, indicated by a high expression of infiltrating macrophages, an increase of IL6 e GRO/KC protein in serum and increased gene expression of inflammatory cytokines/chemokines in peripheral nerves. Therefore, IL6-and/or GRO/KC, after validation in extended studies, could be a useful biomarker to assess neurotoxic effects of PTX-induced neuropathy.

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Transmission Electron Microscopy is a powerful tool for investigating nanoparticles uptake as a function of shape in U-87 human glioblastoma cells

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Glioblastoma multiforme (GBM) is a highly aggressive and fatal brain tumor, representing one of the most challenging cancers to treat due to its rapid proliferation and resistance to conventional therapies [1]. Nanoparticles (NPs) have emerged as a promising tool for targeted drug delivery in cancer therapy [2]. Transmission electron microscopy (TEM) has been chiefly used to monitor the uptake and relocation of NPs inside tissues and cells. This study aimed to understand, using TEM, the uptake efficiency of Ker-AuNPs as a function of shape by U87-MG human glioblastoma cells. A new generation of AuNPs coated with keratin (Ker-AuNPs) showed high biocompatibility. Spherical (25 nm) and bipyramid (50 nm) Ker-AuNPs will be administered to cultured U87-MG human glioblastoma cells for 24 and 48h; cellular viability and cytotoxicity have been evaluated. After the fixation, samples have been treated for the TEM. Ultrastructural analysis of spherical and bipyramid Ker-AuNPs in U87-MG cells showed a similar uptake, specifically they made contact with the plasma membrane, occurring in plasma membrane invaginations, and entered the cell enclosed in endosomes; Ker-AuNPs internalized via endocytosis/phagocytosis were trapped in vacuolar structures. The internalization of both shapes does not seem to affect cell morphology. In this study, we confirm that such objects present interesting properties for the future design of bioimaging agents for cancer therapy, and the scattering features of the AuNPs allow one to locate the nano-objects within the cell precisely. There are no significant differences between the shapes on the uptake. However, it appears that sharp and edgy structures are of great interest thanks to extensive local electric field enhancements at the sharp ends, and their local surface plasmon resonance depends even more on the dielectric environment than in the case of more usual shapes. Consequently, structures such as bipyramids Ker-AuNPs are very promising for biosensing.

References

1) Ostrom, Q.T.; Bauchet, L.; Davis, F.G.; Deltour, I.; Fisher, J.L.; Langer, C.E.; Pekmezci, M.; Schwartzbaum, J.A.; Turner, M.C.; Walsh, K.M.; Wrensch, M.R.; Barnholtz-Sloanet, J.S. The epidemiology of glioma in adults: a "state of the science" review. Neuro Oncology 2014, 16, 896–913.
2) Messina, E.; D'Urso, L.; Fazio; E.; Satriano, C.; Donato, M.G.; D'Andrea, C.; Marago, O.M.; Gucciardi, P.G.; Compagnini, F.; Neri F. Tuning the structural and optical properties of gold/silver nano-alloys prepared by laser ablation in liquids for optical limiting, ultra-sensitive spectroscopy, and optical trapping. Journal of Quantitative Spectroscopy and Radiative Transfer 2012, 113, 2490–2498.

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Role of the AP1G1 gene in clathrin-mediated vesicular trafficking. Usmani-Riazuddin syndrome and possible new therapeutic approaches

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The Adaptor Protein-1 (AP-1) complex plays a crucial role in the intracellular trafficking of vesicles, regulating the transport of proteins between various cellular compartments. In human, mutations in the *AP1G1* gene, which encodes the γ-adaptin subunit of the AP-1 complex, are associated with neurological disorders and multiorgan phenotypes. Usmani-Riazuddin syndrome (OMIM AR 619548)[1] is characterized by variants of the AP1G1 gene.

Recent research has shown that: 1) AP1G1 is essential for normal development and cognitive function in humans and for embryonic development in the zebrafish model; 2) the pathogenic variants in AP1G1 probably have a dominant negative toxic effect on the wild type allele resulting in over-expression of the anomalous protein.

In this study, we investigated the effects of a new pathogenic variant in *AP1G1* (c.196G>A p.Gly66Arg) on fibroblast taken from a young patient with motor and language delay and a moderate form of intellectual disability.

Using immunofluorescence techniques, we analyzed the intracellular distribution of AP-1, GM130 (cis-Golgi network marker), Clathrin, TGN46 (trans-Golgi network marker) and RAB5 (marker for early endosomes) in affected and healthy fibroblasts. Our observations revealed significant differences in the subcellular localization and distribution of vesicular trafficking markers. In *AP1G1*$^{+/-}$ patient's fibroblast, we observed alterations in the perinuclear distribution of AP1G1 and a marked disorganization of vesicular trafficking markers compared to control.

These findings suggest a critical role of the *AP1G1* subunit in regulating the intracellular trafficking of clathrin-coated vesicles. Analysis of specific mutations on *AP1G1* provides significant insights into the pathogenetic mechanisms underlying disorders associated with mutations of the AP-1 complex. Ongoing studies are clarifying the role of this variant in the development of the nervous system in Zebrafish

Further studies are necessary to fully understand the effects of *AP1G1* pathogenic variant on cellular functionality and to develop potential targeted therapeutic strategies for these disorders.

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References

1) Usmani, M.A.; Ahmed, Z.M.; Magini, P.; et al. De novo and bi-allelic variants in AP1G1 cause neurodevelopmental disorder with developmental delay, intellectual disability, and epilepsy. Am. J. Hum. Genet. 2021, 108(7), 1330-1341. doi: 10.1016/j.ajhg.2021.05.007.

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Symmetric and asymmetric organization of the human body

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Symmetry is mostly used to describe body shapes. In humans symmetry can be investigated at microscopic and macroscopic level. At molecular level life is not symmetric. Most compounds are chiral, such as carbohydrates, aminoacids, and nucleic acids. Triglycerides are often chiral. Since in living organisms only one of the two enantiomers of a chiral molecule predominates, this concept is referred as homochirality. Aminoacids appear almost exclusively in the left-handed form, whereas sugars appear in the right-handed form.

At cellular level, some examples of symmetric structures can be found. One is the mitotic spindle, which allows cell division. Another is represented by the primary organizer, with the formation of the notochord, which represents the future axis of bilateral symmetry of the embryo.

At macroscopic level, the sagittal plane divides an organism into roughly mirror image halves, with respect to external appearance only. Animals with bilateral symmetry belong to Bilateria, a large clade of animals called bilaterians. Asymmetry is often an indication of unfitness, including defects during development or injuries throughout a lifetime. Facial symmetry influences human judgments of attractiveness. Nevertheless, nearly all organs of the thorax and abdomen are asymmetric. Another interesting topic is represented by cerebral hemispheres, which look roughly symmetric at first glance. Nevertheless, there are anatomical asymmetries, such as the *planum temporale* of the temporal cortex, which is larger in the left hemisphere. This difference correlates with right-handedness. The studies of Broca and Wernicke in the left hemisphere of right-handed people opened the study on the functional asymmetry of the cerebrum. Intriguing dilemmas occur when interrupting the connection of the two hemispheres, as in the split-brain syndrome: when patients are shown an image only in the left half of each visual field, they cannot verbally name what they have seen, because the language area is contralateral.

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Astrocyte clasmatodendrosis in an *in vitro* model of ischaemia: machine-learning analysis

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Neuroinflammation consists of a concerted set of responses that astrocytes and microglia enact under altered conditions of homeostasis in the central nervous system (CNS), the exacerbation of which contributes to the pathological effects of many neurodegenerative diseases. In previous studies we described, in various animal models of neuroinflammation, the fragmentation of cytoplasmic processes of astrocytes, i.e. clasmatodendrosis, with consequent fragmentation of their functional syncytium. These damage patterns preceded the activation of the reactive response of astrocytes, and were correlated with the expression of a senile-like reactive phenotype in microglia.

In this study, we evaluated the effects of oxygen deprivation, an in vitro model of ischaemia, in organotypic slices of rat hippocampus. We adopted 3D Tausense confocal microscopy (a technique based on photon counting) to separate autofluorescent emission from immunolabeling, and machine-learning procedures to assess the expression of glial and neuronal markers, and for morphometric analyses. We showed significant clasmatodendrosis in slices subjected to oxygen-glucose deprivation, corresponding to marked alterations in microglial morphology. Our data suggest that also in this model of neurodegeneration, fragmentation of the functional astrocyte syncytium significantly influences the reactive response of microglia and, consequently, neuroinflammation. We have also isolated an autofluorescence emission from neurons, and other cells with neuron-like morphology that were however negative for the classical neuronal marker Neuronal Nuclei (NeuN). These preliminary analyses indicate that organotypic slices, obtained from newborn rats, may represent a valid model for studying differentiation processes in the CNS.

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Hippocampus-derived cells: an in vitro model to study the neuroprotective effects of CoQ10 Acetate

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Neurodegeneration represents a complex constellation of conditions characterized by the progressive decline in structure, function, and viability of neurons, the fundamental units of the brain and central nervous system. As society ages, the prevalence of NDs is expected to increase, highlighting the critical need for enhanced understanding and development of innovative preventive strategies.

Several events, including synaptic dysfunction, neuronal loss, and brain atrophy characterize ND. Altered molecular mechanisms in ND include mitochondrial dysfunction, oxidative stress, and low-grade inflammation. Key molecular players in these processes include NF-κB, and the Bcl-2 family proteins, including Bcl-2 and Bax. Nrf2 is a critical regulator of the antioxidant response. Nutritional patterns rich in polyphenols and healthy fats may mitigate the risk of neurodegenerative diseases, decreasing oxidative stress and low-grade inflammation.

The study of phytochemicals like flavonoids and curcumin has shown their ability to affect key molecular pathways involved in neurodegeneration.

Amidst these complex interactions, Coenzyme Q10 (CoQ10) and its derivatives stand out for their dual role in mitochondrial function and antioxidant defense. The neuroprotective potential of CoQ10, bolstered by its involvement in ATP production and its capacity to neutralize reactive oxygen species, presents a promising avenue for therapeutic intervention. However, challenges in bioavailability and optimal dosing underscore the need for further research to harness CoQ10's full potential in combating neurodegenerative diseases.

This study introduces a pioneering *in vitro* comparison of CoQ10 and its derivative CoQ10 Acetate, focusing on their neuroprotective effects and underlying mechanisms in HT22 neuronal cells. Our investigation delves into mitochondrial protection, the modulation of the Bcl/Bax ratio, NF-κB activity, and interleukin regulation, aiming to illuminate the analog's superior efficacy and mechanism profiles. This work represents a significant stride in overcoming CoQ10's dosing challenges, potentially setting a new benchmark in neuroprotective therapeutic strategies.

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High expression of SMN circ4-2b-3 in nusinersen treated SMA I children is associated with improved motor outcomes

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Spinal muscular atrophy (SMA) is a neuromuscular disorder that results in the loss of motor neurons' function and progressive muscle weakness and atrophy. It occurs in infancy or early childhood and is one of the most common genetic causes of death in children. SMA is caused by genetic mutations or deletions that affect the Survival Motor Neuron 1 (SMN1) gene, which is necessary for motor neuron function and survival. SMN2 gene is a nearly identical copy of SMN1 but is unable to produce a functional full-length protein, and alone is not sufficient to avoid the motor neuronal loss that occur in SMA. However, it is related to the severity of the disease since the higher is the number of SMN2 copies, the lower is the severity of symptoms. Therefore, many therapies for SMA treatment aim to correct the splicing defect that underlies the differences between SMN1 and SMN2, allowing the production of a greater amount of the functional protein. Among the therapies that are based on this principle, Spinraza (Nusinersen) acts on SMN2 by modifying its splicing and allows to produce a greater amount of SMN functional protein and this consequently lead to an improvement of motor functions in the treated patients.

With the present work, through the analysis of a court of SMA I children in treatment with Nusinersen, we have identified a molecule whose expression increases in super responders, which are those patients with a certain improvement of motor functions after the beginning of the therapy. This molecule is a circular RNA (circRNA), SMN circ4-2b-3, that is one of the many circRNAs produced from the SMN locus through the back-splicing process, but it is also the only SMN circRNA to be secreted in circulating exosomes and from serum of both controls and patients.

Our observations indicate that in some super responders SMA I patients, the number of copies of circ4-2b-3 is higher compared to the other experimental groups and its levels remain high even when analyzing different samples over time, suggesting its potentiality as biomarker whose expression levels depend on the patients' response to treatment.

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Nanovesicular involvement in the Gut-Brain Axis and their modification after probiotics: in vivo and in vitro evidences

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Dysbiosis, often caused by poor diet or stress, is linked to systemic diseases and mental disorders due to the well-established influence of the gut-brain axis. Probiotic supplements are increasingly recognized for stabilizing gut microbiota and supporting the intricate anatomical structures of the gastrointestinal system, particularly the enteric nervous system and its connection to the central nervous system. This study examines probiotics' impact on the tryptophan pathway, essential for regulating serotonin, thus influencing host physiology and behavior. To do this, nanovesicles were isolated from the plasma of subjects with chronic diarrhea, both before and after 60 days of consuming a probiotic mix (Acronelle®, Bromatech S.r.l., Milan, Italy) and were analyzed for Tryptophan 2,3-dioxygenase 2 (TDO 2). Additionally, HT29 cells were treated with the probiotic mix and H_2O_2 to investigate cytoprotective and anti-stress effects.

In vivo results showed increased TDO 2 levels in nanovesicles post-probiotic treatment, suggesting involvement in the gut-brain axis. *In vitro*, the probiotic mix mitigated H_2O_2-induced stress effects, demonstrating significant cytoprotective properties. The treatment reduced heat shock protein 60 kDa (Hsp60) levels and maintained intestinal integrity and barrier function by restoring tight junction proteins. Furthermore, probiotics increased the expression of TDO 2 and serotonin receptors, crucial for gut-brain communication.

The study provides new insights into the benefits of probiotics on gut microbiota, highlighting their broader health implications. These findings suggest that nanovesicles play a key role in the gut-brain axis, influencing central nervous system function and offering potential therapeutic pathways for neurological disorders. By understanding how probiotics modulate the tryptophan pathway and serotonin levels, the results underscore probiotics' potential as a therapeutic strategy for managing chronic gastrointestinal conditions and improving mental health outcomes through the gut-brain axis. This research emphasizes the importance of maintaining a balanced gut microbiota for overall well-being and presents promising prospects for future clinical applications.

References

1) Aziz, T.; Khan, A.A.; Tzora, A.; Voidarou, C.; Skoufos, I. Dietary Implications of the Bidirectional Relationship between the Gut Microflora and Inflammatory Diseases with Special Emphasis on Irritable Bowel Disease: Current and Future Perspective. Nutrients 2023, 15, 2956.
2) Fucarino, A.; Burgio, S.; Paladino, L.; Caruso Bavisotto, C.; Pitruzzella, A.; Bucchieri, F.; Cappello, F. The Microbiota Is Not an Organ: Introducing the Muco-Microbiotic Layer as a Novel Morphofunctional Structure. Anatomia 2022, 1, 186–203.
3) Cappello, F.; Saguto, D.; Burgio, S.; Paladino, L.; Bucchieri, F. Does Intestine Morphology Still Have Secrets to Reveal? A Proposal about the "Ghost" Layer of the Bowel. Appl. Biosci. 2022, 1, 95–100.
4) Santonocito, R.; Paladino, L.; Vitale, A.M.; D'Amico, G.; Zummo, F.P.; Pirrotta, P.; Raccosta, S.; Manno, M.; Accomando, S.; D'Arpa, F.; et al. Nanovesicular Mediation of the Gut–Brain Axis by Probiotics: Insights into Irritable Bowel Syndrome. Biology 2024, 13, 296.

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Lactoferrin potential to attenuate astroglial reactivity and stimulate the SOX-2 dependent reprogramming into neural precursor cells

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Neurodegenerative diseases are characterized by chronic neuroinflammation and progressive loss of neurons. Upon inflammatory triggers, the astrocytes are activated assuming the neurotoxic A1 phenotype or neuroprotective A2 phenotype. Under pathological conditions, A1 astrocytes subset results to be predominant and neurotoxic effects prevails over supportive functions, playing a key role in the progression of neurodegenerative disorders. However, a portion of reactive astrocytes can be dedifferentiated to acquire a neuronal phenotype in response to injury or chemical inducers.

In the light of this evidence, the attenuation of glial reactivity, along with the astrocyte-to-neuron conversion, may be a promising strategy for the treatment of neurodegenerative disorders.

The iron-binding protein lactoferrin can stimulate neurogenesis, besides performing immunomodulatory functions. This study aims to determine the effects of lactoferrin on astroglial reactivity and its potential to induce the astrocyte-to-neuron conversion. For this purpose, LPS-induced DI-TNC1 cell line was used as an in *vitro* model of prolonged inflammation. Astrocytes were pre-treated with lactoferrin (4μg/ml) for 24 hours followed by LPS (400 ng/ml) and examined after 2-, 9- and 16-days post-treatment. Results prove that lactoferrin attenuates the glial reactivity by reducing the glial fibrillary acidic protein (GFAP) and Toll like receptor 4 (TLR4) expression, as well as improving the expression of the anti-inflammatory cytokine IL-10. Moreover, we found that lactoferrin promotes the astrocytes reprogramming into neural precursor cells by inducing the expression of the reprogramming transcription factor SOX-2. Overall, this study demonstrates that lactoferrin has the potential to attenuate neuroinflammation, in addition to inducing the astrocytes reprogramming into neural precursor cells, suggesting a potential innovative approach for the treatment of neurodegenerative diseases.

References

1) Lawrence, J.M; Schardien, K.; Wigdahl, B.; Nonnemacher, M.R. Roles of neuropathology-associated reactive astrocytes: a systematic review. Acta Neuropathol Commun. 2023, 11(1), 42.
2) Tan, Z.; Qin, S.; Liu, H.; Huang, X.; Pu, Y.; He, C.; Yuan, Y.; Su. Z. Small molecules reprogram reactive astrocytes into neuronal cells in the injured adult spinal cord. J Adv Res. 2023, S2090-1232(23)00176-5.
3) Ma, Y.; Xie, H.; Du, X.; Wang, L.; Jin, X.; Zhang, Q.; Han, Y.; Sun, S.; Wang, L.; Li, X.; Zhang, C.; Wang, M.; Li, C.; Xu, J.; Huang, Z.; Wang, X.; Chai, Z.; Deng, H. In vivo chemical reprogramming of astrocytes into neurons. Cell Discov. 2021, 7(1), 12.

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Prokineticin-2 is highly expressed in colonic mucosa of early Parkinson's disease patients

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Neuroinflammation is emerging as a key factor involved in the pathophysiology of Parkinson's disease (PD). In this context, prokineticin-2 (PK2), a chemokine-like protein, can modulate immune/inflammatory responses and neuronal damage in PD. Of interest, several studies support the contention that gut alterations, including dysbiosis, gut barrier impairments, colonic inflammation and α-synuclein deposition, represent early events in PD that can contribute to central pathology via gut-brain axis. In this context, PK2 has been implicated in gastrointestinal functions and gut inflammation. However, the role of enteric PK2 in PD remains unexplored. On these bases, the aim of the present study was to investigate the expression and tissue localization of PK2 in colonic biopsies from PD patients, shedding light on its implications in the disease progression. Mucosal biopsies from the descending colon were obtained in 11 PD patients, who, according to the disease duration, were divided into two groups: the early one (5 EPD: disease duration 0-5 years) and the long one (6 LPD: more than 5 years disease duration), and 5 asymptomatic subjects. Biopsy samples were processed for PK2 immunofluorescence and western blot. Double-immunofluorescences (PK2-GFAP and PK2-CD68) was also performed to characterize intestinal cells expressing PK2. An increased PK2 expression was detected in colonic biopsies from EPD patients as compared with controls and LPD, while no significant difference was observed among controls and LPD. Of note, GFAP-positive glial cells and CD68-positive macrophages in the colonic mucosa from EPD patients displayed PK2 positivity, thus suggesting a PK2 increase in neurogenic/immune inflammatory cells. A linear correlation between PK2 expression and disease duration was also observed. In conclusion, PK2 is highly expressed within neurogenic/inflammatory cells of colonic mucosa from early PD patients, suggesting a potential role of PK2 as early marker of gut inflammation in PD.

References

1) Schirinzi T, Maftei D, Passali FM, et al. Olfactory Neuron Prokineticin-2 as a Potential Target in Parkinson's Disease. Ann Neurol. 2023; 93:196–204.
2) Pellegrini C, Antonioli L, Colucci R, Blandizzi C, Fornai M. Interplay among gut microbiota, intestinal mucosal barrier and enteric neuro-immune system: a common path to neurodegenerative diseases? Acta Neuropathol. 2018 Sep;136(3):345-361.

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Proteome changes in extracellular vesicles content of human glioblastoma stem cells induced by P2X7 receptor stimulation

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Extracellular vesicles (EVs) are secreted by many tumors, including glioblastoma multiforme (GBM), the most frequent and aggressive brain tumor in adults, characterized by high resistance to conventional therapies, an unfavorable prognosis for the patient with frequent unfortunate outcome. Given the high relevance of the information provided by the tumor cell secretome, we performed a proteomic analysis of the contents of microvesicles (MVs) and exosomes (EXOs) released from GBM-derived stem cells (GSCs) into the culture medium. The cells, obtained from the brains of GBM patients, expressed P2X7 receptors (P2X7R), which are positively correlated with the growth and invasiveness of the GBM itself. P2X7R stimulation of GSCs induced significant changes in EV content, mostly by inducing or de novo upregulating the expression of proteins related to cytoskeletal reorganization, cell motility/spreading, energy supply, protection against oxidative stress, chromatin remodeling and transcriptional regulation. Most of the induced/upregulated proteins have already been identified as diagnostic/prognostic factors of GBM itself, while others have only been reported in peripheral tumors. Our results indicate that stimulation of P2X7R enhances the transport and, therefore, the possible intercellular exchange of proteins that may increase the aggressiveness of GBM by GSC-derived EVs. Therefore, P2X7Rs could be considered a novel drug target of human GBM.

References

1) Kalluri, R.; McAndrews, K.M. The role of extracellular vesicles in cancer. Cell 2023, 186, 1610–1626.
2) Braganhol, E.;Wink, M.R.; Lenz, G.; Battastini, A.M.O. Purinergic Signaling in Glioma Progression. Adv. Exp. Med. Biol. 2020, 1202, 87–108.
3) Lee, Y.J.; Seo, C.W.; Lee, D.; Choi, D. Proteomics of Extracellular Vesicle in Glioblastoma. Brain Tumor Res Treat. 2022, 10, 207–214.

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Effect of extracellular TDP-43 on glial cells

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TAR DNA-binding protein 43 (TDP-43) is a crucial nuclear RNA/DNA-binding protein that plays multiple roles as transcriptional and translational regulator. While its physiological functions are essential, TDP-43 aggregation and deposition in central nervous system is a prevalent hallmark of numerous neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and limbic predominant age-related TDP-43 encephalopathy (LATE). Elevated levels of TDP-43 have been observed in the biological fluids of ALS patients indicating that TDP-43 can be released into the extracellular space, where it can interact with both neuronal and glial cells. Microglia and astrocytes have a crucial role in ALS-related neurotoxicity. While these cells are essential for brain homeostasis, their dysregulation can lead to the release of harmful substances, disrupting neuron support mechanisms and contributing to neurotoxicity.

This study aims to investigate *in vitro* the impact of TDP-43 on glial cells by evaluating cell viability, ROS production and mitochondrial functionality in MMGT12 microglia cells and rat primary astrocytes. Cells were exposed to increasing concentrations of full-length TDP-43 (0.03-0.3 mM) and various parameters were analyzed at different time points (up to 24 hours) by MTT and DCFDA assays, JC-1staining and immunocytochemistry.

Results indicate that TDP-43 induces an activated phenotype both in astrocytes and in microglia. Particularly, in astrocytes, we observed a rapid increase of ROS levels within 2 hours, along with a decrease in mitochondrial membrane potential and Cytochrome C Oxidase Subunit 4 (COX4) expression. In MMGT12 cells the effect was slower, reaching its maximum after 24 h.

In conclusion, these preliminary findings demonstrate that exogenous full-length TDP-43 can affect the normal functioning of glial cells, initiating various intracellular toxic mechanisms that, in turn, could lead to neurotoxicity.

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Forensic clinical anatomy of pediatric abusive head trauma

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Forensic Clinical Anatomy is "the practical application of anatomical knowledge and methods (from ultrastructural to macroscopic aspects), endowed with substantial clinical/surgical implications, to the ascertainment and evaluation of medico-legal problems" (1-2). Pediatric abusive head trauma is an injury to the intracranial contents or skull of an infant or child younger than 5 years old due to violent shaking (shaken baby syndrome) and/or blunt impact. The outcome ranges from complete recovery to significant brain damage or to death. Abusive head trauma with shaking mechanism is frequently characterized by the so-called triad: encephalopathy, intracranial subdural haemorrhage, and retinal haemorrhages. Other pathological findings include bruises/ecchymoses, fractures, lesions of the spinal ligaments, spinal subdural haemorrhages (3), hypoxic-ischaemic/haemorrhagic injuries of the spinal cord, focal haemorrhages of intraorbital adipose tissue or in meningeal spaces of optic nerves. The different aspects of pediatric abusive head trauma are strictly anatomical in nature, with regards to their pathophysiologic mechanisms and correlated methods of investigation in a forensic context.

In the present work, we addressed Forensic Clinical Anatomy issues with reference to our forensic casistics of six cases (one with recovery, one with significant brain damage, four autoptic cases) of pediatric abusive head trauma.

In our experience, from a dissective methodological point of view, judicial autopsies in these cases must be integrated with anatomical dissections to specifically evaluate the different anatomical structures 'layer-by-layer'. Post mortem imaging of sampled anatomical structures (long bones, spinal structures) may be useful to better define in vivo imaging. Sampling for histopathological studies must be performed through strictly anatomical methods, in order to permit the identification of the injured structures (nerves, vessels, muscles). An integrated forensic-anatomical approach in pediatric abusive head trauma is not only useful for better definition of the single case but may also widen the knowledge about the anatomical bases of some findings.

References

1) Porzionato, A.; Macchi, V.; Stecco, C.; Loukas, M.; Tubbs, R.S.; De Caro, R. Forensic clinical anatomy: A new field of study with application to medico-legal issues. Clin. Anat. 2017, 30, 2-5.
2) Porzionato, A.; Macchi, V.; Loukas, M.; De Caro, R. Forensic clinical anatomy – Definitions, Methods and Fields. In: Ferrara SD, editor. P5 Medicine and Justice. Innovation, Unitariness and Evidence. Berlin: Springer; 2017, 377-394.
3) Porzionato, A.; Macchi, V.; De Caro, R. Physical Abuse of Children. N. Engl. J. Med. 2017, 377, 398-399.

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The Sarcoglycan sub-complex expression in MPTP rat's brain

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The sarcoglycan subcomplex is a multimember protein system that mediates the interaction between extracellular matrix and cytoskeleton. This system was considered muscle specific for long time; although that the sarcoglycan sub-complex seems to be expressed in a a variety of tissue such as nervous tissue [1]. Although the role of epsilon sarcoglycan in nervous tissue is well known, the expression and the role of other isoforms remains unclear. Here we aimed to analyse the sarcoglycan expression in animal model of Parkinson disease, MPTP. Rat's brains were processed by histological and immunohistochemistry techniques; antibodies anti each sarcoglycan have been used and their expression have been evaluated in substantia nigra. Results have shown that in sham rats the expression of sarcoglycan sub-complex is localized at plasmalemma level of neuronal cells; the sarcoglycans expression show to be decreased in Parkinson rats if compared to the sham group. These data strongly support a role of all sarcoglycan sub-complex in nervous tissue, although their role has to be further defined.

References

[1] Cutroneo G., Bramanti P., Ananstasi G., Bruschetta D., Favaloro A., Vermiglio G., Trimarchi F., Di Mauro D., Rizzo G. Sarcoglycans and GABAa receptors in rat central nervous system: an immunohistochemical study. IJAE, 2015, 120 (2): 105-116.

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Chronic subdural hematoma: histochemical and ultrastructural study

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Introduction: Chronic Subdural Hematoma (CSDH) significantly impacts a broad population, with an annual incidence in the elderly estimated between 1.72% and 20.6% per 100,000 individuals. The clinical presentation of CSDH varies, depending on the pressure the hematoma exerts on the underlying brain tissue. Initial symptoms can include headache, altered mental status, hemiparesis, and gait disturbances, potentially progressing to coma. The mechanisms underlying the pathophysiology of CSDH are complex and largely unknown, despite various theories attempting to explain them. Our study aims to explore the relationship between cortical atrophy and the development of CSDH by correlating clinical data with immunohistochemical and ultrastructural analyses.

Materials and Methods: Twenty outer and inner membrane samples from CSDH patients were analyzed. Their morphology was investigated by immunohistochemistry and transmission electron microscopy.

Results: Histological examination using histochemical Trichrome staining shows a considerable increase in the fibrotic component of the outer and inner membrane of the subdural hematoma. Immunohistochemical analysis showed that in addition to fibrosis, there is evidence of marked neoformation of capillary vessels, CD31+, which display a different pattern of distribution from vessels CD34+ in terms of number (increase) and arrangement. TEM images showed that those vessels were fragile and hyper permeabilized: the CSDH inner membrane showed the presence of a chronic inflammatory state. New interesting ultrastructural findings observed in the CSDH inner membrane were, for the first time, also reported: (A) the presence of very large cells with the dilated rough endoplasmic reticulum, swollen mitochondria, and autophagic bodies; (B) the presence of a large number of apoptotic bodies, macrovesicles, and exosomes in the extracellular matrix; (C) the presence of multivesicular bodies in the cytoplasm of dural border cells; (D) the presence of neo formed irregular and dilated microvessels located mainly in the inner membrane facing the hematoma cavity; (E) the presence of large numbers of macrovesicles and exosomes in the space between endothelial cells and surrounding pericytes, in most of the capillaries observed; (F) the presence of multivesicular bodies in the endothelial cells.

Conclusions: In summary, the ultrastructural observations of CSDH inner membrane highlight the presence of a chronic inflammatory state, which is probably the causative factor of hematoma formation, simultaneously with a minimal traumatic event.

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The impact of S100B silencing on Multiple Sclerosis model: the role of glial cells

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It has been demonstrated that S100b actively participate in neuroinflammatory processes of different diseases of central nervous system (CNS) (1), such as Experimental Autoimmune Encephalomyelitis (EAE), a recognized animal model for Multiple Sclerosis (MS). We previously showed that the inhibition of S100B activity using pentamidine and of S100B astrocytic synthesis using arundic acid determined an amelioration of clinical and pathologic parameters of the disease: the symptoms were milder and delayed (2-4). This study further goes in detail on the role of S100B, and of astrocytic S100B in these neuroinflammatory processes. To this aim we have purchased S100B KO mice. EAE induction on this mouse strain resulted in an amelioration of the clinical and pathological parameters. To dissect the potential mechanisms that could explain the role of S100B in the development of EAE we sorted, cultured and compared neural subpopulations (astrocytes, microglia and oligodendrocytes) deriving from S100B KO and wild type mice, through flow cytometric panels and ELISA. Neural cells were analysed for proinflammatory molecules showing a significant reduction of TNFa protein in mice where S100B was silenced. As expected also S100B protein was significantly lower in this strain, although the gene expression of this molecule was not different from wild type. The possible explanation of this discrepancy resides in the fact that the gene includes a non-coding cassette that allow its encoding but that avoid the generation of a fully functional protein. We also cultured ACSA2+ cells (astrocytes) sorted and enriched from the brains of EAE affected animals, both KO and wild type animals. The usage of S100B inhibitors demonstrate the direct impact of these molecules on specific subpopulation of neural cells, such as Astrocytes and microglia.

The present results further individuate astrocytic S100B as a key factor and as a potential therapeutic target for EAE neuroinflammatory processes.

Keywords: Astrocytes, Nueorinflammation, Multiple Sclerosis

References

1. Michetti F, Di Sante G, Clementi ME, Sampaolese B, Casalbore P, Volonté C, Romano Spica V, Parnigotto PP, Di Liddo R, Amadio S, Ria F. Growing role of S100B protein as a putative therapeutic target for neurological- and nonneurological-disorders. Neurosci Biobehav Rev. 2021 Aug;127:446-458. doi: 10.1016/j.neubiorev.2021.04.035. Epub 2021 May 7. PMID: 33971224.
2. Di Sante G, Amadio S, Sampaolese B, Clementi ME, Valentini M, Volonté C, Casalbore P, Ria F, Michetti F. The S100B Inhibitor Pentamidine Ameliorates Clinical Score and Neuropathology of Relapsing-Remitting Multiple Sclerosis Mouse Model. Cells. 2020 Mar 18;9(3):748. doi: 10.3390/cells9030748. PMID: 32197530; PMCID: PMC7140642.
3. Camponeschi C, De Carluccio M, Amadio S, Clementi ME, Sampaolese B, Volonté C, Tredicine M, Romano Spica V, Di Liddo R, Ria F, Michetti F, Di Sante G. S100B Protein as a Therapeutic Target in Multiple Sclerosis: The S100B Inhibitor Arundic Acid Protects from Chronic Experimental Autoimmune Encephalomyelitis. Int J Mol Sci. 2021 Dec 17;22(24):13558. doi: 10.3390/ijms222413558. PMID: 34948360; PMCID: PMC8708367.
4. Barros C, Barateiro A, Neto A, Soromenho B, Basto AP, Mateus JM, Xapelli S, Sebastião AM, Brites D, Graça L, Fernandes A. S100B inhibition protects from chronic experimental autoimmune encephalomyelitis. Brain Commun. 2022 Mar 25;4(3):fcac076. doi: 10.1093/braincomms/fcac076. PMID: 35620168; PMCID: PMC9128388.

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A potential marker for anorexia nervosa: exploring the role of SIRT1

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This study investigated a molecule called SIRT1, which plays a role in regulating energy balance in cells and influences hormones like leptin and ghrelin. Researchers investigated SIRT1 levels as a potential blood marker for Anorexia Nervosa (AN), an eating disorder characterized by low body weight and unhealthy eating habits.

The study involved 54 participants, including 32 individuals with AN and 22 healthy controls. Blood levels of SIRT1, along with other molecules like leptin (regulates appetite) and ghrelin (stimulates hunger) were evaluated. In addition, autoantibodies targeting hypothalamic cells involved in regulating eating stimulus were measured.

Results are highlighted that people with AN had higher levels of SIRT1 in their blood compared to the healthy group. However, these levels decreased in relation to duration of illness. SIRT1 levels were negatively related to leptin and body mass index (BMI), meaning they went down as leptin and BMI increased. Conversely, SIRT1 levels were positively correlated with ghrelin and autoantibodies against hypothalamic antigens.

These findings suggest that measuring SIRT1 in the peripheral blood might be a helpful tool for diagnosing or monitoring AN. In addition, the positive correlation with autoantibodies points to a possible connection between SIRT1 and immune response disregulation in AN. Thus, reducing the production of autoantibodies specific for hypothalamic cells could be a sign of improvement in the condition.

Further research is needed to confirm these initial findings and explore the exact role of SIRT1 in AN. However, this study highlights the potential of SIRT1 as a biomarker and opens doors for investigating how the immune system might be involved in AN.

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Time-dependent characterization of cortical projection neuron's alterations and development in a murine model of Spinal Muscular Atrophy

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Spinal Muscular Atrophy (SMA) is a severe neurodegenerative disease of the early childhood, caused by the mutation/deletion of the survival motor neuron (SMN1) gene. The lack of functional SMN protein determine the degeneration of lower motor neurons (MNs) in the spinal cord; however, recent research in animal models and patients revealed that the brain is also impacted by SMN deficiency. Thus, the involvement of cortical alterations in SMA needs to be clarified. We focused on the sensorimotor cortex of SMAΔ7 mice, a severe SMA model, to examine the effect of SMN deficiency on cortical projection neuron survival and cytoarchitecture. We analyzed early (postnatal day 5) and late (P11) symptomatic animals, comparing SMA mice with their wild-type (WT) littermates. We used immunofluorescence to identify projection neuron subtypes and retrograde tracers for morphological analysis. To investigate whether SMN depletion can affect projection neurons since cortical development, we performed thymidine-analogues (EdU) labeling in the dams at different embryonic time points (E12, E14, E15) and mapped EdU+ cell distribution in the cortical layers of pups. We found that both corticospinal (Ctip2-positive) and callosal (Satb2-positive) neurons are reduced at P11 in SMA cortex, suggesting that SMN reduction affects upper MNs as well. SMA cortical cells also show alteration in some morphological traits. Although the same analyses at P5 suggest that these changes occur concurrently with spinal MN death and not earlier, the evaluation of cell birthdating and distribution by EdU are helping us in revealing possible alterations in SMA cortex already at developmental stages. Indeed, preliminary data show a different distribution of EdU+ cells born at E14 and E15 in the cortical layers of SMA brain compared to WT. Overall, knowing the involvement of cerebral cortex in SMA will contribute to unravelling the dynamics of progressive degeneration and will help to understand any developmentally-related deficits.

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Human iPSCs as a model to study peripheral neurotoxicity: a comparison with rat DRG neurons

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A very disabling side effect of several effective antineoplastic drugs is the induction of peripheral sensory neuropathy, mainly targeted on Dorsal Root Ganglia (DRG), which often causes the anticancer treatment interruption. This clinical problem, known as Chemotherapy-Induced Peripheral Neuropathy (CIPN), is currently without a therapy despite ongoing research efforts. Besides the CIPN complexity, a limiting factor for progress is the lack of reliable in vitro human models to screen putative neuroprotective molecules without interfering with the anticancer drug effect; so far, the data have been obtained by using murine DRG.

In this way, human induced pluripotent stem cells (iPSCs) could represent a revolutionary approach for drug screening. In the last decade, the advances made into the reprogramming iPSCs led to great improvements towards their use as models of diseases, with establishment of iPSC-derived peripheral nervous cell models still in its infancy.

Here we evaluated the suitability of human iPSC-derived sensory neurons as peripheral system neuron model. In particular, we analyzed the dynamic expression of neuronal markers along 30 days of culture of iPSCs-derived sensory neurons provided by FUJIFILM Cellular Dynamics (iCell Sensory Neurons), with a comparison to the classical in vitro rat DRG neuron models. We also compared drug responsiveness to different antineoplastic drugs between the two models.

We observed in iPSC-derived sensory neurons the expression of both morphological and functional sensory neuronal markers, with a molecular pattern similar to rat DRG neurons, and a differentiation more oriented towards a nociceptive phenotype. Moreover, antineoplastic drugs had a toxic effect on iPSC-derived sensory neurons at the same concentrations that resulted toxic in rat DRG neurons.

These results confirm the suitability of human iPSC-derived sensory neurons as a model to study neurotoxicity and to push forward efforts to identify effective neuroprotective molecules.

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NFATc1 regulates cell signaling and mitochondria functionality through the modulation of intracellular cholesterol abundance

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NFAT is a family of five different transcription factors (NFATc1-5), and the first four are activated by the Ca2+/calmodulin-dependent phosphatase calcineurin. Notably, the calcineurin/NFAT signaling pathway, which is fundamental to maintaining normal T-cell physiology, has been found to be deregulated both in B/T-cell lymphomas and leukemias, as well as in solid tumors (i.e prostate, breast and pancreatic cancer). Starting from the evidence of an involvement of NFATc1 in the resistance to glucocorticoid (GC) treatment in T cell acute lymphoblastic leukemia cells, we aimed to unveil the biological processes driven by NFATc1, involved in GC resistance. To achieve this goal, we applied Gene Expression Profile and Nuclear Magnetic Resonance analysis on NFATc1 knockdown cells, and we observed, among the most significant biological processes, the downregulation of the intracellular cholesterol abundance. Additionally, by Chromatin Immune Precipitation we revealed that NFATc1 can directly control the transcription of HMGCS1, EBP and DHCR7, key enzymes of cholesterol biosynthesis process. In addition, since cholesterol is a key component of the plasma membrane lipid raft (LR) elements by immunofluorescence we demonstrated that its downregulation decreases the number of LRs, as well as the anchoring and activation of key proteins and co-receptors such as the lymphocyte-specific protein tyrosine kinase (LCK) and CD4 and CD8, thus impairing the TCR signaling cascade. Finally, 3% of intracellular cholesterol is located into the mitochondria membrane, we wondered whether the decrease in cholesterol levels could impact on mitochondria functionality. Intriguingly, through membrane polarization and mitochondrial ROS production assays, we unveiled a loss of mitochondria functionality as well as an increase in autophagy markers. Transmission Electron Microscopy observations are ongoing to further confirmed these evidences. All together these results reveal a novel role for NFATc1 in which trough the regulation of cholesterol intracellular abundance can modulate the intracellular cell signaling and the mitochondria functionality.

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The human major sublingual gland and its neuropeptidergic and nitrergic innervations

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The human sublingual gland tissue mass is not a single organ but consists of a large segment, the major sublingual gland, and a cluster of small independent glands, the minor sublingual glands. The two types of sublingual glands differ in embryonic histories and composition of mucous and seromucous cells. They are drained by separate duct systems: the major sublingual gland by the duct of Bartholin, accompanying the submandibular duct, and each of the minor glands by a separate duct emptying directly into the mouth. Only recently, the adrenergic and cholinergic innervation of the major sublingual gland was reported, while information regarding the non-adrenergic and non-cholinergic innervation is still lacking. Hence, we investigated the possible presence of nerve fibres using neuropeptides and nitric oxide (NO) as transmitters, which may affect the functions of the major sublingual gland.

Bioptic and autoptic specimens of the human major sublingual gland were examined using immunohistochemistry for the presence of vasoactive intestinal peptide (VIP)-, neuropeptide Y (NPY)-, substance P (SP)-, calcitonin gene related-peptide (CGRP)-, and neuronal nitric oxide synthase (nNOS)-labelled neuronal structures.

Regarding the neuropeptidergic innervation of secretory cells (here in the form of mucous tubular and seromucous cells), the findings showed many VIP-containing nerves, few NPY- and SP-containing nerves, and a lack of CGRP-labeled nerves. Regarding the neuropeptidergic innervation of vessels, the number of VIP-containing nerves was modest, while, among the other neuropeptide-containing nerves under study, only few (SP and CGRP) to very few (NPY) nerves were observed. Regarding the nitrergic innervation, nNOS-containing nerves were very few close to secretory cells and even absent around vessels.

In conclusion, the different innervation patterns may suggest potential transmission mechanisms involved in secretory and vascular responses of the human major sublingual gland.

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Fabrication and characterization of bi-layered scaffolds based on bioactive oxidized polyvinyl alcohol and 3D printed polylactic acid for osteochondral defect repair

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Osteochondral defects are a significant challenge in orthopedic surgery, due to cartilage complexity and its exposure to high pressure/motion. Within this scenario, osteochondral tissue engineering has shown an increasing improvement, looking for valuable options. The aim of this study was to develop scaffolds for osteochondral regeneration. As for cartilage, oxidized polyvinyl alcohol (OxPVA) was combined with gelatin microspheres (porogen, 10%, 15% and 25% w/w) by mechanical incorporation. After systems cross-linking by freeze-thawing and gelatin removal by particle-leaching, the supports were characterized for their ultrastructure/mechanical behaviour/interaction with stem cells (HM1-SV40). In parallel, articular cartilage from Donors enrolled in Body Donation Program of Padua University was decellularized, evaluated to assess treatment effectiveness, and homogenized before incorporation into OxPVA (25% w/w of extracellular matrix (ECM) + different amounts of porogen). Derived scaffolds were compared for ultrastructure/ECM distribution/bioactivity *in vitro*/biocompatibility *in vivo*. Morphometric study showed that structure complexity was greater at higher porogen percentages; moreover, higher porosity had a negative impact over material stiffness (reduction) but not on cells adhesion/proliferation (increase). For a balance between compressive strength and scaffolds bioactivity, 15% and 25% w/w gelatin microspheres were chosen to be combined with cartilage ECM into OxPVA, respectively. The addition of decellularized/homogenized ECM furtherly improved porous scaffolds bioactivity without *in vivo* inflammation, thus suggesting scaffolds biocompatibility. Regarding bone, 3D printing was used to fabricate three different polylactic acid (PLA) supports; these were compared for ultrastructure and interaction with cells (HM1-SV40). All the three geometries sustained a good cell viability with a statistically significant difference in proliferation in presence of a higher porosity (64.2%) (lower porosity, 28.5%). Scanning Electron Microscopy showed cells distribution both over and inside the PLA scaffolds, suggesting their full-thickness colonization. Despite future studies will be necessary, combining bioactive OxPVA and 3D printed PLA scaffolds may lead to promising device for osteochondral regeneration.

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Machine learning analysis of brain neuroimaging for prediction of Alzheimer's disease

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Worldwide, there are approximately 10 million new cases of dementia each year, of which Alzheimer's disease (AD) is the most common. A treatment given at an early stage of AD is more effective and causes less harm than a treatment given at a later stage. In its early stages, AD is difficult to predict. To improve the diagnosis of individuals with cognitive impairment due to various etiologies requires the implementation and use of new technologies is necessary.

The development of morphological models of the hippocampus can represent a starting point in identifying the tools allowing an early diagnosis of neurodegenerative disorders.

This study has explored the application of ensemble machine-learning techniques to predict AD progression based on clinical and demographic information and a neuroimaging approach. From a cohort of patients enrolled for ASCOMALVA trial over 48 months, the dataset included hippocampal volume, cognitive test scores and demographic variables. There were three predictive models employed, namely Linear Regression (LR), Neural Networks (NN), and Gradient Boosting Machines (GBM).

There is substantial predictive performance across the models based on the results. There is a strong linear relationship between disease progression and predictors in the LR model, as demonstrated by the R-squared value of 0.84. NN exceeded this with an R^2 of 0.91, demonstrating its ability to capture complex nonlinear relationships in the data, while GBM achieved an R^2 of 0.93, demonstrating enhanced ensemble learning capabilities in the iterative refinement of weak learners to boost model accuracy.

In this study, the effectiveness of ensemble machine-learning approaches for AD prediction is highlighted, emphasizing GBM as one of the most promising approaches.

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Histological assessment of spinal cord and medulla from embalmed anatomical donors

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Tissue from donors routinely embalmed for anatomical examination is often considered to be unsuitable for histological investigations. This is based on the belief that the embalming procedure, while preserving tissue and allowing dissection over months, is not sufficient to preserve microscopic tissue architecture. This may be particularly relevant for nervous tissue.

We studied the extent to which CNS tissue from anatomical donors may be sufficiently preserved by embalming to enable histological examination. We examined medulla and spinal cord (cervical, thoracic, lumbar) samples obtained with appropriate permissions from four donors from the Anatomy Gift Programme of the Royal College of Surgeons in Ireland in Dublin. Donors were embalmed according to a standard procedure by a mixture containing methylated spirit, formaldehyde, glycerin, and phenol. After harvesting, tissue was processed for paraffin embedding and routine histological staining.

Tissue was analysed qualitatively and scored for the level of histological preservation based on seven different parameters (scores: 1=poor, 2=medium, 3=good). Parameters considered were: tissue integrity, possibility to examine leptomeninges along the entire section surface, distinction/adherence grey/white matter, neuronal identification, nuclei of other cells, neuropile pattern, capillaries and other intraneural vessels.

Most samples showed clearly preserved histological architecture. Cellular details, such as tigroid substance (Nissl bodies), were well distinguishable in some large neurons. On average, of the 4 regions examined, medulla, cervical, and lumbar spinal cord showed similar scores of approximately 75-77% of the maximum possible for quality of histological preservation. Thoracic spinal cord, in contrast, showed on average approximately 52% of the maximum possible score.

In conclusion, nervous tissue of donors, routinely embalmed for anatomical examination, can be suitable for histological studies. This offers a considerable source of tissue for in-depth studies of human CNS and considerably expands the impact of the generosity of anatomical donors for the advancement of biomedical knowledge.

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Novel radiological technologies to study the anatomy of the peripheral nervous system: anatomical variants and pathological correlations

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Non-invasive evaluation of Peripheral Nervous System (PNS) is an open challenge in the field of radiologic studies, often tackled with ultrasound, which is limited to superficial fibers. High-resolution imaging techniques, such as magnetic resonance neurography (MRN) and multishell DTI, have revolutionized the visualization of peripheral nerves, allowing for the detection of subtle anatomical variations and abnormalities previously elusive with conventional methods.

Multishell DTI, an advanced form of diffusion imaging, captures data at multiple b-values, enhancing the sensitivity and specificity of nerve imaging. This technique maps the diffusion of water molecules along nerve fibers with greater precision, offering critical information on nerve integrity and microstructure. Such detailed imaging facilitates the identification of anatomical variants, crucial for surgical planning and avoiding iatrogenic injuries.

Moreover, these imaging modalities are invaluable in diagnosing and understanding peripheral neuropathies. We present three clinical cases demonstrating the utility of these advanced technologies. The first case involves the avulsion of the chord of C7 of the right brachial plexus following a motorbike accident, where multishell DTI provided detailed visualization of the traumatic nerve disruption, guiding surgical intervention. The second case describes impingement of nerve roots between the scalene muscles in a waiter, with MRN and multishell DTI identifying the exact location and nature of the compression, facilitating effective treatment. The third case highlights an anatomical variant where a sciatic root passes through the obturator muscle, identified via MRN, critical for understanding patient-specific anatomy and avoiding surgical complications.

In conclusion, the integration of advanced radiological technologies, particularly multishell DTI, has transformed the study of the PNS, leading to improved diagnostic accuracy and patient outcomes, and paving the way for innovative therapeutic interventions.

References

1) Gasparotti R, Salvalaggio A, Corbo D, Agazzi G, Cacciavillani M, Lozza A, Fenu S, De Vigili G, Tagliapietra M, Fabrizi GM, Pareyson D, Obici L, Briani C. Magnetic resonance neurography and diffusion tensor imaging of the sciatic nerve in hereditary transthyretin amyloidosis polyneuropathy. J Neurol. 2023 Oct;270(10):4827-4840.
2) Castellaro M, Moretto M, Baro V, Brigadoi S, Zanoletti E, Anglani M, Denaro L, Dell'Acqua R, Landi A, Causin F, d'Avella D, Bertoldo A. Multishell Diffusion MRI-Based Tractography of the Facial Nerve in Vestibular Schwannoma. AJNR Am J Neuroradiol. 2020 Aug;41(8):1480-1486

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The superior colliculus in the MPTP rat model of Parkinson's disease: a microscopic study

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The superior colliculus represents an important center of multimodal information and sensorimotor transformation. It has been shown that visuomotor impairments in Parkinson's disease, such as hypometria of visually guided saccades, can occur through colliculus functional alteration. Many studies have extensively studied and described the laminar structure of the colliculus in various animal species, characterizing the neuronal types and neuronal density in each lamina [1]; at the same time, the involvement of superior colliculus in visuomotor impairments of Parkinson have been investigated mainly from a functional point of view. The literature does not show enough microscopic structural data of the superior colliculus in association with Parkinson. By that, the aim of the present study was to analyse the microscopic structure of the superior colliculus of rats in which Parkinson's disease has been experimentally induced by MPTP injection. Rat's brains have been processed for histological staining and immunohistochemistry techniques. Through NeuN antibody we carried out a neuronal count in each layer of the colliculus both in sham and MPTP rat's brain. The most interesting result shows that in MPTP superior colliculus there is a significant reduction of neurons in the superficial layers, an area dedicated to the exclusive reception of visual stimuli. This preliminary data, together with those we are going to collect, could help the understanding of visuomotor impairments in Parkinson's disease.

[1] Jianhua Cang,1 Elise Savier,1 Jad Barchini and Xiaorong Liu. Visual Function, Organization, and Development of the Mouse Superior Colliculus. Annu. Rev. Vis. Sci. 2018. 4:239–62.

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Glioblastoma Connections within the human neuro-glial network: friends and foes

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High-grade gliomas (HGGs), including astrocytoma and glioblastoma (GBM), are the most common primary tumors of the central nervous system (CNS). Patients with glioblastoma often face poor prognoses. At present, the main treatment of glioma is tumor resection, followed by radiotherapy and chemotherapy. However, postoperative recurrence is common along with epilepsy, due to the heterogeneity of the mass, the unbounded combination of the tumor and normal tissue, and the high invasiveness of glioma cells,

Exploring new concepts and novel molecular targets for the treatment of HGGs is urgent. The interplay of GBM-resident CNS could be based on connections. Many connexin (CX) molecules have been explored in the CNS, and CX43 seems to be a marker for HGGs from mouse models.

We prepared acute and organotypic slices from the human peritumoral cortex to characterize the role of Cx43 in the human neuroglial network within the HGGs. Human primary glioblastoma cells were tagged using lentiviral transduction and injected into organotypic slices. Glioblastoma cells were tracked until DIV 7 to test the tumor progression as well as their response to Gap19, a selective blocker of the Cx43 hemichannels. Astrocytes, microglia/macrophages, and extracellular matrix were studied using morpho-molecular techniques.

Implanted glioblastoma cells integrated into the neuroglial network and invaded the peritumoral tissue, inducing functional modifications. Connections within the peritumoral tissue of patients with astrocytoma lead to a major molecular remodeling of the tissue. The blockage of Cx43 hemichannels implied further alterations of the peritumoral microenvironment, accompanied by a reduction of the epileptic discharges and polarization of GBM cells.

GBM connections may contribute to the spread, and recurrence of the tumor, revealing a complex dynamic. The present evidence prompts a paradigm shift, challenging the perception of GBM as a foreigner within the brain. Accordingly, extra-CNS metastasis remains a rare clinical entity.

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High expression of SMN circ4-2b-3 in SMA I children treated with Nusinersen is associated with improved motor outcomes

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Spinal muscular atrophy (SMA) is a neuromuscular disorder that results in the loss of α-motor neurons with progressive muscle atrophy. SMA is caused by deletions of *Survival Motor Neuron 1* (*SMN1*) gene, encoding for SMN protein, which is necessary for motor neuron function and survival. *SMN2* gene is a nearly identical copy of *SMN1* but is unable to produce a functional full-length protein due to a splicing defect, and alone is not sufficient to avoid the motor neuronal loss that occurs in SMA patients. However, *SMN2* gene is possibly related to the severity of the disease since the higher is the number of *SMN2* copies, the lower is the severity of symptoms. Therefore, at least two therapies for SMA treatment aim to correct the splicing defect of *SMN2*, allowing the production of a greater amount of the functional SMN protein from this gene. Among these, *Nusinersen* is an antisense oligonucleotide intrathecally administered to all pediatric and adult SMA patients. Although the treatment shows important neuromuscular improvements, not all SMA patients equally respond to the therapy. In this scenario, the identification of biomarkers might pave the ground for better clinical management of the SMA patients. Herein, we evaluated the expression of SMN circRNAs in human SMA fibroblasts and detected one of them, the SMN circ4-2b-3, in exosomes of blood serum from age-matched healthy individuals and SMA type I patients. Interestingly, high copy number of SMN circ4-2b-3 occurs in a small subgroup of SMA type I patients who have been clinically defined as *super-responder* group, based on their optimal response to the *Nusinersen*. Interestingly for most of them, the levels of SMN circ4-2b-3 remain high even when analyzing different samples over time, suggesting the potential for this molecule as biomarker to predict the course of pathology for *Nusinersen*-treated SMA type I patients.

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Salivary biomarkers in Parkinson's Disease and Synucleinopathies

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Parkinson's Disease (PD) is characterized by the accumulation of misfolded alpha-synuclein (a-syn) and by the activation of different molecular pathways, converging in neuronal death and synaptic loss. Clinical diagnosis and treatment of PD are hampered by the progressive deterioration of target neuronal circuits and by the mismatch between clinical and neuropathological onset. Molecular biomarkers are of unreplaceable importance to couple neuropathological and clinical features. Salivary glands are richly innervated by *viscero-sensory* and *viscero-moror* fibres which enter in strict contact with the serous and mucous adenomeres, reversing neuronal-derived molecules in the context of saliva by extracellular vesicles (EVs). For this reason, saliva – an easily accessible biofluid, whose collection is free of pain and discomfort for the patient – has recently demonstrated a great potential as source of biomarkers for synucleinopathies.

ELISA analysis [1,2] and Real-Time Quaking Induced Conversion (RT-QuIC) assays [3] have been applied to detect a-syn aggregates, tau and phosphorylated aggregates, as well as inflammation and autophagy biomarkers, in the saliva of patients affected by synucleinopathies and healthy subjects. Molecular data have been correlated with clinical features of the patients and used for molecular clustering through principal component analysis (PCA). Immunofluorescence for a-syn and phosphorylated a-syn have been employed on skin biopsies of PD patients and healthy subjects and analysed by confocal microscopy to correlate molecular alterations in saliva with peripheral nerve fibres degeneration.

Altered salivary biomarkers have been demonstrated through ELISA in the saliva of patients affected by synucleinopthies and they were able to predict clinical prognosis. Moreover, RT-QuIC assay demonstrates seeding competent a-syn species in the saliva of PD patients and RT-QuIC kinetic parameters correlated with disease severity. Finally, autophagic markers and inflammatory markers were increased in the saliva of PD patients and were responsible of their molecular clustering. In skin biopsies, a-syn aggregates were detected in sensory intra-epidermic amyelinic fibres and in autonomic fibres around sweating glands and pilo-erector muscles and partially correlated with the molecular alterations detected in saliva.

In conclusion, saliva represents a key biofluid candidate for the detection of biomarkers in synucleinopathies and could be also used for clustering different clinical subtypes and to predict disease prognosis.

References

1. Vivacqua et al. PLoS One. 2016; 11(3): e0151156
2. De Bartolo et al. Ann Neurol. 2023; 93(3): 446-459
3. Vivacqua et al. Mov Disord. 2023; 38(1): 153-155

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Prenatal overactivation of cannabinoid receptor CB2 affects offspring male germ cells development via epigenetic mechanisms

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Cannabis use among pregnant women has increased in the last years. Cannabinoids can cross the placenta, enter the fetal bloodstream, and distribute to fetal tissues causing several adverse outcomes. However, the impact on germline development of the offspring is still poorly known. During fetal life, primordial germ cells (PGCs) migrate from the base of allantois to the gonadal ridges at ~10.5 dpc and at ~13.5 dpc they differentiate on the basis of sex. In male, germ cells enter a period of quiescence from 13.5 dpc and after birth, they resume mitotic proliferation and initiate meiosis at around PND7. This process is regulated by both genetic and epigenetic mechanisms. In this study, we investigated the effects of prenatal exposure to cannabinoid JWH-133, a selective CB2 receptor agonist, on male germ cell development and spermatogenesis. We show that exposure to JWH-133 during fetal life, through administration of the drug to pregnant females, causes a delay of spermatogonia differentiation. Histological examination of testis of P30 offspring showed the presence of round spermatids in exposed mice while more differentiated cells, elongating spermatids and spermatozoa, were present in unexposed mice. In order to identify the involved molecular mechanisms we focused on histone epigenetic methylation of H3K27me3 and H3K4me3 that are known to silence or activate gene expression respectively, playing a pivotal role in cell differentiation. We found that prenatal exposure to JWH-133 was associated with an enrichment of H3K27me3 in spermatogonia stem cells (Plzf+), while H3K4me3 level did not change. Methylation of H3K27me3 is an important mechanism in the maintenance of self-renewing of embryonic stem cells (ESCs) by repressing the differentiation pathway (1). Our findings suggest that prenatal cannabinoid exposure can induce accumulation of aberrant histone marks (H3K27me3) in spermatogonia stem cells that lead to altered gene expression and delay of cell differentiation (2).

References

1) Das, P., Taube, J.H. Regulating methylation at H3K27: a trick or treat for cancer cell plasticity. Cancers (Basel) 2020, 12 (10).
2) Zucchi, A., Innocenzi, E., Onorato, A., Dolci, S., Colopi, A., Balistreri, C.R., Grimaldi, P. Prenatal exposure to CB2 receptors agonist differentially impacts male and female germ cells via histone modification. Mechanisms of Ageing and Development, 2023, 213,111840.

Histogenesis, functions and dysfunctions of the musculoskeletal system

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Anticancer effect of Omomyc peptide on cellular models of human osteosarcoma

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Osteosarcoma (OS) is the most common primary malignant bone tumor that usually affects children and adolescents. It is highly malignant, with irregular bone growth and distant metastases that are commonly observed in the lungs1. Although the 5-year survival rate of patients with localized OS was improved to 65–70% by chemotherapy combined with surgical resection, the overall survival rate in osteosarcoma has remained virtually unchanged over the past 30 years2. Therefore, novel strategies are urgently needed to improve the clinical outcome in osteosarcoma. A potential therapeutic target could be c-Myc which is a frequently deregulated oncogene in cancer and commonly amplified in OS too. It works as a pleiotropic transcription factor coordinating transcriptional programs involved in cell proliferation, cell growth, metabolism, apoptosis and immune suppression. Omomyc3, the best-characterized direct c-Myc inhibitor to date, functions as a dominant negative for c-Myc transcriptional activity. Omomyc sequesters c-Myc away from DNA and occupies target gene promoters in the form of inactive homodimers or heterodimers with Max, shutting down their transcription. Our data demonstrate Omomyc ability in modulating the c-Myc proliferative effects in OS, in term of onco-suppressor increase and cyclins reduction. Our preliminary results support that inhibition of c-Myc significantly reduces osteosarcoma cell growth, migration and, therefore, represents a promising strategy in OS treatment.

References

1) Akkawi, R.; Hidmi, O.; Haj-Yahia, A.; Monin, J.; Diment, J.; Drier, Y.; Stein, G.S.; Aqeilan, R.I. WWOX promotes osteosarcoma development via upregulation of Myc. Cell Death Dis. 2024, 15, 13.
2) Jiang, k.; Zhang, Q.; Fan, Y.; Li, J.; Zhang, J.; Wang, W.; Fan, J.; Guo, Y.; Liu, S.; Hao, D.;Wang, Y.; Wang, L.; Shan, L. MYC inhibition reprograms tumor immune microenvironment by recruiting T lymphocytes and activating the CD40/CD40L system in osteosarcoma. Cell Death Discov. 2022, 8, 117.
3) Massó-Vallés, D.; Soucek, L. Blocking Myc to Treat Cancer: Reflecting on Two Decades of Omomyc. Cells 2020, 9, 883.

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The sarcoglycan subcomplex in chondrocytes: an in vitro study

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The sarcoglycan complex (SGC) is essential for maintaining the structural integrity of muscle fibers and muscle function. It comprises six subunits: alpha, beta, gamma, delta, epsilon, and zeta sarcoglycan (SG), which are transmembrane glycoproteins located mainly in the sarcolemma of skeletal muscles. These proteins are part of the dystrophin-glycoprotein complex (DGC) and play a crucial role in linking the cytoskeleton of muscle cells to the extracellular matrix. This stabilizes the sarcolemma of myofibrils, protecting muscle fibers from damage during repeated cycles of contraction and relaxation. Mutations in sarcoglycan genes are linked to various forms of muscular dystrophy, including limb-girdle muscular dystrophy (LGMD) and myoclonus-dystonia syndrome. Research indicates that the sarcoglycan complex is not exclusive to muscle tissue; it is also found in the brain, kidneys, and lungs. Our research group has studied sarcoglycans in tissues such as the brain, prostate, gingival tissue, and breast tissue. This widespread distribution suggests that sarcoglycans have roles beyond muscle function, including cell adhesion and signal transduction. Studies show that sarcoglycans interact with integrins, highlighting a bidirectional signaling pathway between sarcoglycans and the integrin adhesion system, which may be vital for cell adhesion and tissue integrity. In this work we wanted to investigate the presence of sarcoglycans in cultured chondrocyte cells, considering that there are no data in the literature. Chondrocytes, the primary cellular component of cartilage tissue, are crucial for maintaining its structure and function. They synthesize and maintain the extracellular matrix, providing cartilage with its unique mechanical properties. Our results showed the presence of sarcoglycans at the focal plaques. Understanding chondrocytes' role in cartilage biology is vital for elucidating the mechanisms of cartilage development, homeostasis, and repair.

References

1) Anastasi, G. Sarcoglycan[s] are not muscle-specific: hypothetical roles. Ital J Anat Embryol. 2010;115(1-2):19-24
2) Cutroneo,G.; Anastasi, G.; Bramanti, P.; Bruschetta, D.; Favaloro, A.; Vermiglio, G.; Trimarchi, F., Di Mauro, D.; Rizzo, G. Sarcoglycans and gaba(a) receptors in rat central nervous system: an immunohistochemical study. Ital J Anat Embryol. 2015;120(2):105-16.

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RAGE engagement at myofiber level plays a determinant role in cancer-induced muscle wasting

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Cachexia is a highly debilitating multifactorial syndrome affecting patients with advanced cancer characterized by progressive muscle wasting. We observed that RAGE (receptor for advanced glycation end-products) is re-expressed in atrophic myofibers in tumor-bearing mice, and high amounts of RAGE ligands released from tumor and inflammatory cells act as pro-cachectic factors hyperstimulating RAGE and amplifying signaling pathways involved in protein degradation. RAGE signaling in mice sustains hallmarks of cancer cachexia, including skeletal muscle catabolism, inflammation, and tumor progression. Tumor-bearing mice lacking RAGE (*Ager*–/– mice) showed surprisingly increased survival and delayed loss of muscle mass and strength compared to controls. To understand the specific contribution of RAGE expressed at the muscle level to cachexia, the effects of subcutaneous injection of Lewis lung carcinoma (LLC) cells were evaluated in a conditional mouse model in which the RAGE gene is selectively deleted in skeletal muscles (*Ager*mKO mice), generated by crossing *Ager*flx/flx with tamoxifen-inducible HSA-MerCreMer mice. LLC-bearing *Ager*mKO *vs* LLC-bearing control (*Ager*flx/flx) mice showed: i) almost complete resistance to muscle wasting; ii) slow-down of body weight loss and increased survival, although to a lesser extent than LLC-bearing *Ager*–/– mice; iii) reduced cachexia-inducing factors in serum and muscles. The absence of RAGE in muscles of tumor-bearing mice resulted in maintenance of muscle mass and performance by reducing the ubiquitin-proteasome system-dependent myosin-heavy chain (MyHC)-II degradation, and increasing the expression of the slow isoform MyHC-I, which confers resistance against cancer-induced myofiber atrophy. Muscle proteomic analyses revealed similar pathways modulated in *Ager*mKO and *Ager*–/– *vs* control mice in cancer conditions. Our results suggest that RAGE engagement at myofiber level appears as a determinant for muscle atrophy in cancer conditions, although total ablation of RAGE results in the highest protection against cancer cachexia. Thus, molecular targeting of RAGE might represent a useful approach to counteract the cachectic syndrome in cancer patients.

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The role of Protein-Kinase C theta in ALS disease progression

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Neuromuscular junction (NMJ) represents the morphofunctional interface between muscle and nerve. The impairment of muscle-nerve communication is a typical characteristic of several diseases, including Amyotrophic Lateral Sclerosis (ALS). ALS is characterized by NMJs dismantlement, myofiber type switch, metabolic defect and immune cell infiltration that leads to spinal cord inflammation and finally to motor neuron degeneration. In a previous work we have characterized a transgenic mouse model that over-expresses the ALS human mutant gene, the SOD1G93A one, selectively in the skeletal muscle (MLC/SOD1G93A mice) (1). These mice exhibit several features of the pre-symptomatic phase of ALS disease, including the impairment of muscle metabolism and NMJs defects. Recently, we have demonstrated a causal link between the aberrant activation of the Protein kinase C-θ (PKCθ) and the NMJ dismantlement in the skeletal muscle of the MLC/SOD1G93A mice (2). Therefore, here aimed to better clarify the role of PKCθ activity in the mouse model of ALS disease represented by the SOD1G93A mice, that overexpress ubiquitously the human SOD1 mutant gene. We firstly demonstrated the aberrant activation of PKCθ in muscle tissue of SOD1G93A mice and then we evaluated whether the pharmacological inhibition of PKCθ activity by Compound 20 (C20) could ameliorate disease progression in ALS mice. We observed that the C20 treatment, was able to significantly elongate lifespan of SOD1G93A treated mice, improving locomotor activity, attenuating muscle atrophy, metabolic defects and finally ameliorating NMJ morphology and functionality. Moreover, we observed that C20 treatment was able to attenuate immune cells recruitment in both skeletal muscle and spinal cord which may play a critical role in NMJ dismantlement. Overall, these results suggest that C20 can counteract different pathological aspects of ALS diseases and thus represents a promising pharmacological tool to counteract ALS disease in human patients.

References

1) Dobrowolny, G.; Aucello, M.; Rizzuto, E.; Beccafico, S.; Mammucari, C.; Boncompagni, S.; Belia, S.; Wannenes, F.; Nicoletti, C.; Del Prete, Z.; Rosenthal, N.; Molinaro, M.; Protasi, F.; Fanò, G.; Sandri, M.; Musarò, A. Skeletal muscle is a primary target of SOD1G93A-mediated toxicity. Cell Metab. 2008, 8(5),425-36.
2) Dobrowolny, G.; Martini, M.; Scicchitano, BM.; Romanello, V.; Boncompagni, S.; Nicoletti, C.; Pietrangelo, L.; De Panfilis, S.; Catizone, A.; Bouchè, M.; Sandri, M.; Rudolf ,R.; Protasi, F.; Musarò, A. Muscle Expression of SOD1G93A Triggers the Dismantlement of Neuromuscular Junction via PKC-Theta. Antioxid Redox Signal. 2018, 28(12),1105-1119.

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Effects of mechanical stress on signaling pathways and LINC complex in skeletal muscle cells from young and old subjects

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Skeletal muscle cells are characterized by their ability to withstand mechanical stress, which is correlated with a relatively high risk of rupture at the junctional sarcolemma level, especially in the elderly. Through mechano-signaling, tension forces are sensed by membrane receptors and transduced to the nucleus, leading to changes in cellular structure, gene expression, and signaling pathways, which govern myofibril size. Specifically, external signals (mechanical stimuli) are converted into biochemical signals and transmitted through the cytoskeleton to the nuclear envelope via the LINC (Linker of Nucleoskeleton and Cytoskeleton) complex, a protein complex that connects the cytoskeleton to nuclear envelope proteins. The LINC complex, in turn, interacts with various signaling pathways, leading to changes in cell architecture, maintenance of proper nuclear morphology, nuclear positioning, DNA repair, cell migration, and gene expression.

This study aims to identify the main molecular interactions and modulations of signaling pathways and LINC complex components in differentiating skeletal muscle cells collected from young and old healthy subjects under uniaxial mechanical strain conditions (4 Hz for 4 hours) that mimic physiological conditions. Notably, the ability of cells to respond to mechanical stimuli changes with aging and after injury, although the specific players and signaling pathways involved are still not fully identified.

We show that, upon induction of mechanical strain, young skeletal muscle cells react to uniaxial stretch stimuli by activating mechano-signaling-related pathways, including Akt/mTOR, Wnt/β-catenin, and YAP, altering some LINC complex proteins and releasing inflammatory cytokines, while muscle cells collected from older individuals fail to do so.

These preliminary results pave the way for a more comprehensive understanding of mechano-signaling-related pathways that affect skeletal muscle cells in aging.

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Pericyte dysfunction in collagen VI-related myopathies: Implications of collagen VI-NG2 binding

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Collagen VI-related myopathies (COLVI-RMs) are rare genetic conditions characterized by defects in the assembly and secretion of collagen VI (COLVI), a remarkable protein essential for maintaining extracellular matrix (ECM) integrity. Mutations in genes encoding the α1, α2, and α3 chains of COLVI lead to a spectrum of muscle disorders ranging from mild Bethlem myopathy (BM) to severe Ullrich congenital muscular dystrophy (UCMD), which vary in clinical manifestations, inheritance patterns, and severity. Specifically, COLVI deficiency disrupts the ECM' structure and biomechanical properties, resulting in muscle weakness, joint contractures, and progressive muscle fiber atrophy. However, the precise molecular and cellular mechanisms underlying muscle atrophy in COLVI-RMs remain poorly understood.

Recent studies highlight the emerging impact of pericytes in supporting skeletal muscle regeneration. Indeed, in response to muscle injury, pericytes are activated, migrate from the capillary basement membrane, and differentiate into muscle cells to promote tissue repair. Therefore, this study aims to investigate the involvement of pericytes in the development of COLVI-RMs, focusing on elucidating the cellular and molecular mechanisms governing their regenerative capacity. Of note, the research emphasized the functional implications of COLVI-NG2 binding in affected patients compared to healthy individuals.

The findings here revealed that the aberrant COLVI secretion disrupts pericyte function by impairing the balance between proliferation and quiescence in COLVI-RMs patients. Defects in signaling pathways are primarily linked to alterations of NG2-COLVI interaction. Collectively, results indicate that NG2-COLVI binding plays a critical role in regulating pericyte proliferation and quiescence. Disruption of this interaction significantly impairs the myogenic potential of pericytes, thereby hindering muscle regeneration and repair mechanisms.

Overall, understanding the molecular mechanisms underlying pericyte dysfunction in COLVI-RMs may open new avenues for therapeutic interventions aimed at restoring ECM homeostasis and improving clinical outcomes for patients with these debilitating muscle disorders.

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Novel insights into the extracorporeal shock wave therapy in musculoskeletal disorders

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Introduced in the 1980s into routine clinical practice for treating urinary stones (i.e., lithotripsy), in the last decades extracorporeal shock wave therapy (ESWT) has emerged as an effective therapeutic approach for managing various musculoskeletal disorders [1]. Due to the negligible side effects, ESWT has received increasing attention for its potential beneficial effects on various bone and soft tissue pathologies, yielding promising outcomes for pain relief and functional recovery. Indeed, this therapy represents a safe, advantageous, well-tolerated approach alternative to surgery.

Several clinical data evidence a complete or partial fragmentation of calcification, pain relief, and a significant improvement of joint movement in patients with calcifying tendinitis following ESWT [2]. Furthermore, increasing evidence shows that the beneficial effects of ESWT on the locomotor system may be way beyond a mere mechanical disintegrative effect. For instance, wound healing, bone remodelling, and inflammatory and anti-angiogenic effects were detected after ESWT [3].

In line with this, our studies demonstrate encouraging results of ESWT for treating several non-calcifying pathologies such as bone non-union fractures, plantar fibrosis, greater trochanteric pain syndrome, and ulnar nerve subluxation at the elbow.

Although the precise molecular mechanisms of shock waves are still largely unknown, increasing evidence indicates that the application of ESWT to the locomotor system may exert its effects via mechanotransduction pathways. At the molecular level, it has been shown that shock wave therapy promotes cell growth, cell proliferation, and cell differentiation, and suppresses the production of proinflammatory cytokines. Further in-depth studies, including a morphological approach, could clarify the mechanobiological effects exerted by EWST at the ultrastructural level.

References

[1] Lawler, A.C.; Ghiraldi, E.M.; Tong, C.; Friedlander, J.I. Extracorporeal ShockWave Therapy: Current Perspectives and Future Directions. Curr. Urol. Rep. 2017, 18, 25.
[2] Cacchio, A.; Paoloni, M.; Barile, A.; Don, R.; de Paulis, F.; Calvisi, V.; Ranavolo, A.; Frascarelli, M.; Santilli, V.; Spacca, G. Effectiveness of radial shock-wave therapy for calcific tendinitis of the shoulder: Single-blind, randomized clinical study. Phys. Ther. 2006, 86, 672-682.
[3] Wang, C.J. Extracorporeal shockwave therapy in musculoskeletal disorders. Journal of Orthopaedic Surgery and Research. 2012, 7, 11.

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Mesenchymal stem cell-derived extracellular vesicles as a therapeutic treatment for osteosarcopenia: crosstalk among neurons, muscle and bone

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Osteoporosis and sarcopenia are widespread geriatric conditions and, when osteoporosis and sarcopenia occur together, osteosarcopenia can be established. Given the close, reciprocal influence also of muscle on nerve, and vice versa, there are corresponding aging changes in the biochemistry and morphology of the neuromuscular junctions (NMJ). Oxidative stress plays an important role in the pathogenesis of many clinical conditions and aging, including osteosarcopenia and NMJ impairment, therefore a treatment with a biological system, containing antioxidant effects, could be useful to counteract this multi-tissues pathology (1).

Mesenchymal stem cell-derived extracellular vesicles (EVs) have been under investigation as potential treatments for many diseases, based on their anti-aging mechanisms. EVs have antioxidant properties, which can also explain their anti-inflammatory and cytoprotective effects (2).

To study cell interactions in healthy and pathological conditions occurring in neuromuscle-skeletal apparatus, we developed a three culture system in which osteoblasts could be treated to induce osteoporosis before the co-culture with both myotubes and neurons, eventually treated with EVs.

Preliminary results showed that the previous induction of osteoporosis modulated the secretome of myotubes, i.e. myostatin, FGF-2, TGFβ1, IL6, and IL7. Osteokines secreted by these cells, i.e. FGF-23, OCN, and IL-15, were regulated in osteoporotic condition and can be implicated in bone–muscle crosstalk. Aside from the paracrine interaction, modifications in morphology and in protein expression have been explored. The neurites reach the myotubes passing through the membrane of the insert; when the co-culture was exposed to osteoporotic osteoblasts, the number of healthy myotubes and of neurites contacting myotubes was affected. Indeed, the expression of typical markers of sarcopenia and neurodegeneration were observed. The EVs treatment reverted at least in part all these events, suggesting a potential role in slowing down the modifications induced in osteoblasts during bone disorders determining a cascade in the muscle and neuron parts.

References

1) Larsson, L.; Degens, H.; Li, M.; Salviati, L.; Lee, Y.I.; Thompson, W.; Kirkland, J.L.; Sandri, M. Sarcopenia: Aging-Related Loss of Muscle Mass and Function. Physiol Rev. 2019 Jan 1;99(1):427-511.
2) Zhang, W.; Wang, T.; Xue, Y.; Zhan, B.; Lai, Z.; Huang, W.; Peng, X.; Zhou, Y. Research progress of extracellular vesicles and exosomes derived from mesenchymal stem cells in the treatment of oxidative stress-related diseases. Front Immunol. 2023 Aug 14;14:1238789.

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Enhancing micro-dystrophin gene therapy: the role of Sirtuin1-activating compound SRT2104 for the treatment of Duchenne Muscular Dystrophy (DMD)

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Duchenne Muscular Dystrophy (DMD) is an X-linked recessive disorder caused by mutations in the dystrophin gene. The lack of dystrophin protein disrupts the dystrophin-glycoprotein complex eliciting structural degeneration and functional impairments of myofibers. Currently, there is no cure for DMD and the standard treatment consists of the use of corticosteroids. However, progress has been made in gene therapies to restore dystrophin and among the different trialed strategies, the approach based on AAV-delivered micro-dystrophin (MD) is the last to be approved despite some limits. Indeed, the dystrophic muscle milieu exhibits chronic inflammation and early sarcolemmal fragility that do not support MD engraftment and its preservation over time. Therefore, to enhance gene therapy efficacy, it is crucial to develop conservative therapies that preserve dystrophic muscles.

For this purpose, the NADH-dependent deacetylase Sirtuin1 (SIRT1) is emerging as a suitable target. In mdx mice, SIRT1 overexpression tends to counteract the dystrophic muscular and cardiac phenotype. Among the new SIRT1-activating compounds, SRT2104 has never been tested in DMD. We assessed its efficacy on mdx mice demonstrating that, after 12 weeks of administration, treated mice show functional, metabolic and histological improvements compared to the controls.

Overall, given its effects on crucial hallmarks of DMD, SRT2104 could be a promising candidate to sustain MD-based gene therapy in a combined treatment. Noteworthy, this dual approach could also allow to reduce the required AAV dose and, consequently, reduce the dangerous adverse effects related to viral vector immunogenicity. In a dose-response study, we selected the minimum sub-optimal doses of AAV-MD able to restore at least 20% of dystrophin expression in mdx mice and two of these doses have been injected into mice previously treated with SRT2104 to assess whether this can promote a recovery superimposable to the optimal dose of MD, thus demonstrating the advantages of a combined therapy.

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β3 adrenoceptor agonism prevents skeletal muscle alterations in a rat model of hyperoxia-induced damage

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Premature birth, defined as birth occurring before 37 weeks of gestation, is the primary cause of death in children under 5 years of age, and it is associated with numerous short- and long-term complications related to immature organ systems. Premature birth exposes newborns to a relatively hyperoxic environment compared to the intrauterine one, causing increased production of reactive oxygen species (ROS). The oxidative stress could alter the postnatal development of the organs, leading to diseases such as retinopathy of prematurity (ROP), bronchopulmonary dysplasia (BPD), and necrotizing enterocolitis (NEC) [1]. While the impact of preterm birth on skeletal muscles has been less explored, existing evidence suggests its association with muscle fiber atrophy, fiber type shifting, and impairment in muscle function [2]. Considering that β3-adrenoreceptor (β3-AR) is expressed on the skeletal muscle [3], is regulated by environmental oxygen levels, and exerts an antioxidant effect [1], it is conceivable that this receptor could be involved in the skeletal muscle damage induced by hyperoxia, and its activation could provide a protective effect. In an effort to explore this hypothesis, a study was conducted using Sprague–Dawley rat pups exposed to ambient oxygen levels (21%) or hyperoxia (85%) and treated with different doses of BRL37344, a selective β3-AR agonist, or left untreated during the first 14 days after birth. At the conclusion of the study, samples of the gastrocnemius muscle were collected for analysis. The morphologic evaluations revealed that high oxygen levels induce muscle fiber damage, chronic inflammation, and accumulation of type I collagen, while treatment with BRL37344 was able to prevent these alterations. These findings further pave the way for β3-AR pharmacological targeting as an approach for diseases caused by hyperoxia, which are typical of prematurity disorders. Further research in this area could lead to innovative interventions aimed at counteracting the impact of premature birth on skeletal muscle.

References

1. Filippi, L.; Cammalleri, M.; Amato, R.; Ciantelli, M.; Pini, A.; Bagnoli, P.; Dal Monte, M. Decoupling Oxygen Tension From Retinal Vascularization as a New Perspective for Management of Retinopathy of Prematurity. New Opportunities From β-Adrenoceptors. *Front Pharmacol* 2022, *13*, 835771, doi:10.3389/fphar.2022.835771.
2. Deprez, A.; Orfi, Z.; Radu, A.; He, Y.; Ravizzoni Dartora, D.; Dort, J.; Dumont, N.A.; Nuyt, A.M. Transient Neonatal Exposure to Hyperoxia, an Experimental Model of Preterm Birth, Leads to Skeletal Muscle Atrophy and Fiber Type Switching. *Clin Sci (Lond)* 2021, *135*, 2589–2605, doi:10.1042/CS20210894.
3. Martin, C.A.; Advenier, C. Beta 3-Adrenoceptors and Airways. *Fundam Clin Pharmacol* 1995, *9*, 114–118, doi:10.1111/j.1472-8206.1995.tb00270.x.

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Assessment of the impairment of the tendon microenvironment following corticosteroid treatments

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Disorders and inflammatory states affecting musculoskeletal tissues are frequent and widely associated with sports activities, age-related condition, and chronic diseases. Injections of corticosteroids, like Triamcinolone acetonide (TCA) and Dexamethasone (DEXA), represent one of the clinical strategies to hamper the inflammatory mediators during these processes. However, the effectiveness of corticosteroid treatments is still debated as injections of TCA and DEXA can lead to tendon rupture and/or compromised healing (1,2). Typical features of damaged tendons are the alterations in cell morphology as well as extracellular matrix (ECM) composition and arrangement resulting in impaired functionality. Among tendon cells, tenocytes are the main responsible for tissue homeostasis, and the presence of a heterogeneous pool of stem and progenitor cells represents its intrinsic regenerative potential (3). Here we have explored the actual detrimental effects of corticosteroids in the tendon microenvironment, to optimize new therapeutic strategies. With this purpose, we treated immortalized tenocytes from Achilles' tendon (hAT1-tert cells) with TCA and DEXA, also focusing on the paracrine effect of corticosteroid-treated tenocytes on periosteal-derived stromal cells (PDSCs). Our results indicated that TCA and DEXA had harmful effects on hAT1-tert cells in a time and dose-dependent way, reducing cell viability, altering morphology and affecting the expression of main tendon markers. On the contrary, PDSC morphology and viability do not seem to be affected by the direct administration of TCA and DEXA nor by the paracrine effect of damaged tenocytes.. However, in PDPCs we detected an increase in the autophagic flux, probably as a conservative response to an imbalance in oxidative stress defences.

Overall, our data indicate that corticosteroid treatments can damage tendons, without affecting the regenerative potential of the stromal surrounding compartment, suggesting an encouraging context in the repair process of tendons.

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References

1) Millar, N.L.; Silbernagel, K.G.; Thorborg, K.; Kirwan, P.D.; Galatz, L.M.; Abrams, G.D.; Murrell, G.A.C.; McInnes, I.B.; Rodeo, S.A. Tendinopathy. Nat Rev Dis Primers. 2021, 7, 7(1);1.
2) Tanaka, T.; Ogawa, T.; Yanai, T.; Okano, E.; Kohyama, S.; Ochiai, N. Disruption of Flexor Tendon after Intrasheath Triamcinolone Acetonide Injection for Trigger Digits: Two Case Reports. J Hand Surg Asian-Pac Vol. 2017, 22(03), 380–3.
3) Citro, V.; Clerici, M.; Boccaccini, A.R.; Della Porta, G.; Maffulli, N.; Forsyth, N.R. Tendon tissue engineering: An overview of biologics to promote tendon healing and repair. J Tissue Eng. 2023, 14, 20417314231196275.

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Employment of a novel phytotherapic formulation in the recovery of muscle damage: an in vitro study on a model of statin-induced myopathy

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Muscle damage induced by physical exercise or other stimuli is characterized by cytoskeletal injury and mitochondrial dysfunction which could in turn impair muscle function and morphology [1,2]. Statins are among the most effective treatments to prevent cardiovascular diseases by decreasing plasma triglycerides and inducing a modest increase in high-density lipoprotein (HDL) cholesterol. Although statins are generally considered well-tolerated, their wide use has shed light on adverse effects. It has been reported that statins, by inhibiting hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase within the mevalonate pathway, can reduce CoQ10 levels, inhibit mitochondrial electron transport chain (ETC) complexes and increase reactive oxygen species (ROS) production leading to myopathy[1,2]. Natural products have been widely employed in the treatment of various diseases including skeletal muscle disorders, thanks to their antioxidant and anti-inflammatory properties [3]. The purpose of this study was to evaluate the effects of a novel phytotherapic formulation composed by *Curcuma* and *Boswellia* essential oils, *Harpagophytum procumbens root* and *Bromelain* on human AC16 cardiomyocytes in an *in vitro* model of atorvastatin-induced myopathy. Our results showed that atorvastatin decreases cell viability, induces ROS production and mitochondrial structural damage. Interestingly, the synergic combination of the phytotherapic extracts is able to improve muscular recovery upon atorvastatin injury, reducing oxidative stress and improving mitochondrial reshape. These results highlight a new insight into statin-induced myopathy in human AC16 cells suggesting the employment of the novel phytotherapic formulation as a promising agent in the recovery of damaged muscle.

References

1) Liu, A.; Wu, Q.; Guo, J.; Ares, I.; Rodríguez, J. L.; Martínez-Larrañaga, M. R.; Yuan, Z.; Anadón, A.; Wang, X. & Martínez, M. A. Statins: Adverse reactions, oxidative stress and metabolic interactions. Pharmacol Ther. 2019, 195, 54–84.
2) Turner, R. M. & Pirmohamed, M. Statin-Related Myotoxicity: A Comprehensive Review of Pharmacokinetic, Pharmacogenomic and Muscle Components. J Clin Med. 2019, 9(1):22.
3) Huang, L.; Li, M.; Deng, C.; Qiu, J.; Wang, K.; Chang, M.; Zhou, S.; Gu, Y.; Shen, Y.; Wang, W.; Huang, Z. & Sun, H. Potential Therapeutic Strategies for Skeletal Muscle Atrophy. Antioxidants (Basel). 2022, 12(1):44. (2022).

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Muscular function before and after causal therapy in periodontal pathology: a clinical and electromyographic study

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Periodontitis is a multifactorial chronic condition that induces the loss of tooth supportive structures and neuroreceptors located within them, leading to tooth mobility and impaired proprioceptive function of the periodontium.

This can impact the activity of masticatory muscles, thereby affecting their function during clenching and chewing. Causal therapy aims to resolve periodontal inflammation. The study aims to investigate, through standardized superficial electromyographic examination(ssEMG), how the electrical activity of the masticatory muscles, masseter and anterior temporalis, is altered during maximum intercuspation and chewing in a clinical scenario of diagnosed periodontitis before and after causal therapy.

Methods. Five subjects with stage III grade B periodontitis, were enrolled. Patients underwent screening and analysis of their periodontal conditions as well as of their clenching and kinematic activity using ssEMG during the initial visit before performing causal periodontal therapy (T0), after three months (T1), and after one year (T2). A descriptive statistic of the ssEMG and clinical parameters was performed for each timepoint.

After therapy, the severity of periodontal disease decreased from a stage III grade B to a grade A. At ssEMG, during clenching, at T0, the muscular symmetry (POC%) was low for both masseters (73.80%) and temporalis (76.52%), while after therapy, levels gradually returned in a normal range (83.40% masseters, 84,62% temporalis at T2). The global muscular activity was lower at T0 (84.32%) than at T2 (95.94%). Regarding chewing, the muscle recruitment repeatability improved after therapy and a normalization of the ellipse positions within the correct quadrants appeared. At T2, the quadrants of 4 out of 5 patients were correctly aligned.

To conclude, after periodontal therapy, masticatory muscle exhibited enhanced activity and coordination during clenching and chewing. Besides revealing the utilization of protective mechanisms by the masticatory system, the study seems to illustrate a continual adaptation of the masticatory system.

References

1) Lamba, A.K.; Tandon, S.; Faraz, F.; Garg, V.; Aggarwal, K.; Gaba, V. Effect of periodontal disease on electromyographic activity of muscles of mastication: A cross-sectional study. J Oral Rehabil. 2020, 47, 599-605.

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Myofascial junction: Emerging insights into the connection between deep/muscular fascia and muscle

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Muscles and fasciae are interconnected and influenced by force transmission, yet the specifics of their anatomical and histological connections remain unclear. This study aimed to investigate the connections between muscles and deep/muscular fasciae across various regions in human cadavers and mice.

The findings revealed that myofascial junctions (MFJ) are composed of collagen I immune-positive structures, with an average area of 5.11 ± 0.81 μm². These structures were located at the muscle-fascia interface, showing an average density of 9.7 ± 2.51 MFJ/mm and an average inclination angle of 35.25 ± 1.52°. Additionally, the MFJs exhibited immunopositivity for collagen III and hyaluronic acid (HA), along with the presence of elastic fibers.

These results illustrate that myofascial junctions are visible, offering new insights into the anatomical and functional connections between deep/muscular fascia and muscle.

References

1. Tidball, J. G. Force transmission across muscle cell membranes. Journal of Biomechanics, 1991; 24(Suppl. 1), 3–52.
2. Patel, T. J., & Lieber, R. L. (). Force transmission in skeletal muscle: From actomyosin to external tendons. Exercise and Sport Sciences Reviews, 1997; 25, 321–363.

3D models and organoids, tissue engineering and regenerative medicine

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A NSC-34 cell line-derived spheroid model: potential and challenges for in vitro evaluation of neurodegeneration

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Three-dimensional (3D) spheroid models aim to bridge the gap between traditional two-dimensional (2D) cultures and the complex *in vivo* tissue environment. These models, created by self-clustering cells to mimic a 3D environment with surrounding extracellular framework, provide a valuable research tool. The NSC-34 cell line, generated by fusing mouse spinal cord motor neurons and neuroblastoma cells, is essential for studying neurodegenerative diseases like Amyotrophic Lateral Sclerosis (ALS), where abnormal protein accumulation, such as TAR-DNA-binding protein 43 (TDP-43), occurs in affected nerve cells. However, NSC-34 behavior in a 3D context remains underexplored, and this study represents the first attempt to create a 3D model to determine its suitability for studying pathology. We generated NSC-34 spheroids using a non-adhesive hydrogel-based template and characterized them for 6 days. Light microscopy revealed that NSC-34 cells in 3D maintained high viability, a distinct round shape, forming stable membrane connections. Scanning electron microscopy identified multiple tunnel-like structures, while ultrastructural analysis highlighted nuclear bending and mitochondria alterations. Using inducible GFP-TDP-43-expressing NSC-34 spheroids, we explored whether 3D structure affected TDP-43 expression, localization, and aggregation. Spheroids displayed nuclear GFP-TDP-43 expression, albeit at a reduced level compared to 2D cultures and generated both TDP-35 fragments and TDP-43 aggregates. This study sheds light on the distinctive behavior of NSC-34 in 3D culture, suggesting caution in the use of the 3D model for ALS or TDP-43 pathologies. Yet, it underscores the spheroids' potential for investigating fundamental cellular mechanisms, cell adaptation in a 3D context, future bioreactor applications, and drug penetration studies.

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Bio-hybrid scaffolds based on polyvinyl alcohol and decellularized human cartilage for the recovery of articular focal lesions in haemophilic patients

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Haemophilic Arthropathy (HA) is one of the major complications of Haemophilia and is caused by repeated joint bleeding (hemarthrosis) resulting in the intra-articular deposition of iron, which leads to the damage to both the articular cartilage (AC) and subchondral bone [1]. Current therapies can slow down osteochondral damage, but do not stimulate cartilage regeneration. Tissue Engineering can offer valid alternative strategies for the treatment of the AC damage. Therefore, this work investigated the fabrication of bio-hybrid polyvinyl alcohol (PVA) scaffolds, assuring for mechanical support, combined with decellularized human AC to enhance polymer bio-activity. Human AC was harvested from cadaver donors and minced into fragments which underwent decellularization by detergent-enzymatic treatment. The quality of acellular AC was assessed by DNA quantification assay and histological/histomorphometric analyses, confirming that decellularization correctly removed the immunogenic tissue components (i.e., cells, DNA), while preserving the structural biomolecules of the extracellular matrix (i.e., collagen, elastic fibers, glycosaminoglycans). In parallel, PVA hydrogels at two different concentrations (15%, 20%) were investigated by mechanical tests, showing that the almost-instantaneous compressive behavior of PVA varies with the hydrogel concentration. Finally, PVA/AC hybrid supports were fabricated by two methods: a) the mechanical incorporation of the homogenized acellular AC matrix into the polymer or b) the cross-linking of a layer of homogenized and freeze-dried matrix onto the hydrogel to obtain a double-layer support. The ultrastructure of the PVA/AC scaffolds was studied by scanning electron microscopy, revealing different characteristics of roughness and porosity, depending on how the matrix was combined with PVA. The cytocompatibility of the hybrid supports was tested by seeding mesenchymal stem cells on PVA/AC scaffolds and verifying cell growth at 7 and 14 days, with better results obtained on double-layer scaffolds. These data highlight some promising properties of PVA/AC bio-hybrid scaffolds for cartilage regeneration in haemophilic patients with HA.

References

1) Gualtierotti, R.; Solimeno, L. P.; Peyvandi F. Hemophilic arthropathy: Current knowledge and future perspectives. Journal of Thrombosis and Haemostasis, 2021, 19, 2112–2121.

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Evaluating Microplastic and Nanoplastic emissions from orthodontic clear aligners: a study on 3D Printed vs. Thermoformed devices

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The introduction of clear aligners transforms orthodontic treatments, offering a less conspicuous alternative to traditional metal braces. Among them, directly printed aligners use advanced 3D printing technology to customize devices with biocompatible resins. Despite their advantages, concerns regarding the potential release of micro (MPs) and nanoplastics (NPs) in oral conditions are increasing, posing unanswered questions about their short- or long-term human safety implications.

Based on this, the aim of this study is to examine and compare the release and dispersion of plastic particles from traditional thermoformed aligners (TFA) and 3D printed clear aligners (DPA) under simulated oral conditions, such as chemical interactions with simulated saliva pH and simulated chewing. Using a combination of imaging techniques, including optical microscopy, transmission electron microscopy (TEM), and atomic force microscopy (AFM), NPs and MPs were classified and quantified based on their size: microplastics (> 10 μm), sub-microplastics (from 40 nm to 10 μm) and nanoplastics (<40 nm). The results obtained indicate significant differences in the size and distribution of microplastic particles between the types of aligners. The mass of MPs and NPs separated after rubbing was 0.001g/200μl and 0.004g/200μl for TFA and DPA samples, respectively. In particular, TEM analysis demonstrated that DPA samples had larger and more numerous particles (203.08±2651.65mm^2) compared to TFA (0.23±27.53mm^2) and AFM analysis indicated a bigger root mean square gran size for DPA (159.89±350.72nm) than TFA (5.48±1.88nm).This variations highlight the influence of manufacturing techniques and material choices on the release of micro and/or nano particles, suggesting areas of potential improvement in the manufacturing processes of 3D printed aligners.

This preliminary study focused only on particle production, without addressing potential biological effects. Therefore, further research is needed to deepen the implications of exposure to micro- and nanoplastics on human health to optimize new orthodontic technologies and ensure safety and effectiveness.

References

1) Panayi, NC.; Efstathiou, S.; Christopoulou, I.; Kotantoula, G.; Tsolakis, IA. Digital orthodontics: Present and future. AJO-DO Clinical Companion. 2024;4(1):14-25.
2) Tartaglia, G.M.; Mapelli, A.; Maspero, C.; Santaniello, T. Serafin, M.; Farronato, M. et al. Direct 3D Printing of Clear Orthodontic Aligners: Current State and Future Possibilities. Materials (Basel). 2021;14(7).
3) Wulff, J.;Schweikl, H.; Rosentritt, M. Cytotoxicity of printed resin-based splint materials. J Dent. 2022;120:104097
4) Fang, C.; Awoyemi, O.S.; Luo, Y.; Naidu, R. Investigating Microplastics and Nanoplastics Released from a Rubber Band Used for Orthodontic Treatment with Improved Raman Imaging Algorithms. Environment & Health. 2023;1(1):63-71.

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Morphological evaluation of nerves and muscles of the upper limb in a nerve transfer surgery patient: preliminary results

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The modern approach to tetraplegia patients' treatment includes, in selected cases, the surgical technique of nerve transfer, i.e., the transfer of a functional nerve (donor) to a muscle innervated by an injured nerve (recipient), which is of great importance for the patient's autonomy. The literature highlights how a neurosurgical study of nerve transfer surgery supported by a morphological and molecular analysis fits into a niche where knowledge is lacking or even absent.

The aim of this study is to describe the morphostructural profile of the donor nerve and the recipient muscle of five patients to optimize functional recovery after surgery. This is a preliminary report analyzing, in the upper limb, a donor nerve (branch of the musculocutaneous nerve for brachialis muscle) and an innervated muscle (biceps brachii), and a recipient nerve (posterior interosseous) and a denervated muscle (triceps brachii) from one patient who underwent triple nerve transfer surgery (branch for brachialis muscle to anterior interosseous nerve transfer, branch for supinator muscle to posterior interosseous nerve transfer and branch for teres minor muscle to one radial nerve branch for triceps brachii nerve transfer). The samples were fixed in formalin, embedded in paraffin and cut at the microtome. General morphology was assessed by haematoxylin-eosin staining; MCOLL staining was employed for collagen and myelin evaluation in nerve, while Sirius red staining was used for collagen evaluation in muscle. The results showed a well-preserved myelin sheath with a predominance of type I collagen fibers in the donor nerve, whereas the recipient nerve showed more disorganized nerve fibers with myelin segmentation and increased type III collagen fibers. A similar effect in collagen composition was seen in the muscle samples. The results of this study may address new strategies supporting nerve regeneration.

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References

1) Bellity, L.; Le Hanneur, M.; Boudjemaa, S.; Bachy, M.; Fitoussi, F. Histopathological nerve assessment in brachial plexus birth injuries. Hand Surg. Rehabil. 2021, 40, 400-404.
2) Carriel, V.; Garzón, I.; Alaminos, M.; Campos, A. Evaluation of myelin sheath and collagen reorganization pattern in a model of peripheral nerve regeneration using an integrated histochemical approach. Histochem. Cell Biol. 2011, 136, 709-717.
3) Arruda, E.M.; Mundy, K.; Calve, S.; Baar, K. Denervation does not change the ratio of collagen I and collagen III mRNA in the extracellular matrix of muscle. Am. J. Physiol. Regul. Integr. Comp. Physiol. 2007, 292, R983-R987.

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Spatial transcriptomics: bridging anatomy and molecular techniques for precision medicine in rare diseases

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Mitochondrial Neurogastrointestinal Encephalomyopathy (MNGIE) is a rare genetic disorder caused by defects in the thymidine phosphorylase (TP) enzyme. These defects result in fatal mitochondrial dysfunction, which can be reversed by providing a stable source of TP through a liver transplant[1]. Although transplantation is a life-saving technique, it does not restore damage to the digestive tract that manifests as gastrointestinal fibrosis, hypoxia, abnormalities in the longitudinal muscle layer, and can lead to fatal gastrointestinal bleeding. TP is essential to form the new blood vessels pre-lumen and the MNGIE-related absence of TP leads to an aberrant vascularization characterized by smaller and fragile vessels[2]. Understanding the key players in vascularization altered by the absence of TP is crucial for finding a targeted therapy for MNGIE patients. To identify these factors, we employed Visium CytAssist spatial transcriptomics on full-thickness jejunal tissue from two MNGIE patients and two healthy controls. Despite the challenges posed by the disease's rarity and the limited sample size, the applied method effectively identified distinct coding patterns across all tissue layers. Control samples exhibited consistent patterns within each layer, markedly different from those observed in MNGIE samples. Focusing on blood vessels allowed us to identify a set of genes significantly altered between controls and patients. Pathway enrichment analysis revealed seven dysregulated genes specifically involved in the abnormal blood vessel morphology; eleven genes responsible for an aberrant collagen fibril organization and twenty genes associated to an altered extracellular matrix organization. While further validation of these findings is necessary, spatial transcriptomics, despite its application to a limited sample size, enabled the identification of several potential molecular targets. Applying spatial transcriptomics to rare samples could represent a significant advancement in precision medicine for cases where large-scale sampling is unachievable.

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References

1) De Giorgio R.; Pironi L.; Rinaldi R.; Boschetti E.; Caporali L.; Capristo M.; Casali C.; Cenacchi G.; Contin M.; D'Angelo R.; D'Errico A.; Gramegna LL.; Lodi R.; Maresca A.; Mohamed S.; Morelli MC.; Papa V.; Tonon C.; Tugnoli V.; Carelli V.; D'Alessandro R and Pinna AD Liver transplantation for mitochondrial neurogastrointestinal encephalomyopathy Annals of Neurology 2016 80:448-55. doi: 10.1002/ana.24724
2) Boschetti E.; D'Angelo R.; Tardio L.M.; Costa R.; Giordano C..; Accarino A.; Malagelada C.; Clavenzani P.; Tugnoli V.; Caio G.; Righi V.; Garone C.; D'Errico A.; Cenacchi G.; Dotti M.T.; Stanghellini V.; Sternini C.; Pironi L.; Rinaldi R.; Carelli V. De Giorgio R. Evidence of enteric angiopathy and neuromuscular hypoxia in patients with mitochondrial neurogastrointestinal encephalomyopathy Am J Physiol Gastrointest Liver Physiol. 2021 doi.org/10.1152/ajpgi.00047.2021

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Differential behavior of gingival fibroblasts, endothelial cells and periodontal ligament stem cells on implant abutment: a comparison of surface topographies and treatments

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Titanium is widely used for dental implants due to its favorable mechanical properties and osseointegration capability. The long-term success of dental implants requires both hard and soft-tissue integration. Surface micro structuring and functionalization are two major strategies for modifying implant surfaces to enhance osteointegration and connective tissue healing (1). The aim of this study was to directly compare the behavior of gingival fibroblasts (HGF-1), endothelial cells (HUVEC), and periodontal ligament stem cells (PDLSCs) on titanium substrates with different surface topographies. Grade 4 titanium was machined to a diameter of 14 mm x 4.22 mm thickness, half coated in PVD TiN, with different textures such as: polished, patterns with pitches of 100 mm, 50 mm, and xy grid with pitch of 200 mm. After the specific surface treatment, all specimens were packaged and sterilized by gamma irradiation. The morphology of materials was examined using a High-Resolution Scanning Electron Microscope (HRSEM mod CrossBeam 350 ZEISS – Germany), @ 5 KVolt of energy. Images were acquired with secondary electron signal. The hydrophilicity was determined by water dynamic contact angle measurements by Contact Angle Meter, CAM 200 (KSV Instrument LTD, Helsinki, Finland). The cytotoxic effects of the samples were excluded by the MTT assay. Cell proliferation and morphology were evaluated by counting the DAPI-stained nuclei and phalloidin staining, respectively, at days in vitro (DIV)10 using fluorescence microscopy (Leica DM IL LED, Leica Microsystems, Milan, Italy). Immunofluorescence analysis of vinculin was performed to assess the effect of different surfaces on cell adhesion at DIV10. Our results demonstrate that cellular responses on Ti surfaces consisting of periodic arrays of grooves with spacings ranging from 50 mm to 200 mm were significantly enhanced when compared to polished surfaces, with 50 mm spacing offering the best outcome. The PVD TiN coating slightly influence cell response.

References

1) Ripszky Totan, A.; Imre, M.M.; Parvu, S.; Meghea, D.; Radulescu, R.; Enasescu, D.S.A.; Moisa, M.R.; Pituru, S.M. Autophagy Plays Multiple Roles in the Soft-Tissue Healing and Osseointegration in Dental Implant Surgery – A Narrative Review. Materials 2022, 15, 6041.

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In vitro evaluation of a new copper and zinc-doped implant surface: effects on cytocompatibility and antibacterial properties

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Dental implant has become the standard for rehabilitating edentulous sites. Nevertheless, implant failures, primarily caused by peri-implant diseases, still affect many patients.

Plasma Electrolytic Oxidation (PEO) is a versatile process used on light metals to generate hydrophilic oxide coatings, allowing the incorporation of bioactive ions. Copper (Cu) and zinc (Zn), known for their antibacterial properties and roles in physiological processes are promising doping agents.

This project supported by PRIN-2022, aimed to evaluate cytocompatibility, adhesion, functionality, hemocompatibility, and antibacterial properties of titanium surfaces treated with PEO and doped with Zn and Cu.

MC3T3 cells and human dermal fibroblasts were used to evaluate disks *in vitro* for titanium grade 2, PEO, and PEO doped with Cu and Zn. Doping was achieved using electrical parameters of 10-5 and 20-5. Surfaces were analyzed for stability in water and air using Energy Dispersive X-ray Spectroscopy at intervals up to 28 days. Direct and indirect viability tests were performed, morphological analyses and antibacterial effects on Staphylococcus aureus. Blood cloth stability was tested using thromboelastography.

The incorporation of Cu and Zn into titanium dioxide layer showed high stability. Co-doped surfaces had favorable cytocompatibility, with an inverse relationship between doping agent amounts and cellular metabolic activity. PEO 10-5's superior cytocompatibility was due to lower Cu content, while PEO 20-5 reduced cellular metabolism. Low roughness and high microporosity enhanced cellular adhesion. Cu and Zn did not affect extracellular matrix secretion and mineralization, though microporosity might accelerate matrix calcification.

PEO 10-5, with balanced doping agents, favored stable blood clotting. In vitro antibacterial testing showed superior bacteriostatic effects in PEO 20-5.

PEO co-doped surfaces showed promise, suggesting Cu and Zn modulation can tailor surfaces for specific clinical contexts. PEO 10-5, with high cytocompatibility and hemostatic capacity, suits general clinical use, while PEO 20-5 is ideal for high antibacterial protection.

References

1) Fabbro, M. D.; Taschieri, S.; Canciani, E.; Addis, A.; Musto, F.; Weinstein, R.; Dellavia, C. Osseointegration of Titanium Implants With Different Rough Surfaces: A Histologic and Histomorphometric Study in an Adult Minipig Model. Implant dentistry. 2017, 26(3), 357–366.
2) Canciani, E.; Ragone, V.; Biffi, C. A.; Valenza, F.; D'Ambrosi, R.; Olimpo, M.; Cristofalo, A.; Galliera, E.; Dellavia, C. Understanding the Role of Surface Modification of Randomized Trabecular Titanium Structures in Bone Tissue Regeneration: An Experimental Study. Medicina (Kaunas, Lithuania). 2022, 58(2), 315.
3) Pavarini, M.; Moscatelli, M.; Candiani, G.; Tarsini, P.; Cochis, A.; Rimondini, L.; Najmi, Z.; Rocchetti, V.; De Giglio, E.; Cometa, S. ;De Nardo, L.; Chiesa, R. Influence of frequency and duty cycle on the properties of antibacterial borate-based PEO coatings on titanium for bone-contact applications. Applied Surface Science. 2021, 567, 150811, ISSN 0169-4332.

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Identification of laminin-binding oligopeptides guiding cardiac differentiation

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The extracellular matrix (ECM) plays a key role in tissue regeneration, in particular interacting with integrins and focal adhesions (FAs) which facilitate cell adhesion, guiding cell behavior. FAs link to actin stress fibers and include transmembrane integrin receptors, specifically integrins α and β heterodimers, binding the intracellular environment to the ECM. Activating pathways like Focal Adhesion Kinase (FAK) and mitogen-activated protein kinases (MAPKs), integrins induce hypertrophic and anabolic responses in cardiac myocytes (1-3). Despite partial knowledge of FAs, studies highlight the pathophysiological roles of integrins in cardiac cells, acting as major mediators between cardiomyocytes and the extracellular environment. In adult cardiac cells, one of the mainly expressed integrins is α7β1, binding specifically to laminin-1, -2, and -4; this integrin has a protective effect on cardiomyocytes and is reduced after a myocardial infarction (MI), indicating an important role in cardiac functionality (4–7). To evaluate the cardiomyogenic potential of the laminin-specific integrin α7β1 derived peptides, KKGSYNNIVVHV (A2G2), YAIFLNKGRLEV (A2G52) (8), and neonatal mouse ventricular cardiomyocytes were used.

Staining with phalloidin and vinculin revealed morphological changes with enhanced focal adhesion and stress-fibers formation compared to untreated cells, mainly evident at a concentration of 50 μg/ml for both peptides. Morphometric analysis revealed an increased length of FA in primary cardiomyocytes. Cardiac and adhesion markers analyses confirmed the role of peptides in cardiomyogenic differentiation and commitment, in which treated cells shows higher expression of cytoskeletal cardiac marker genes (GATA-4, cardiac troponin I, desmin).

Cardiac growth and differentiation involve complex interactions among growth factors, proteins, receptors, and specific ligands. Here, we demonstrated how integrin binding peptides influence cell behavior in primary cardiomyocytes, guiding cardiomyogenic cytoskeletal changes. The peptides derived from cellular matrices may also be used to functionalize matrices such as hydrogel and used for regenerative purposes in post cardiac ischemia.

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References

1) B. Geiger, A. Bershadsky, "Assembly and mechanosensory function of focal contacts.", Curr Opin Cell Biol. 13, 584–592 (2001), doi: 10.1016/s0955-0674(00)00255-6.
2) R. S. Ross, T. K. Borg, "Integrins and the myocardium.", Circ Res. 88, 1112–1119 (2001), doi: 10.1161/hh1101.091862.
3) B. Kovacic-Milivojević et al., "Focal adhesion kinase and p130Cas mediate both sarcomeric organization and activation of genes associated with cardiac myocyte hypertrophy.", Mol Biol Cell. 12, 2290–2307 (2001), doi: 10.1091/mbc.12.8.2290.
4) H. Okada et al., "Integrins protect cardiomyocytes from ischemia/reperfusion injury.", J Clin Invest. 123, 4294–4308 (2013), doi: 10.1172/JCI64216.
5) N. Maitra et al., "Expression of alpha and beta integrins during terminal differentiation of cardiomyocytes.", Cardiovasc Res. 47, 715–725 (2000), doi: 10.1016/s0008-6363(00)00140-1.
6) P. Oliviéro et al., "Expression of laminin alpha2 chain during normal and pathological growth of myocardium in rat and human.", Cardiovasc Res. 46, 346–355 (2000), doi: 10.1016/s0008-6363(00)00034-1.
7) H. Yu et al., "A bio-inspired platform to modulate myogenic differentiation of human mesenchymal stem cells through focal adhesion regulation.", Adv Healthc Mater. 2, 442–449 (2013), doi:10.1002/adhm.201200142.
8) S. Urushibata et al., "Identification of biologically active sequences in the laminin alpha2 chain G domain.", Arch Biochem Biophys. 497, 43–54 (2010), doi: 10.1016/j.abb.2010.03.006.

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eNOS mediated activation of angiogenic potential of endothelial cells by Complex Magnetic Field

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The application of Complex Magnetic Fields as a non-invasive technology for several biological alterations' treatment is becoming diffuse. A novel device committed to produce a special symphony of waveforms, named Complex Magnetic Fields (CMFs) has been developed and applied to treat different pathological conditions. Thus, the aim of this research was to understand the influence of CMFs system on the different steps of angiogenesis in an *in vitro* EA.hy926 endothelial cell line. A Regenerative Tissue Program (RTP) was applied and three different CMFs applications appeared to positively affect the metabolic activity of EA.hy926 cells: two consecutive RTP cycles, one RTP cycle at T0 and an additional cycle after 8h (T0+T8) and one cycle at T0 followed by an additional cycle after 24h (T0+T24). The application of these three conditions to EA.hy926 cells increased cell metabolic activity up to 20% after 24h and 48h of exposure, compared to the control along with the number of cells/ml. Secondly, the three conditions of RTP were found to markedly improve the expression of Matrix Metalloproteinase (MMP) 1 and MMP-9 and, at the same time, to significantly influence the migratory capability of endothelial cells, assessed by a wound healing. In parallel an increase of endothelial Nitric Oxide Synthase (eNOS) expression was recorded.

In conclusion, these preliminary results demonstrate that CMFs application on endothelial cells enhances the different steps of the angiogenic process by promoting ECM remodeling through an increased expression of MMPs and an increased migratory capability of cells, which induces cell proliferation.

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Integrated design based on 3D Bioprinting and Bioreactor for skeletal muscle tissue engineering

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In recent years, great efforts have been spent to create engineered muscle constructs recapitulating the 3D architecture and applying external stimulations. In this regard, tissue engineering approaches could be very promising in regenerating skeletal muscle, in which bioprinting techniques have produced encouraging results especially regarding tissue 3D architecture and geometry [1]. Tensile stimuli showed a fundamental role in regulating the behavior of muscle cells both in terms of 3D organizations and protein expression [2, 3]. Despite this promising premise, the combination of 3D bioprinting and mechanical stimulation in muscle tissue has been poorly investigated. To this aim, the present work proposes the design, manufacturing, and benchmarking of a bioprinting-integrated mechanical platform conceived for mechanically stimulating a 3D muscle model directly printed into the bioreactor to promote the integration of 3D bioprinting and stimulation. The study consists of three main steps: 1) the design, fabrication, and mechanical characterization of stretchable supports suitable for bioprinting and long-term cell culture; 2) the design, assisted by computational tools, and the fabrication of the smart petri dish containing the stimulation mechanism and of the final cyclic mechanical platform; 3) the *in-vitro* validation of the proposed platform in terms of transmission of the mechanical stimulation to the constructs and the biological effect of dynamic culture on 3D bioprinted murine muscle cells. The results highlighted excellent viability and demonstrated that the external stimulus influences the murine myoblasts (C2C12 cells) behavior already after 7 days of culture. In conclusion, prototypes are now available of a mechanical platform that integrates the 3D bioprinting and is capable of stimulating 3D biological constructs for applications in the field of muscle tissue engineering.

References

1) Moyle, Louise A., Erik Jacques, and Penney M. Gilbert. "Engineering the next generation of human skeletal muscle models: from cellular complexity to disease modeling." Current Opinion in Biomedical Engineering 16 (2020): 9-18.
2) Potyondy, Tyler, et al. "Recent advances in 3D bioprinting of musculoskeletal tissues." Biofabrication 13.2 (2021): 022001.
3) Mueller, Claudia, et al. "Effects of external stimulators on engineered skeletal muscle tissue maturation." Advanced Materials Interfaces 8.1 (2021): 2001167.

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Mimicking glioblastoma multiforme microanatomical architecture via patient-derived 3D spheroids

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Glioblastoma multiforme (GBM) represents one of the most malignant forms of brain tumors (1). Despite advances in treatment modalities, prognosis remains poor, due to the remarkable resistance to therapies attributed to molecular intertumoral and intratumoral heterogeneity within GBM (2). Consequently, it is necessary to develop novel experimental models able to mimic the in vivo spatial and molecular organization of GBM, to better understand its biological complexity. Among *in vitro* models, 3D spheroids mimic the morphological and molecular complexity and heterogeneity of the tumor, as they recapitulate the cell-cell and cell-extracellular matrix interactions present *in vivo*. Patient-derived 3D spheroids are increasingly considered more physiologically relevant.

In this study, our focus lies on regional intratumoral differences and on the potential use of patient-derived 3D spheroids as model able to recapitulate the GBM niches.

In GBM patient specimens, we assessed the architecture of vascular niche characterized by increased VEGF (vascular endothelial growth factor) expression level and hypoxic niche that contributes to tumor growth and resistance, wherein HIF (hypoxia-inducible factor) contributes to the upregulation of VEGF and supports cell proliferation in hyperproliferative and invasive niche, overexpressing PCNA (proliferating cell nuclear antigen) and c-KIT (tyrosine-protein kinase KIT). Subsequently, primary cell lines were isolated from GBM tumor tissues and characterized. To investigate the architecture and cellular heterogeneity of GBM patient-derived 3D spheroids were generated, particularly focusing on the c-KIT pathway. C-KIT, also known as stem cell factor receptor (SCF), is a proto-oncogene implicated in both normal growth and development of neoplastic processes, frequently overexpressed and amplified in gliomas. Uncontrolled activity of the SCF/c-KIT pathway by glioma cells can activate brain microvascular endothelial cells, supporting proliferation, angiogenesis, stemness, and metastasis (3). In summary, we have developed a 3D culture model that faithfully mimics the *in vivo* architecture of GBM, providing a valuable tool for future mechanistic studies.

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References

1) Louis, D.N.; Perry, A.; Wesseling, P.; Brat, D.J.; Cree, I.A.; Figarella-Branger, D.; Hawkins, C.; Ng, H.K.; Pfister, S.M.; Reifenberger, G.; Soffietti, R.; von Deimling, A.; Ellison, D.W.; The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. Neuro Oncol. 2021, 23(8):1231-1251.
2) Sottoriva, A.; Spiteri, I.; Piccirillo, S.G.; Touloumis, A.; Collins, V.P.; Marioni, J.C.; Curtis, C.; Watts, C.; Tavaré, S. Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics. Proc Natl Acad Sci U S A. 2013, 110(10):4009-14.
3) Cetin, N.; Dienel, G.; Gokden, M. CD117 expression in glial tumors. J Neurooncol. 2005, 75(2):195-202.

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Excessive stiffness of meshes for oral guided bone regeneration may cause mucosal dehiscence: an in-vitro comparative loading study between titanium alloy and polycaprolactone

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Tooth loss leads to anatomical modification of the alveolar bone impairing the rehabilitation of the masticatory function by endosseous dental implants. Guided bone regeneration (GBR) aim to rebuilt lost bone with CAD designed 3D printed titanium alloy (Ti6Al4V) or polycaprolactone (PCL) meshes, which act both as space maintainers and barriers to separate the oral mucosa from the growing bone[1]. Nevertheless, a common drawback in GBR occurring in 30-50% of cases is mesh exposure (ME) through the mucosal tissue after the surgical procedure[2], which may cause of oral bacteria spreading in the wound resulting in infection and lost of the regenerated bone. A possible explanation could be the accidental loading of covering oral mucosa associated with an excessive stiffness of the underlying mesh. To investigate this occurrence we designed and 3D printed five Ti6Al4V and ten PCL meshes. All meshes were 10 mm x 30 mm, while thickness was 0.2 mm for Ti6Al4V and 0.8 mm for PCL, respectively. Before loading, five PCL meshes were sterilized using ethanol solution (70%). All meshes were fixed in four point at the ends and loaded centrally with a universal testing machine (MTS 810) at 130N and 10 mm/min speed using a spherical point of 10 mm diameter until the first failure, i.e. a fracture of a part of the mesh. First failure of not-sterilized and sterilized PCL meshes occurred at similar loading value, although slightly higher for the former, while was almost twice for Ti6Al4V. PCL showed also low stiffness compared with Ti6Al4V, but it is more than adequate to act as space maintainer. Furthermore, PCL and keratinized mucosa stiffness values reported in literature appeared comparable[3]. In conclusion, similar stiffness between meshes for GBR and the covering oral mucosa appears to be a prerequisite to avoid dehiscence and ME.

References

1) Ciocca L, Lizio G, Baldissara P, Sambuco A, Scotti R, Corinaldesi G. Prosthetically CAD-CAM-Guided Bone Augmentation of Atrophic Jaws Using Customized Titanium Mesh: Preliminary Results of an Open Prospective Study. J Oral Implantol. 2018 Apr;44(2):131-137. Ù
2) Briguglio F, Falcomatà D, Marconcini S, Fiorillo L, Briguglio R, Farronato D. The Use of Titanium Mesh in Guided Bone Regeneration: A Systematic Review. Int J Dent. 2019 Feb 7;2019:9065423.
3) Choi JJE, Zwirner J, Ramani RS, Ma S, Hussaini HM, Waddell JN, Hammer N. Mechanical properties of human oral mucosa tissues are site dependent: A combined biomechanical, histological and ultrastructural approach. Clin Exp Dent Res. 2020 Dec;6(6):602-611.

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Enhanced biocompatibility and equivalent angiogenic properties of two innovative bioactive glasses, enriched with magnesium and strontium, offering a potential replacement for the golden standard bioactive glass 45S5

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The progressive aging of the population and the increasing incidence of musculoskeletal diseases have led bone tissue engineering towards innovative biomaterials that could sustain regenerative processes. One of the main concerns in the clinical scenario is the disruption of vascularization following critical-size fractures that causes their inability to repair spontaneously. In fact, a functional vascular network is a necessary prerequisite to allow the regenerative processes, particularly osteogenesis. Thus, the impact of bioactive glasses (BAGs) stands out as potential bone substitutes, especially exerting their effects through the dissolution of active ions that can stimulate osteoprogenitors and endothelial cells in the osteogenic process. However, under certain conditions BAGs such as the well-known 45S5 Bioglass® could be cytotoxic, likely due to a strong increase in pH, caused by a burst release of sodium ions. In this work, two novel BAGs formulations enriched in Strontium and Magnesium ions and improved crystallization temperature were evaluated. The effects of BGMS10 and Bio_MS on cell adhesion and viability were analyzed both in presence of bioglasses and in indirect settings (i.e. with of medium from BAGs cultures) to assess the impact of ionic dissolution products, compared to 45S5 bioglass, demonstrating significant higher biocompatibility of the novel formulations. The angiogenic potential was evaluated by means of the Chorio-Allantoic Membrane (CAM) assay, an ethical model to simulate the *in vivo* conditions. All the BAGs demonstrated to be significantly pro-angiogenic compared to control, showing a significant upregulation of genes with key roles in angiogenic pathways. These data were in line with the histological analyses which demonstrated the higher biocompatibility of the novel BAGs, displaying remarkable proliferation of CAM tissues without inflammatory reactions. In conclusion, these results show that the new formulations are extremely effective in promoting adhesion, proliferation and angiogenesis in *in vitro* and *in vivo* settings, deserving further experimental investigations.

Keywords: Bioactive glasses; Therapeutic ions; Biocompatibility, CAM assay; Angiogenesis; Bone tissue engineering.

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Biological response of human ureter primary epithelial and muscular cells to a PLC nanostructured biomaterial

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Surgery is able to face some clinical conditions as urolithiasis, chronic inflammation, infection, and ureteral carcinoma that may seriously damage ureter (1) but the widespread application of surgery has greatly increased the incidence of iatrogenic ureteral injuries (2). In addition, although different surgical techniques may be employed for the reconstruction of ureteral injuries, these procedures exhibit several complications (3). Ureter tissue regeneration (TR) is the most encouraging approach to avoid surgical interventions. Therefore, the aim of this *in vitro* study is to characterize a resorbable scaffold for ureteral TR made of synthetic polycaprolactone (PCL) and to test its effects on cell adhesion, proliferation and protection from bacterial invasion. The in vitro biological effects of this scaffold (in the presence of rifampicin and plasma) were evaluated on primary epithelial and muscular cells derived from human ureter. Cell proliferation, cytotoxicity, inflammation and morphology were assayed at day 4 and 7 either on each primary cell culture or on the epithelial/muscle cell co-culture to analyze paracrine effects between the two cells types. The results showed that cytotoxicity, in both cell culture conditions, was higher in the presence of rifampicin if compared to control, whereas plasma treatment showed a similar number of viable cells as in untreated controls, suggesting that plasma many counteract the effect of rifampicin. The release of PGE-2 increased in both muscle and epithelial cells in the presence of rifampicin only after 7 days of treatment, even though this effect was remarkably reduced by plasma. Cellular morphology did not show any significant changes. Overall, our preliminary data suggest that plasma could significantly improve TR success of rifampicin-treated PCL scaffolds due to the lack of cytotoxicity and inflammation. Nonetheless further studies are required to clarify mechanisms involved.

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References

1) Lucas JW, Ghiraldi E, Ellis J, Friedlander JI. Endoscopic Management of Ureteral Strictures: an Update. Curr Urol Rep. 2018 Mar 2;19(4):24.
2) Traxer O, Thomas A. Prospective evaluation and classification of ureteral wall injuries resulting from insertion of a ureteral access sheath during retrograde intrarenal surgery. J Urol. 2013 Feb;189(2):580-4.
3) Benson MC, Ring KS, Olsson CA. Ureteral reconstruction and bypass: experience with ileal interposition, the Boari flap-psoas hitch and renal autotransplantation. J Urol. 1990 Jan;143(1):20-3.

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Establishment of 3D cultures of uterine leiomyosarcoma cells with different bioinks

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Uterine leiomyosarcoma (LMS) represents the most common type of uterine sarcoma. LMS is an uncommon malignant tumor with a very poor prognosis. Consequently, the development of *in vitro* methodologies that mimic LMS pathophysiology is fundamental to comprehend tumor progress and find successful therapeutic strategies. Three-dimensional (3D) cell cultures have materialized as effective approaches in the study of tumor pathophysiology, offering considerable improvements upon traditional two-dimensional cell culture methods. In 3D systems, the growth of cancer cells occurs in an environment that more closely simulates the 3D architecture and complexity of *in vivo* tumors. This method has reformed cancer research by giving a more precise version of the tumor microenvironment and allowing the study of tumor comportment and response to treatments in a more appropriate framework. This study presents the results of obtaining 3D cultures of SK-LMS-1 cells, a LMS cell line with fibroblast morphology commonly used in the research of this type of tumors, using different biocompatible alginate matrices and the method of 3D printing, with an Inkredible+ (Cellink) bioprinter. Three alginate-based biocompatible matrices were used to obtain the 3D cell cultures: Commercial Alginate (Cellink), 10% Alginate with stirrer and 10% Alginate with deposition. The bioinks were mixed with the cells using the double syringe method, subsequently printed in 6-well plates and later was added the culture medium. The plates were photographed for several days under the optical microscope, to monitor the growth of the formed spheroids. DAPI and Hematoxylin-Eosin staining were also performed. The evidence obtained allows us to affirm that the three matrices studied constitute valid supports for obtaining 3D cell cultures with LMS cells, which opens a spectrum of possibilities for the study of different aspects of the tumoral biology, as well as for carrying out *in vitro* trials with potential antitumor therapies.

References

1) Greco, S.; Zannotti, A.; Pellegrino, P.; Giantomassi, F.; Delli Carpini, G.; D'Agostino, M.; Goteri, G.; Ciavattini, A.; Donati, Ch.; Bernacchioni, C.; Petraglia, F.; La Teana, A.; Ciarmela, P. High levels of hypusinated eIF5A in leiomyoma and leiomyosarcoma pathologies: a possible novel therapeutic target. Reproductive BioMedicine Online. 2023, 47, 1, 15-25.
2) Greco, S.; Pellegrino, P.; Giampieri, F.; Capocasa, F.; Delli Carpini, G.; Battino, M.; Mezzetti, B.; Giannubilo, S. R.; Ciavattini, A.; Ciarmela, P. The in vitro effects of Romina strawberry extract on 3D uterine leiomyosarcoma cells. Nutrients. 2023, 15, 11, 2557.
3) Greco, S.; Pinheiro, J.; Cardoso-Carneiro, D.; Giantomassi, F.; Pellegrino, P.; Scaglione, G.; Delli Carpini G.; Ciavattini, A.; Zannoni, G. F., Goteri, G.; Martinho, O.; Ciarmela, P. Raf kinase inhibitor protein expression in smooth muscle tumours of the uterus: a diagnostic marker for leiomyosarcoma? Reproductive BioMedicine Online. 2024, 48, 6, 103816.

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Morphological and morphometrical evaluation of hemostatic topical formulations

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The process of cutaneous wound healing, even if superficial, is incredibly complex and engages numerous highly regulated factors working in concert to restore injured skin towards barrier function[1,2]. This sequence of events plays out "normally" in the vast majority of superficial wounds. However, when wound healing does not progress "normally", especially due to underlying disease states, a chronic non-healing wound may result with a significant patient's discomfort and burdening the medical system[3]. To date, traditional materials are potentially effective in the cutaneous wound healing process and various types of hemostatic formulations can be used in the clinical application and in home-setting; however, due to the economic and patient care impacts of wound healing, it comes as no surprise that the field of wound healing research is incredibly active.

In this study was compared the hemostatic effect of the two different Nova Argentia Srl (Gorgonzola, Milan, Italy) formulations: (1) NOVA.emoSTOP powder and (2) NOVA.emoSTOP pad. We observed that both hemostatic formulations promote a rapid formation of a medium/small clot. Disproportionate clot formation in the hemostasis phase may result in excessive accumulation of extracellular matrix so altering the wound healing process. The morphological and morphometrical analyses performed in the present study showed that the NOVA.emoSTOP pad promotes the formation of a blood clot richer in platelets respect to NOVA.emoSTOP powder. Interestingly, the NOVA.emoSTOP pad used in the nosebleed simulation promotes a very rapid formation of a platelet-rich clot underlining its valuable hemostatic effect together with the capability to absorb blood.

In conclusion, the NOVA.emoSTOP pad formulation may represent a functional, effective, safe and easy-to-use hemostatic innovative-tool for healing superficial skin wounds, such as abrasions, excoriations, and small surgical wounds. Furthermore, it has promising potential application for stopping nosebleed.

References

1) Han, G.; Ceilley, R. Chronic wound healing: A review of current management and treatments. Adv. Ther. 2017, 34, 599-610.
2) Reinke, J.M.; Sorg, H. Wound repair and regeneration. Eur. Surg. Res. 2012, 49, 35-43.
3) Bhoyar, S.D.; Malhotra, K.; Madke, B. Dressing materials: A comprehensive review. J. Cutan. Aesthet. Surg. 2023, 16, 81-89.

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Oxidative stress and nicotine effect in the management and progression of Mycobacterium tuberculosis infection: the first steps towards a 3D model a 3D-cellular model

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The impact of smoking on tuberculosis (TB) has been assessed by several recent studies. Among all the components, nicotine is the main immunomodulatory molecule in cigarettes: data has already demonstrated its interaction with the lung immune system. However, limited results have been collected on the impact of cigarette smoking-induced oxidative stress on the broader pulmonary milieu and on its epithelial compartment [1]. Moreover, lung epithelial cells were considered for years only a passive component of the lung environment, whereas, more recently, an active emerging role has been assigned to them, during the first phases of *Mycobacterium tuberculosis* (Mtb) infection.

The aim of our study was to investigate the molecular mechanisms through which nicotine exposure affect the establishment of Mtb infection, in an in-vitro model, mainly focussing on the morphological and structural alterations that may occur in the lung's epithelial compartment [2].

Two non-immortalised cell lines have been cultured separately: a keratinocyte cell line obtained from human lung bronchiole (HBEC3-KT) and a human lung fibroblast cell line (LF-hTERT). They were exposed to a treatment with nicotine at a concentration ranging from 1 μM to 100 μM for 24, 48, 72 and 120 hours.

Cell viability was evaluated by Alamar blue assay. For a first morphological evaluation cells have been fixed with methanol and subsequently stained with cresyl violet.

Concentration-time profiles showed that nicotine affects cell viability after 24 hours of incubation at concentrations of 10 μM showing an effect directly proportional to the increase in nicotine concentration, reaching a maximum effect at a concentration of 40 μM.

The results obtained confirm a direct effect of nicotine on both cell lines, allowing the definition of exposure times and concentrations that are non-lethal for the cells. Moreover, they lay the background for the more complex model to be developed in the near future.

References

1) Pulici, P.; Cazzaniga, R.; Mozzini, R.; Salvadori, R.; Pecci, E.; Roccotelli, G.; Sala, P.; Sala, C. Effect of Poly-L-Lysine Coating on Titanium Osseointegration: From Characterization to in Vivo Studies. J. Oral Implantol. 2015, 41, 626–631.
2) Bertocchi, M.; Santin, N.; Pelosin, M.; Zaccarelli, R.; Graziani, F.; Radice, L. Understanding the Role of Surface Modification of Randomized Trabecular Titanium Structures in Bone Tissue Regeneration: An Experimental Study. Medicina 2022, 58, 315.

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Morphological and ultrastructural characterization of 3D spheroids of leiomyosarcoma

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In recent years, there has been an interest in studies of three-dimensional (3D) cell culture models. Studying drugs, identifying potential anticancer therapeutic compounds, and reproducing tissue and microtumor characteristics have been possible thanks to 3D models based on spheroid formation. Like tumors, spheroids create structures that expose mitotically active cells on the surface, while within them are non-proliferating cells and a hypoxic necrotic core (1). The organization of spheroids therefore contributes to a better understanding of the mechanism of tumorigenesis of cancer models *in vitro* (2). To date, the observation of live cells in a 3D physiological environment is very complicated, for this reason the focus of our study was to implement a detailed morphological and ultrastructural characterization of the spheroids. In this study we used the Cellink Inkredible+ bioprinter and a biocompatible natural polymer as bioink. The bioink was mixed with leiomyosarcoma cell lines. Uterine leiomyosarcoma is a rare but aggressive tumor as it metastasizes, derived from the smooth muscle cells of the uterus, recognized and diagnosed based on histological criteria of hypercellularity and is also characterized by the involvement of the extracellular matrix (ECM) (3). The analysis in this study allowed us to define in detail the evolution and growth of the spheroids at different times and days, and to define the structural organization by histochemical stains, including hematoxylin and eosin and Masson's trichrome staining, from which it emerged that there was the formation of ECM fibers. The ultrastructural analysis using the transmission electron microscope (TEM) and scanning microscope (SEM) confirmed the presence of fibrosis, in addition to this, it emerged that the spheroids could establish intercellular connections through the formation of vesicles. From the results obtained we hypothesize that this type of analysis could be used to propose therapeutic targets and for the development of potential therapies.

References

1. Antezana PE, Municoy S, Álvarez-Echazú MI, Santo-Orihuela PL, Catalano PN, Al-Tel TH, et al. The 3D Bioprinted Scaffolds for Wound Healing. Pharmaceutics. 2022;14(2).
2. Pinto B, Henriques AC, Silva PMA, Bousbaa H. Three-Dimensional Spheroids as In Vitro Preclinical Models for Cancer Research. Pharmaceutics. 2020;12(12).
3. Greco S, Zannotti A, Pellegrino P, Giantomassi F, Delli Carpini G, D'Agostino M, et al. High levels of hypusinated eIF5A in leiomyoma and leiomyosarcoma pathologies: a possible novel therapeutic target. Reprod Biomed Online. 2023;47(1):15-25.

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Developing 3D corneal epithelium-on-chip for the study of diabetic keratopathy

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Diabetic keratopathy (DK) is a degenerative corneal disease occurring in more than 50% of diabetic patients. This ocular pathology is due to the hyperglycemic state resulting in morphological and functional changes in corneal layers. Currently, most studies related to the cornea are made on in vitro models, or on animal models. The first ones have the advantage to provide large amounts of data at low cost. However, two-dimensional in vitro models poorly represent the complex pathophysiology of the human cornea. On the other hand, the in vivo studies guarantee to reproduce the complexity of the biological events occurring in humans but present ethical problems and high costs. Therefore, it is necessary to identify new avenues and models that can integrate the information validly and effectively, to ultimately reduce the number of animals used. To this end, we developed a three-dimensional (3D) corneal epithelial barrier, by culturing corneal epithelial cells in a micro fluidic device through the technology organ-on-chip. The 3D corneal epithelium was subjected to high-glucose conditions to generate a model of DK. Our model showed well-established molecular and cellular features of DK, such as (1) epithelial defects and delayed wound repair; (2) inflammation, with increased expression of interleukin-1β (IL-1β), tumor necrosis factor- (TNF-), and nuclear factor-kappa-B (NF-kB). The data provided highlight the utility of 3D corneal epithelium-on-chip in modeling DK. This offers new avenues in drug screening, as well as in precision and personalized medicine.

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Light-colored radiations on mechanobiology of human skin cells

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Skin cells contain various pigments and biomolecules that absorb different wavelengths of light, leading to structural changes or generating reactive species, which can trigger cellular responses or damage. This research aims to examine the effects of different light wavelengths on adult human skin cells and the resulting photodynamic changes. We sought to elucidate the mechanisms underlying the potential risks or benefits associated with different wavelengths of light. We utilized an in vitro model involving two cell lines: keratinocytes (HaCaT) and fibroblasts (HDF) (1). The cells were exposed to two specific wavelengths: 530 nm and 780 nm, with fluence values of 1.5 J/cm² and 3 J/cm², respectively (2). The study focused on assessing cell viability, migration, and proliferation, as well as examining the extracellular matrix (ECM). HaCaT cells showed increased viability when treated with both wavelengths compared to the control group, while no significant changes were observed in the viability of HDF cells. HDF cells exhibited an increased migration speed when exposed to the 780 nm wavelength at 3 J/cm², compared to the control, whereas the migration speed of HaCaT cells remained unchanged. No significant differences in cell proliferation were observed in either keratinocytes or fibroblasts. Regarding the ECM, we observed increased type I collagen deposition in cells irradiated with 530 nm at a fluence of 1.5 J/cm² compared to those treated with 780 nm at a fluence of 3 J/cm². This highlights the potential of PBM at 530 nm to enhance ECM remodeling and wound healing (3). The results indicate that photobiomodulation could effectively promote wound healing and tissue repair. By enhancing cell viability, migration, and ECM remodeling, as well as modulating inflammatory responses, PBM could offer a promising avenue for therapeutic intervention in various pathological conditions.

References

1) F. Rossi, G. Magni, F. Tatini, M. Banchelli, F. Cherchi, M. Rossi, E. Coppi, A.M. Pugliese, D. Rossi degl'Innocenti, D. Alfieri, F.S. Pavone, R. Pini, P. Matteini, Photobiomodulation of Human Fibroblasts and Keratinocytes with Blue Light: Implications in Wound Healing, Biomedicines, 9 (2021).
2) D. Bennet, B. Viswanath, S. Kim, J.H. An, An ultrasensitive biophysical risk assessment of light effect on skin cells, Oncotarget, 8 (2017) 47861-47875.
3) Y.S. Tian, N.H. Kim, A.Y. Lee, Antiphotoaging effects of light-emitting diode irradiation on narrow-band ultraviolet B-exposed cultured human skin cells, Dermatol Surg, 38 (2012) 1695-1703.

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In vitro remodeling of decellularized human dermal matrix by resident human cardiac progenitor cells for cardiac tissue regeneration

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The extracellular matrix (ECM) acts as a complex scaffold where cells reside and engage in vital interactions that govern cellular behavior. The ECM profoundly shapes cellular dynamics and maintains tissue equilibrium. Decellularized extracellular matrix (d-ECM) has rapidly emerged as an attractive biomaterial for tissue engineering and recent evidence suggests that decellularized human skin (d-HuSk) represents an excellent biomaterial for cardiac tissue engineering (CTE). To better understand the interactions between ECM and cells in d-Husk and to evaluate whether d-Husk is remodeled *in vitro* by cells, human dermal skin from patients undergoing abdominoplasty was decellularized and cryosectioned to obtain d-ECM scaffolds that were recellularized with human cardiac progenitor cells (hCPCs) isolated from adult human hearts. The scaffolds were cultured for two weeks to allow cell engraftment and proliferation, then hCPCs were induced to myogenic differentiation and cultured for two more weeks. The hCPC-d-HuSk bioconstructs were then analyzed by real-time PCR, immunofluorescence, SEM, dye-binding specific assays to evaluate the differentiation of hCPCs and their effects on d-HuSk composition and organization, using d-HuSk cell-free scaffolds as a reference. Molecular analyses by Real-time PCR revealed a significant up-regulation of markers typical of differentiating and mature cardiomyocyte, with a preserved transcription of mesenchymal, endothelial, and smooth muscle cell genes. Microscopic observation by SEM demonstrated a more structured network of elastic fibers in d-HuSk scaffolds recellularized with hCPCs compared to acellular d-HuSk, which exhibited higher collagen fiber density. Furthermore, analyses of scaffold indicated a reduction in collagen content in recellularized d-HuSk scaffolds, while elastin and GAG content did not change significantly. Additionally, a higher mean fluorescence intensity for fibronectin and tenascin was observed by immunofluorescence in recellularized d-HuSk. Our study suggests that d-HuSk constitutes an extremely valuable biomaterial for CTE scaffolds since resident cells can rearrange and replace it by synthesizing their own matrix.

References

1) Belviso, I.; Romano, V.; Sacco, A.M.; Ricci, G.; Massai, D.; Cammarota, M.; Catizone, A.; Schiraldi, C.; Nurzynska, D.; Terzini, M.; Aldieri, A.; Serino, G.; Schonauer, F.; Sirico, F.; D'Andrea, F.; Montagnani, S.; Di Meglio, F.; Castaldo, C. Decellularized Human Dermal Matrix as a Biological Scaffold for Cardiac Repair and Regeneration. Front Bioeng Biotechnol. 2020, 8:229

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Development of a technological platform for the study and use of Normal and Tumour Patient-Derived Colon Organoids

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Organoid production from ES (normal or iPSCs) and ASCs (Adult Stem Cells) has been established as a major technological breakthrough in biomedical research models. As a "patient in the lab", Patient-Derived Organoids (PDOs) from ASCs spatially and dynamically summarize the native organ, representing the cellular and physio-pathological components of patients from which they derive, and can be maintained long-term in culture without losing the original phenotype and genetic stability. ASCs from intestinal or colon biopsies can self-organize in PDOs, recapitulating both the stem and the functional cellular components of the crypt-villus structure of the epithelium. This near-physiological 3D model facilitates *in vitro* investigation of a range of *in vivo* biological and biochemical processes including tissue renewal, stem cell/niche functions, tissue homeostasis, and tissue responses to molecules. Colorectal cancer (CRC) represents the third most common type of cancer in the world. In a complementary way to the normal component, Patient-derived Tumor Organoids (PDTOs) reproduce many biological and biochemical aspects of the primary tumor, including histology and biomarker expression patterns.

To fill the gap between the laboratory and the clinic, we set up a Platform to produce Colon Normal and Tumoral PDOs from the same patient. In order to propose a robust in vitro experimental model, we are validating this Platform to investigate the onset and maintenance of the physio-pathology, to test clinical drug responses of the related patient, and to assess the inter-patient variability in treatments. IHC and fluorescent vital-dyes *in vivo* time-lapse analysis[1] showed that PDOs and PDTOs recapitulate the phenotypical features of the patient-derived tissue.

Finally, we used an innovative analytical flow-based millifluidic cytometer[2] (W8, CellDynamics) to measure different parameters (i.e. diameter, weight, and mass density) and characterize the biophysical behavior of PDOs and PDTOs during drug and molecule testing.

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References

1) Di Franco, E.; Costantino, A.; Cerutti, E.; D'Amico, M.; Privitera, AP.; Bianchini, P.; Vicidomini, G.; Gulisano, M.; Diaspro, A.; Lanzanò, L. SPLIT-PIN software enabling confocal and super-resolution imaging with a virtually closed pinhole. Sci Rep. 2023 13(1):2741. doi: 10.1038/s41598-023-29951-9
2) Sargenti, A.; Musmeci, F.; Cavallo, C.; Mazzeschi, M.; Bonetti, S.; Pasqua, S.; Bacchi, F.; Filardo, G.; Gazzola, D.; Lauriola, M.; Santi, S. A new method for the study of biophysical and morphological parameters in 3D cell cultures: Evaluation in LoVo spheroids treated with crizotinib. PloS one 2021, 16(6), e0252907. doi: 10.1371/journal.pone.0252907

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Development of three-dimensional composite scaffolds for *in vitro* testicular organoid growth

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Preserving spermatogonial cells is an expanding necessity for prepubertal male patients undergoing gonadotoxic therapies, which may potentially deplete these cells [1].

Creating testicular organoids [2] appears to be a promising strategy to provide the right supportive environment for the development of spermatogonial stem cells (SSCs). Several proposals have been suggested, including strategies based on the use of extracellular matrix (ECM) as a biological scaffold for tissue engineering applications [3]. ECM is known to play an important role in modulating cell adhesion, signaling, migration, proliferation, and three-dimensional arrangement. As a result, ECM-based materials can be used in a variety of tissue engineering and regenerative medicine approaches to tissue reconstruction [3].

Aim of this study was to develop two exploratory 3D scaffolds for testis organoid growth together with chitosan-alginate mixtures of different concentrations and porosities or with poly(propylene azelate) (PPAz), with the addition of a decellularized ECM (dECM) from porcine prepubertal tunica albuginea, previously characterized in our laboratory.

The process has been analyzed step by step, combining non-invasive microscopic (scanning electron microscope) and spectroscopic analysis (Brillouin and Raman micro-spectroscopy, ATR-FTIR spectroscopy), along with genomic DNA and total RNA analysis and histomorphological assessment, to obtain a complete evaluation of the morphology and mechano-chemistry properties of the scaffolds. Moreover, to assess the cytotoxicity of alginate-chitosan and PPAZ scaffolds, in the presence or absence of 5% dECM, an indirect toxicity assay was adopted on primary cultures of prepubertal porcine Sertoli cells.

Analysis of morphology, mechano-chemistry properties and cytotoxicity showed that the PPAZ scaffold, with 5% dECM, could be the best prototype for *in vitro* spermatogenesis studies in the nearest future.

References

1) Stukenborg, J.B.; Jahnukainen, K.; Hutka, M.; Mitchell, R.T. Cancer treatment in childhood and testicular function: the importance of the somatic environment. Endocr. Connect. 2018, 7, R69–R87.
2) Baert, Y.; De Kock, J.; Alves-Lopes, J.P.; So¨der, O.; Stukenborg, J.B.; Goossens, E. Primary Human Testicular Cells Self-Organize into Organoids with Testicular Properties. Stem Cell Rep. 2017, 8, 30-38.
3) Vermeulen, M.; Del Vento, F.; de Michele, F.; Poels, J.; Wyns, C. Development of a Cytocompatible Scaffold from Pig Immature Testicular Tissue Allowing Human Sertoli Cell Attachment, Proliferation and Functionality. Int. J. Mol. Sci. 2018, 19, 227.

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Understanding the crosstalk between AML cells and bone marrow stromal cells to overcome treatment resistance

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Although tremendous progress has been made in developing and applying in clinics molecular targeted therapies for acute myeloid leukemia (AML), drug resistance and relapses, mostly due to clonal selection and the protective effect of the leukemic bone marrow microenvironment, are still major issues. We developed a strategy based on a combination of drugs inducing proteotoxic and oxidative stress. We demonstrated that it efficiently leads to the death of AML cell lines and primary leukemic stem cells (LSCs) bearing the mutation FLT3-ITD, without affecting normal HSCs, *in vitro* and *in vivo*. However, bone marrow stromal cells (BMSCs) protect AML cells by reducing the amount of oxidative stress generated by the treatment in a co-culture system. Our focus is to investigate the mechanisms contributing to the protective abilities of the BMSCs. Furthermore, aiming to optimize the combination of drugs to increase their translational potential, we evaluated the efficacy of combining proteotoxic stress with different drugs at the cutting edge in clinical trials for AML, among which is the BCL-2 inhibitor Venetoclax.

We tested the sensitivity of FLT3-ITD+ AML cell lines and primary LSCs to different treatments in monoculture or in coculture with BMSCs, in 2D or 3D models. In parallel, we evaluated the efficacy of the combination RBA plus Venetoclax in an *in vivo* orthotopic murine model of AML.

We demonstrate that the combination of proteotoxic stress and Venetoclax is effective against FLT3-ITD+ AML cells *in vitro*, overcoming the protection provided by BMSCs in a coculture system without affecting their viability, and *in vivo* significantly prolonging the life span of a murine model of FLT3-ITD+ AML. Importantly, our investigations into the crosstalk between AML cells and BMSCs upon different treatments reveal the involvement of mechanotransduction signaling in the BMSCs for the first time. Thus, our finding opens new paths for research and potential therapeutic strategies.

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Mitochondrial transfer from adipose stem cells to breast cancer patient derived organoids

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The knowledge of Breast cancer (BC) tumor microenvironment (TME) is crucial to improve BC patients' outcomes.

Apart from immune infiltrate, BC cells (BCCs) are surrounded by mammary adipose tissue which is composed of adipose cells and stromal cells such as adipose mesenchymal stem cells (ASCs).

Recently, it was shown that cancer cells acquire mitochondria from stem cells to compensate the loss of mitochondrial function in a process called Mitochondrial Transfer (MT). We previously showed that ASCs donate their mitochondria via tunneling nanotubes (TNTs) to 2D BCCs, promoting chemoresistance. Our objective was to evaluate if MT occurs in 3D patient-derived organoids (PDOs) in co-culture with ASCs.

We generated and characterized via immunohistochemistry PDOs from luminal BC consenting patients, obtaining organoids from both tumor and peritumor samples. Furthermore, we generated primary ASCs from the same patient which were characterized by flow cytometry. We set up a hybrid co-culture model with 3D PDOs and 2D ASCs, employing both commercially available and primary autologous lines. Thanks to immunofluorescence, we verified the occurrence of MT, for both tumoral and peritumoral organoids, with commercial and primary homologous ASCs. Thereafter, to evaluate potential mechanisms driving this phenomenon, we treated co-cultured cells with Cytochalasin B, showing that, unlike previously demonstrated in our 2D models, MT in organoids occurs in a different mechanism than TNTs.

MT appears as a key process that could drive tumor aggressiveness in patient-derived models, whose better understanding could help to design more effective treatment strategies to overcome drug resistance.

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3D human epidermal model in vitro to assess the effects of Boron Neutron Capture Therapy on keratinocyte proliferation

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Boron Neutron Capture Therapy (BNCT) is an innovative radiotherapy for treating solid tumors unresponsive to traditional therapies. The administration of a compound labelled with boron-10 (^{10}B, non-radioactive) is a pre-treatment, followed by irradiation with low-energy neutrons. The neutron beam penetrates the patient across the skin, making it a limiting tissue. It thus needs to study the dose-effect relation in the skin. [1, 2, 3]

The SkinEthic™ model, an in-vitro reconstructed human epidermal model, was used for this purpose. Several studies [4] have confirmed the reliability of its response compared to the native tissue.

Samples were incubated with boronophenylalanine (BPA), a ^{10}B delivery agent, and irradiated at three different power levels: 4.5, 11.4 and 22.7 Gy. Models were fixed at subsequent observation times, embedded in paraffin, and sliced. The percentage of proliferating cells was assessed using Bromo-Deoxy-Uridine incorporation assay (BrdU) and the expression of Proliferation-Cell Nuclear Antigen (PCNA). Same analyses were performed on constructs irradiated both with the neutron beam without BPA and with a photon beam to evaluate BNCT's effectiveness.

Morphological and immunohistochemical analyses showed progressive changes in the tissue structure of the constructs over time and in response to radiation doses. The cell proliferation activity varied, with low BrdU-positive-cells in samples treated with BNCT for 2 days. The anti-PCNA assays also showed variable positivity trends, peaking on the second day in culture. The reduction of proliferating cells was linked to a high presence of protein involved in reparative mechanisms, consistent with previous in-vitro studies.

These preliminary experiments aim to refine the study protocol and improve the characterization of constructs and their responses to radiation. The high variability in results makes the standadization difficult, suggesting the need to increase sample number for statistically significant results. Observations at shorter times and lower radiation doses would complete this dose-effect study.

References

[1] Malouff, T. D., D. S. Seneviratne, D. K. Ebner, William C. Stross, M. R. Waddle, D. M. Trifiletti, and S. Krishnan. 2021. «Boron Neutron Capture Therapy: A Review of Clinical Applications». Frontiers in Oncology 11: 601820.

[2] Suzuki, M. 2020. «Boron Neutron Capture Therapy (BNCT): A Unique Role in Radiotherapy with a View to Entering the Accelerator-Based BNCT Era». International Journal of Clinical Oncology 25 (1): 43–50.

[3] Bortolussi, S., I. Postuma, N. Protti, L. Provenzano, C. Ferrari, L. Cansolino, P. Dionigi, et al. 2017. «Understanding the potentiality of accelerator based-boron neutron capture therapy for osteosarcoma: dosimetry assessment based on the reported clinical experience». Radiation Oncology (London, England) 12: 130.

[4] Pellevoisin, C., C. Videau, D. Briotet, C. Grégoire, C. Tornier, A. Alonso, A. S. Rigaudeau, C. Bouez, e N. Seyler. 2018. «SkinEthicTM RHE for in Vitro Evaluation of Skin Irritation of Medical Device Extracts». Toxicology in Vitro 50: 418–25.

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Fabrication and characterization of bi-layered scaffolds based on bioactive oxidized polyvinyl alcohol and 3D printed polylactic acid for osteochondral defect repair

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Osteochondral defects are a significant challenge in orthopedic surgery, due to cartilage complexity and its exposure to high pressure/motion. Within this scenario, osteochondral tissue engineering has shown an increasing improvement, looking for valuable options. The aim of this study was to develop scaffolds for osteochondral regeneration. As for cartilage, oxidized polyvinyl alcohol (OxPVA) was combined with gelatin microspheres (porogen, 10%, 15% and 25% w/w) by mechanical incorporation. After systems cross-linking by freeze-thawing and gelatin removal by particle-leaching, the supports were characterized for their ultrastructure/mechanical behaviour/interaction with stem cells (HM1-SV40). In parallel, articular cartilage from Donors enrolled in Body Donation Program of Padua University was decellularized, evaluated to assess treatment effectiveness, and homogenized before incorporation into OxPVA (25% w/w of extracellular matrix (ECM) + different amounts of porogen). Derived scaffolds were compared for ultrastructure/ECM distribution/bioactivity *in vitro*/biocompatibility *in vivo*. Morphometric study showed that structure complexity was greater at higher porogen percentages; moreover, higher porosity had a negative impact over material stiffness (reduction) but not on cells adhesion/proliferation (increase). For a balance between compressive strength and scaffolds bioactivity, 15% and 25% w/w gelatin microspheres were chosen to be combined with cartilage ECM into OxPVA, respectively. The addition of decellularized/homogenized ECM furtherly improved porous scaffolds bioactivity without *in vivo* inflammation, thus suggesting scaffolds biocompatibility. Regarding bone, 3D printing was used to fabricate three different polylactic acid (PLA) supports; these were compared for ultrastructure and interaction with cells (HM1-SV40). All the three geometries sustained a good cell viability with a statistically significant difference in proliferation in presence of a higher porosity (64.2%) (lower porosity, 28.5%). Scanning Electron Microscopy showed cells distribution both over and inside the PLA scaffolds, suggesting their full-thickness colonization. Despite future studies will be necessary, combining bioactive OxPVA and 3D printed PLA scaffolds may lead to promising device for osteochondral regeneration.

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Regenerative potential of bioresorbable collagen membranes: ultrastructural profile of Human Periodontal Ligament Fibroblasts exposed to Bovine Pericardium Membranes

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Regenerative dentistry has seen promising advancements in the development of biomaterials that can guide and stimulate the regeneration of dental tissues. Unique chemical, mechanical and biological properties, with a specific mention to osteoinductivity and osteoconductivity, are the main features of these class of biomaterials, which make them suitable to interact with living tissue [1]. A significant contribute to regenerative dentistry derived from resorbable and non-resorbable membranes. Resorbable membranes made of collagen are promising biomaterials, and serum bovine pericardium membranes are the most representative resorbable membranes. The aim of this study is to investigate the ultrastructural profile of human periodontal ligament fibroblasts (HPLFs) cultured in standard conditions and exposed to bovine pericardium membranes of different thicknesses (0.2 mm and 0.4 mm) for 24 hours. The HPLFs underwent transmission electron microscopy (TEM) standard preparative. The ultrastructural profile of HPLFs displayed peculiar changes, when exposed to bovine pericardium membranes for 24 hours. HPLFs in fact showed a large nucleus, prominent nucleoli, clustered rod-like shaped mitochondria, richly developed endoplasmic reticulum (ER) and Golgi apparatus, and extensive tapering cytoplasmic projections. The protein synthesis and metabolism cellular compartments increased, when compared to the control: Unpaired *t*-test and one-way ANOVA showed that HPLFs exposed to membranes displayed an increase in the number of mitochondria (89.23 ± 7.44 vs 66.90 ± 9.58; T1 and control; $p < 0.01$ and 84.05 ± 14.01 vs 66.90 ± 9.58; T2 and control; $p < 0.05$).

This new ultrastructural evidence ascertains the stimulation of the protheosynthetic active state of HPLFs triggered by the collagen bovine membrane, confirming the active role played by this type of biomaterial in the dynamics of tissue regeneration [2].

References

1) Mehta, D.; Deshpande, N.; Dandekar, S. Comparative Evaluation of Platelet-Rich Fibrin Membrane and Collagen Membrane along with Demineralized Freeze-Dried Bone Allograft in Grade II Furcation Defects: A Randomized Controlled Study. J. Indian Soc. Periodontol. 2018, 22, 322-327.
2) Bianchi, S.; Bernardi, S.; Simeone, D.; Torge, D.; Macchiarelli, G.; Marchetti, E. Proliferation and Morphological Assessment of Human Periodontal Ligament Fibroblast towards Bovine Pericardium Membranes: An In Vitro Study. Materials 2022, 15, 8284.

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In vitro maturation and structural enhancement of three-dimensional cardiac bioconstructs via physiological mechanical stimulation

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Successfully recapitulating the physiological stimuli of cardiac tissue environment *in vitro* is crucial for cardiac tissue engineering (CTE). Biochemical cues and mechanical forces play a vital role in orchestrating tissue patterning and growth in the body as they influence intracellular biochemistry and gene expression. Stretch bioreactors are commonly used in CTE to provide the most appropriate signals from the microenvironment for the development of functionally mature tissue constructs. On this basis we prepared three-dimensional cardiac bioconstructs by repopulating decellularized human skin (d-HuSk) scaffolds obtained from waste material of patients undergoing abdominoplasty with human cardiac progenitors cells (hCPCs) isolated from adult human hearts. The bioconstructs were cultured in standard conditions for the first 7 days to allow cell engraftment and then in a stretch bioreactor applying a 10% cyclic strain at 1 Hz. Subsequently, the cardiac bioconstructs were analyzed by SEM, histochemistry, immunofluorescence, and Real-time PCR, using bioconstructs cultured in static conditions for 2 weeks as a reference. Microscopic analysis showed a layered organization of hCPCs on the surface of d-HuSk in both conditions, although in constructs cultured under dynamic conditions h CPCs migrated from the surface into the internal layers of the d-ECM.. Furthermore, the mechanical stimulation guided the alignment of the cells which were arranged parallel to each other and orthogonal to the direction of the stretch. Finally, gene expression analyses showed the up-regulation of transcripts for cardiac cell lineages and mesenchymal cells in hCPCs cultured in dynamic conditions. These findings suggest that the mechanical stimulation enhanced the functional maturation and differentiation of d-HuSk-based cardiac constructs, thus strengthening the suitability of the dermal matrix as a substitute for the cardiac ECM.

References

1) Putame, G.; Gabetti, S.; Carbonaro, D.; Di Meglio, F.; Romano, V.; Sacco, A.M.; Belviso, I.; Serino, G.; Bignardi, C.; Morbiducci, U.; Castaldo, C.; Massai, D. Compact and tunable stretch bioreactor advancing tissue engineering implementation. Application to engineered cardiac constructs. Med Eng Phys. 2020, 84:1-9.
2) Belviso, I.; Romano, V.; Sacco, A.M.; Ricci, G.; Massai, D.; Cammarota, M.; Catizone, A.; Schiraldi, C.; Nurzynska, D.; Terzini, M.; Aldieri, A.; Serino, G.; Schonauer, F.; Sirico, F.; D'Andrea, F.; Montagnani, S.; Di Meglio, F.; Castaldo, C. Decellularized Human Dermal Matrix as a Biological Scaffold for Cardiac Repair and Regeneration. Front Bioeng Biotechnol. 2020, 8:229.

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Application of gellan gum-based patches to explore skin regenerative and remodeling effects of ascorbic and tannic acids

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Skin regeneration and remodeling, following injuries and external biological, chemical, and mechanical threats, are crucial in maintaining its integrity and functionality. Rapid wound healing and tissue repair prevent infections, prolonged inflammation, scars, and chronic wound formation. Recently, numerous innovative strategies have been developed to ensure and accelerate these processes. Biomaterials offer a promising solution by providing scaffolds mimicking the natural extracellular matrix and allowing cell attachment, growth, migration, and differentiation to facilitate tissue repair and regeneration.

In our study, methacrylated gellan gum (GGMA)-based patches were synthesized via substitution of hydroxyl groups in the GG repeating units with methacrylic moieties (MA) to produce photo-crosslinkable biomaterial ink. Afterward, GGMA was bio-functionalized by incorporating two bioactive compounds, i.e. ascorbic acid (AA) and tannic acid (TA), known for their antioxidant and anti-inflammatory properties and their ability to promote collagen synthesis. Both acids were loaded into the synthesized patches by diffusion-filling method.

The characterized bio-functionalized GGMA-based patches were then employed to evaluate, *in vitro*, their biocompatibility as per ISO 10993, using a human dermal fibroblasts (HDFs) cell line. To this end, cell viability was evaluated directly by applying the designed patches on cells for 24h hours (test by direct contact) and indirectly by growing cells for 24 hours in a culture medium conditioned with the same patches (test by indirect contact). Moreover, the cell migration rate was evaluated by performing a wound-healing assay in the presence of the conditioned culture medium.

The obtained preliminary results confirm the biocompatibility of the bio-functionalized GGMA-based patches and suggest a possible synergistic effect of AA and AT in promoting cell viability and motility.

Further investigations will allow clarifying the potential of these patches and their promising applications in the field of regenerative medicine.

References

1) Downer, M.; Berry, C.E.; Parker, J.B.; Kameni, L.; Griffin, M. Current Biomaterials for Wound Healing. Bioengineering (Basel) 2023, 10, 1378.
2) D'Amora, U.; Ronca, A.; Scialla, S.; Soriente, A.; Manini, P.; Phua, J. W.; Ottenheim, C.; Pezzella, A.; Calabrese, G.; Raucci, M. G.; Ambrosio, L. Bioactive Composite Methacrylated Gellan Gum for 3D-Printed Bone Tissue-Engineered Scaffolds. Nanomaterials (Basel) 2023, 13, 772.
3) Michalak, M.; Pierzak, M.; Kręcisz, B.; Suliga, E. Bioactive Compounds for Skin Health: A Review. Nutrients 2021, 13, 203.

Ageing and degenerative diseases

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Evaluation of the time stability of rat aortic rings after a 7-day incubation period

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Vascular disorders pose a significant challenge due to the incomplete understanding of their pathophysiology. *In vitro* isolated vascular tissues represent a valuable technique for investigating novel pharmacological approaches and discover new vasoactive drugs.

This study aimed to evaluate the functional activity and histological morphology of thoracic and abdominal rat aorta rings cultured for 7 days as compared to fresh control preparations.

In cultured rings, a general reduction of the contractile activity in response to depolarizing stimuli (60 mM KCl) was observed in both the abdominal (7 days 197 ± 88 mg, control 962 ± 310 mg, *n=3-6*; P=0,1382) and thoracic aorta (7 days 403 ± 75 mg, control 1513 ± 222 mg, *n=4-6*; P=0,0045). Similar results were obtained in preparations challenged by the α_1 adrenergic agonist phenylephrine (thoracic 7 days 238 ± 107 mg, control 1153 ± 183 mg, *n=4-6*; P=0,0056; abdominal 7 days 343 ± 168 mg, control 797 ± 279 mg, *n=3-6*; P=0,3186).

This effect was associated with an electromechanical hyperresponsiveness to low concentrations of KCl. However, the concentration-response curves to phenylephrine recorded in the two experimental models overlapped.

The histological analysis did not detect significant differences in the luminal diameter, wall thickness, and the number of elastic lamellae between fresh and cultured rings.

These findings demonstrate that 7 days of culture reduce the contractile function of rat aorta rings, though without abolishing it. Further anatomical and functional investigations are ongoing to explore the mechanisms underpinning this phenomenon, focusing on the potential involvement of voltage-gated calcium channels, potassium channels, and adrenergic receptors.

References

1) Durante, M.; Frosini, M.; Fusi, F.; Gamberucci, A.; Chegaev, K.; Rolando, B.; Riganti, Fruttero, R.; C.; Saponara, S. *In vitro* vascular toxicity assessment of NitDOX, a novel NO-releasing doxorubicin. Eur. J. Pharmacol. 2020, 5, 880, 173164.
2) Durante, M.; Frosini, M.; Chiaino, E.; Fusi, F.; Gamberucci, A.; Gorelli, B.; Chegaev, K.; Riganti, C.; Saponara, S. Sdox, a H_2S releasing anthracycline, with a safer profile than doxorubicin toward vasculature. Vascul Pharmacol. 2022, 143, 106969.

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Alpha-Synuclein pathology in the Enteric Nervous System is a promising biomarker for Parkinson's Disease

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The role of the gut-brain axis has been recently highlighted as a major contributor to Parkinson's Disease (PD) pathophysiology, with numerous studies investigating bidirectional transmission of pathological alpha-synuclein (αSyn). However, the extent and the characteristics of pathology in the enteric nervous system (ENS) have not been fully investigated.

We characterized αSyn pathology, glial responses and immune-cell populations in gastric and duodenal biopsies of PD patients via conformation-specific antibodies and histopathology, and evaluated seeding activity via Real-Time Quaking Induced Conversion (RT-QuIC) assay.

We examined 20 patients with advanced PD who underwent PEG-J placement, 6 untreated patients with early PD, as well as 18 matched healthy controls undergoing routine diagnostic endoscopy. Immunohistochemistry was performed for anti-aggregated αSyn (5G4), enteric glial markers (GFAP, SOX10, S100B) and immune-population markers followed by morphometrical semi-quantitative analysis. RT-QuIC analyses were performed to evaluate alpha-synuclein seeding activity.

Immunoreactvity for aggregated αSyn, presenting a typical thread-like pattern, was identified in all PD patients (early and advanced) and colocalized with neuronal marker beta-III-tubulin; statistically-significant quantitative differences between early and advanced PD patients were also detected. Evaluation of enteric glial cells revealed increased size and density when compared to controls, suggesting reactive gliosis. Similarly, increased T- and B-lymphocyte densities, as well as higher expression of HLA-DR, was detected in the gut of PD patients. The accuracy of αSyn RT-QuIC was 67.4% (20.7%-63.7%) in duodenum, and 80.0% (64.4%-90.9%) in gastric biopsies. Assay's sensitivity was significantly higher in advanced than early PD for all matrices.

We found evidence of αSyn pathology and gliosis in the enteric nervous system of PD patients, which was accurate in discerning PD patients from healthy controls. Future studies are required to evaluate how early in the disease process enteric nervous system pathology occurs, and whether it plays any role in mediating dopamine treatment efficacy in advanced patients.

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Unveiling a role for Mitogen Activated Protein Kinase 15 in hepatic steatosis

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Metabolic dysfunction-associated steatotic liver disease (MASLD), previously called non-alcoholic fatty liver disease (NAFLD), is the latest term for steatotic liver disease associated with metabolic syndrome, characterized by hepatic steatosis associated with a higher risk of developing chronic kidney disease. Meanwhile, NAFLD/MASLD has become one of the most common cause of chronic liver disease worldwide. NAFLD/MASLD comprises a spectrum of liver conditions ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) which can progress to cirrhosis and hepatocellular carcinoma. One of the crucial events involved in NAFLD/MASLD progression is the excessive accumulation of fatty acids inside the hepatocytes.

Here, we report data correlating the expression of the atypical Mitogen Activated Protein kinase 15 (MAPK15) to NAFLD/MASLD. MAPK15 is an atypical MAP kinase implicated in several cellular processes, such as cell proliferation, genomic integrity, autophagy, mitophagy, oxidative stress, ageing and cellular senescence (Franci et al, 2022, Franci et al, 2024). Although MAPK15 has been associated to obesity through genome-wide association studies (https://www.ebi.ac.uk/gwas/genes/MAPK15), the role of MAPK15 in mammalian lipid metabolism has not been addressed yet. To explore the role of MAPK15 in lipid homeostasis, we analysed MAPK15 knockout (KO) mice. Results showed that MAPK15 KO mice accumulated more abundant subcutaneous and abdominal visceral fat compared to wild-type (WT) and liver histological sections demonstrated a higher lipid content. Upon feeding MAPK15 KO mice with a Western diet (with higher content of lipids), KO liver sections showed more severe hepatic steatosis compared to WT. In agreement, *in vitro* experiments of MAPK15 downregulation in HEPG2 cells demonstrated an intracellular increased lipid storage.

Taken together, our results demonstrated an increased propensity of MAPK15 KO mice to develop hepatic steatosis, suggesting an increased risk of developing obesity and NAFLD/NASH when the MAPK15 protein is reduced.

References

1) Franci, L., Tubita, A., Bertolino, F. M., Palma, A., Cannino, G., Settembre, C., Rasola, A., Rovida, E., & Chiariello, M. (2022). MAPK15 protects from oxidative stress-dependent cellular senescence by inducing the mitophagic process. Aging Cell, *21*(7), e13620. doi: 10.1111/acel.13620.
2) Franci L, Vallini G, Bertolino FM, Cicaloni V, Inzalaco G, Cicogni M, Tinti L, Calabrese L, Barone V, Salvini L, Rubegni P, Galvagni F, Chiariello M. MAPK15 controls cellular responses to oxidative stress by regulating NRF2 activity and expression of its downstream target genes. Redox Biol. 2024. doi: 10.1016/j.redox.2024.103131.

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High fiber diet to counteract early signs of heart damages: an ultrastructural morphological study to beating dysbiosis effects

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Cardiovascular diseases (CVDs) increase with aging and are often comorbidities of degenerative diseases. Recent clinical and experimental evidence in human microbiota studies sheds light on the contribution of gut microbiota alteration, called dysbiosis, to the development and progression of CVDs, also suggesting diet intervention as a potential strategy to mitigate dysbiosis effects at the cardiac level. In this context, we investigated in an in vivo experimental mouse model the effects of gut microbiota homeostasis alteration, induced by the antibiotic vancomycin (VAN) dissolved in drinking water, on the cardiac muscle tissue, and the potential protection of a high-fiber diet (HFiber) following antibiotic treatment against heart damage.

Light microscopy analysis of heart tissue structure did not reveal any morphological alteration induced by VAN. Analysis of digitalized transmission-electron microscopy (TEM) images of cardiomyocytes ultrastructure showed that the regular arrangement of myofibrils was preserved, but mitochondria in the VAN group were significantly larger, more elongated and, above all, damaged compared with untreated control (CT). No significant alteration has been detected in the HFiber group. Molecular characterization of mitochondria dynamics assessed by Real-time PCR indicated that antibiotic administration induced expression of genes driving mitochondria fusion (OPA1, DNM1, MFS1), oxidative damage (e.g. SOD2) and a trend of reduction of biogenesis related-genes (e.g. NRF1, PPRargc1). Proteomic analysis by FT-Orbitrap highlighted alterations of the mitochondrial respiratory chain in dysbiotic mice and also an increase of interstitial collagen, suggestive of a potential onset of fibrosis, not observed with the followed high-fiber diet administration. Analysis of the circulating levels of markers of CVDs is ongoing.

In conclusion, i) antibiotic treatment induces early ultrastructural alterations of intermyofibrillar mitochondria in cardiomyocytes; ii) TEM analysis is useful to detect ultrastructural alterations in absence of evident histological tissue damage; iii) Hfiber diet can counteract the detrimental effect triggered by dysbiosis induced by antibiotics administration.

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Epiretinal membranes: a matrix ground substance study

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Epiretinal membranes (ERMs) are fibrocellular membranes that form at the vitreoretinal interface. They could be secondary to ocular or systemic diseases (e.g. retinal detachment, diabetes) or idiopathic, and in the latter case they are mostly ageing pathologies. They are composed of different cell types and extracellular matrix and they can be either asymptomatic or sight-threatening. Through the years, many studies have been dedicated to ERMs cell characterization and different cells derived from different populations have been identified in ERMs samples, including Müller cells, astrocytes, retinal pigmented epithelial cells and hyalocytes. On the other hand, the extracellular matrix (ECM) has received less attention with a few studies focusing on the collagen family or on matrix-associated proteins such as thrombospondin and fibronectin, while some molecules have been almost entirely neglected; in particular, this is the case of proteoglycans (PGs) and glycosaminoglycans (GAGs), which are of particular interest, not only for their role in extracellular matrix organization but also because they can be involved in the migration process and therefore in ERMs formation. Indeed, thanks to a bioinformatic approach on previously reported ERM ECM proteins, we identified Cluster of Differentiation 44 (CD44) as a central regulator of ERMs aberrant dynamics.[1] It is worth to note that CD44 is a hyaluronic acid (HA) receptor. Having this as a starting point we investigated the presence and the distribution of HA and of some PGs such as decorin (DEC) and Proline And Arginine Rich End Leucine Rich Repeat Protein (PRELP) in our samples, trying to understand their relationship with other ECM components (e.g. collagens) and with ERMs cells, in order to shed light on some unknown ERM aspects.

References

1) Bianchi, L.; Altera, A.; Barone, V.; Bonente, D.; Bacci, T.; De Benedetto, E.; Bini, L.; Tosi, G.M.; Galvagni, F.; Bertelli, E. Untangling the Extracellular Matrix of Idiopathic Epiretinal Membrane: A Path Winding among Structure, Interactomics and Translational Medicine. Cells 2022, 11, 2531.

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Ultrastructural and cytofluorimetric analyses of blood-derived human NK cells cultured under metastatic-calcification-like conditions

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Natural killer (NK) cells are innate lymphoid cells involved in the immune response against virally infected or malignant cells, playing a regulatory role in adaptive immunity in both physiological and pathological conditions. NK cells are also reported to infiltrate both atherosclerotic plaques and mineralizing aortic valve leaflets[1], but whether they play a pathophysiological or compensatory role in cardiovascular calcific diseases still needs more elucidation. Here, propensity of peripheral blood human NK cells to undergo mineralization was assayed culturing cells up to 8 days with a validated pro-calcific medium simulating metastatic calcification. Cells were examined by electron microscopy, for their morphological evaluation, and multiparametric flow cytometry, for the analysis of NK cell subsets as well as major HLA class I (NKG2A, KIRs) and non-HLA class I specific (i.e. NCRs) NK receptor expression. Ultrastructurally, NK cells not exposed to the pro-calcific milieu exhibited preserved organelles. Conversely, NK cells from pro-calcific cultures showed suffering signs, but appeared free from calcification. Actually, they showed not typical features of pro-calcific degeneration, such as accumulation of membrane-derived lipid material and its layering at cell edges, as previously described for mineralizing valve interstitial cells cultured under the same pro-calcific conditions[2,3]. Cytofluorimetric analyses showed that treatment with the pro-calcific medium reduced NK cell counts, but without altering the ratio between cell subsets ($CD56^{bright}CD16^{dim/-}$ and $CD56^{dim}CD16^{+}$) and the expression of the assayed HLA receptors, compared to NK cells cultured without the pro-calcific medium. Moreover, the pro-calcific treatment seemed to slightly upregulate the NCR NKp44, without significantly affecting the expression of the other NCRs NKp30 and NKp46. Overall, these preliminary data revealed that NK cells do not undergo mineralization as well as significant phenotypic alterations even if cultured under severe pro-calcific conditions. Further investigations are ongoing to improve knowledge on possible involvement of NK cells in calcification processes.

References

1) Passos, L.S.A.; Lupieri, A.; Becker-Greene, D.; Aikawa E. Innate and adaptive immunity in cardiovascular calcification. Atherosclerosis 2020, 306, 59-67.
2) Bonetti, A.; Della Mora, A.; Contin, M.; Tubaro, F.; Marchini, M.; Ortolani, F. Ultrastructural and spectrophotometric study on the effects of putative triggers on aortic valve interstitial cells in in vitro models simulating metastatic calcification. Anat. Rec. 2012, 295, 1117-1127.
3) Bonetti, A.; Allegri, L.; Baldan, F.; Contin, M.; Battistella, C.; Damante, G.; Marchini, M.; Ortolani, F. Critical involvement of calcium-dependent cytosolic phospholipase A2a in aortic valve interstitial cell calcification. Int. J. Mol. Sci. 2020, 21, 6398.

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The anti-fibrotic effect of Lactose-Modified Hyaluronic Acid molecules on primary pulmonary fibroblasts

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Galectin-3 (Gal-3) is a b-galactoside binding lectin highly expressed in a variety of chronic inflammatory and fibrotic diseases and its capability to enhance extracellular matrix (ECM) proteins synthesis by lung fibroblasts has been recently demonstrated.

This study aims to evaluate *in vitro* the ability of HA-based molecules functionalized with D-glucose and D-galactose (Hylach®), able to bind Gal-3, to reduce inflammation and fibrosis on human primary pulmonary fibroblasts obtained from normal and idiopathic pulmonary fibrosis (IPF) subjects.

For this purpose, cells were either exposed to the conditioned medium (CM) of U937 monocytes to induce inflammation or treated with TGF-β to promote fibrosis. Changes in cell viability, and pro-inflammatory mediators and extracellular matrix (ECM) molecules expression were analyzed at both gene and protein levels.

The *results* revealed that Hylach compounds with 10% and 30% of lactosylation administrated to TGF-β - stimulated lung fibroblast cultures, significantly downregulated α- 2 smooth muscle actin (α-SMA) gene expression and decreased collagen type I, collagen type III, elastin, fibronectin gene and protein expression to near baseline values. This anti-fibrotic activity was accompanied by a strong anti-inflammatory effect when cells were exposed to CM of activated monocytes and by a downregulation of the gene expression of Smad2 for both Hylachs, in comparison to the native HA. Moreover, HA and Hylach at different percentages of lactosylation, do not affect the viability of lung fibroblasts.

In conclusion Hylach attenuated and TGF-β -induced over-expression of α-SMA and ECM protein expression by primary human lung fibroblasts. These data may be relevant for preventing pulmonary deterioration and the progression of pulmonary diseases, providing a step toward the development of new therapeutic treatments for chronic pulmonary diseases.

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Skin-derived cells in the study of aging processes: investigating the potential of *Curcuma caesia* Roxb.

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Aging is a complex biological process influenced by genetic, environmental, and lifestyle factors, including UV radiation exposure. This process not only leads to visible signs of skin aging, such as wrinkles and elasticity loss but also predisposes individuals to a spectrum of age-related diseases. The oxidative stress theory, emphasizing the role of reactive oxygen species (ROS) in cellular aging, provides a foundational framework for investigating potential anti-aging interventions.

This study aims at studying the antiaging potential of a Phytocomplex (CURoxPlex) obtained by the rizhome of *Curcuma caesia* Roxb, on HaCaT cells, a human keratinocyte model.

Through HPLC-ESI-MS/MS, the chemical analysis identified key phenolic compounds, including (-)-epicatechin, procyanidin B2, p-Coumaric acid. HaCaT cells were treated by CURoxPlex, both in normal and UV-induced conditions, to assess its impact on various subcellular and molecular parameters indicative of oxidative stress and aging.

Results demonstrated a significant reduction in mitochondrial superoxide anion levels and improvements in mitochondrial membrane potential, indicating enhanced cellular resilience to oxidative stress. Additionally, CURoxPlex modulated the expression of cytokines such as IL-1 and TNF-α, crucial in inflammatory pathways associated with aging. Notably, CURoxPlex treatment also mitigated UV-induced cell cycle arrest, further underscoring its potential in preserving cellular function in the face of environmental stressors.

In conclusion, CURoxPlex emerges as a promising natural agent for anti-aging skincare. Its array of bioactive compounds, exhibiting strong antioxidative and anti-inflammatory effects, positions it as a valuable ingredient for formulations aimed at combating the signs of skin aging and enhancing overall skin health. This study paves the way for further exploration of Curcuma caesia's applications in skincare and nutraceuticals, contributing to healthier, more resilient skin and an improved quality of life amidst aging populations.

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Morphological and functional aspects in the gut of aged mice and the associations with anti-inflammatory treatments

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The gastrointestinal (GI) barrier represents one of the primary interfaces between organism and the external environment.

Thus, maintaining the structural and functional integrity of the GI barrier is crucial for overall well-being, as it helps prevent systemic inflammation and oxidative stress, which are major contributors to age-related diseases (1). All the cells of the body release at least two subtypes of Extracellular vesicles (EVs), the medium/large EVs (m/lEVs) and small EVs (sEVs). EVs released by microglia play an important role in brain patrolling in physio-pathological processes, for example EVs released by proinflammatory-antitumoral microglia counteract the progression of brain tumors (2). The nervous and the GI systems show a mutual relationship, forming a bidirectional route called the gut-brain axis. In addition, aged mice exhibit impaired intestinal barrier function (3). The aim of the study is to evaluate the morpho-functional differences in the intestinal wall of male aged mice with or without an anti-inflammatory treatment and relative involvement of the enteric nervous system (ENS) and the epithelial barrier. For this purpose, we used C57BL/6J mice intranasally infused with vehicle (control) or m/lEVs-IL4 released by anti-inflammatory microglia (BV2 microglial cell line stimulated with IL-4) to shift microglia toward a "younger" morphology, affecting their inflammatory profile. Specimens of small intestine were collected, and sections underwent to: (i) histological staining using hematoxylin & eosin and PAS staining, and (ii) immunohistochemical procedures using specific nervous system markers, such as α-synuclein (α-syn), 5-hydroxytryptamine (5-HT), vasoactive intestinal peptide (VIP) and marker of epithelial barrier, such as Occludin and Claudin family. In this preliminary study the small intestinal mucosa revealed significant differences in villi size and in epithelial tight junction architecture. Our observations may elucidate the possible connections between the GI barrier and the ENS to restore functionality of the gut-brain axis and slow down neuroinflammation processes in aging.

References

1) Homolak J. Gastrointestinal redox homeostasis in ageing. Biogerontology. 2023 Oct;24(5):741-752. doi: 10.1007/s10522-023-10049-8. Epub 2023 Jul 12. PMID: 37436501.
2) Serpe C, Monaco L, et al. Microglia-Derived Small Extracellular Vesicles Reduce Glioma Growth by Modifying Tumor Cell Metabolism and Enhancing Glutamate Clearance through miR-124. Cells. 2021 Aug 12;10(8):2066. doi: 10.3390/cells10082066. PMID: 3444083.
3) Nalapareddy K, Nattamai KJ, et al. Canonical Wnt Signaling Ameliorates Aging of Intestinal Stem Cells. Cell Rep. 2017 Mar 14;18(11):2608-2621. doi: 10.1016/j.celrep.2017.02.056. PMID: 28297666.

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LRRK2 at the crossroads between intestinal epithelial barrier alterations and enteric gliotic processes in Parkinson's disease

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Patients with Parkinson's disease (PD) show an impairment of the intestinal epithelial barrier (IEB), presence of enteric gliosis and signs of enteric inflammation that could contribute to the onset of gastrointestinal symptoms [1,2]. In this context, alterations in the expression and activity of leucine rich-repeat kinase 2 (LRRK2) have been associated with the development of PD and related intestinal inflammation [3]. However, the actual role of LRRK2 in the gut alterations, with particular regard for the IEB impairment, associated with PD remain unclear. In the present study, we investigated the role of LRRK2 in IEB changes associated with PD, focusing the attention to its role in the interplay between enteric glia and intestinal epithelial cells.

Human A53T α-synuclein transgenic mice at 9 months of age were employed as a model of PD. Age-matched non-transgenic mice were used as controls. Colonic tissue samples were assessed for α-synuclein and LRRK2 expression. Enteric gliosis was evaluated by immunostaining of colonic GFAP-positive cells, which in part expressed LRRK2; IEB condition was tested by histochemical detection of neutral/acidic mucins and ZO-1 staining along with autophagy signaling (parameters involved in its homeostasis).

PD mice showed colonic α-synuclein accumulation along with an increase in LRRK2 and GFAP expression, which co-localized in immunostained glial cells of both *tunica mucosa/submucosa* and *tunica muscularis*. Of interest, PD animals also displayed an impairment of IEB as documented by altered mucus production in goblet cells as well as reduction of ZO-1 staining and alterations in autophagy signaling pathways in lining epithelial cells.

In conclusion, the upregulation of LRRK2 in enteric glial cells may contribute to compromise intestinal mucosal barrier integrity *via* the process of autophagy. These changes could influence both bowel symptoms as well as central neurodegeneration associated with PD.

References

1) Pellegrini C, Fornai M, D'Antongiovanni V, Antonioli L, Bernardini N, Derkinderen P. The intestinal barrier in disorders of the central nervous system. Lancet Gastroenterol Hepatol. 2023, 8, 66-80.
2) Bellini G, Benvenuti L, Ippolito C, Frosini D, Segnani C, Rettura F, Pancetti A, Bertani L, D'Antongiovanni V, Palermo G, Del Prete E, Antonioli L, Nardini V, Morganti R, Pellegrini C, Bernardini N, Ceravolo R, Fornai M, Bellini M. Intestinal histomorphological and molecular alterations in patients with Parkinson's disease. Eur J Neurol. 2023, 30, 3440-3450.
3) De Guilhem de Lataillade, A., Pellegrini, C., Neunlist, M., Rolli-Derkinderen, M., Derkinderen, P. Are LRRK2 mysteries lurking in the gut? Am J Physiol Gastrointest Liver Physiol. 2023, 325, G429-G435.

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Glutathione S-transferase P influences cellular proteostasis and the apoptotic response to uremic solutes in peripheral blood leukocytes

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Glutathione S-transferase P (GSTP) is an inducible protein important for cellular electrophiles detoxification and redox modulation of signaling proteins. Since the blood cells of uremic patients express increased levels of this protein, in this study we investigated whether uremic retention solutes are responsible for *GSTP* gene induction in peripheral blood mononuclear leukocytes (PBL) also exploring the role of this induction response in the apoptotic signaling of these cells.

These aspects were evaluated both in PBL isolated from uremic patients and healthy controls (u-PBL and c-PBL, respectively), as well as in GSTP-manipulated cell models including mononuclear leukocytes and murine embryonic fibroblasts (MEF), that were exposed to high-molecular weight uremic retention solutes (u-HMW) isolated from patients' plasma by ultrafiltration with 50 kDa cut-off membrane microconcentrators.

Compared to c-PBL, freshly isolated u-PBL showed higher levels of apoptosis and increased expression of GSTP protein that was characterized by extensive denaturation as determined by immunoblot carried out after electrophoretic separation under non-reducing conditions. Exposure to u-HMW further increased GSTP expression in PBL and induced GSTP protein also in mononuclear cell lines also leading to higher levels of H_2O_2, cellular GSH, and protein glutathionylation. Increasing GSTP expression in THP1 macrophages by *hGSTP1* gene transfection induced H_2O_2 production and the pro-apoptotic activity of u-HMW exposure, while *GSTP* gene ablation in MEF produced opposite effects. In MEFs, GSTP expression was also critical for the autophagy and JNK activation response to u-HMW.

In conclusion, uremic retention solutes interfere with the expression, stability, and signaling functions of GSTP of PBL. These findings suggest a role for GSTP protein in the proteostasis and pro-apoptotic response of peripheral immune cells to uremic solutes.

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References

1) Galli, F.; Bartolini, D.; Ronco, C. Oxidative stress, defective proteostasis and immunometabolic complications in critically ill patients. Eur J Clin Invest. 2024, e14229.
2) Bartolini, D.; Grignano, M.A.; Piroddi, M.; Chiaradia, E.; Galeazzi, G.; Rende, M.; Ronco, C.; Rampino, T.; Libetta, C.; Galli, F. Induction of Vesicular Trafficking and JNK-Mediated Apoptotic Signaling in Mononuclear Leukocytes Marks the Immuno-Proteostasis Response to Uremic Proteins. Blood Purif. 2023, 52(9-10):737-750.

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Innovative approaches in treating Aortic Stenosis: the EndoTAVI System 1.0

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Innovative treatment and diagnostic methods are required since cardiovascular illnesses continue to be a major cause of death and disability in the Western world. If left untreated, aortic stenosis – the most common valvular condition in developed nations – can result in serious consequences. There are now two treatment methods available: transcatheter aortic valve implantation (TAVI), which is less invasive than traditional aortic valve replacement (AVR), and both are carried out by different specialists in various settings. This division hinders comprehensive evaluation, particularly regarding their impact on endothelial function, a critical factor in patient prognosis.

Our project introduces the EndoTAVI System 1.0, an integrated solution designed for hybrid hospital environments to perform TAVI, while simultaneously evaluating endothelial function through advanced diagnostics. This multifunctional system allows conventional surgery, percutaneous approaches, and extensive endothelial assessments, including flow-mediated dilation (FMD) and molecular markers.

The innovation focuses on identifying novel macro- and micro-markers of endothelial function. Macromarkers include ultrasound evaluations of FMD, reflecting the brachial artery's response to ischemia-reperfusion. Micro-markers involve assessing inflammatory cytokines (IL-1 beta, IL-2, IL-4, IL5, IL-6, IL-8, IL-10, GM-CSF, IFN gamma, TNF alpha).

A prospective study with 150 patients undergoing TAVI will evaluate these markers immediately post-procedure and during follow-up. Preliminary results suggest significant insights into the comparative impacts of these treatments on endothelial health.

The project's originality lies in its comprehensive approach, combining advanced imaging, molecular diagnostics, and hybrid procedural capabilities. This innovative system represents a significant advancement in managing aortic stenosis, with potential for broader applications in cardiovascular medicine.

References

1. Moscarelli, Marco, et al. "The effect of surgical versus transcatheter aortic valve replacement on endothelial function. An observational study." *International Journal of Surgery* 63 (2019): 1-7. https://doi.org/10.1016/j.ijsu.2019.01.014
2. Devito, F., Moscarelli, M., Ciccone, M.M., Rizzo, P., Ferrari, R., Speziale, G. (2018). The Impact of Surgical and Trans-Catheter Aortic Valve Replacement on Endothelial Function: New Markers Ahead. In: Fattouch, K., Lancellotti, P., Vannan, M., Speziale, G. (eds) Advances in Treatments for Aortic Valve and Root Diseases. Springer, Cham. https://doi.org/10.1007/978-3-319-66483-5_25
3. Serradifalco C, Catanese P, Rizzuto L, Cappello F, Puleio R, Barresi V, Nunnari CM, Zummo G, Di Felice V. Embryonic and foetal Islet-1 positive cells in human hearts are also positive to c-Kit. European journal of histochemistry: EJH. 2011 Oct 10;55(4).

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Clinical use of ultrasonography for evaluation of anatomical structures in the aesthetic medicine of the face: a Review

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Ageing leads to facial volume loss, as the soft tissues become lax, thin, and lose elasticity. Changes in the fat compartments, in addition to gravity and muscle tug, display the appearance of wrinkles and aesthetic defects. The request for fillers and bio-stimulator injections and suspension threads for face rejuvenation is progressively increasing[1]. Reported complications arising from these procedures include erythema, necrosis, blindness and even death[2]. The meticulous identification of risk anatomical structures of the face is crucial, considering also the individual variability. The present review aimed to assess if the use of ultrasonography (US) would help clinicians in the identification of the anatomical structures in the aesthetic medicine of the face[3].

A systematic search was performed, from 2000 to 2024, on biomedicine bibliographic databases using the Keywords 'ultrasonography face cosmetic injections' OR ((‘ultrasonography’/exp OR ultrasonography) AND (‘face’/exp OR face) AND (‘cosmetic’/exp OR cosmetic) AND (‘injections’/exp OR injections)). The retrieved studies were screened, and risk of bias was assessed.

Of a total of 2299 identified studies, 30 illustrating 1578 patients were included in the final analysis: 3 Case Reports, 19 Case Series, 4 Observational Studies, 1 Retrospective Chart, 1 Narrative Review, 1 Prospective Randomized Study and 1 Prospective Case-Control Study. Among these, 17/30 employed US for the description of different anatomical structures: 41% the facial artery, 23% the orbital area, 17% the facial vein, 29% the frontal, 17% the temporal and 17% the labio-mental area. Thirteen papers used US for clinical purposes and performed pre-, intra or post-operative evaluations for botulinum toxin injections (46%), filler volumization (38%) and plastic surgery (16%). In all studies, the authors declared that US may help in the identification and description of anatomical structures.

In conclusion, the use of US intraoperatively could help clinicians to respect the anatomical structures according to the variabilities.

References

1) Friedman O. Changes associated with the aging face. Facial Plast Surg Clin North Am. 2005;13(3):371-380. doi:10.1016/j.fsc.2005.04.004
2) Signorini M, Liew S, Sundaram H, et al. Global Aesthetics Consensus: Avoidance and Management of Complications from Hyaluronic Acid Fillers-Evidence- and Opinion-Based Review and Consensus Recommendations. Plast Reconstr Surg. 2016;137(6):961e-971e. doi:10.1097/PRS.0000000000002184
3) Cral WG. Ultrasonography and Facial Aesthetics. Aesthetic Plast Surg. 2022;46(2):999-1000. doi:10.1007/s00266-021-02458-2

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Sdox, a H2S releasing anthracycline, with a safer profile than doxorubicin toward vasculature

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Sdox is a synthetic H_2S-releasing doxorubicin (Dox) less cardiotoxic and more effective than Dox in preclinical, Dox-resistant tumour models. The well-known anthracycline vascular toxicity, however, might limit Sdox clinical use. This study aimed at evaluating Sdox vascular toxicity *in vitro*, using Dox as reference compound.

The studies on vascular anatomy and cell sensitivity, show that both vascular smooth muscle A7r5 and endothelial EA.hy926 cells were more sensitive to Dox than Sdox, although both drugs equally increased intracellular free radical levels. Sdox released H_2S in both cell lines. The H_2S scavenger hydroxocobalamin partially reverted Sdox-induced cytotoxicity in A7r5, but not in EA.hy926 cells, suggesting a role for H_2S in smooth muscle cell death.

Markers of Sdox-induced apoptosis were significantly lower than, in A7r5 cells, and comparable to those of Dox in EA.hy926 cells. In A7r5 cells, Dox increased the activity of caspase 3, 8, and 9, Sdox affecting only that of caspase 3. Moreover, both drugs induced comparable DNA damage in A7r5 cells, while Sdox was less toxic than Dox in Ea.hy926 cells.

In conclusion, Sdox may represent the prototype of an innovative anthracycline, effective against Dox-resistant tumours, displaying a more favourable vascular toxicity profile compared to the parent compound.

This work was performed within the framework of COST (European Cooperation in Science and Technology, www.cost.eu) Action CA17104 STRATAGEM – "New diagnostic and therapeutic tools against multidrug resistant tumors".

References

1) Fusi, F.; Durante, M.; Spiga, O.; Trezza, A.; M.; Frosini, M.; Floriddia, E.; Teodori, E.; Dei, S.; Saponara, S. In vitro and in silico analysis of the vascular effects of asymmetrical N,N-bis(alkanol)amine aryl esters, novel multidrug resistance-reverting agents. Naunyn Schmiedeberg's Arch. Pharmacol. 2016, 389, 9, 1033-1043.
2) Durante, M.; Frosini, M.; Fusi, F.; Neri, A.; Sticozzi, C.; Saponara, S. In vitro vascular toxicity of tariquidar, a potential tool for in vivo PET studies. Basic Clin Pharmacol Toxicol. 2017, 44, 241-247.
3) Fusi, F.; Durante, M.; Gorelli, B.; Perrone, MG.; Colabufo, NA.; Saponara, S. MC225, a novel probe for P-glycoprotein PET imaging at the blood-brain barrier: in vitro cardiovascular safety evaluation. J. Cardiovasc. Pharmacol. 2017, 70, 6, 405-410.
4) Fusi, F.; Durante, M.; Sticozzi, C.; Frosini, M.; Perrone, MG.; Colabufo, NA.; Saponara, S. Vascular toxicity risk assessment of MC18 and MC70, novel potential diagnostic tools for in vivo PET studies. 2017, 120, 5,434-441.
5) Durante, M.; Frosini, M.; Fusi, F.; Gamberucci, A.; Chegaev, K.; Rolando, B.; Riganti, C.; Saponara, S. In vitro vascular toxicity assessment of NitDOX, a novel NO-releasing doxorubicin. Eur. J. Pharmacol. 2020, 5, 880, 173164.

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Alpha-Synuclein pathology in the Enteric Nervous System is a promising biomarker for Parkinson's Disease

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The role of the gut-brain axis has been recently highlighted as a major contributor to Parkinson's Disease (PD) pathophysiology, with numerous studies investigating bidirectional transmission of pathological alpha-synuclein (αSyn). However, the extent and the characteristics of pathology in the enteric nervous system (ENS) have not been fully investigated.

We characterized αSyn pathology, glial responses and immune-cell populations in gastric and duodenal biopsies of PD patients via conformation-specific antibodies and histopathology, and evaluated seeding activity via Real-Time Quaking Induced Conversion (RT-QuIC) assay.

We examined 20 patients with advanced PD who underwent PEG-J placement, 6 untreated patients with early PD, as well as 18 matched healthy controls undergoing routine diagnostic endoscopy. Immunohistochemistry was performed for anti-aggregated αSyn (5G4), enteric glial markers (GFAP, SOX10, S100B) and immune-population markers followed by morphometrical semi-quantitative analysis. RT-QuIC analyses were performed to evaluate alpha-synuclein seeding activity.

Immunoreactvity for aggregated αSyn, presenting a typical thread-like pattern, was identified in all PD patients (early and advanced) and colocalized with neuronal marker beta-III-tubulin; statistically-significant quantitative differences between early and advanced PD patients were also detected. Evaluation of enteric glial cells revealed increased size and density when compared to controls, suggesting reactive gliosis. Similarly, increased T- and B-lymphocyte densities, as well as higher expression of HLA-DR, was detected in the gut of PD patients. The accuracy of αSyn RT-QuIC was 67.4% (20.7%-63.7%) in duodenum, and 80.0% (64.4%-90.9%) in gastric biopsies. Assay's sensitivity was significantly higher in advanced than early PD for all matrices.

We found evidence of αSyn pathology and gliosis in the enteric nervous system of PD patients, which was accurate in discerning PD patients from healthy controls. Future studies are required to evaluate how early in the disease process enteric nervous system pathology occurs, and whether it plays any role in mediating dopamine treatment efficacy in advanced patients.

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Choko-age project: *in vitro* bioactivity evaluation of a new Vitamin E-funzionalized chocolate product

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The idea of functionalizing a chocolate product with Vitamin E (VE) and using it to treat malnutrition of the elderly respond to the need of facing suboptimal intake of this micronutrient vitamin in this specific population thus enhancing the protection against age-dependent degenerative processes associated with major endocrine defects, immune dysfunction, oxidant stress and their correlates. These include protein-energy malnutrition (PEM) and the subsequent muscle wasting (sarcopenia) that are important risk factors for frailty, dementia and many other ageing complications [(1)]. In this project we hypothesized that dark chocolate rich in coca polyphenols can be utilized as a palatable food to increase calory intake and to supplement VE to pre-dementia elderly subjects thus reducing the risk of PEM, muscle loss and frailty; such a challenging hypothesis is at present under verification in a randomized clinical trial [(2)] and through in vitro studies. For these in vitro studies different lots of the VE-enriched food product utilized for the clinical trial were preliminary investigated for the content and activity of the main bioactives, namely VE and polyphenols, and acetonic extracts rich in phenolics were utilized to treat (24-hr exposure) human mononuclear leukocytes (PBMCs) after exposure (4-hr pre-treatments) to different types of challenges that recapitulate adverse events of aging and PEM. These included the immunosuppressant cortisol (Cort) and the bacterial endotoxin LPS (1μg/mL) which activates inflammatory genes and the innate immunity response. PBMCs were isolated from buffy coats of six non-smoking healthy subjects (3 M/3 F) aged ≥50 years. α-tocopherol acetate (α-TOH) and Epicatechin were utilized as control molecules to assess food extract activity. Cortisol induced apoptosis in PBMCs, and the phenolic extracts of both the control and VE-enriched chocolate reduced the levels of apoptosis. This antiapoptotic effects was also observed for α-TOH but not Epicatechin treatment. Moreover, phenolic extracts reduced the LPS and Cort-induced production of cellular ROS indicating a protective effect against oxidative stress. Also, phenolic extracts, α-TOH and Epicatechin reduced the levels of membrane thiols induced by the pre-treated with LPS and Cort. In conclusion, phenolic extracts of dark chocolate and VE are effective in reducing indices of oxidative stress and apoptotic death in PBMCs challenged with endocrine and immunoinflammatory stimuli that mimic those encountered in age-dependent PEM and frailty.

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References

1) Martocchia A, Gallucci M, Noale M, et al.. The increased cortisol levels with preserved rhythmicity in aging and its relationship with dementia and metabolic syndrome. Aging Clin Exp Res 2022;34:2733–40.
2) Pedrinolla A, Isanejad M, Antognelli C, Bartolini D, Borras C, Cavedon V, Di Sante G, Migni A, Mas-Bargues C, Milanese C, Baschirotto C, Modena R, Pistilli A, Rende M, Schena F, Stabile AM, Telesa NV, Tortorella S, Hemmings K, Vina J, Wang E, McArdle A, Jackson MJ, Venturelli M, Galli F. Randomised controlled trial combining vitamin E-functionalised chocolate with physical exercise to reduce the risk of protein-energy malnutrition in predementia aged people: study protocol for Choko-Age. BMJ Open. 2023;13(12):e072291.

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Implementing endothelial cells from women affected by gestational diabetes and humor vitreous as a novel *in vitro* model of diabetic retinopathy

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Diabetic Retinopathy (DR) is the most common microvascular complication of diabetes and a major cause of blindness. Due to chronic exposure to hyperglycemia, retinal endothelial cells (ECs) undergo a variety of morphological and functional changes during DR, leading to vascular alterations. Several studies have shown that these abnormalities persist even after proper glycemic control is restored, due to the activation of adaptive responses and the acquisition of epigenetic modifications[1].

Human umbilical vein endothelial cells (HUVECs) from women with gestational diabetes (GD-HUVECs) represent a useful platform to mimic *in vitro* the pathological alterations induced by chronic hyperglycemia. In this frame, vitreous humor from patients affected by proliferative DR (PDR) may provide insights into disease pathogenesis and may be exploited for the preclinical evaluation of novel drug candidates[2].

Here, we characterized the angio-inflammatory behavior of GD-HUVECs compared to HUVECs isolated from healthy donors (HD-HUVECs). Our results indicate that GD-HUVECs present increased cell proliferation and sprout formation compared to HD-HUVECs under basal conditions. Moreover, GD-HUVECs display a stronger pro-inflammatory potential, as indicated by the overexpression of inflammasome components, pro-inflammatory cytokines, and adhesion molecules implicated in leukostasis. Interestingly, while GD-HUVECs maintain the capacity to respond to the prototypic pro-angiogenic mediator Vascular Endothelial Growth Factor (VEGF), our results suggest a limited effect of the pro-inflammatory mediator Tumor Necrosis Factor a, suggesting that constant exposure to hyperglycemia may desensitize these cells to further stimulation.

Lastly, we analyzed the effect of PDR vitreous treatment on GD-HUVEC pro-angiogenic responses. Our preliminary results demonstrate that PDR vitreous induces GD-HUVEC proliferation and sprouting, with a significantly stronger effect compared to VEGF.

In conclusion, although further studies are required, we anticipate that the combination of GD-HUVECs and PDR vitreous will provide a valuable platform for investigating the mechanisms involved in the pathogenesis of DR and for identifying novel therapeutic strategies.

References

1) Kowluru, RA.; Mohammad, G. Epigenetics and Mitochondrial Stability in the Metabolic Memory Phenomenon Associated with Continued Progression of Diabetic Retinopathy. Sci Rep. 2020 Apr 20;10(1):6655. doi: 10.1038/s41598-020-63527-1.
2) Nawaz, IM.; Rezzola, S.; Cancarini, A.; Russo, A.; Costagliola, C.; Semeraro, F.; Presta, M. Human vitreous in proliferative diabetic retinopathy: Characterization and translational implications. Prog Retin Eye Res. 2019 Sep;72:100756. doi: 10.1016/j.preteyeres.2019.03.002.

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New insights into the role of Secosterol-B in human endothelial cell dysfunction

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The endothelium is a single layer of endothelial cells responsible for maintaining the structure and tone of blood vessels. Oxysterols are a family of 27-carbon cholesterol oxidation derivatives found in low-density lipoproteins (LDLs) and atherosclerotic plaques where they trigger several biological responses involved in the initiation and progression of atherosclerosis. Several evidence suggest that oxysterols contribute to endothelial dysfunction (ED) due to their ability to alter membrane fluidity and permeability leading to inflammation and oxidative stress[1,2]. Moreover, some oxysterols have been shown to induce endoplasmic reticulum (ER) stress involved in apoptosis of endothelial cells[3]. Our research aims to investigate the effects of Secosterol-B (SEC-B), a specific autoxidation product of cholesterol, on human umbilical vein endothelial cells (HUVEC) to identify the mechanisms involved in ED. The results obtained demonstrated that treatment with SEC-B leads to an early increase of nitric oxide (NO) and reactive oxygen species (ROS) production along with a reduction in glutathione levels. Additionally, SEC-B induces ER stress as demonstrated by the modulation of ATF4, XBP1, and Bip protein levels. Fluorescence and electron microscopy analysis confirmed ER membrane enlargement and disorganization. Furthermore, confocal microscopy analysis reveals the presence of protein aggregation in the cells treated for 24 hours with SEC-B.

Interestingly, we demonstrated that at low doses of SEC-B, HUVEC attempt to manage ER stress by activating autophagy and the ubiquitin-proteasome system. On the other hand, at higher doses, cell apoptosis occurs through a pathway involving early phosphorylation of eIF2α and NF-kB activation. In conclusion, SEC-B induces ER stress and protein aggregation in HUVEC, which, if not resolved via the ubiquitin-proteasome system, could lead to apoptosis. These findings provide additional insights into the role of oxysterols in ED and its potential involvement in atherosclerotic pathophysiology.

References

1) Gargiulo S, Gamba P, Testa G, Leonarduzzi G, Poli G. The role of oxysterols in vascular ageing. J Physiol. 2016;594(8):2095-2113.
2) Poli G, Biasi F, Leonarduzzi G. Oxysterols in the pathogenesis of major chronic diseases. Redox biology. 2013;1:125-30.
3) Tao YK, Yu PL, Bai YP, Yan ST, Zhao SP, Zhang GQ. Role of PERK/eIF2alpha/CHOP Endoplasmic Reticulum Stress Pathway in Oxidized Low-density Lipoprotein Mediated Induction of Endothelial Apoptosis. Biomedical and environmental sciences: BES. 2016;29(12):868-76.

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Gastrum preventive spaces: emerging roles of foods derived substances in the field of oncology

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Grape pomace represents an important portion of winery-by-products. In this work we developed a method for the extraction of a standardized phytocomplex based on grape pomace, named UrbX. It was characterized by High-Performance Liquid Chromatography coupled with Mass Spectrometry (HPLC-MS) and Electrospray Ionization (ESI). The anticancer activities were evaluated in AGS, KATO III, and SNU-1 gastric carcinoma cell lines and cytotoxicity was also assed in epithelial gastric cells GES-1[1].

Concerning the chemical characterization, several polyphenols, such as flavonoids, phenolic acids and dihydostilbenes were identified[2]. Also oligopeptides and amminoacids were detected. In vitro experiments demonstrated UrbX ability to favour apoptosis and autophagy affecting a plethora of biochemical pathways, such as those regulated by caspases 3, 9, LC III and beclin 1. Furthermore, UrbX, at the EC_{50} concentrations, showed not only not to be detrimental towards GSE-1, but also to improve their vitality, indicating a sharp selective cytotoxicity against cancer cells. These data suggest the potential application of UrbX in gastric cancer prevention and represent the starting step for repurposing winemaking by-products.

Through chemical characterization and in vitro evaluation of its selective cytotoxicity against gastric cancer cells, this study lays the foundations for developing a novel nutraceutical substance potentially contributing to the broader preventive strategies against gastric cancer.

References

1. Micucci, M., Stella Bartoletti, A., Abdullah, F.O., Burattini, S., Versari, I., Canale, M., D'Agostino, F., Roncarati, D., Piatti, D. Sagratini, G., Caprioli, G., Mari M., Retini, M., Faenza, I. & Salucci, S. Paradigm Shift in Gastric Cancer Prevention: Harnessing the Potential of Aristolochia olivieri Extract. *Int. J. Mol. Sci.* 24, 16003 (2023).
2. Mallamaci, R., Budriesi, R., Clodoveo, M.L., Biotti, G., Micucci, M., Ragusa, A., Curci, F., Muraglia, M., Corbo, F. & Franchini, C. Olive Tree in Circular Economy as a Source of Secondary Metabolites Active for Human and Animal Health Beyond Oxidative Stress and Inflammation. *Molecules* 26, 1072 (2021).

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FFAs cause mitochondrial damage in obesity related glomerulopathy

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Concurrent with the global obesity epidemic, there is an increasing number of people of all ages developing chronic kidney disease associated with obesity. Reduced insulin sensitivity and hyperinsulinemia are among the most important factors in obesity contributing to renal injury. Emerging evidence suggests that dysfunctional mitochondria play a primary role in the development of CKD and co-morbidities related to CKD. It's known that palmitate at concentrations seen in the serum of obese patients induces podocyte dysfunctions, and podocyte insulin resistance, all events that are involved in the reduction of kidney function. All these effects are mediated by the palmitate induced increase of ROS generation. Therefore we hypothesis that palmitate can increase ROS production by directly inducing mitochondrial dysfunction and by altering mitochondrial dynamics. Mitochondrial functions can be altered by excessive mitochondrial fission or decreased fusion with a subsequent increase of mitochondrial ROS production. Mitochondrial dynamics are necessary not only for mitochondrial morphology maintenance but also for maintaining mtDNA integrity, regulating cellular survival and death, transmitting redox-sensitive signals, and participating in metabolic processes. In order to evaluate the effect of palmitate on mitochondrial dynamics we analyzed the mitochondrial fission and fusion in podocytes treated with palmitate. Mitochondrial fusion involves fusion of both the OMM and IMM, depending on Mfn1, Mfn2, and the dynamin family GTPase OPA1. Dynamin-related protein Drp1 and its downstream protein fission protein 1 (Fis1) are responsible for mitochondrial fission, which is involved in mitochondrial recruitment and segregation. In order to evaluate the effect of palmitate on mitochondrial dynamics we analyzed the mitochondrial fission and fusion in podocytes treated with palmitate. Palmitate exposure increased the expression of Fis-1 and this palmitate effect was maintained up to 48h when the palmitate was removed. At the same time palmitate exposure reduce OPA-1 expression and also this effect was maintained up to 48h when palmitate was removed. Taken together these results show that the palmitate altered mitochondria dynamics.

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Novel diagnostic biomarkers for Parkinson's Disease

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Parkinson's disease (PD) is a neurodegenerative disorder that impairs essential nerve functioning and cannot be diagnosed in its early stages. The VGF gene [1] encodes a VGF precursor protein which produces a variety of VGF peptides [2]. In both the plasma from untreated PD patients and the substantia nigra of PD animal models, there is a decrease in the VGF peptides containing the pro VGF C-terminus [3]. The study's aim was to determine if the C-terminal region was the only part of the proVGF involved (vs other regions) and specifically changed in PD. Plasma was collected from PD patients (n= 23 naive, n=50 equally distributed between those with 1-5 and >5 years of disease) as well as neurodegenerative-, neurological-, autoimmune- and psychiatric-diseases. These were: multiple sclerosis (n=16), dystonia (n=16), systemic lupus erythematosus (n=49), major depression disorder (n=37), bipolarism disease (n=40) and schizophrenia (n=40). Using specialized antibodies against the proVGF C-terminus and surrounding peptides containing the NAPP-, TLQP-, and AQEE-sequences as well as a peptide at the N-terminus portion and at the meddle of the proVGF sequence (encompassing the GGEE sequence), ELISA was performed. In naïve PD patients, three out of four VGF C-terminal peptides were shown to be reduced: NAPP, TLQP, and the C-terminus [3]. In advanced PD, the C-terminus peptide was the only one that declined; however, this loss only occured in the first five years of the condition before levels returned to normal. Interestingly, when the amounts of the VGF C-terminus peptide were investigated in the other disorders, no differences were found. In conclusion, early in the course of PD, certain peptides at the VGF C-terminus region are reduced. These alterations seem to be disease-selective, making them crucial for supporting the diagnosis.

References

1. Levi, J. D. Eldridge, and B. M. Paterson, Science 229:393-395, 1985;
2. Trani E, Giorgi A, Canu N, Amadoro G, Rinaldi AM, Halban PA, Ferri GL, Possenti RSME, Levi A (2002) Isolation and characterization of VGF peptides in rat brain. Role of PC1/3 and PC2 in the maturation of VGF precursor. J Neurochem 81(3):565–574
3. Cocco C, Corda G, Lisci C, Noli B, Carta M, Brancia C, Manca E, Masala C, Marrosu F, Solla P, Manconi B, Bongioanni P, Ferri GL. VGF peptides as novel biomarkers in Parkinson's disease. Cell Tissue Res. 2020 Jan;379(1):93-107. doi: 10.1007/s00441-019-03128-1. Epub 2019 Nov 12. PMID: 31713727;

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Monitoring locus coeruleus degeneration in Alzheimer's Disease: a follow-up neuroimaging analysis

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Introduction: In this study, we used longitudinal magnetic resonance imaging (MRI) to investigate the occurrence of progressive changes in the Locus Coeruleus (LC) and its association with clinical progression in Alzheimer's Disease (AD) continuum. **Methods:** A group of 12 AD dementia (ADD) patients and 45 Mild Cognitively Impaired (MCI) subjects underwent LC-MRI scans, both at baseline and after a 2.5-year follow-up. MCI individuals were categorized as converters (cMCI), who developed dementia (n=19) or non-converters (ncMCI), who remained stable (n=26). Standardized template-based analysis quantified LC-MRI parameters: Locus Coeruleus Contrast Ratio (LC_{CR}) and Locus Coeruleus-belonging voxels (LC_{VOX}). **Results:** Both LC_{CR} and LC_{VOX} showed significant reductions ($p<0.001$) across the entire population and within each diagnostic group, except for LC_{VOX} in the AD group. A more pronounced reduction was found in both ADD patients (reduction of LC_{CR} = -140%, Effect size: 1.820) and the cMCI group (reduction of LC_{CR} = -112%, Effect size: 1.610; reduction of LC_{VOX}= -62%, Effect size: 0.910) compared to non-converters (reduction of LC_{CR} = -81%, Effect size: 1.370; reduction of LC_{VOX}= -41%, Effect size: 0.670). Linear mixed models (LMMs) confirmed the progressive decline in LC-MRI parameters over time ($p<0.001$), but not the association with disease severity. **Conclusions:** This study provides the first *in vivo* evidence in humans for progressive LC degeneration during AD clinical progression. Our findings align with prior post-mortem neuropathological data [1,2], supporting the utility of LC-MRI as a reliable tool to assess the integrity of the central noradrenergic system in AD patients.

References

1) Kelly SC, He B, Perez SE, Ginsberg SD, Mufson EJ, Counts SE. Locus coeruleus cellular and molecular pathology during the progression of Alzheimer's disease. Acta Neuropathol Commun. 2017 Jan;5(1):8.
2) Theofilas P, Ehrenberg AJ, Dunlop S, Di Lorenzo Alho AT, Nguy A, Leite REP, et al. Locus coeruleus volume and cell population changes during Alzheimer's disease progression: A stereological study in human postmortem brains with potential implication for early-stage biomarker discovery. Alzheimer's and Dementia. 2017 Mar;13(3):236–46.

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Expression of Cx26 on human cardiac-derived and plasma extracellular vesicles and its possible role in miRNAs's loading

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Connexins (Cxs) are transmembrane proteins forming hemichannels and gap junctions in almost every cell. Cx43 and Cx26 represent the predominant human cardiac Cxs. Cx43 is mainly localized in intercalated discs (IDs) where it is mostly involved in regulating electrical impulse transmission. Cx26 differs from other cardiac Cxs in its cellular localization, being absent at IDs. Cx26 has been found in several cytoplasm organelles including multivesicular bodies and on the surface of rat cardiomyocyte-derived EVs. EV-mediated communication between different cells of the cardiovascular system has been implicated in the regulation of normal tissue function and the propagation of injury or cardioprotective signals during cardiovascular diseases. Although a modulation of Cx26 expression in cardiomyocytes has been observed during ageing or upon drug treatments, a specific Cx26 cardiac function is still unknown. However, its cellular localization could suggest its involvement in the EV-mediate intercellular communication at longer distance.

To investigate this possibility, we verified the presence of Cx26 both on EVs derived from hiPSC cardiomyocytes and on EVs isolated from human plasma, characterizing those derived from cardiomyocytes. At the same time, we evaluated, in *in vitro* rat model of cardiac hypoxia, the possible EV-Cx26 involvement in the loading of the miRNAs into EVs.

Immunogold and western blot showed that EVs released from human cardiomyocytes and collected from cell culture supernatant or plasma had Cx26 on their surface. Moreover, an alteration of both EV-Cx26 and miRNAs expression was observed in hypoxia compared to normoxia suggesting that Cx26 on EVs can participate in EV-mediated communication and in miRNAs recruitment. Further studies are underway to validate these assumptions and to understand if EV-Cx26 could become a non-invasive predictive plasma biomarker of cardiovascular disease onset and progression.

References

1) Moscato, S.; Cabiati, M.; Bianch,i F.; Vaglini, F.; Morales, MA., Burchielli, S.; Botta, L.; Sabbatin,i ARM.; Falleni, A.; Del Ry, S.; Mattii, L. Connexin 26 Expression in Mammalian Cardiomyocytes. Sci Rep. 2018, 8, 13975-13986.
2) Falleni, A.; Moscato, S.; Sabbatini, ARM.; Bernardeschi, M.; Bianchi, F.; Cecchettini, A.; Mattii L. Subcellular Localization of Connexin 26 in Cardiomyocytes and in Cardiomyocyte-Derived Extracellular Vesicles. Molecules. 2021, 26, 6726-6739.
3) Martins-Marques, T.; Girão, H. The good, the bad and the ugly: the impact of extracellular vesicles on the cardiovascular system. J Physiol. 2023, 601, 4837-4852.

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Evaluation of the effects of a novel NLRP3 inflammasome inhibitor in a spontaneous model of accelerate senescence and a transgenic model of Alzheimer's disease

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Increasing evidence suggests that gut dysbiosis, impaired gut barrier and enteric inflammation may represent early events in Alzheimer's disease (AD) and contribute to brain pathology, via microbiota gut-brain axis. In this context, the nucleotide-binding oligomerization domain leucine-rich repeat and pyrin domain-containing protein 3 (NLRP3) inflammasome has been found to be involved in the shaping central and systemic (including the gut) immune/inflammatory responses in AD 1. Herein, we examined the effects of a novel gut-directed locally acting NLRP3 inhibitor (INF176), in two different animal models of AD: the SAMP8 mouse, that develops spontaneously AD, and the 5xFAD, a transgenic mouse model characterized by extremely aggressive AD pathology. SAMR1 and C57BL/6 were used as controls, respectively. Animals were treated with INF176 50mg/kg/day, MCC950 (as a standard comparator) 20mg/kg/die or vehicles for two months (n=6/group) to evaluate the effect of a gut-directed therapy in AD at different disease severity stages. During the last week of treatment, mice underwent the Morris water maze test to evaluate the effect of drugs on cognitive functions. Upon sacrifice, brain and colonic tissues were excised and processed for the evaluation of: 1) AD-related protein deposition; 2) inflammasome signaling activation; 3) microgliosis and astrogliosis. SAMP8 and 5xFAD mice displayed cognitive dysfunctions, central p-tau and Aβ1-42 accumulation along with astrogliosis/microgliosis and central/enteric inflammation, characterized by activation of NLRP3 pathways. Treatment with INF176 counteracted cognitive impairment, decreased central AD-related protein deposition as well as colonic and brain NLRP3 signalling activation. INF176 also counteracted the increase in the number of microglia and astrocytes in the brain. Thus, INF176 exerts beneficial effects on AD mice, suggesting that a gut-directed inflammasome inhibition could represent an additional therapeutical strategy for the treatment of AD.

References

1) Pellegrini C, Antonioli L, Calderone V, Colucci R, Fornai M, Blandizzi C. Microbiota-gut-brain axis in health and disease: Is NLRP3 inflammasome at the crossroads of microbiota-gut-brain communications? Prog Neurobiol. 2020;191:101806.

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The effects of caloric restriction on inflammatory targets in the prostates of aged rats

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Numerous animal models have demonstrated that caloric restriction (CR) is an excellent tool to delay aging and increase the quality of life, likely because it counteracts age-induced oxidative stress and inflammation. The aging process can affect the prostate in three ways: the onset of benign prostatic hyperplasia, prostatitis, and prostate cancer. In this study, we used 14 aged male Sprague Dawley rats, which were allocated into two groups, at the age of 18 months old. One group was fed ad libitum (a normal diet (ND)), and the other group followed a caloric restriction diet with a 60% decrease in intake. The rats were sacrificed at the age of 24 months. By immunohistochemical (IHC) and Western blot (WB) analyses, we studied the variations between the two groups in immune inflammation and fibrosis-related markers in aged prostate tissues. Morphological examinations showed lower levels of prostatic hyperplasia and fibrosis in the CR rats vs. the ND rats. The IHC results revealed that the prostates of the CR rats exhibited a lower immune proinflammatory infiltrate level and a reduced expression of the NLRP3 inflammasome pathway, together with significantly reduced expressions of mesenchymal markers and the profibrotic factor TGFβ1. Finally, by WB analysis, we observed a reduced expression of ERα, which is notoriously implicated in prostate stromal proliferation, and increased expressions of SOD1 and Hsp70, both exerting protective effects against oxidative stress. Overall, these data suggest that CR brings potential benefits to prostatic tissues as it reduces the physiological immune–inflammatory processes and the tissue remodeling caused by aging.

References

1) Holmannova, D.; Borsky, P.; Parova, H.; Stverakova, T.; Vosmik, M.; Hruska, L.; Fiala, Z.; Borska, L. Non-Genomic Hallmarks of Aging-The Review. Int. J. Mol. Sci. 2023, 24, 15468.
2) López-Otín, C.; Blasco, M.A.; Partridge, L.; Serrano, M.; Kroemer, G. Hallmarks of Aging: An Expanding Universe. Cell 2023, 186, 243–278.
3) Guerra, L.H.A.; Campos, S.G.P.; Taboga, S.R.; Vilamaior, P.S.L. Prostatic Morphological Changes throughout Life: Cytochemistry as a Tool to Reveal Tissue Aging Markers. Microsc. Res. Tech. 2024, 87, 1020–1030.

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Early aging in childhood cancer survivor: role of mitochondrial dynamics and antioxidant defenses

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Survival rates for pediatric cancers have improved enormously, but Childhood Cancer Survivors (CCS) often display long-term clinical complications related to chemo/radiotherapy, consistent with early aging. However, the cellular and molecular bases of these symptoms remain unclear. Given the pivotal role of mitochondrial metabolism and oxidative stress in aging, we evaluated oxidative stress/antioxidant defenses balance and mitochondrial dynamics markers in CCS.

Analyses were performed on mononuclear cells (MNCs) isolated from the peripheral blood of 96 CCS aged 5-20 years. Data were compared with those obtained from MNCs isolated from 74 healthy subjects aged between 5 and 106 years. We assessed the expression of proteins involved in mitochondrial dynamics (i.e., mTOR, 4EBP1, DRP1, and FIS1) and antioxidant response (i.e., NRF2 and KEAP1), as well as the activity of antioxidant enzymes (i.e., catalase, glutathione reductase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase), and oxidative damage markers (i.e., malondialdehyde, 8idroxy deoxyguanosine, and nitrotyrosine).

Our results show that CCS MNCs exhibit hyperphosphorylation of mTOR, 4EBP1, and DRP1 compared to healthy controls and a hyper-expression of FIS1, indicating an imbalance between mitochondrial fission and fusion. Additionally, antioxidant enzyme activity appeared low in CCS, which correlated with reduced NRF2 expression and normal level of KEAP1 compared to the age-matched control and elderly subjects, causing the accumulation of oxidative damage.

These findings suggest that altered energy metabolism in CCS may be attributed to mitochondrial hyperfission and a compromised ability to activate antioxidant defenses. This dysfunction could contribute to the early aging observed in CCS. Understanding these molecular mechanisms is crucial for developing targeted therapies to mitigate long-term complications in CCS.

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References

1) Ravera, S.; Vigliarolo, T.; Bruno, S.; Morandi, F.; Marimpietri, D.; Sabatini, F.; Dagnino, M.; Petretto, A.; Bartolucci, M.; Muraca, M.; Biasin, E.; Haupt, R.; Zecca, M.; Fagioli, F.; Cilloni, D.; Podestà, M.; Frassoni, F. Identification of Biochemical and Molecular Markers of Early Aging in Childhood Cancer Survivors. Cancers 2021, 13, 5214.

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Ferroptosis in autism spectrum disorders: a possible therapeutic approach through melatonin

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Autism spectrum disorders (ASD) are a heterogeneous series of neurodevelopmental disorders compromising social and communicational abilities and are associated to various abnormalities that affect different organs including liver. The etiology underlying ASD is multifactorial and involves genetics, environmental and immunological factors. Among others, oxidative stress, neuroinflammation and apoptotic mechanisms seem to be strictly related with its pathogenesis[1]. Ferroptosis is a recently discovered form of cell death, characterized by reactive oxygen species (ROS) accumulation in cells, iron deposition and lipid peroxidation[2]. Among the causes leading to ferroptosis is autophagy, a metabolic process that maintains intracellular homeostasis and increases the survival rate of cells under stress conditions. When excessive or compromised, autophagy contributes to the accumulation of ROS and the reduction of antioxidant activity[3].

Melatonin (N-acetyl-5-methoxytryptamine) is an endogenous multitasking indoleamine useful for its ability to scavenge free oxygen radicals and also for the beneficial effects shown on liver injuries[1].

The aims of the present study were to investigate liver alterations in an autistic mouse model BTBR T+Itpr3tf/J (BTBR) mice, focusing on oxidative stress, inflammation, ferroptosis and autophagy and then identify the potential therapeutic strategy for attenuate hepatic damages through melatonin oral administration.

BTBR mice and healthy control mice (C57BL6/J) have been randomly divided in 4 groups and treated and not treated respectively with melatonin. For each group, we studied hepatic cytoarchitecture and specific markers of oxidative stress, inflammation, ferroptosis and autophagy. We observed modified hepatic morphology in BTBR mice compared to control mice, as well as altered signs of oxidative stress, inflammation, ferroptosis and autophagy.

Notably, beneficial effects on hepatic cytoarchitecture and metabolic functions were observed in BTBR mice treated with melatonin, suggesting the potential therapeutic impact of this multitasking indoleamine against ASD comorbidities.

References

1) Rezzani, R.; Gianò, M.; Pinto, D.; Rinaldi, F.; van Noorden, C.J.F.; Favero, G. Hepatic Alterations in a BTBR T + Itpr3tf/J Mouse Model of Autism and Improvement Using Melatonin via Mitigation Oxidative Stress, Inflammation and Ferroptosis. Int J Mol Sci. 2024, 25, 1086.
2) Zhou, B.; Liu, J.; Kang, R.; Klionsky, D.J.; Kroemer, G.; Tang, D. Ferroptosis is a type of autophagy-dependent cell death. Semin Cancer Biol. 2020, 66, 89-100.
3) Qian, Z.B.; Li, J.F.; Xiong, W.Y.; Mao, X.R. Ferritinophagy: A new idea for liver diseases regulated by ferroptosis. Hepatobiliary Pancreat Dis Int. 2024, 23, 160-170.

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Western diet consumption induces muscle atrophy and worsens cancer cachexia. A help from *Vaccinium macrocarpon* extract

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The worldwide nutritional transition to a "Western diet" (WD) has promoted several diseases by sustaining local and systemic inflammation and oxidative stress. Advanced glycation end-products (AGEs) are non-enzymatic adducts, especially glycosylated proteins, endogenously formed or introduced with the diet (dAGEs) by consumption of ultra-processed and high sugar/fat foods typical of WD. AGEs damage tissues by altering proteins' function and promote the progression of multiple disorders by interacting with their receptor, RAGE. High levels of AGEs are detectable in plasma and tumors of cancer patients and RAGE signaling induces the loss of muscle mass and strength (i.e. muscle wasting, MW) in several conditions, including cancer. About half of patients with advanced cancer, especially lung and gastrointestinal cancers, are affected by cancer cachexia (CC) an unresolved multifactorial debilitating syndrome characterized by progressive MW and poor prognosis. Here, we report that the consumption of WD containing high dAGEs *vs* standard diet (SD) in male adult mice induced: i) increased body, fat and liver weights; ii) increased presence of thin myofibers and reduction of myosin heavy chain (MyHC)-II expression in muscles; iii) reduced muscle performance; iv) muscle and plasma AGE accumulation; and, v) activation of RAGE signaling and ubiquitin-proteasome system in muscles. The subcutaneous injection of procachectic Lewis lung carcinoma (LLC) cells in WD- *vs* SD-fed mice translated into: i) similar tumor growth; ii) more severe cachexia as demonstrated by a greater loss of body weight and the presence of atrophic myofibers; and, iii) increased MyHC-II degradation. Administration of a *Vaccinium macrocarpon* (*VM*) extract counteracted WD-dependent muscle atrophy and restrained CC in WD-fed tumor-bearing mice by reducing dAGE accumulation/activity. Collectively, our data demonstrate that WD induces MW *per se* and predisposes to a more severe CC. *VM* might be used as a phytotherapy ally to prevent WD-dependent detrimental effects in muscles, even in cancer conditions.

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Role of ferroptosis in the pathophysiology of the biliary epithelium

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Ferroptosis is an iron-dependent regulated cell death event characterized by iron accumulation, lipid peroxidation, and the production of ROS that depends on the activity of NADPH oxidase (1). An alterated homeostasis of iron may induce ferroptosis determining cellular death, release of DAMPs and activation of the inflamamtory process. The liver represents the primary site of iron-overload injury for its critical role in iron metabolism (2). The involvement of ferroptosis in liver fibrosis was recently investigated at the level of hepatocytes and hepatic stellate cells (HSCs); however, its role in biliary epithelium is yet unknown. With the present study we aimed: (i) to study the possible activation of ferroptosis *in vivo* in cholestatic mouse liver (BDL) compared to control samples by means of the expression of specific ferroptosis markers, (ii) to highlight, in a normal cell line of murine cholangiocytes, the effects of ferroptosis inducers and inhibitors, including cannabis terpenoids, such as β-Caryophyllene and β-Caryophyllene oxide, described as inhibitors of the growth of hepatoma cell lines by regulating the level of cellular oxidative stress and iron metabolism (3), and (iii) to create an *in vitro* pro-fibrotic model to evaluate the changes in ferroptosis process and the possible protective effect of Lactoferrin (Lf). *In vivo*, we found alterations in the expression of the several markers between normal and BDL mice. *In vitro*, we showed a block of Erastin-induced ferroptosis through the pretreatment with β-Mercaptoethanol (βME). Consequently, considering iron chelators to impede ferroptosis may present a promising therapeutic approach for hepatic pathological disorders, such as fibrosis.

References

1) Galluzzi L, Vitale I, et al. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death Differ. 2018 Mar;25(3):486-541.
2) Casini A, Leone S, et al. The emerging role of ferroptosis in liver cancers. Life (Basel). 2022 Dec 16;12(12):2128.
3) Xiu Z, Zhu Y et al. Caryophyllene oxide induces ferritinophagy by regulating the NCOA4/FTH1/LC3 pathway in hepatocellular carcinoma. Front Pharmacol. 2022 Jul 11:13:930958.

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Study of heat shock proteins modulators to assist the folding of ΔF508CFTR protein

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Cystic Fibrosis (CF) is a genetic disease caused primarily by the malfunction of the chloride channel cystic fibrosis transmembrane conductance regulator (CFTR). The most common mutation is the ΔF508 which is responsible for a severe folding defect in CFTR leading to a nonfunctional channel. Clinically, the mutation causes the unbalanced flow of salts and fluids across cell membranes, particularly in the lungs, leading to chronic infections and inflammation. The Heat Shock Proteins (HSPs) belong to the molecular chaperone family of proteins able to assist other proteins in folding and transport into the functional subcellular compartment. The expression of HSP90, HSP70, and HSP60 have shown an increase in several lung diseases, including CF, and the activity of HSPs has been described as pivotal for CFTR biogenesis. Furthermore, HSPs are induced by several stress conditions, and particularly the HSP60 is well-known for its involvement in the inflammatory processes. However, the HSPs' role in regulating inflammation and immunity is controversial and not yet well known.

In this study, we screened *in silico* a library of compounds on HSP70 and HSP60. IB3.1 CF cells have been treated with the half maximal inhibitory concentration (IC_{50}) and the expression of HSP90, HSP70, and HSP60 was assessed through western blot analysis. CFTR intracellular localization has been evaluated through immunofluorescence assay and the inflammatory-associated interleukins have been investigated. Further experiments to understand how the compounds may improve the HSPs expression are currently ongoing, however, our preliminary results suggest the potentiality to modulate the HSPs network for the development of possible pharmacological chaperone therapy able to promote the ΔF508-CFTR rescue/folding and membrane re-localization. These preliminary data may contribute to developing a model for studying alterations in the homeostasis of the tissues most affected by the pathology.

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References

1) Hutt, D. M.; Mishra, S. K.; Roth, D. M.; Larsen, M. B.; Angles, F.; Frizzell, R. A.; Balch, W. E. Silencing of the Hsp70-Specific Nucleotide-Exchange Factor BAG3 Corrects the F508del-CFTR Variant by Restoring Autophagy. J. Biol. Chem. 2018, 293, 13682–13695.
2) Chanoux, R. A.; Rubenstein, R. C. Molecular Chaperones as Targets to Circumvent the CFTR Defect in Cystic Fibrosis. Front. Pharmacol. 2012, 3, 1–10.
3) Nieddu, E.; Pollarolo, B.; Merello, L.; Schenone, S.; Mazzei, M. F508del-CFTR Rescue: A Matter of Cell Stress Response. Curr. Pharm. Des. 2013, 19, 3476–3496.

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miRNAs as biomarkers of cardiovascular conditions in breast cancer long term survivors

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Breast cancer represents the most common cause of cancer-related mortality in the female sex. The need of tests to early predict cardiovascular conditions in breast cancer long term survivors is increasing since the risk of developing cardiac complications such as ischemic heart disease, heart failure or cardiomyopathy, atherosclerosis (1).

In our study we aimed to evaluate if long-survivor breast cancer women with cardiovascular complications show a different circulating miRNA expression profile than long-survivors without complications (2).

The study involved recruitment of breast cancer long term survivors treated with hormone therapy after surgery, free of disease for at least 5 years after the end of the therapies i.e. 10 years after the diagnosis, with or without cardiovascular disease. Patients were meshed by age, breast cancer grade, treatment received, and conventional cardiovascular risk factors (i.e., ischemic heart disease, carotid endarterectomy, stroke, heart failure), atrial fibrillation, or venous thromboembolism. A panel of 13 microRNAs involved in both pathologies was then selected. RNA extraction, Real-Time PCR and ELISA assays were performed on blood samples to assess differences in both circulating microRNAs, classified as Onco/Tumor suppressors (miR19-29-125-143-187-195); onco-miRNA (miR21-24-210) and Tumor suppressors (miR141-200-205) and cytokines anti-inflammatory (IL-10; IL-4); pro-inflammatory (IL-18) and adipokines (Leptin, Asprosin, Adiponectin). The results obtained in this pilot study show different expression levels of microRNAs. In particular, miRNAs 125 and 210 showed significant differences within the two groups analyzed, thus making them good candidates as biomarkers, whereas no particular significant differences were found in the analysis of the cytokines.

These results are promising and of great interest since miRNAs. represent excellent candidates for new biomarkers capable of early predicting alterations and suitable of repeated analysis for follow up on patients.

References

1) Strongman, H.; Gadd, S.; Matthews, A.; Mansfield, K. E.; Stanway, S.; Lyon , A.R.; Dos-Santos-Silva, I.; Liam Smeeth, L.; Bhaskaran, K . Medium and long-term risks of specific cardiovascular diseases in survivors of 20 adult cancers: a population-based cohort study using multiple linked UK electronic health records databases. Lancet. 2019, 1041-1054.
2) Gupta, D.; Lee Chuy, K.; Yang, JC.; Bates, M.; Lombardo, M.; Steingart, RM. Cardiovascular and Metabolic Effects of Androgen-Deprivation Therapy for Prostate Cancer. *J Oncol Pract*. 2018;14(10):580-587.

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Exacerbated renal fibrosis and oxidative changes in sirtuin 1 heterozygous mice fed hypercaloric high fat diet

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Chronic kidney disease impact on 10% of the world's population and the terminal stage of reduced renal function is strongly associated with fibrosis [1] that actually has no effective treatment.

Sirtuin 1 is the most studied isoform of sirtuin family, histone III deacetylase enzymes involved in metabolism, aging and epigenetic changes. Recent studies in sirtuin1 heterozygous mice (HET), fed hypercaloric high fat diet (60% Energy from lard-HFD) for 16 weeks, reported aberrant autophagy, mitochondrial oxidative damage and steatosis in the liver and heart [2,3]. The present study aims to define glomerular and tubular changes in the same model. Maintenance rodent diet (8.5% Energy from fat) was administered to HET mice and wild type C57BL6/J mice (WT) for comparison. Indirect calorimetry indicated lower energy expenditure at night in HET HFD mice. Renal fibrosis was analysed by Masson, PAS stainings and type III and type IV collagen immunoreactions. Lipid peroxidation, ER stress, inflammation and autophagic flux were assessed by 4 HNE, CHOP, caspase1, p62 immunostainings. Mitochondrial mass and size were analysed by TOM20 and mitofusin 2 immunostainings and TEM. In semithin and ultrathin sections, cortical proximal tubules of HET HFD mice showed lysosomal changes, abnormal donut-like mitochondria and myelin figures. Glomerulomegaly, few podocytes with fused pedicels, high basal membrane thickness characterized HET mice vs C57BL6/J littermates placed on the fatty regimen. Melatonin intake (10 mg/kg), in drinking water, alleviated fibrosis and restored tubular mitofusin 2 expression in WT but not in HET HFD mice. These findings suggest that sirtuin 1 may represent a therapeutic target to counteract renal profibrotic status and mitochondrial damage in obesity. However, melatonin copes with full sirtuin 1 expression to act as a safe reliable dietary supplement in this model.

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References

1) Liu, Y.; Lei, W.; Zhang, W.; Xing, Q.; Liu, R.; Wu, S.; Liu, Z.; Yan, Q.; Li, W.; Liu, X.; Hu, Y. Pyroptosis in renal inflammation and fibrosis:current knowledge and clinical significance. Cell Death Disease 2023, 14, 474.
2) Stacchiotti, A.; Grossi, I.; Garcia-Gomez, R.; Patel, G.; Salvi, A.; Lavazza, A.; De Petro, G.; Monsalve, M.; Rezzani, R. Melatonin effects on non-alcoholic fatty liver disease are related to MicroRNA-34a-5p/Sirt1 axis and Autophagy. Cells 2019, 8, 1053.
3) Favero, G.; Golic, I., Arnaboldi, F.; Cappella, A.; Korac, A.; Monsalve, M.; Stacchiotti, A.; Rezzani, R. Cardiometabolic changes in sirtuin1-heterozygous mice on high -fat diet and melatonin supplementation. Int J Mol Sci 2024, 25, 860.

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Brain iron accumulation: a shared hallmark of aging and Alzheimer's disease?

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Iron is the most physiologically abundant transition metal in nearly all living organisms and it is required in various fundamental biological processes essential for life. Indeed, iron is critical to physiological cellular homeostasis since it works as a cofactor for proteins involved in essential (ATP production, DNA biosynthesis/repair, cell division) and specialized (oxygen transport, neurotransmission) cellular functions, associated mainly to mitochondria.

In the brain iron is involved in a variety of neurological processes such as myelination of axons, neuronal cells division and dopaminergic neurotransmitters synthesis, especially of monoamines. However, during aging, iron can deposit and accumulate in the nervous tissue inducing neuroinflammation and toxicity, leading to neuronal death by ferroptosis.

Intensive research is ongoing in the field of aging and neurodegenerative disorders to decipher the role of iron and mitochondria in the maintenance of cellular homeostasis and genomic integrity. Indeed, a functional mitochondrial metabolism is known to be essential for the normal physiology of neuronal function. Perturbations of the system due to aging, genetics or environmental factors, induce alterations of the physiological balance and lead to mitochondrial dysfunctions contributing to disease. Interestingly, mitochondrial dysfunctions, oxidative stress and iron dyshomeostasis are some of the earliest neuropathological features observed also in Alzheimer's disease (AD) which led to the suggestion that it might play a causative role in the disease. We believe that mitochondria and iron homeostasis could represent new therapeutic targets and potential biomarkers of aging and neurodegeneration for early diagnosis, to monitor the progression of the disease and, in the future, to test new treatments' efficacy to prevent and/or cure AD.

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References

1) Mezzanotte M, Ammirata G, Boido M, Stanga S, Roetto A. Activation of the Hepcidin-Ferroportin1 pathway in the brain and astrocytic-neuronal crosstalk to counteract iron dyshomeostasis during aging. Sci Rep. 2022 Jul 9;12(1):11724.
2) Stanga S, Caretto A, Boido M, Vercelli A. Mitochondrial Dysfunctions: A Red Thread across Neurodegenerative Diseases. Int J Mol Sci. 2020 May 25;21(10):3719.

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Anatomical optimization of steatosis induction in HepG2 cell cultures using oleic and palmitic acids: a comparative study

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Background: Steatosis, characterized by lipid accumulation in hepatocytes, can be experimentally induced in HepG2 cell cultures using fatty acids. Ricchi et al. demonstrated the use of oleic acid (OA) and palmitic acid (PA) for this purpose. This study evaluates the efficacy of different OA and PA concentrations to determine optimal conditions for inducing steatosis in HepG2 cells.

Methods: Experiments were conducted at the Department of Biomolecular Sciences, University of "Carlo Bo" of Urbino. HepG2 cells were treated with OA and PA (0.125-2 mmol/L) and their combinations in ratios (3:1, 2:1, 1:1) at final concentrations of 0.5-1 mmol/L. Lipid accumulation was assessed using Oil Red O staining, triglyceride (TG) content measurements, and mRNA/protein expression of mTOR, S6K1, and SREBP-1c via qRT-PCR and Western blotting.

Results: OA and PA induced dose-dependent increases in TG content, with PA being more effective at lower concentrations (0.25 and 0.5 mmol/L) and OA at higher concentrations (0.75 and 1 mmol/L) (Moravcová et al., 2015). The combination of OA and PA resulted in significant lipid accumulation and reduced cytotoxicity compared to PA alone. The 2:1 OA/PA mixture induced effective lipid deposition with lower cytotoxicity and apoptosis markers (Gómez-Lechón et al., 2007). OA significantly influenced lipid synthesis pathways, increasing mTOR/S6K1/SREBP-1c expression (Zhou et al., 2018).

Conclusion: The 2:1 OA/PA combination is effective in inducing steatosis in HepG2 cells while minimizing cytotoxicity, providing a robust model for studying lipid metabolism and liver diseases in vitro.

References

1. Moravcová, A., Červinková, Z., Kučera, O., Mezera, V., Rychtrmoc, D., & Lotková, H. (2015). The effect of oleic and palmitic acid on induction of steatosis and cytotoxicity on rat hepatocytes in primary culture. Physiological research, 64 Suppl 5, S627-36. doi:10.33549/PHYSIOLRES.933224
2. Gómez-Lechón, M., Donato, M., Martínez-Romero, A., Jiménez, N., Castell, J., & O'Connor, J. (2007). A human hepatocellular in vitro model to investigate steatosis. Chemico-biological interactions, 165(2), 106-16. doi:10.1016/J.CBI.2006.11.004
3. Zhou, Y. P., Wu, R., Shen, W., Yu, H. H., & Yu, S. (2018). Comparison of effects of oleic acid and palmitic acid on lipid deposition and mTOR/S6K1/SREBP-1c pathway in HepG2 cells. Zhonghua gan zang bing za zhi = Zhonghua ganzangbing zazhi = Chinese journal of hepatology, 26(6), 451-456. doi:10.3760/cma.j.issn.1007-3418.2018.06.012
4. Ricchi, M., Odoardi, M. R., Carulli, L., Anzivino, C., Ballestri, S., Pinetti, A., … & Lonardo, A. (2009). Differential effect of oleic and palmitic acid on lipid accumulation and apoptosis in cultured hepatocytes. Journal of Gastroenterology and Hepatology, 24(5), 830-840. doi:10.1111/j.1440-1746.2008.05733.x

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Morphofunctional characteristics of kidney tissue during hypothyroidism

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Thyroid hormones exhibit numerous biological effects on various tissues and organs. They are essential for proper growth and differentiation of kidney tissue, as well as for maintaining water and electrolyte homeostasis. On the other hand, the kidneys participate in the metabolism and elimination of thyroid hormones and are the target organ for the action of iodothyronines. 35 Wistar rats at a mean age of seven months were prospectively analyzed, divided into two groups - experimentally reduced thyroid function and a control group. The group with experimentally induced hypothyroidism through the application of pure substance propylthiouracil in the drinking water included 25 individuals, while the control group included 10 individuals without treatment.

After removal of both kidneys, standard micrometer paraffin sections with appropriate staining and histological preparations are used for further microscopic analysis. Changes in the cortex and medulla were analyzed on an Olympus Pro light microscope at x10, x20 and x40 magnification using A cell processing software, while the obtained results were photographically documented. In hypothyroidism, the ratio of body weight to kidney weight decreases. Investigating the effects of thyroid hormones on the structure of the kidneys indicate morphological changes in the glomeruli in hypothyroidism such as: thickening of the glomerular basement membrane and deposition of mucopolysaccharides in the mesangial matrix. After appropriate treatment with L-thyroxine these changes show complete reversibility.

The control group showed normal kidney tissue morphology without significant variations.

In conclusion, hypothyroidism causes changes in the cytoarchitectonics of the renal tissue that are reversible.

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Psychophysical, electrofunctional, and morphological evaluation in naïve neovascular AMD patients treated with intravitreal anti-VEGF

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The aim of this study was to investigate the retinal morpho-functional characteristics of patients with neovascular wet age-related macular degeneration (nAMD) treated with intravitreal injection (IV) of aflibercept (AFL).

The study was conducted on 35 patients previously diagnosed with type 1 nAMD who received a fixed-dosing regimen of aflibercept injections over 12 months. The goal was to assess trends in visual abilities over time by measuring visual acuity (VA), contrast sensitivity (CS), visual evoked potentials (VEPs), and spectral domain-optical coherence tomography (SD-OCT). The same psychophysical, electro-functional, and morphological tests administered at baseline (T0) were repeated 4 to 8 weeks after the last aflibercept injection (Tn), resulting in a total of six examinations.

At Tn, all subjects exhibited improved VA for both far and near distances compared to values detected at T0. Similarly, VEP amplitude and latency values at Tn showed a greater P100 improvement than those observed at T0. Additionally, the CS examination at Tn demonstrated improvement, particularly at high spatial stimulation frequencies. The Tn SD-OCT results highlighted a reduction in macular thickness compared to T0 values.

This exploratory research indicates that intravitreal injections of AFL, following a fixed-dosing regimen, represent a valuable therapeutic approach for enhancing visual performance. This conclusion is supported by comprehensive statistical analysis of psychophysical, electro-functional, and morphological examinations within the same group of patients with nAMD, as demonstrated for the first time.

References

1) Xi L. Pigment epithelium-derived factor as a possible treatment agent for choroidal neovascularization. Oxidative Med Cell Longev. 2020;2020:8941057.
2) Li E, Donati S, Lindsley KB, Krzystolik MG, Virgili G. Treatment regimens for administration of anti-vascular endothelial growth factor agents for neovascular age-related macular degeneration. Cochrane Database Syst Rev. 2020;5(5):CD012208.
3) Altinbay D, Sahli E, Idil A. Comparison of two different contrast sensitivity testing methods in patients with low vision. J Current Ophthalmol. 2022;34(1):60-66.

Epithelial tissues: epithelium-mesenchymal transition in organogenesis and carcinogenesis

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Polylactic acid (PLA) vs. polystyrene (PS): internalization efficiency and biological effects in human intestinal-derived cells

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Microplastics (MPs) and nanoplastics (NPs) have become ubiquitous in the environment due to the extensive use of plastics and inadequate waste management. They can enter the human body through inhalation, ingestion, and skin contact. Concerns about the environmental and public health impacts of plastics have driven the development of bioplastic like polylactic acid (PLA) [1], however, our understanding of the PLA health impact remains limited, as most research has focused on non-biodegradable polymers. This has prompted us to investigate the potential toxicity of newly developed PLA-NPs on human health intestinal-derived cells *in vitro*. We also assessed the toxicity of polystyrene (PS) MPs in the same experimental conditions to compare the effects of biodegradable and non-biodegradable plastics. We used commercially purchased PS-MPs conjugated with FITC and in-house synthesized PLA-NPs conjugated with Rhodamine.

We examined cellular uptake of PS-MPs and PLA-NPs in HT29 and Caco-2 (human colon adenocarcinoma) cells at non-toxic concentrations of 100 μg/mL and 300 μg/mL from 24 to 72 hours via flow cytometry. The cellular uptake analysis revealed that PS-MPs were taken up from 20 to 50% of cells depending on the concentration used, while PLA-NPs showed 100% internalization. Immunofluorescence analysis corroborated these results showing a perinuclear and intracytoplasmic distribution of both kinds of plastics. We also assessed pro-inflammatory cytokine release by determining IL-8 production via ELISA. In this case, no significant changes were observed, indicating that neither the type of plastic nor the concentration induced pro-inflammatory reactions. Protein analysis of oxidative stress markers will be also presented. Our preliminary results suggest that both PS and PLA can cross cell membrane potentially affecting cell functions. However, further investigations are needed to provide a more comprehensive understanding of the potential impact of these plastics on human health.

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References

1) Lamparelli, EP.; Marino, M.; Szychlinska, M.A.; Della Rocca, N.; Ciardulli, M.C.; Scala, P.; D'Auria, R.; Testa, A.; Viggiano, A.; Cappello, F.; Meccariello, R.; Della Porta, G.; Santoro, A. The Other Side of Plastics: Bioplastic-Based Nanoparticles for Drug Delivery Systems in the Brain. Pharmaceutics. 2023, 15, 2549.

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Cholangiocarcinoma subtypes imply morphological differences in ducts of origin and are characterized by specific features at spatial molecular analysis

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The term cholangiocarcinoma (CCA) defines tumours arising from any given point of the biliary tree and comprises malignancies with diverse anatomical/histological background. The prominent heterogeneity of intrahepatic (i) CCA subtypes reflects that observed in the putative cells and tissues of origin. Nevertheless, besides diagnosis confirmation, histology currently does not have a part in the management of subjects affected by CCA. We aimed to study the clinical relevance of histological and molecular heterogeneity of CCA.

Intrahepatic CCA samples (N=96) were obtained from six centres participating to the ENSCCA clinical registry. Histological and immunohistochemical stains were performed. A selection of cases (N=12) was processed for spatial transcriptomics analysis on the NanoString GeoMx DSP.

Based on haematoxylin and eosin stain, small bile duct (SBD) and large bile duct (LBD) type accounted for 40% and 45% of iCCAs, respectively. No significant differences in 5-year overall survival (OS) were found in SBD vs LBD-type iCCA. When we classified cases based on periodic acid-Schiff (PAS) positivity (mucus content), PAS^{HIGH} LBD type iCCA showed a significantly worse 5-year OS compared to PAS^{LOW} iCCA. Spatial molecular analysis of the tumoral epithelial compartment individuated EPCAM and HIF1A as the most up- and down-regulated genes in PAS^{HIGH} LBD vs PAS^{LOW} SBD type iCCA. By immunohistochemistry, EpCAM positivity was confirmed in the whole cohort; moreover, we observed differences in microvascular density in tumour stroma between histologic subtypes, parallelling HIF1A gene expression. Multivariate Cox regression individuated PAS^{HIGH} LBD type iCCA phenotype as predictor of a worse OS compared to other histological subtypes, independently from age, gender, stage, and tumour grade.

In conclusion, the histologic iCCA subtypes recapitulate putative duct of origin and are characterized by different molecular characteristics and pathogenetic background. Our data underline the importance of individuating morphological subclasses in iCCA for pathogenetic implications, risk stratification and prognosis.

References

1) Banales J.M.; Marin J.J.G.; Lamarca A.; Rodrigues P.M.; Khan S.A.; Roberts L.R.; et al. Cholangiocarcinoma 2020: the next horizon in mechanisms and management. Nat Rev Gastroenterol Hepatol 2020;17(9):557-588.
2) Izquierdo-Sanchez L; Lamarca A; La Casta A; Buettner S; Utpatel K; Klümpen HJ; et al. Cholangiocarcinoma landscape in Europe: Diagnostic, prognostic and therapeutic insights from the ENSCCA Registry. J Hepatol. 2022 May;76(5):1109-1121.

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The Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) approach unveils the proteomic landscape of prostate cancer cells treated with Capivasertib

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Prostate Cancer (PC) is the second cause of cancer death in men worldwide. Because androgen receptor has a crucial role in PC and disease progression, all tumors initially respond to androgen depletion therapy (ADT), therefore castration remains the first therapeutic choice. Sadly, most patients become resistant to ADT and progress to castration-resistant prostate cancer (CRPC). However, novel therapeutic options are missing for CRPC patients.

Preclinical studies have associated phosphoinositide 3-kinase (PI3K)/protein kinase (AKT)/phosphatase and tensin homolog (PTEN) signaling with the development of mCRPC and resistance to chemotherapy, paving the way to PI3K/AKT targeted treatments (1). AKT inhibition by capivasertib, recently trialled in CAPItello 280 and CAPItello 281 clinical studies (2) (3), is giving encouraging results in PTEN-null CRPC patients associated to conventional docetaxel+ADT+prednisone therapy.

Therefore, we undertook a proteomic dissection of the AKT target landscape to improve our understanding of the mechanism of action of capivasertib in PC context.

SILAC-based LC-MS/MS is a powerful approach to proteome mapping and signal transduction studies. To this purpose PTEN-null PC3 prostate cancer cells were incubated with labelled or unlabelled aminoacids and subjected to analysis upon treatment with capivasertib or with vehicle. Compared to the untreated control, the abundance of 986 proteins was downregulated while 200 proteins were upregulated following Akt inhibition. Evaluation through the Kyoto Encyclopedia of Genes and Genomes (KEGG) identified several biological processes associated to the physiological function of these proteins. In particular, a number of proteins were associated with a significant reduction in glucose metabolism through glycolysis and gluconeogenesis modulation. Moreover, ubiquitin and proteasome-mediated degradation was widely downregulated by AKT inhibition, in contrast to the upregulation of the Hippo pathway.

Confirmation of the above findings by western Blot and by rt-qpcr is ongoing, aiming to identify for the first time the biological processes involved in capivasertib mechanism of action, opening up new study perspectives.

References

1) Roudsari NM et al., Inhibitors of the PI3K/Akt/mTOR Pathway in Prostate Cancer Chemoprevention and Intervention. Pharmaceutics. Pharmaceutics 2021 3;13(8).
2) Simon J. Crabb et al., CAPItello-280: A phase III study of capivasertib and docetaxel versus placebo and docetaxel in metastatic castration-resistant prostate cancer. JCO 2023, 41, TPS287-TPS287.
3) Karim Fizazi et al., A phase III trial of capivasertib and abiraterone versus placebo and abiraterone in patients with de novo metastatic hormone-sensitive prostate cancer characterized by PTEN deficiency (CAPItello-281). JCO 2021, 39, TPS178-TPS178.

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Role of SAM68 on transcription termination regulation in prostate cancer

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Prostate cancer (PC) is a highly lethal malignancies in men worldwide. Its onset and progression rely on androgen receptor (AR) signaling and hormone deprivation therapy (HDT) remains the primary therapeutic option. Although HDT is initially effective, this benefit is temporary because PC relapses with castration-resistance forms that is currently incurable. Thus, the identification of novel therapeutic targets is required.

Several mechanisms have been implicated in PC progression. Relapsed PC presents persistently over-activation of AR and up-regulation of the oncogenic transcription factor MYC. Hence, PC acquired addiction on aberrant transcriptional programs mediated by MYC and AR might represent a feasible therapeutic option.

We recently reported that MYC targets SAM68 and XRN2 form a complex that play a significant role in globally modulating the transcriptome of PC cells through the regulation of 3'end processing.

Given the functional link between 3'end processing and transcription termination (TT) we investigated the role of SAM68 on TT process in PC cells. Our results revealed that SAM68 interacts with transcription termination factors (TTFs) that showed altered localization in its absence. Furthermore, SAM68 depletion induces the accumulation of both 5' uncapped RNA and RNAPII in the termination zone consistent with TT defects. Due the kinetic competition between RNAPII processivity and TT, we investigated the RNAPII kinetic in absence of SAM68. Interestingly, we observed an altered phosphorylation pattern of RNAPII on chromatin and nuclear matrix as well as an increase of RNAPII processivity in highly transcribed genes. Collectively, our data suggest that SAM68 interacts with TTFs and acts as a transcription termination factor to ensure efficient transcription termination of highly transcribed genes.

We believe that the outcome from our studies might provide new insight on gene expression regulation in PC pathogenesis and novel therapeutic options to counteract transcriptional addiction mediated by MYC.

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Unraveling the relationships between tumor cells and their microenvironment using high-resolution spatial transcriptomics

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During the last decade, single-cell RNA sequencing (scRNA-seq) methods paved the way for deep molecular analysis of normal and pathological tissues at unprecedented resolution. Single-cell molecular analyses allow to identify different cell types, including some never discovered before, and to have an insight to their ontogenetic relationships. Moreover, these data can also be used to infer the signals that different types of cells exchange with each other.

Despite these advances, scRNA-seq data do not contain a crucial set of information, which is the spatial architecture of the analysed tissues and organs, as they are obtained through the dissociation of the original specimens at single-cell level.

Without the knowledge of the spatial relationship between cells, it is difficult to get any insight on their functional interrelations and the workings of the entire cell ecosystem that compose a particular tissue. This problem is even more conspicuous for tumors, for which there is no prior knowledge on the structure of the tissue, as it varies from patient to patient.

In recent years, new methods, generally referred as "spatial transcriptomics" have been developed to chart the high-detailed picture emerging from scRNA-seq analyses into tissue sections. Here I present a new study showing how high-resolution spatial transcriptomics helps to unravel the structure of tumor tissues, not only by charting the cell types identified by scRNA-seq on the tissue, but also allowing to make educated guesses on their functional relationships.

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Che-1/miR-590-3p/TAZ axis sustains multiple myeloma disease

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Multiple myeloma (MM) is a blood disease characterized by the malignant accumulation of monoclonal plasma cells in the bone marrow. Among the pathological consequences of the MM, defects in osteogenesis characterized by osteolytic lesions, osteopenia, and pathologic fractures are frequently described.

Che-1/AATF (Che-1) is a co-transcriptional factor involved in MM transformation and proliferation. Here, we show that Che-1 expression in MM contributes to maintaining low level of WWTR1 (TAZ), a transcriptional coactivator downstream of the Hippo-signaling pathway. Previous results have demonstrated that low levels of TAZ in MM are mainly due to hypermethylation of its promoter and post-translational modifications. The results shown in this study identify a further mechanism TAZ regulation in this pathology, thus underlining how it is relevant for MM to control the expression of this protein.

Che-1 didn't bind TAZ promoter. Therefore, to further clarify the mechanism by which Che-1 inhibits TAZ expression in MM, we focused our study on the post-transcriptional mechanisms responsible for the downregulation of TAZ. We report that the miR-590-3p, deriving from the mRNA splicing of the EIF4H host gene, targets TAZ, contributing to downregulating its expression in MM. Furthermore, we demonstrate by in vivo mouse model and in vitro experiments that Che-1 transcriptionally induces EIF4H gene. We provide data to support that miR-590-3p is secreted by MM cells in vitro and in vivo and that it downregulates TAZ levels and the physiological transcriptional expression of osteogenic-related genes, in mesenchymal stem cells.

In MM patients we show low expression of TAZ which is anti-correlated with high expression of Che-1 and miR-590-3p. Furthermore, low level of TAZ was associated to poor outcome of MM patients.

Our findings unveil an unexplored novel Che-1/miR-590-3p/TAZ axis in MM tumorigenesis and metastatic process by providing a rationale to explore the therapeutic potential of metastatic bone lesions.

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References

1) Bruno, T., Catena, V., Corleone, G., Cortile, C., Cappelletto, M. C., Bellei, B., De Nicola, F., Amadio, B., Gumenyuk, S., Marchesi, F., Annibali, O., Blandino, G., Fanciulli, M., Di Agostino, S. Che-1/miR-590-3p/TAZ axis sustains multiple myeloma disease. Leukemia 2024. 38(4), 877–882.
2) Donato, E., Biagioni, F., Bisso, A., Caganova, M., Amati, B., Campaner, S. YAP and TAZ are dispensable for physiological and malignant haematopoiesis. Leukemia 2018. 32(9), 2037–2040.
3) Bruno, T., De Nicola, F., Corleone, G., Catena, V., Goeman, F., Pallocca, M., Sorino, C., Bossi, G., Amadio, B., Cigliana, G., Ricciardi, M. R., Petrucci, M. T., Spugnini, E. P., Baldi, A., Cioce, M., Cortese, G., Mattei, E., Merola, R., Gianelli, U., Baldini, L., … Fanciulli, M. Che-1/AATF-induced transcriptionally active chromatin promotes cell proliferation in multiple myeloma. Blood advances 2020. 4(22), 5616–5630.

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The IRONic face of EMT in pancreatic ductal adenocarcinoma: preliminary results from IRONy study

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Iron can contribute to cancer initiation and progression since rapidly proliferating cancer cells have a greater metabolic demand for iron than normal cells and hence express high levels of transferrin receptor (TfR1) to internalize circulating iron. Indeed, iron chelators can inhibit cell growth and have been considered for the therapy of tumors (1), including pancreatic ductal adenocarcinoma (PDAC) (2).

Since during PDAC progression cells undergo epithelial-to-mesenchymal transition (EMT), characterized by the loss of epithelial and the acquisition of mesenchymal features, we aimed at investigating the possible relationship between EMT and iron homeostasis in three PDAC cell lines exhibiting different EMT-related phenotype (3,4).

For this purpose, HPAC, BxPC-3 and MiaPaCa2 cells were grown in 3D spheroids, that better recapitulate tumor microarchitecture. Gene expression for ferritin (FtH), TfR1, ferroportin (FPN) and hepcidin (HAMP) was assessed by real time PCR.

Our results show higher TfR1 mRNA levels in the more epithelial HPAC and BxPC-3 cells, while, in the more mesenchymal MiaPaCa2 cells, lower TfR1mRNA levels were detected. According with these data, preliminary results showed higher FtH protein expression (a relevant indicator of iron accumulation) in MiaPaCa2. HAMP and FPN gene expression seem inversely related, as expected, in the more epithelial PDAC cells. Interestingly, in MiaPaCa2 cells both FPN and HAMP mRNA levels were almost undetectable: this pattern is consistent with low endogenous expression hepcidin and likely associated to a different EMT-related phenotype, suggesting to further evaluate if this local expression is sufficient to allow FPN expression at the plasma membrane in these cells.

Overall, our preliminary results suggest a possible relationship between the expression of genes involved in iron homeostasis and the EMT-related phenotype in PDAC cells. Further investigations are needed to confirm these results to fully ascertain whether the EMT process influences the mechanisms involved in iron metabolism in PDAC.

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References

1) Lui, G. Y.; Kovacevic, Z.; Richardson, V.; Merlot, A.M.; Kalinowski, D.S.; Richardson, D.R. Targeting cancer by binding iron: Dissecting cellular signaling pathways. Oncotarget 2015, 6: 18748–18779.

2) Kalinowski, D.S.; Richardson, D.R. The evolution of iron chelators for the treatment of iron overload disease and cancer. Pharmacol. Rev. 2005, 57:547–83.

3) Bianchi, F.; Sommariva, M.; Cornaghi, L.B.; Denti, L.; Nava, A.; Arnaboldi, F.; Moscheni, C.; Gagliano, N. Mechanical cues, E-Cadherin expression and cell "sociality" are crucial crossroads in determining pancreatic ductal adenocarcinoma cells behavior. Cells 2022, 11, 1318.

4) Galy, B.; Conrad, M.; Muckenthaler, M. Mechanisms controlling cellular and systemic iron homeostasis. Nat. Rev. Mol. Cell. Biol. 2024, 133-155.

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TCam-2 Seminoma cells respond to HGF mesenchymal signal by triggering secretome modification

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Type II Testicular Germ Cell Tumours (TGCTs) represent the most frequent malignancy in Caucasian males (20–40 years). It is commonly accepted that these pathologies arise from an imbalance of the testicular embryonic niche that leads the block of gonocyte differentiation. HGF is available in the testicular microenvironment, from early embryonic development to adult stage, and c-Met/HGF system is well known in the epithelial-mesenchyme crosstalk as well as in the onset and progression of various human cancers. In previous studies we found that c-Met and HGF are expressed both in seminoma and non-seminoma lesions as well as in seminoma (TCam-2) and non-seminoma (NT2D1) cell lines with different scores [1,2]. Notably, we found that NT2D1 cells increase their proliferation, polarized migration, and invasion in response to HGF administration whereas TCam-2 cells do not in spite the presence of c-Met receptor [1].

Herein, we report the secretome analysis on TCam-2, and NT2D1 cells cultured with or without HGF. Surprisingly, we found that TCam-2 cells change their secretome with higher extent with respect to NT2D1 cells, in spite their apparent negligible behavioural response to HGF administration. In particular we found in TCam-2 cells an up-regulation of the secretion of 1) TGFα and βs, 2) the neurotrophins NGF, GDNF, NT3, NT4, 3) the growth factors EGF, FGF, PDGFs, VEGF, G-CSF, M-CSF, GM-CSFIGF and IGFBP. Conversely, the secretory response of NT2D1cells is limited to the up-regulation of NGF and G-CSF.

This observation let us to conclude that HGF stimulates TCam-2 to open an epithelial/mesenchyme molecular dialogue and let us speculate that the availability of HGF can significantly modify the molecular niche and, in turn the behavioural features of seminoma cells. However, this effect does trigger significant changes in proliferation, polarized migration, and invasion of cultured TCam-2 cells since the interaction with the mesenchyme is lost.

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References

1) Scheri, K.C.; Leonetti, E.; Laino, L.; Gigantino, V.; Gesualdi, L.; Grammatico, P.; Bizzari, M.; Franco, R.; Oosterhuis, J.W.; Stoop, H.; Looijenga, L.H.J.; Ricci, G.*; Catizone, A. c-MET receptor as potential biomarker and target molecule for malignant testicular germ cell tumors. Oncotarget. 2018 7;9(61):31842-31860. *corresponding author
2) Gesualdi, L.; Leonetti, E.; Cucina, A.; Scicchitano, B.M.; Sorrentino, S.; Tarsitano, M.G.; Isidori, A.; Bizzarri, M.; Filippini, A.; Riccioli, A.; Cammarota, M.; Gigantino, V.; Ricci, G.*; Catizone, A. The PI3K/AKT Pathway Is Activated by HGF in NT2D1 Non-Seminoma Cells and Has a Role in the Modulation of Their Malignant Behavior. Int J Mol Sci. 2020 17;21(22):8669. *corresponding author

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Subtype-dependent down-modulation of akt signaling induced by garlic in breast tumor cells with a triple negative phenotype

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Breast cancer includes tumor subgroups with morphological, molecular, and clinical differences. Intrinsic heterogeneity especially characterizes breast tumors with a triple negative phenotype, that are characterized by the lack of expression of ER, PR, HER2, and their related genes, and a high incidence of TP53 mutations, losses of RB1 and BRCA1, and elevated activation of the PI3K/AKT pathway (1). Although recent technologies have made it possible to better characterize triple negative breast cancers (TNBCs), these classifications are still not sufficient to effectively treat this group of breast tumors. It is currently of great interest to identify new strategies adjuvating conventional therapies, and there is increasing consideration of the use of natural substances, due to their potent biological activities and mild side effects (2). In this context, garlic (*Allium sativum*) shows anti-cancerous potential, interfering with the proliferation, motility, and malignant progression of both non-invasive and invasive breast tumor cells (3). Despite the increasing number of studies on the role of garlic on features of breast tumor cells, they do not consider that tumor heterogeneity could be at the basis of the variable response to this natural compound. To try to deal with this problem, the main objective of this study was to evaluate the anti-tumoral activity of an organosulfur-enriched garlic extract in breast cancer cells with a triple negative phenotype. Established TNBC cell lines from patient-derived xenografts (PDXs) were used, revealing subtype-dependent effects on morphology, cell cycle, and invasive potential, correlated with the peculiar down-modulation of Akt signaling, a crucial regulator in solid tumors. Our results first demonstrate that the effects of garlic on TNBC breast cancer are not unique and suggest that only more precise knowledge of the mechanisms activated by this natural compound in each tumor will allow for the inclusion of garlic in personalized therapeutic approaches to breast cancer.

References

1) Iacopetta, D.; Ceramella, J.; Baldino, N.; Sinicropi, M.S.; Catalano, A. Targeting Breast Cancer: An Overlook on Current Strategies. Int. J. Mol. Sci. 2023, 24, 3643.
2) Bhattacharya, T.; Dutta, S.; Akter, R.; Rahman, M.H.; Karthika, C.; Nagaswarupa, H.P.; Murthy, H.C.A.; Fratila, O.; Brata, R.; Bungau, S. Role of Phytonutrients in Nutrigenetics and Nutrigenomics Perspective in Curing Breast Cancer. Biomolecules 2021, 11, 1176.
3) Brugnoli, F.; Tedeschi, P.; Grassilli, S.; Maietti, A.; Brandolini, V.; Bertagnolo, V. Ethanol-Based Garlic Extract Prevents Malignant Evolution of Non-Invasive Breast Tumor Cells Induced by Moderate Hypoxia. Biomed. Pharmacother. 2021, 142, 112052

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Immunohistochemical expression of novel biomarkers in Fluoro-Edenite-Induced Malignant Mesothelioma: A Preliminary Study

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Malignant Mesothelioma (MM) is strongly associated with exposure to asbestiform fibers. Fluoro-edenite (FE), a silicate mineral belonging to the amphibole group, has been identified as a potential risk factor for MM. Unfortunately, this cancer has often a poor prognosis, and current diagnostic and prognostic biomarkers are inadequate. In this study, based on the hypothesis that pituitary adenylate cyclase-activating polypeptide (PACAP) and PACAP-preferring receptor (PAC1R) expressions probably dysregulate in MM tissues and that they could potentially act as diagnostic or prognostic biomarkers, we aimed to investigate the immunohistochemical expression of PACAP and PAC1R in pleural biopsies from MM patients exposed to FE fibers. A total of 12 patients were included in this study, and their biopsies were processed for immunohistochemical analysis to evaluate the expression of PACAP and PAC1R. The study revealed a correlation between the overexpression of PACAP and PAC1R and the shorter overall patient's survival. These findings suggest that PACAP and PAC1R expression levels could serve as potential prognostic biomarkers for MM. Furthermore, the immunohistochemical analysis may provide valuable information for clinicians to guide therapeutic decisions and identify patients with poorer prognosis.

References

1) Ledda C, Senia P, Rapisarda V. Biomarkers for Early Diagnosis and Prognosis of Malignant Pleural Mesothelioma: The Quest Goes on. Cancers (Basel). 2018 Jun 15;10(6):203.
2) Barone AD, Mastrantonio M., Romeo L. Environmental risk factors in pleural malignant mesothelioma. Medicina Lavoro2012;103:29-42.

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A role for TPC2 endolysosomal / melanosomal channel in malignant melanoma progression

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Two-pore channel 2 (TPC2) is an ion channel (Na^+/Ca^{2+}) localized on the membrane of intracellular acidic organelles such as endo-lysosomes and melanosomes. It is well known to play a role in the tumor progression of several type of cancers including melanoma[1,2]. Melanoma is one of the most aggressive and treatment-resistant tumors, often characterized by the mutation in the serine/threonine kinase BRAF[3].Our project investigates the role of TPC2 in relation with the state of the disease (primary or metastatic) to asses whether this channel may be a novel druggable target for tailored therapy of melanoma patients. We analyzed two different pairs of human melanoma cell lines (IGR and WM) each pair derived from the same patient at two different stages of tumor progression and genetically characterized by the mutation in BRAF gene. We found that all these human melanoma cell lines show a heterogeneous expression of the EMT markers, and different behaviors in terms of migratory capability and ability to adhere to type 1 collagen. Of note, cells derived from the primary site of the tumor (IGR39 and WM115) have a lower TPC2 expression than the metastatic cells (IGR37 and WM266-4). To investigate the role of TPC2 in melanoma progression we used two different drugs, SG-094, a TPC2 pharmacological inhibitor, and TPC2-A1-N, a TPC2 pharmacological agonist. Surprisingly, both treatments abated the migratory ability of IGR and WM cell lines and the adhesivity of IGR lines to type 1 collagen, strongly indicating the involvement of TPC2-dependent intracellular Ca^{2+} signaling. These data demonstrate that TPC2 activity is relevant for the aggressive traits of melanoma and prompt further insight to uncover its complex regulation, in view of possible therapeutic strategies combining TPC2 and BRAF inhibition to overcome therapy resistance.

References

1) D'Amore, A., Hanbashi, A. A., Di Agostino, S., Palombi, F., Sacconi, A., Voruganti, A., Taggi, M., Canipari, R., Blandino, G., Parrington, J., & Filippini, A. (2020). Loss of Two-Pore Channel 2 (TPC2) Expression Increases the Metastatic Traits of Melanoma Cells by a Mechanism Involving the HippoSignalling Pathway and Store-OperatedCalcium Entry. *Cancers*, *12*(9), 2391.
2) Nguyen, O. N., Grimm, C., Schneider, L. S., Chao, Y. K., Atzberger, C., Bartel, K., Watermann, A., Ulrich, M., Mayr, D., Wahl-Schott, C., Biel, M., &Vollmar, A. M. (2017). Two-Pore Channel Function Is Crucial for the Migration of Invasive Cancer Cells. *Cancer research*, *77*(6), 1427–1438.
3) Kozar, I., Margue, C., Rothengatter, S., Haan, C., & Kreis, S. (2019). Many ways to resistance: How melanoma cells evade targeted therapies. *Biochimica et biophysica acta. Reviews on cancer*, *1871*(2), 313–322.

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Tailoring graphene nanoparticles for bio-medical application: structural precision and biological assays

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Graphene nanoparticles (GNPs), thanks to their large surface areas and easy functionalization, are attracting materials for pharmaceutical and bio-medical applications, particularly in the field of cancer research where their targeted use may increase the effectiveness of chemotherapy and bypass the issue of multidrug resistance. GNPs apparently similar may possess very different characteristics as average particles size, size distribution, kind and degree of functionalization and morphology, leading to a variety of responses when tested in biological environment. Therefore, a structural/functional characterization of such materials to rationalize their performances is mandatory.

Here we evaluated the toxicity of two different non-functionalized GNPs, BOTTOM60 and TOP60, *in vitro* in HT1376 urothelial carcinoma and U373 astrocytoma cell lines, human cells of different origin and with different features in terms of endocytic capability as well as drug sensitivity. Moreover, considering the potential immunological effect of GNPs, we investigated the ability of our nanoparticles to activate human THP-1 derived macrophages.

In HT1376 culture cells, the high number of carboxylic groups caused widespread BOTTOM60 aggregation and precipitation, a concentration- and time-dependent decrease of cell viability and uptake failure, leading to consider these GNPs unsuitable for bio-medical applications.

TOP60, with fewer carboxylic groups, showed a slight rate of aggregation. In HT1376 cells, TOP60 affected cell viability in a concentration- and time-dependent manner, impaired cell proliferation only at the highest concentration, but no intracellular uptake was detected. In U373 cells, TOP60 were internalized in membrane bounded organelles and decreased cell viability only at the highest concentrations, without affecting cells proliferation. Finally, TOP60 do not trigger an inflammatory response in THP-1-derived macrophages.

These findings highlight the differential behaviour and cytotoxic effects of TOP60 depending on the cell type and paves the way for further investigations to the set-up our GNPs based drug delivery platforms.

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Immunofluorescence and quantitative analysis of sarcoglycans subcomplex in epithelial tissues

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Sarcoglycans are transmembrane glycoproteins that play a key role in maintaining sarcolemma stabilization during muscle contraction. Several studies have demonstrated that this complex is not muscle specific and that it is also expressed in epithelial tissues as gingival, breast [1] and prostatic epithelia [2] and recently in the adipose tissue, demonstrating that these proteins are involved in cell-cell and cell-matrix interactions. In the present study, we investigated sarcoglycans expression in the epithelia of digestive, respiratory and urinary tracts. We performed immunofluorescence reactions using antibody against a-, b-, g-, d-, e- and -sarcoglycans. Moreover, in the same samples, quantitative real-time PCR was carried out to analyze gene expression of sarcoglycans. Total RNA was extracted using Trizol following standard protocol. Quantitative analysis of extracted RNA was carried out with the use of Nanodrop 1000. Reverse transcription was performed using 1 μg of total RNA with SuperScript IV Reverse Transcriptase kit (Invitrogen) and random primers according to standard protocol. cDNA obtained was used to evaluate gene expression of sarcoglycans, using β-actin as housekeeping gene for relative quantification. Real Time PCR was performed in duplicate in 96 well. Real Time PCR was monitored using CFX Opus 96 Dx and amplified products were quantified measuring target genes and housekeeping gene cycle threshold (Ct). After normalization control mean value was used as calibrator and results were expressed using $2^{-\Delta\Delta ct}$ compared to the average of control. Our results show the expression of sarcoglycans in the basal, lateral, and apical epithelial cell's surface; moreover, sarcoglycans show to colocalize with each other. These results, supported by quantitative molecular analysis, demonstrated the role of sarcoglycans in cell-cell and cell-matrix interaction. Moreover, it is important to analyse the same proteins in epithelial during pathological conditions in order to evidence the real role of sarcoglycans in interaction processes.

References

1) Arco, A.; Favaloro, A.; Gioffrè, M.; Santoro, G.; Speciale, F.; Vermiglio, G.; Cutroneo, G. Sarcoglycans in the normal and pathological breast tissue of humans: an immunohistochemical and molecular study. Cells Tissues Organs. 2012, 195, 550-562.
2) Cutroneo, G.; Bramanti, P.; Favaloro, A.; Anastasi, G.; Trimarchi, F.; Di Mauro, D.; Rinaldi, C.; Speciale, F.; Inferrera, A.; Santoro, G.; Arena, S.; Patricolo, M.; Magno, C. Sarcoglycan complex in human normal and pathological prostatic tissue: an immunohistochemical and RT-PCR study. Anat. Rec. (Hoboken). 2014, 297, 327-336.

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Potential role of nuclear Phospolipase C beta 1 as a predictive biomarker in lung cancer

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Lung cancer (LC) is one of the most common types of neoplasms, representing the 11.4% of all new cancer cases, and accounting for the 18% of all cancer-related deaths. Despite consistent progress in LC treatment, the overall prognosis remains poor, due to the low efficacy of therapies on drug-resistant cancer cells[1]. Therefore, there is a strong need to understand the molecular bases involved in LC onset and progression, in order to find new reliable biomarkers for diagnosis, prognosis and targeted therapy. The potential role of Phospholipase C (PLC) enzymes in tumor growth and metastasis has been recently proposed[2]. Our analyses evaluated the involvement of PLC isoforms in lung cancer patients. mRNA expression of all PLC isoforms was estimated by Real-Time PCR, which showed a dysregulation of many PLCs. Notably, an overexpression of PLCB1 gene was observed in the majority of the patients enrolled. Non-Small Cell Lung Cancer Tissue Microarray was used to determine PLCb1 expression with immunocytochemistry. The results prove that PLCb1 is purely localized in the nucleus and confirm that its expression is increased in all tumor tissue samples, compared to their adjacent normal tissue. In addition, correlations with tumor histotype and stage were observed. These findings demonstrate the involvement of PLC family enzymes, and in particular of PLCb1, in lung cancer, suggesting their emerging role as potential biomarkers. Further studies are needed to establish how Phospholipase C enzymes regulate cancer-associated cellular processes and their influence on cell motility and cell proliferation in specific lung cancer histotypes.

References

1) Thandra, K.C.; Barsouk, A.; Saginala, K.; Aluru, J.S.; Barsouk, A. Epidemiology of Lung Cancer. Contemp Oncol 2021, 25(1), 45–52.

2) Salucci S.; Aramini B.; Bartoletti-Stella A.; Versari I.; Martinelli G.; Blalock W.; Stella F.; Faenza I. Phospholipase Family Enzymes in Lung Cancer: Looking for Novel Therapeutic Approaches. Cancers 2023, 15(12):3245

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A novel mechanism of adaptive resistance to AKT targeting in a mouse xenograft model of prostate cancer

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Prostate cancer (PC) is the third cause of deaths in European men. Although all prostate tumors are initially responsive to androgen depletion therapy, recurrence associated with reactivation of the androgen receptor pathway and progression to a metastatic castration-resistant prostate cancer (CRCP) phenotype is frequent, and these patients have short survival and limited treatment options. Furthermore aberrant activation of the PI3K/AKT pathway is often observed in PC, especially in patients with *PTEN* deletion. Therefore recent clinical studies associated abiraterone androgen depletion therapy to the AKT inhibitors capivasertib or ipatasertib, with encouraging results. However, it is very well known that adaptive signaling can cause attenuation of PI3K/AKT inhibition efficacy, limiting clinical outcome.

In vitro studies from our laboratory show that AKT inactivation in prostate cancer cell lines may prompt resistance due to adaptation of signaling through a previously undescribed AKT/miR-145/RAS circuit. Our findings indeed demonstrate for the first time that, in the *PTEN* null PC3 cell line, pharmacological inactivation of AKT completely downregulates miR-145 while triggering a ~20 folds increase of its target RAS. In turn, RAS can cause paradoxical reactivation of signaling, possibly driving resistance. To confirm these results, we established a xenograft mouse model of PC, represented by NOD/SCID mice injected with PC3 cells to reproduce the neoplastic lesion. After 3 weeks of tumor engraftment, mice were treated with capivasertib up to 4 weeks. Starting from the first week of treatment, capivasertib significantly reduced tumor growth in comparison to vehicle-treated control group. However, the efficacy of the drug to reduce the tumor size decreased over time. Immunoistochemical analysis revealed that capivasertib increased the expression of RAS and others targets negatively regulated by miR-145 such as SENP1 and MTDH. These preliminary *in vivo* results are in agreement with our previous *in vitro* studies confirming the emergence of resistance to AKT-inhibition therapy, elicited by miR145-RAS pathway.

Innovation in teaching strategies and multimedia technologies: present and future

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Digital teaching devices for the study of human anatomy as part of the degree course in Exercise and Sports Sciences: Primal Pictures and Anatomage

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In-depth knowledge of musculoskeletal anatomy is a fundamental requirement for Exercise and Sports Sciences students. The high number of students at our university does not allow to organize large-scale lessons on mannequins, gold standard methodology for the study of human anatomy. Therefore, in the academic year 2023/24, we made use of two innovative digital teaching tools (Primal Pictures and Anatomage) to achieve a deep understanding of the subject, and an easy transposition of theoretical knowledge into practical activities.

First year students, in addition to textbook, utilizing Primal Pictures, a free and user-friendly software, could selectively examine the anatomical structures' feature of their interest. Indeed, the program allows to stratify and analyze in detail the locomotor components (like bones, ligaments, and skeletal muscles).

Subsequently, second year students, under the guidance of an expert tutor, using Anatomage, delved deeper their knowledge about the adaptations of joint structures in dynamic conditions. The program, in fact, can generate the image of a joint during a movement, allowing to observe the movement-induced adaptations of the different articular components and facilitating the application of such notions to physical exercises in which the same joint positions are assumed.

The use of Primal Pictures and Anatomage allows Exercise and Sports Sciences students to have a more detailed and immediate understanding of musculoskeletal anatomy and joint biomechanics applied to physical exercises, pivotal factor to improve the connection between theoretical notions and practical activities.

Such tools represent a useful resource in the training of professionals, both oriented towards physical exercise for performance purposes and towards physical activity for prevention and health. The poster that will be presented at the congress will demonstrate how, by studying any human body's joint, textbook comprehension can be facilitated and implemented thanks to the different point of view obtained using such digital teaching tools.

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The hidden danger: rethinking visceral adipose tissue in anatomical education

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While often overlooked, visceral adipose tissue (VAT), commonly known as "*visceral fat*", plays a critical role in various diseases. Studies highlight its significant contribution to cardiovascular, metabolic, and inflammatory conditions, as well as a high prevalence of VAT exceeding that of subcutaneous adipose tissue (SAT). Traditional clinical measurements like BMI, waist-hip ratio, and waist circumference, while useful, cannot accurately quantify VAT mass. This limitation can lead to misclassification of individuals regarding their metabolic risk. Despite this, current anatomical education often fails to adequately address the possible existence of an excessive VAT and to highlight its significance. Computed Tomography (CT) and Magnetic Resonance (MR), on the other hand, offers a precise discrimination and quantifications of both VAT and SAT. Despite the availability of these imaging techniques and growing research emphasizing VAT's role in disease, anatomical textbooks currently depict splanchnic cavities with minimal visceral fat. This discrepancy between clinical reality and anatomical education leaves medical students with a limited knowledge of VAT until they encounter it during surgical procedures or internal medicine studies. To bridge this gap, we propose integrating CT and MR imaging into the education of topographic anatomy to familiarize students with VAT and SAT. By visualizing epicardial, mesenteric, and pelvic VAT in various planes (axial, coronal, and sagittal) across a spectrum of body compositions, students can develop a comprehensive understanding of VAT normal or abnormal distribution. Furthermore, comparing CT/MR images with laparoscopic views and cadaveric specimens can solidify their understanding of VAT's anatomical relationships and its often-overlooked abundance. In conclusion, revising anatomical education to accurately reflect the clinical significance of VAT is crucial. By incorporating advanced imaging techniques and acknowledging its prevalence, we can equip future healthcare professionals with the knowledge to identify VAT and to address the health risks associated with this often-hidden danger.

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Ultrasonography face anatomy for a safe aesthetic medicine practice

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The growing demand of minimally invasive aesthetic procedures has increased the size of the aesthetic medicine market. The market is benefiting from the greater popularity of minimally invasive aesthetic procedures. Patients increasingly prefer aesthetic medicine treatments due to their reduced risks, short recovery times and positive results.

The incidence of complications in aesthetic medicine procedures is low and most adverse events are mild. Risk can be minimized through careful patient and product selection and the use of safe approaches. Ultrasound is a significant imaging technology the applications of which in medicine is increasing.

The present study has investigated the applications of ultrasonography analysis on cadaver faces to assess the feasibility of this approach for a safe aesthetic medicine practice.

Analysis was performed on fresh samples of cadaveric faces. The study included anatomical dissection, highlighting structures of interest, photography of structures and their ultrasound analysis. The areas of interest were 7, namely the great auricular nerve, the temporal (frontal) branch of the facial nerve, the marginal mandibular, zygomatic, and buccal branches of the facial nerve, supraorbital and supratrochlear nerves, infraorbital nerve, and mental nerve.

Ultrasound analysis highlights the precise localization and morphology of critical facial nerve structures. Detailed ultrasound images provide a clear view of the soft tissue arrangement, which closely correlates with data obtained from anatomical dissections. This can contribute to prevent damage during aesthetic procedures.

Using ultrasound on cadaver faces in anatomy courses provides a dynamic learning experience. It allows students to correlate ultrasound images with actual anatomical structures, enhancing both theoretical understanding and practical skills.

The integration of dissection and ultrasound in anatomy courses may represent a significant evolution in medical teaching. It provides a comprehensive platform to understand anatomy in a more interactive and contextualized way. This can contribute remarkably to a safe aesthetic medicine practice.

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Using augmented reality to enhance anatomical education: the *AEducAR 2.0* experience

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Anatomical education is foundational in medical training, with augmented reality (AR) offering novel methods to enhance learning experiences. This study presents *AEducAR 2.0*, an advanced AR tool designed to improve anatomical education through interactive and immersive learning. An interdisciplinary team developed and tested *AEducAR 2.0* with 130 second-year medical students at the University of Bologna. The tool encompasses two applications, "Track & Explore" and "Place & Check," focusing on the orbit zone, the facial bones, and the mimic muscles. Students engaged in explorative and interactive activities, subsequently assessed through targeted quizzes. Data were collected via questionnaires and interviews to evaluate students' perceptions and learning outcomes. Results indicate significant improvements in both knowledge acquisition and student engagement. *AEducAR 2.0* not only facilitated high quiz scores but also received positive feedback for its interactive features, highlighting the potential of AR in medical education. The study's findings suggest that *AEducAR 2.0* can bridge the gap between traditional methods and modern technological advancements, making anatomical education more effective and engaging. Students appreciated the hands-on, practical components, which enhanced their understanding and retention of complex anatomical structures. Furthermore, the interdisciplinary approach ensured a comprehensive evaluation of the tool's educational impact. In conclusion, this study underscores the efficacy of AR in enhancing traditional anatomical education methods, suggesting a blended learning approach to optimize educational outcomes. The integration of AR technologies in medical curricula not only prepares students for clinical practice but also aligns with contemporary advancements in medical training, paving the way for future innovations in medical education [1].

The research leading to these results has received funding from the European Union – NextGenerationEU through the Italian Ministry of University and Research under PNRR – M4C2-I1.3 Project PE_00000019 "HEAL ITALIA" to Alessandra Ruggeri CUP J33C22002920006 of the University of Bologna, Italy.

References

1) Neri, I.; Cercenelli, L.; Marcuccio, M.; Lodi, S.; Koufi, F.D.; Fazio, A.; Marvi, M.V.; Marcelli, E.; Billi, A.M.; Ruggeri, A.; et al. Dissecting human anatomy learning process through anatomical education with augmented reality: AEducAR 2.0, an updated interdisciplinary study. Anat Sci Educ 2024, 17, 693-711.

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Innovation and tradition: synergies between digital teaching and anatomical dissection in medical education

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The relationship between traditional dissection in human anatomy and multimedia anatomy is complementary, combining hands-on experience with advanced technological tools to enhance medical education and understanding. While dissection offers irreplaceable hands-on experience and an understanding of real anatomical variability, virtual anatomy provides accessible, repeatable, and detailed visual learning. Together, they form a robust framework for anatomical education, ensuring that students gain a comprehensive understanding of human anatomy through both theoretical and practical approaches. For three years, the University of Trieste and Padua have been cooperating and exchanging students (from CLOMPD) that have learned both the dissections of the head and neck as well as the digital anatomy using Technologies of Advanced Visualization.

The purpose of this presentation is to make you participate in our collaborative project analyzing the integration of multimedia anatomical teaching in synergy with dissection to train professionals increasingly competent and prepared.

References

1) Chytas D, Johnson E, Piagkoub M, Mazarakisb M et al. Annal of Anatomy 2020, 1-6
2) Preim B, Saalfeld P. A survey of virtual anatomy education systems. Computer and Graphics 2018, 132-153

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3D Digital tools in human anatomy teaching from *peer-to-peer* tutoring to clinically oriented approaches: a single center experience

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First year med students from the Italian (n=170) and International Course (n=48) were provided with the opportunity to exploit *Anatomage* table while studying: fourth- and fifth-year med students were trained as *peer-to-peer* tutors to facilitate them.

Participating students were divided into groups (10-12 students each); they met with a tutor for at least 3 sessions lasting 2 hours with a predefined topic: thorax (topic 1), abdomen/pelvis (topic 2), neuroanatomy (topic 3). Moreover, each group participated in a session without a predefined topic during which they freely explored the teaching tool under supervision. This was not a compulsory activity and was carried out in students' free hours during the 2nd semester. A questionnaire was filled at the end of each session rating satisfaction (0: no satisfaction at all; 10: the highest possible satisfaction) and collecting information on the impact on exam preparation.

One-hundred-twenty-eight students (105 from the Italian course and 23 from the international course) decided to participate. Overall, more than 95% of students rated the whole initiative as highly satisfactory (i.e., score equal or higher than 7) and 100% of students would suggest others to participate. More than 90% of students replied that this initiative increased their willingness to use the Anatomy room for independent study if *Anatomage Table* was available.

Based on these results, a further implementation is planned for the next year, also exploiting DICOM data (i.e., radiological images the professor is gaining during her clinical duties) to present the students with real clinical cases bearing a highly didactive anatomy content. Moreover, in the international course, given the peculiar nature of human anatomy teaching (based on a vertical track approach) we are also strongly relying on flipped-classroom activities via *Anatomage-Lessons*. Furthermore, these tools are being implemented for clinically oriented teaching for residents/consultants.

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Enhancing anatomy learning: a study of emerging educational technologies

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Anatomy teaching is experiencing a notable shift as a result of the integration of cutting-edge educational software that utilizes advanced technologies like virtual reality (VR), augmented reality (AR), and interactive 3D models. This study seeks to analyze and assess the most cutting-edge software solutions presently accessible for anatomy education. We performed a thorough examination and comparison of four prominent anatomy software platforms: 3D Organon, Anatomage Table, Anatomyka, and BioDigital Human. The evaluation criteria encompassed various aspects such as the quantity and quality of anatomical models, interactive functionalities and user interface, compatibility with different platforms and accessibility, integration with current educational systems, and the efficacy in improving student learning and involvement. Almost 10,000 realistic and interactive anatomical models are available from 3D Organon. Immersion learning sessions using VR headsets are supported by these models. They shine at enabling in-depth investigation of spatial linkages and anatomical features. Modern 3D anatomical tables like the Anatomage Table enable virtual dissections. With it, anatomical models can be realistically manipulated in a safe and contemporary substitute for conventional dissection techniques. Anatomyka is unique in that it is widely available on several platforms and uses a customized learning style. Intricate 3D models and accurate medical explanations are used to improve the educational process. Comprising over 8,000 anatomical features and 600 diseases, the BioDigital Human is an extensive resource. It is notable for its smooth connection with assessment tools and learning management systems (LMS), which offers a lively and interesting educational experience. The successful implementation of these technologies presents certain challenges, including the requirement for teacher training and upgrades to infrastructure. Nevertheless, the clear advantages in enhancing student learning and involvement make these advancements essential for the future of anatomical education. Additional research is necessary to measure the lasting effects of these tools on student performance and knowledge retention.

References

1. Estai, M.; Bunt, S. Best teaching practices in anatomy education: A critical review. Ann Anat 2016, 208, 151–157.
2. Adnan, S.; Xiao, J. A Scoping Review on the Trends of Digital Anatomy Education. Clin. Anat. 2023, 36, 471–491.

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Connecting pre-cinical knowledge to modern imaging techniques in gross anatomy learning

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Teaching human anatomy is a fundamental pillar of medical education. Traditional methods include classroom "frontal lessons" where the teacher instructs students, allowing the acquisition of basic gross anatomy knowledge. Beyond textbooks, images are essential for learning the anatomical discipline, with anatomical atlases containing classic didactic drawings. However, modern textbooks and atlases now include more "living anatomy" images from medical imaging techniques, such as radiological, echographic, computed tomography, magnetic resonance, and positron emission tomography. In this regard, the Human Anatomy course at the University of Pisa designed a pilot experience to better connect basic knowledge to modern imaging. This involved two learning laboratory sessions for medical students, where an expert radiologist introduced practical concepts of medical imaging, focusing on computed tomography. This allowed revisiting certain anatomical topics, such as the knee joint, heart, and abdominal cavity, through the lens of this imaging approach. The anatomical structures were video-examined under different planes, magnifications, resolutions, and radio-opaque contrast media, improving the value of this didactic strategy by exploring anatomical variations and mild pathological conditions. Students have expressed great appreciation for this teaching methodology in the study of human anatomy. This feedback suggests that vertical connections between pre-clinical and clinical disciplines can represent a methodological tool of great educational potential, also with a view to greater flexibility within the medical degree program.

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The Anatomia Universa (1823) of Paolo Mascagni (1755-1815): the memory of a masterpiece in the history of anatomy and a modern approach to medical education

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Two hundred years ago, the first of the nine volumes of Paolo Mascagni's monumental *Anatomia universa* was published posthumously. This project, which consumed a significant portion of Mascagni's life, aimed to create an unparalleled anatomical atlas. Envisioned as a perfect reflection of human dissection on paper, this work aimed to revolutionize the teaching of anatomy, a field heavily reliant on this practical skill.

The authors embark on a captivating exploration, tracing key moments in the life of the great anatomist Paolo Mascagni. Through this journey, they pay homage to his extraordinary work, the *Anatomia universa*. Drawing upon historical accounts and showcasing evocative anatomical plates, meticulously preserved to this day in the prestigious Museum of Siena, they illuminate the enduring legacy of this masterpiece in the history and teaching of medicine.

Mascagni's *Anatomia universa* revolutionized anatomical illustration with its unique approach.

The plates are organized to reveal the body from the superficial muscle layer down to the skeleton, as in the process of dissection. For the first time in the history of anatomy, the plates were life-size. Furthermore, in an original manner, and again for the first time, these plates showed the network of lymphatic vessels that Mascagni had brought to light a few years earlier. The beauty and perfection of these drawings are the result of Mascagni's knowledge and his ability to recruit the most expert artists and engravers of the time.

The future of anatomy education likely lies in a combination of traditional methods, like cadaver dissection, and innovative technologies like virtual dissection software. Mascagni's pioneering work with the *Anatomia Universa* serves as a reminder of the importance of continually seeking new and effective ways to teach students about the complexities of the human form.

Stem cells, histogenesis and differentiation

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Morpho-functional characteristics of semen in men with varicocele

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Purpose: Patients with varicocele often experience changes in sperm production. An uncontrolled, long-standing varicocele can lead to a significant reduction in male fertility. The purpose of this study is to show differences in sperm quality in men with varicoceles when compared with healthy men.

Material and methods: The study was performed on 30 human semen samples divided into control and experimental groups A, B. The experimental group A included semen samples of men with varicoceles, while experimental group B included semen samples of healthy men. Semen samples were analysed with CASA system (computer assisted semen analysis).

Results: Analysis of semen samples in group A showed changes in sperm production in a way of decreased total number of spermatozoa in semen. Sperm motility was affected also, showing decreased motility. Sperm morphology showed presence of multiple deviations on spermatozoa, affecting sperm heads in first place.

Conclusion: The analysis of the ejaculate in patients with varicocele provides a detailed insight into the production of spermatozoa, sperm motility and sperm morphological structure. Continuous analysis of the ejaculate in every three to six months in these patients is of great importance for the treatment of varicocele and the maintenance of their fertility.

Keywords: sperm quality, varicocele, men fertility

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Modulation of Vav1 in differentiation of hiPSCs to β cells: a potential strategy to enhance outcomes of *in vitro*-generated insulin producing cells transplantation

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Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by destruction of pancreatic β cells, responsible for reduced insulin production [1]. Pancreatic islet transplantation represents a promising solution to restore insulin levels in T1D patients, but this strategy is limited by donor scarcity and immune rejection complications. Human-induced pluripotent stem cells (hiPSCs) have emerged as a promising source for generating transplantable insulin producing cells; however, despite continuous improving, production of fully functional β cells remains a significant challenge [2].

This study focuses on β cell differentiation from iPSCs of fibroblast origin, with particular efforts on the role of the guanosine exchange factor Vav1, based on our previous studies indicating that Vav1 plays a crucial role during the early stages of human biliary stem cells (hBTSCs) maturation into β cells [3]. Our data revealed that Vav1 expression peaks at the endocrine progenitor (EP) stage, declining to minimal levels by the end of the differentiation process, corresponding to β-like cells, in which immunocytochemical analyses shown an inverse Vav1/insulin correlation. Preliminary results obtained by downmodulating Vav1 levels with a specific pool of siRNAs in EP cells, demonstrated a further increase of insulin production, suggesting a stage-related role for this multidomain protein in the mechanism leading to β cell differentiation from hiPSCs, similarly to what we already demonstrated for hBTSCs.

Our data can help in better understanding the mechanisms leading to generation of β-like cells starting from different precursors and, despite further studies are necessary to establish the involved signalling pathways, suggest that a stage-specific modulation of Vav1 could constitute a potential strategy to improve the insulin production in cells generated *in vitro* for transplantation in T1D patients.

References

1) Robertson, C. C.; Elgamal, R. M.; Henry-Kanarek, B. A.; Arvan, P.; Chen, S.; Dhawan, S.; Eizirik, D. L.; Kaddis, J. S.; Vahedi, G.; Parker, S. C. J.; Gaulton, K. J.; & Soleimanpour, S. A. Untangling the genetics of beta cell dysfunction and death in type 1 diabetes. Molecular metabolism. 2024, 101973.
2) Pellegrini, S.; Zamarian, V.; & Sordi, V. Strategies to Improve the Safety of iPSC-Derived β Cells for β Cell Replacement in Diabetes. Transplant international: official journal of the European Society for Organ Transplantation. 2022, 35, 10575.
3) Brugnoli, F.; Grassilli, S.; Cardinale, V.; Carpino, G.; Gaudio, E.; Alvaro, D.; Capitani, S.; & Bertagnolo, V. Vav1 Sustains the In Vitro Differentiation of Normal and Tumor Precursors to Insulin Producing Cells Induced by all-Trans Retinoic Acid ATRA. Stem cell reviews and reports. 2021, 17, 673–684.

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The impact of Calr-mutation on hematopoietic microenvironment remodeling and MK differentiation

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The somatic Calreticulin (CALR) mutation is a key mutation in Myeloproliferative Neoplasms (MPNs) such as Essential Thrombocythemia and Primary myelofibrosis. The CALR mutant protein leads to the constitutive alteration of JAK2 downstream target, causing cellular transformation and microenvironment abnormalities in both bone marrow (BM) and spleen, as megakaryocytes proliferation and bone marrow fibrosis.

However, the altered megakaryocytic differentiation which occurs upon CALR mutation, and their effect is not completely understood.

In the present study, we have investigated the BM and spleen microenvironment form *Calr* 10-month-old haploinsufficient mice.

Calr$^{+/-}$ mice present morphological abnormalities as observed for the MPN phenotype. The bone marrow from *Calr*$^{+/-}$ mutated mice was characterized by ineffective megakaryopoiesis and altered platelets production. Electron microscopy (T.E.M.) revealed that most of the *Calr*$^{+/-}$ MKs remain immature and are characterized by ultrastructural abnormalities in both mitochondria and rough endoplasmic reticulum. The morphological observation highlighted the formation of vesicular-like structures in the cytoplasm of MKs, which are likely released by the affected mitochondria in the extracellular microenvironment. The immature MKs were characterized by a poorly developed demarcation membrane system and few platelet territories. Terminally maturated MKs were mostly identified in spleen from *Calr*$^{+/-}$ mice. The released platelets phenotypically exhibit increased granularity and adhesive activity. Platelets aggregation and in adhesion with endothelial cells were immunohistochemically confirmed by P-selectin staining. An increased infiltration of neutrophils and macrophages was observed either in bone marrow as in the spleen. The granulocytic series were mostly immature characterized by the presence of the Auer bodies and no chemotaxis was observed. Furthermore, aged *Calr*$^{+/-}$ mice, develop focal fibrosis, as confirmed by the reduced presence of activated fibroblasts (TEM observations).

In conclusion, by our findings, the CALR mutation induce alteration in both hematopoietic organs, and the *Calr*$^{+/-}$ mice may represent a suitable animal model for the study of MPNs disease.

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The role of the moonlighting protein Clathrin in fully-grown germinal vesicle mouse oocytes

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Clathrin is a well-known moonlighting protein that changes function according to the cell cycle. It is involved in membrane trafficking during interphase and spindle assembly. Clathrin is essential for maturation up to MII in mammalian oocytes, localizing at the spindle level.

Immunofluorescence and transmission electron analysis on fully-grown germinal vesicle (GV) mouse oocytes shows Clathrin localization on the cortical region with three peculiar patterns: complete, incomplete, and half-moon. The first configuration is characterized by Clathrin lattices along the membrane as dynamic actin-controlled hubs for further endocytosis; the second configuration is represented by Clathrin lattices interrupted by invaginations forming coated vesicles as an indication of active endocytosis. The half-moon profile, instead, consists of Clathrin lattices distributed to one-half of the cell. We analyze organelles' positioning and cytoplasmic rearrangements through time-lapse experiments to shed light on this peculiar pattern. The arrangement of endosomes and lysosomes was followed during oocyte maturation, from the GV to the MII stage. It revealed the disposition of these organelles from the cytoplasm to one side of the cell, opposite to spindle positioning and polar body extrusion. This movement can be due to an extensive anterograde transfer of endo/lysosomes in the peripheral portion of the cytoplasm to form the spindle and to ensure the correct partitioning of organelles after the polar body extrusion. These results helped us investigate the meaning of the Clathrin half-moon profile according to its moonlighting nature. During Germinal Vesicle Breakdown (GVBD), Clathrin detaches from the oocyte cortical region and starts associating around the chromosome congression area in the center of the cell to become a spindle stabilizer during MI and MII. For this reason, before oocytes undergo GVBD, Clathrin localizes on the side of the cell opposite to future spindle migration, thus becoming a marker to predict spindle orientation in mouse oocytes.

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References

1) Veiga, M.; Camia, B.; Diepenbrock, S.; Redi, CA.; Casasco, A.; Monti, M. Artificial intelligence selection of viable oocytes from phase contrast images for in vitro maturation and fertilization. BOR. 2023, 109, 386–389.
2) Monti, M.; Calligaro, A.; Behr, B.; Reijo-Pera, A.; Redi, CA.; Woosidlo, M. Functional topography of the human fully grown oocyte. EJH 2017, 61, 2769.

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Modulation of apoptotic pathways in leukemic hematopoietic stem cells by Venetoclax and Azacytidine combination treatment

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Phospholipases C (PLCs) and their associated pathways, which target BCL-2, can influence the therapeutic response of the combination of Azacytidine (AZA) and Venetoclax (VEN), effective in MDS hematopoietic stem cells (HSCs) [1,2].

This study aimed to evaluate AZA+VEN therapy on MDS/AML cells, focusing on PLCs-related and apoptotic pathways. HSCs from 10 MDS patients, treated with AZA or AZA+VEN, were collected at baseline and during therapy. At baseline, 7 patients exhibited a novel frameshift BCL-2 mutation (p.Ala28Glyfs*14), possibly leading to a truncated protein, whose role is under validation and investigation. Molecular analyses also revealed significant differences, as BAX was downregulated in responders and upregulated in non-responders to AZA alone, while all AZA+VEN patients responded rapidly with early BCL-2 and BAX upregulation, followed by decreased gene expression in later cycles. Notably, one patient showed increased BCL-2 expression before AML progression after AZA+VEN, with no significant BAX changes.

THP-1 and MV4-11 leukemic cells, used as VEN resistant/sensitive *in vitro* models, were treated with AZA/VEN/AZA+VEN for 24 hours. Flow cytometry analysis revealed a significant Sub-G0 phase increase, especially with AZA+VEN. Annexin V assays confirmed apoptosis, with apoptotic markers showing a significant increase in pro-apoptotic markers (BIM, BAK1) only in MV4-11 cells. Treatment also influenced PLCs and mature myeloid marker expression (CD11, CD14) in both cell lines, but only THP-1 cells showed increased PLCG2 protein levels. Furthermore, AZA+VEN upregulated AKT gene expression in MV4-11 cells but reduced phosphorylated AKT (Ser473) without impacting pGSK3, while increasing pGSK3α in both cell lines.

All in all, this study hints at an involvement of inositide pathways in the regulation of BCL2-dependent pathways during AZA+VEN treatment in HSCs from MDS and AML cells, with an induction of apoptosis due to AZA and an involvement of AKT/GSK3a, warranting validation in larger patient cohorts and diverse cell lines.

References

1) Mongiorgi, S.; De Stefano, A.; Ratti, S.; Indio, V.; Astolfi, A.; Casalin, I.; Pellagatti, A.; Paolini, S.; Parisi, S.; Cavo, M.; Pession, A.; McCubrey, J.A.; Suh, P.G.; Manzoli, L.; Boultwood, J.; Finelli, C.; Cocco, L.; Follo, M.Y. A miRNA screening identifies miR-192-5p as associated with response to azacitidine and lenalidomide therapy in myelodysplastic syndromes. Clin Epigenetics. 2023 Feb 20;15(1):27.
2) Bazinet, A.; Bravo, G.M. New Approaches to Myelodysplastic Syndrome Treatment. Curr Treat Options Oncol. 2022 May;23(5):668-687.

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Leukemic cells adherence to mesenchymal stromal cells: a new mechanism for stem cell differentiation in hematopoietic cells?

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Mesenchymal stromal cells (MSCs) are believed to regulate hematopoietic stem cell (HSC) behavior, especially within the bone marrow microenvironment, which plays a pivotal role in leukemic progression.[1]

To analyze the influence of MSCs on leukemic cells, we set up direct contact co-cultures using MSCs obtained from different sources (HS-5 cell line and dental pulp-derived MSCs) and leukemic cells (THP-1 and KG-1 cell lines). Cellular behavior was assessed via both optical and scanning electron microscopy (SEM) examination, while cytofluorimetric analyses evaluated CD11b and CD14 expression in both in suspension cells and cells adherent to MSCs.

Our findings show that MSCs may stimulate cell adhesion and induce leukemic cells to divide in two subpopulations: suspended and adherent to MSCs. Interestingly, cytofluorimetric analyses revealed a progressive increase in CD11b and CD14 expression on KG-1 and THP-1 cells during co-culture, and an initial high percentage of CD34+ KG-1 cells adherent to MSCs, followed by a progressive decrease, even in suspension KG-1 cells up to 144h after co-culture. These results suggest that MSCs may affect myeloid differentiation. This hypothesis is further supported by the observation that THP-1 cells already induced to macrophage differentiation by phorbol myristate acetate (PMA) are less adherent to MSCs. That is why our current transwell experiments are providing insights into the significance of direct cell contact in this molecular mechanism.

As for morphology, SEM observations revealed increased vesicle secretion at later stages of co-culture, suggesting their probable significant role in the cross-talk between the two cellular sub-populations, that could be confirmed by ongoing immunolabeling experiments that will allow us to better detect and localize differentiation markers within the samples.

All in all, our findings may further elucidate the effect of MSCs on HSCs during myeloid differentiation or leukemic progression, possibly leading to novel therapeutic strategies targeting the bone marrow microenvironment.

References

1) Pontikoglou, C. G.; Matheakakis, A.; Papadaki, H. A. The mesenchymal compartment in myelodysplastic syndrome: Its role in the pathogenesis of the disorder and its therapeutic targeting. Front Oncol. 2023, 13, 1102495.

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Activation and reprogramming of human Müller cell line MIO-M1 exposed to high glucose and glucose variability: an in vitro study

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Diabetic retinopathy (DR) is the most frequent complication of diabetes, and it is associated with reactive gliosis and activation of human retinal Müller cells (MCs) [1], characterized by increased glial fibrillary acidic protein (GFAP). In lower vertebrates, gliosis is followed by MCs' acquisition of retinal stem cell properties through Sonic Hedgehog (SHH) and SRY-Box-Transcription-Factor-2 (SOX2) expression. This study investigated the impact of high-glucose and glucose variability (GV) on the activation and reprogramming of human MCs cell line MIO-M1.

Cell-treatments: MIO-M1 cultured in normo-glycemic (NG; 5mM glucose) and hyper-glycemic (HG; 25mM glucose) conditions were exposed to different treatments for 96h: I) constant basal-glucose medium (5mM for NG and 25mM for HG), II) constant high-glucose medium (25mM for NG and 45mM for HG); III) alternating basal and high-glucose every 24h; IV) basal-glucose for 72h followed by high-glucose for the last 24h; V) alternating low (3mM for NG and 5mM for HG) and high (25mM and 45mM for NG and HG) every 24h [2].

Results: The results showed higher GFAP levels in HG cells compared to NG cells. A significant increase in GFAP expression was also observed when NG cells were exposed to sustained high-glucose (II) and GV (III, IV, V) associated with an increased number of hypertrophic cells. In contrast, HG cells maintained a consistent level of GFAP expression under all conditions, with no significant variation in hypertrophic cells number. In basal condition, HG cells exhibited higher levels of SHH and SOX2 levels compared to NG cells. NG cells, exposed to high-glucose (II) and GV showed a significant upregulation of SHH and SOX2. Conversely, HG cells showed a reduced expression of SHH and SOX2 in response to the treatments.

Conclusions: NG cells showed a dynamic adaptation to fluctuating glucose levels in contrast to desensitized HG cells. These findings indicate the existence of a differential response of Müller cells under diabetic conditions.

References

1) Coughlin B. A.; Feenstra D. J.; Mohr S. Müller cells and diabetic retinopathy. Vision Res. 2017, 139:93-100.
2) Picconi F.; Parravano M.; Sciarretta F.; Fulci C.; Nali M.; Frontoni S.; Varano M.; Caccuri A. M. Activation of retinal Müller cells in response to glucose variability, Endocrine. 2019, 65(3):542-549.

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Involvement of GATA3 and TGF-β in trophoblastic development

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GATA3 plays essential roles in the development and function of various tissues and organs, with notable expression in both haematological and non-haematopoietic tissues. Its presence is particularly significant during embryonic and placental development (1). Similarly, TGF-β signaling is critical for placental development and can modulate GATA3 expression (2). Understanding the interactions between these factors is crucial for comprehending both normal and pathological developmental processes.

In our study, we investigated the roles of GATA3 and TGF-β in trophoblast development. Using immunohistochemistry, we evaluated the localization and expression levels of GATA3 and TGF-β in term placentas from normal pregnancies and those complicated by pre-eclampsia. Our findings revealed up-regulation of both GATA3 and TGF-β in pathological placentas, with specific localization in the syncytiotrophoblast, villus stroma, and decidua.

Both GATA3 and TGF-β appear to contribute to aberrant trophoblast development in pre-eclampsia, disrupting the delicate balance between trophoblast proliferation, differentiation, and invasion (3). It is known that GATA3 inhibits GCM1 by binding to it through specific domains. Deletion of GATA3 increases the expression of HtrA4, a GCM1 target gene encoding a serine protease involved in trophoblast cell invasion. This interaction between GATA3 and GCM1 underscores GATA3's role in regulating human placental cell invasion, similar to TGF-β (4).

Based on these findings, a logical next step would be to investigate the interactions between TGF-β, GATA3, GCM1, and other downstream factors such as HtrA4, given the known TGF-β-HtrA1 interaction in the development of various cell types (5).

References

1) C.K. Kaufman, P. Zhou, H.A. Pasolli, M. Rendl, D. Bolotin, K.C. Lim, X. Dai, M.L. Alegre, E. Fuchs, GATA-3: an unexpected regulator of cell lineage determination in skin, Genes Dev, 17 (2003) 2108-2122.
2) B. Afzali, P. Mitchell, R.I. Lechler, S. John, G. Lombardi, Translational mini-review series on Th17 cells: induction of interleukin-17 production by regulatory T cells, Clin Exp Immunol, 159 (2010) 120-130.
3) H. Qu, R.A. Khalil, Vascular mechanisms and molecular targets in hypertensive pregnancy and preeclampsia, Am J Physiol Heart Circ Physiol, 319 (2020) H661-h681.
4) Y.H. Chiu, H. Chen, GATA3 inhibits GCM1 activity and trophoblast cell invasion, Sci Rep, 6 (2016) 21630.
5) A. De Luca, M. De Falco, L. De Luca, R. Penta, V. Shridhar, F. Baldi, M. Campioni, M.G. Paggi, A. Baldi, Pattern of Expression of HtrA1 During Mouse Development, Journal of Histochemistry & Cytochemistry, 52 (2004) 1609-1617.

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Seasonal effects on sarda sheep oocytes quality: ultrastructural evidences

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Seasonality of reproduction, a common feature in sheep of temperate latitudes, has been recognized for a very long time. Sheep are domesticated animals, which in temperate latitudes remain seasonal breeders [1]. In general, the annual breeding season in Sarda sheep is controlled by photoperiod: long days inhibit and short days stimulate sexual activity [2]. Then the season of high sexual activity in Sarda sheep is represented by autumn. The reproductive seasonality of domestic animals is often manipulated to have extended reproductive periods for commercial purposes related to the production of milk and meat. In Sardinia, an extension of the sheep breeding season is finalized to distribute lambing along the year to ensure milk production for several additional months as well as to satisfy the meat market demand. However, these strategies may result in decreased fertility [3]. To date, the effect of season on oocyte developmental competence has not been thoroughly examined [4]. In the present study we evaluated the effects of season, in terms of climate conditions (temperature and humidity), on oocyte quality. Oocytes were collected during winter (January- March, group 1) and summer (May-June and July, group 2) and, to evidence possible morphological alterations, their quality was investigated by light and transmission electron microscopy (LM and TEM). Oocytes of both groups evidenced a round shape with a thin perivitelline space surrounded by a continuous zona pellucida; microvilli covered the oolemmal surface. The ooplasm presented numerous mitochondria clusters, clear vacuoles and high electron-dense lipid droplets that in the group 1 appeared more abundant. In conclusion, our data showed very few differences between the two examined groups; these preliminary findings could be useful to better investigate how ovine oocyte quality could be influenced by season and by environmental conditions.

References

1) Misztal, T.; RomanowicZ, K.; Barcikowski, B. Melatonin - a modulator of the GnRH/LH axis in sheep. Reproductive Biology. 2002, Vol. 2, No. 3..
2) Mara, L.; Sanna, D.; Casu, S.; Dattena M.; Mayorga Muñoz, I.M. Blastocyst rate of in vitro embryo production in sheep is affected by season. Zygote 2013, 22, 366.
3) Serra, E.; Gadau, S.D.; Leoni, G.G.; Naitana, S.; Succu, S. Seasonal Effect on Developmental Competence, Oxidative Status and Tubulin Assessment of Prepubertal Ovine Oocyte. Animals 2021, 11, 1886.
4) Baez ´, F.; N´elida Rodríguez-Osorio, D.; Vinoles, C. Journal of Thermal Biology 2022, 103, 1031712021.

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Role of PLCgamma2 point mutation Associated with Therapy-Resistant Patients in Myelodysplastic Neoplasms

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Nuclear inositide signalling is involved in the regulation of hematopoiesis. A common cluster of point mutations affecting three inositide-specific genes (PLCG2, AKT3 and PI3KCD) has been significantly associated with loss of response to Azacitidine (AZA) and Lenalidomide (LEN) therapy in patients with Myelodysplastic Neoplasms (MDS) at higher risk of leukemic evolution[1]. To investigate the functional role of PLCG2 mutation, KG1 cell line was transduced with the wild type and the mutated form of PLCgamma2. Molecular analyses validated the elevated expression of PLCgamma2 in the transduced cells, thereby confirming the success of the transfection. Subsequent molecular analysis demonstrated a decrease in the expression levels of hematopoietic differentiation markers CD33 and CD14, as well as the pro-apoptotic protein PUMA, only in cells expressing the mutated variant of PLCgamma2. As MDS patients are currently treated even with Venetoclax (VEN)[2], KG1 leukemia cells were also treated with AZA, VEN, and AZA+VEN, and cell viability was evaluated using flow cytometry (FACS). FACS analyses showed that PLCgamma2 overexpression enhanced proliferation in culture and demonstrated reduced mortality and susceptibility to nutrient depletion stress resulting from normal media consumption. Furthermore, under treatment with AZA and VEN, either individually or in combination, the mutated cells exhibited diminished sensitivity to these therapies in comparison to wild-type cells. Indeed, FACS analyses revealed increased proliferation and decreased mortality. These findings suggest that the point mutation in PLCG2 gene may contribute to the resistance to apoptosis and enhance cell growth, potentially explaining the therapy-resistant phenotype observed in MDS patients. This study underscores the importance of targeting PLCgamma2 mutations in the development of more effective therapeutic strategies for patients with hematologic malignancies.

References

1) Follo MY, Pellagatti A, Armstrong RN, Ratti S, Mongiorgi S, De Fanti S, Bochicchio MT, Russo D, Gobbi M, Miglino M, Parisi S, Martinelli G, Cavo M, Luiselli D, McCubrey JA, Suh PG, Manzoli L, Boultwood J, Finelli C, Cocco L. Response of high-risk MDS to azacitidine and lenalidomide is impacted by baseline and acquired mutations in a cluster of three inositide-specific genes. Leukemia. 2019 Sep;33(9):2276-2290
2) Zeidan AM, Borate U, Pollyea DA, Brunner AM, Roncolato F, Garcia JS, Filshie R, Odenike O, Watson AM, Krishnadasan R, Bajel A, Naqvi K, Zha J, Cheng WH, Zhou Y, Hoffman D, Harb JG, Potluri J, Garcia-Manero G. A phase 1b study of venetoclax and azacitidine combination in patients with relapsed or refractory myelodysplastic syndromes. Am J Hematol. 2023 Feb;98(2):272-281

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Autologous cardiomyocytes from differentiating hGMSCs- derived iPS: a new proposal for cardiac regeneration

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The high mortality in the world population due to cardiovascular diseases underscores the urgency of identifying alternative efficient therapies. Technological innovation and advances in regenerative clinical applications are promising tools for this purpose. The core of regenerative medicine is stem cells. The regenerative field has focused on the study and use of multipotent adult stem cells (MSCs). Furthermore, in recent years, to have a greater potential of the cells for therapeutic purposes, and at the same time to bypass the controversies related to the ethical problems of Embryonic stem cells (ESCs) use, a lot of studies are focusing on the induced pluripotent stem cell lines (iPSCs) generation. The aim of the work is to establish a cardiomyocytes primary cell line by differentiating hGMSCs-derived iPS line, a new pluripotency cell line obtained for the first time by reprogramming human gingival mesenchymal stem cells (hGMSCs). The new hGMSCs-derived iPS line was obtained reprogramming hGMSCs through one of the most innovative reprogramming methods without viral vector: StemRNATM 3rd Gen Reprogramming Kit. The new autologous cardiomyocytes primary cell line from hGMSCs-derived iPS line, was established through only with specific culture medium. The characterization of the new iPSCs line was performed by optical and scanning electron microscopies, immunofluorescence and real-time PCR for pluripotency analysis. The derived cardiomyocytes cell lines were characterized through optical microscopy, immunofluorescence and real-time PCR analysis for the cardiac markers expression. The new hGMSCs-derived iPS line demonstrated to be pluripotent in all aspects. The new derived cardiomyocytes line showed cardiomyogenic phenotype in all performed analysis. In conclusion, the new autologous primary cardiomyocytes line obtained from hGMSCs-derived iPS cells could represent an innovative dynamic platform for personalized cardiac tissue regeneration.

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IL-6 drives PD-L1-mediated immunomodulatory properties of human dental pulp stromal cells and synovial fibroblasts

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Human dental pulp stromal cells (hDPSCs) exposed to inflammatory conditions exert immunomodulatory properties through the activation of Fas/FasL and PD1/PD-L1 pathways leading to the reduction of inflammatory cytokines and induction of apoptosis in immune cells. In the same inflammatory settings, hDPSCs have shown the ability to upregulate the expression and release of IL-6, whose involvement in the immunoregulation of hDPSCs awaits clarification. In this study, we demonstrate the role of IL-6 in supporting the expression of PD-L1 by activating the trans-signaling pathway in hDPSCs. The activation of this pathway induced PD-L1 synthesis also in cultured synovial fibroblasts from healthy donors (SFs), revealing that the IL-6/PD-L1 axis is not tissue-specific, likely acting as a general response to inflammatory conditions. Indeed, the joint tissue is a site of immune-mediated events involved in the pathogenesis of different immune-mediated chronic inflammatory diseases, such as rheumatoid arthritis (RA). Many studies have identified synovial fibroblasts (SFs) as key players of RA pathogenesis and IL-6 as a key cytokine in driving their pathogenic transformation. Therefore, we investigated the role of IL-6/PD-L1 axis on SFs isolated from RA patients (RA-SFs). Our results displayed high levels of PD-L1 in RA-SFs compared to normal SFs, which further increased upon IL-6 stimulation. PD-L1 expression was also demonstrated in the inflamed synovium of antigen-induced arthritis (AIA) mice and confirmed in human RA synovium, providing proof-of-concept that PD-L1 is expressed by SFs in inflammatory arthritis. Altogether, our findings uncover the context-dependent nature of the IL-6/PD-L1 axis that becomes activated in different inflammatory settings. This work was kindly supported by the Italian Ministry of Health (prot. no. RF-2019-12370609).

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Functionalization by adsorption of CAPE derivatives improves biomaterial osteogenic properties

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Bone defects resulting from various causes present significant challenges in orthopedic and maxillofacial surgery. Despite the remarkable regenerative abilities of bone, extensive loss often requires intervention. Current approaches, including bone grafts, synthetic and biologically derived tissue-engineered biomaterials aim to facilitate bone healing. Regenerative Medicine (RM) strategies offer promising alternatives by harnessing the body's natural healing mechanisms by introducing exogenous materials to modify the tissue environment and promote osteogenesis.

Small molecules, such as caffeic acid phenethyl ester (CAPE) has shown promising effects in promoting bone regeneration. However, its clinical application is hindered by stability issues. Therefore, new CAPE derivatives were synthesized and the bone regenerative potential of four of them (1a, 1d, 2a, 2d) was investigated. The compounds were selected based on their demonstrated efficacy in skin regeneration (1) and administered (0-5 μM) to dental pulp stem cells (DPSCs) induced to differentiate to osteoblasts for 28 days.

Cell proliferation, Alizarin Red Staining (ARS), alkaline phosphatase (ALP) activity, BMP2, SP7 and DSPP gene expression and antimicrobial properties were evaluated. The results demonstrate that compounds 1a and 1d effectively enhance DPSC differentiation towards an osteogenic lineage and exhibited antimicrobial and antifungal activity. The adsorption of the compounds on a hydroxyapatite-chitosan biomaterial showed no indirect nor direct cytotoxicity on human foetal fibroblasts, as well as improved osteoblast adhesion and proliferation, suggesting that this strategy can represent a promising alternative to conventional therapeutics for regenerative medicine applications.

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References

1) Balaha, M.; Cataldi, A.; Ammazzalorso, A.; Cacciatore, I.; De Filippis, B.; Di Stefano A.; Maccallini, C.; Rapino, M.; Korona-Glowniak, I.; Przekora, A.; di Giacomo, V. CAPE derivatives: Multifaceted Agents for Chronic Wound Healing. Archiv der Pharmazie (accepted for publication)

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The effects of the pesticide Lindane on mouse oocytes: an ultrastructural study

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Exposure to environmental toxicants can impact ovarian follicles, affecting the female reproductive health and influencing that of the offspring. Lindane is one of the most persistent organochlorine pesticides, classified as an endocrine disruptor, and is associated with numerous pathologies of the female reproductive system, including infertility. Lindane is a Cx43 gap-junction blocker that can disrupt folliculogenesis by eliminating the oocyte-directed follicle-organizing activity [1]. *In vitro* studies demonstrated that concentrations of Lindane between 10 and 100 μM affect the first meiotic spindle formation and the polar body extrusion in mice [2]. Its reproductive toxicity likely stems from the gap-junction blocker activity on Granulosa Cells (GCs) or affects intracellular calcium homeostasis, maturation-promoting factor (MPF) activity, and mitotic spindle formation. Recent results evidenced ultrastructural alterations in mouse parietal GCs, exposed to concentrations of Lindane ranging from 1 to 100 μM, as nuclear membrane invagination, cytoplasmic blebbing, reduction of microvilli, intercellular connections and cellular debris [3]. In this study, we evaluated the ultrastructural effects of Lindane on MII-stage mouse oocytes cultured *in vitro* with or without (control) increasing doses of Lindane (1, 10, 100 μM), by using Light and Transmission Electron Microscopies. At both 1 μM and 10 μM concentrations, oocytes showed an intact zona pellucida and an irregular microvilli distribution, compared to controls. Numerous mitochondria and vacuoles were dispersed in the cytoplasm, with few visible cortical granules. At the highest concentration, oocytes exhibited an irregular or reduced distribution of microvilli, a decreased presence/distribution of the organelle and an extensive vacuolization. Differently from the previous groups, hooded mitochondria were observed. Cortical granules were drastically reduced and autophagic vesicles were abundant; these are signs of impaired function of oolemma peripheral domain. These morphological changes may help to elucidate the harmful action of Lindane on female reproduction and its potential impact on fertility.

References

1) Li, R.; Mather, J.P. Lindane An Inhibitor of Gap Junction Formation, Abolishes Oocyte Directed Follicle Organizing Activity in Vitro. Endocrinology. 1997, 38, 4477–80.
2) Picard, A.; Pahlavan, G.; Robert, S.; Pesando, D.; Ciapa, B. Effect of Organochlorine Pesticides on Maturation of Starfish and Mouse Oocytes. Toxicol. Sci. 2003, 73, 141–8.
3) Palmerini, M.G.; Zhurabekova, G.; Balmagambetova, A.; Nottola, S.A.; Miglietta, S.; Belli, M.; Bianchi, S.; Cecconi, S.; Di Nisio, V.; Familiari, G.; Macchiarelli, G. The Pesticide Lindane Induces Dose-Dependent Damage to Granulosa Cells in an in Vitro Culture. Reprod. Biol. 2017, 17, 349-356.

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Exploring the expression pattern of Trop-2 and stemness markers in human amniotic membrane obtained from healthy women

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First discovered in trophoblast cells of placenta, Trop-2 (Trophoblast cell-surface antigen 2) is a transmembrane glycoprotein expressed in normal epithelia and overexpressed in most human carcinomas (1). Because upregulated Trop-2 correlates with tumor aggressiveness and growth, it has become a prognostic marker and a therapeutic target. Interestingly, while in normal adult epithelia, Trop-2 is present in its unprocessed full-length form, it was recently found that the protein can be activated by a proteolytic cleavage at the R87-T88 site (2). This is a cancer-specific phenomenon that promotes tumor progression and metastasis. Here, we propose the human amniotic membrane (hAM) as a model to investigate Trop-2 expression/activation in stem cells, given the stem-cell-like properties of human Amniotic Epithelial Cells (hAECs) (3). According to our previous mapping of hAM (3), we report a comprehensive overview of Trop-2 expression across the four hAM areas, with the aim to evaluate whether the presence of the full-length/cleaved forms of Trop-2 could be different across the hAM regions and somewhat related to the expression of common pluripotency markers. Thus, human placentas were collected from three healthy women (mean age ± SD 33.6±4.8), undergoing caesarian section at the SS. Annunziata Hospital of Chieti, and embedded in OCT to obtain frozen tissue samples preserving antigenic expression. Confocal multi-color immunofluorescence and Western blotting analyses were performed by using anti-Trop-2 antibodies directed against either an immunodominant pan-Trop-2 epitope or the cancer-activated cleaved form in combination with antibodies directed against pluripotency markers. Our findings support a new scenario where Trop-2 shows a heterogenous and specific pattern of expression/activation across the 4 different areas of human amniotic membrane and in relation with stemness markers.

References

1) Trerotola, M.; Cantanelli, P.; Guerra, E.; Tripaldi, R.; Aloisi, A.L.; Bonasera, V.; Lattanzio, R.; de Lange, R.; Weidle, U.H.; Piantelli, M.; Alberti, S. Upregulation of Trop-2 quantitatively stimulates human cancer growth. Oncogene. 2013, 10;32(2):222-33.
2) Trerotola, M.; Guerra, E.; Ali, Z.; Aloisi, A.L.; Ceci, M.; Simeone, P.; Acciarito, A.; Zanna, P.; Vacca, G.; D'Amore, A.; Boujnah, K.; Garbo, V.; Moschella, A.; Lattanzio, R.; Alberti, S. Trop-2 cleavage by ADAM10 is an activator switch for cancer growth and metastasis. Neoplasia. 2021,23(4):415-428.
3) Centurione, L.; Passaretta, F.; Centurione, M.A.; Munari, S.; Vertua, E.; Silini, A.; Liberati, M.; Parolini, O.; Di Pietro, R. Mapping of the Human Placenta: Experimental Evidence of Amniotic Epithelial Cell Heterogeneity. Cell Transplant. 2018;27(1):12-22.

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The proper interplay between the expression of Spo11 splice isoforms and the structure of the pseudoautosomal region promotes XY chromosomes recombination

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XY chromosome missegregation is relatively common in humans and can lead to sterility or the generation of aneuploid spermatozoa. A leading cause of XY missegregation in mammals is the lack of formation of double-strand breaks (DSBs) in the pseudoautosomal region (PAR), a defect that may occur in mice due to faulty expression of Spo11 splice isoforms. Using a knock-in (ki) mouse that expresses only the single Spo11β splice isoform, here we demonstrate that by varying the genetic background of mice, the length of chromatin loops extending from the PAR axis and the XY recombination proficiency varies. In spermatocytes of C57Spo11βki/– mice, in which loops are relatively short, recombination/synapsis between XY is fairly normal. In contrast, in cells of C57/129Spo11βki/– males where PAR loops are relatively long, formation of DSBs in the PAR (more frequently the Y-PAR) and XY synapsis fails at a high rate, and mice produce sperm with sex-chromosomal aneuploidy. However, if the entire set of Spo11 splicing isoforms is expressed by a wild type allele in the C57/129 background, XY recombination and synapsis is recovered. By generating a Spo11αki mouse model, we prove that concomitant expression of SPO11β and SPO11α isoforms, boosts DSB formation in the PAR. Based on these findings, we propose that SPO11 splice isoforms cooperate functionally in promoting recombination in the PAR, constraining XY asynapsis defects that may arise due to differences in the conformation of the PAR between mouse strains.

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Involvement of nuclear Phospholiapse C beta 1 (PLCβ1) in epigenetic regulation in Acute Promyelocytic Leukemia (APL)

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Acute Promyelocytic Leukemia (APL) is a rare subtype of acute myeloid leukemia, predominantly characterized by the presence of the PML-RAR-alpha fusion gene transcript. While the combination of All Trans Retinoic Acid (ATRA) and Arsenic Trioxide (ATO) successfully induces complete remission in most APL cases, a subset of high-risk, relapsed, and refractory patients exhibit resistance to these therapies. This resistance remains a significant challenge across all prognostic subgroups of APL patients, underscoring the need for new therapeutic strategies tailored to this patient category. This study aimed to tackle this challenge by targeting the epigenetic signaling pathway involving PLCβ1 and epigenetic modulators, which regulates H3K9me3 levels and influences the leukemic cells transcriptome. Using the NB4 promyelocytic cell line and its ATRA-resistant derivative, R4, we employed lentiviral vectors to silence the expression of PLCβ1. Initially, low cell survival rates following viral infection necessitated the optimization of viral production and transduction protocols. Our focus was to enhance the efficiency of these processes without compromising cell viability, thus enabling the assessment of gene silencing effects on leukemic cell growth and differentiation. Our results indicate that silencing PLCβ1 leads to decreased cell growth, increased susceptibility to treatments, and enhanced cell differentiation. Understanding these pathways could identify potential molecular targets for developing secondary therapies to overcome ATRA resistance in APL patients. These findings provide a foundation for future research aimed at improving treatment outcomes for high-risk and resistant APL patient subgroups.

References

1) Yokoyama Y. Risk factors and remaining challenges in the treatment of acute promyelocytic leukemia. Int J Hematol. 2024 Feb 22. doi: 10.1007/s12185-023-03696-7. Epub ahead of print. PMID: 38386203
2) Monaghan L, Massett ME, Bunschoten RP, Hoose A, Pirvan PA, Liskamp RMJ, Jørgensen HG, Huang X. The Emerging Role of H3K9me3 as a Potential Therapeutic Target in Acute Myeloid Leukemia. Front Oncol. 2019 Aug 2;9:705. doi: 10.3389/fonc.2019.00705. PMID: 31428579; PMCID: PMC6687838.
3) Ratti S, Evangelisti C, Mongiorgi S, De Stefano A, Fazio A, Bonomini F, Follo MY, Faenza I, Manzoli L, Sheth B, Vidalle MC, Kimber ST, Divecha N, Cocco L, Fiume R. "Modulating Phosphoinositide Profiles as a Roadmap for Treatment in Acute Myeloid Leukemia". Front Oncol. 2021 May 24;11:678824. doi: 10.3389/fonc.2021.678824. eCollection 2021. PMID: 34109125 Free PMC article. Review.

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Influence of mesenchymal stromal cell extracellular vesicles on myofiber repair and regeneration

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Mechanical stresses and myofiber fragility caused by physical activity, aging and/or genetic diseases can seriously damage the skeletal muscles. The process of myofiber repair and regeneration requires a highly coordinated mechanisms based on the action of various intercellular factors such as proteins, cytokines, miRNA and membrane lipids mainly released by extracellular vesicles (EVs). Although the cellular and molecular mechanisms in skeletal muscle regeneration are well known, the influence of EVs in the intercellular communications to coordinate the repair and regeneration of injured myofibers is still under investigation. Recently, it has been suggested that mesenchymal stromal cells (MSCs) showed a great potential in treating muscular damage due to their paracrine factors. However, the role of EVs released by MSCs in myogenic repair and regeneration has not been extensively studied. The aim of this study was to investigate the role of EVs isolated from MSCs on muscle repair and regeneration.

EVs were isolated from cell culture medium by liquid exchange method based on polyethylene glycol precipitation protocol. The EVs isolated from control, differentiating murine myoblasts and MSCs, were characterized for their size, membrane markers and presence of myokines by electron microscopy, western blot, Elisa assays and proquantum immunoassays. EVs isolated from MSCs were then added to the medium of damaged differentiated myoblasts to investigate their influence on the process of myofiber repair. The ability to produce myotubes was investigated by inverted light microscopy and the expression of the muscle differentiation markers MyoD and myogenin were evaluated by western blot.

Results showed the ability of damaged myoblasts to release inflammatory factors enclosed in EVs and the influence of EVs isolated by MSCs in reducing the inflamed microenvironment and promoting the myogenic repair and regeneration.

In conclusion, our data demonstrated the powerful role of MSC - EVS in regulating myogenic differentiation. In the future, we aim to investigate treatments, such as photobiomodulation, that could increase the release of specific myokines to accelerate the repair and regeneration of injured muscle.

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Stem cells from second trimester and full-term amniotic fluid: differentiation potential, proteome and miRNome analysis

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Amniotic fluid (AF) is now known to harbor highly potent stem cells, making it an excellent source for cell therapy. However, most of the studies employs stem cells isolated are from AF of second trimester in which the collection procedure involves an invasive technique termed amniocentesis. This has limited the access in getting the fluid as this technique imposes certain level of risks to the mother as well as to the fetus, therefore it has been replaced by other non-invasive techniques. Alternatively, getting AF from full-term pregnancies during Caesareum deliveries would be a better resolution. The question remains whether full-term AF harbors stem cells of similar potency as of the stem cells of mid-term AF. We collected mesenchymal stem cells derived from the amniotic fluid (hAFSCs) obtained from both the second and the third trimester, to compare these different amniotic cell populations and their secretome. Stemness characteristics and capability were evaluated. Stem cells derived from amniocentesis (Amnio cells) are probably more potent than the AF mesenchymal stem cells isolated from full-term AF (Caesareum cells). Then, we analyzed extracellular vesicles (EVs) secreted by hAFSCs: proteomic analysis and miRNA expression profiling were carried out. The major part of EV proteins was shared by the two groups suggesting a similar protein pattern for Caesareum-EVs and Amnio-EVs, even if a minor part of proteins are unique for each group. miRNA expression analysis highlighted that most of the highest and commonly expressed miRNAs are mainly involved in anti-cancer effects. Moreover, many of the high expressed miRNA in both groups can alleviate oxidative stress. Since distorted redox signaling pathways orchestrate pathologic events inside cancer cells, we can propose that EVs from amniotic cells obtained at mid and term of pregnancy could be both an interesting tool to counteract diseases involving redox unbalance such as tumors.

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Scinderin expression in human placenta and possible role in preeclampsia

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Scinderin (a name derived from the Latin "scindere" meaning "to cut") was first described in chromaffin cells and it is expressed in tissues with high secretory activity (1). Scinderin is a Ca2+-dependent actin-binding proteins that regulates the dynamics of actin filament length (1), suggesting a critical role in the reorganization of actin filaments. The placenta is a unique, autonomous and transient organ that ensures maternal-fetal exchanges. The human placenta is characterized by the intensity and the specificity of its endocrine functions. In addition, placental hormones are required for the establishment and maintenance of pregnancy (2). The endocrine tissue of the placenta is the syncytiotrophoblast, which covers the chorionic villi, and arises from the fusion of the cytotrophoblasts. Preeclampsia, a serious complication of pregnancy, involves hormones, complements, and cytokines contributing to placental and endothelial dysfunction with inflammation (3). We have analysed the expression of scinderin in normal human placentas of first and third trimester of gestation and in placentas complicated with preeclampia. In addition, we investigated the role of cytokines, of oxidative stress and hypoxia, that are associate with preeclampsia, in an in vitro model of placenta trophoblast. The data demonstrated that the scinderin in expressed in the villous cytotrophoblast of the placenta both in normal and pathological conditions and that its expression increased in preeclampsia. The in vitro results suggested that the increase of scinderin expression in pathological conditions could be related to oxidative stress and the TNF-alpha stimulus. In conclusion, these data suggest that scinderin could be a molecule involved in the onset of preeclampsia and that could be play a significant role in molecular process underlying the development of this pathology.

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References

1) J-M. Trifaró, S. D. Rosé, and M. G. Marcu. Scinderin, a Ca2+-Dependent Actin Filament Severing Protein that Controls Cortical Actin Network Dynamics During Secretion. Neurochemical Research, Vol. 25, No. 1, 2000, pp. 133–144.
2) Pathology of the Human Placenta, Kurt Benirschke, Peter Kaufmann, 2000, Iv edition, Springer
3) Yi-Ting Chiang, Kok-Min Seow, Kuo-Hu Chen. The Pathophysiological, Genetic, and Hormonal Changes in Preeclampsia: A Systematic Review of the Molecular Mechanisms. Int J Mol Sci 2024 Apr 20;25(8):4532.

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Synergic role of IL-6 and PD-L1 in affecting biological properties of cholangiocytes when exposed to inflammatory conditions

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Primary damage to cholangiocytes and/or alterations of the biliary epithelial tissue can contribute to the development of cholangiopathies[1,2]. Particularly, proinflammatory signals play a crucial role in the establishment of pathophysiology conditions. In this regard, we studied the immunomodulatory properties of human H69 in an inflammatory microenvironment, mimicked in vitro by an indirect co-culture system with PBMCs activated with anti-CD-3/CD-28 antibodies (aPBMCs).

Immunomodulatory pathways (PD-1/PD-L1), cell cycle, and senescence markers were investigated in H69 by q-PCR, Western Blot, and Immunofluorescence analyses.

Data showed an up-regulation of IL-6, NF-kB/p65, and PD-L1, a cell cycle arrest, and an epithelial-mesenchymal transition in H69 after 48 h of inflammatory stimuli. Moreover, a decreased expression of pro-inflammatory cytokines was detected in aPBMCs after H69 co-culture, probably due to the compensatory pathways that synergistically allow H69 to modulate the inflammatory microenvironment and support fibrosis establishment and neoplastic progression of cholangiopathies. The interaction between NFkB/p65 and pSTAT3 such as IL-6/PD-L1 correlation in inflammatory conditions was supported by exposing H69 to the IL-6/sIL-6r complex in aPBMCs co-culture. IL-6/sIL-6r alone wasn't able to induce an up-regulation of PD-L1, which was observed in H69 after aPBMCs co-culture with IL-6/sIL-6r. Interestingly, IL-6 enhanced the PD-L1 up-regulation in H69 when the inflammatory milieu was established, likely due to PD-L1 stabilization at a post-transcriptional level.

Based on the experimental evidence described above, H69 cells were treated with a selective IL-6 receptor inhibitor for 48 h. WB analysis confirmed that H69 cells showed a decreased expression of all the analyzed markers when treated with IL-6 receptor inhibitor resembling those of H69 cultured alone, suggesting the synergic role of IL-6, PD-L1, and NFkB/p65 in promoting immunomodulatory effects of H69 under inflammatory conditions.

Our results pave the way for future insights into pathophysiological mechanisms responsible for cholangiopathies to provide the basis for new therapeutic tools.

References

1) Carpino, G.; Cardinale, V.; Folseraas, T.; Overi, D.; Grzyb, K.; Costantini, D.; Berloco, P. B.; Di Matteo, S.; Karlsen, T. H.; Alvaro, D.; Gaudio, E. Neoplastic Transformation of the Peribiliary Stem Cell Niche in Cholangiocarcinoma Arisen in Primary Sclerosing Cholangitis. Hepatology 2019, Feb; 69(2):622-638.
2) Macias, R. I. R.; Cardinale, V.; Kendal, T. J.; Avila, M. A.; Guido, M.; Coulouarn, C.; Braconi, C.; Frampton, A. E.; Bridgewater, J.; Overi, D.; Pereira, S. P.; Rengo, M.; Kather, J. N.; Lamarca, A.; Pedica, F; Forner, A.; Valle, J. W.; Gaudio, E.; Alvaro, D.; Banales, J. M.; Carpino, G. Clinical relevance of biomarkers in cholangiocarcinoma: critical revision and future directions. Gut. 2022, Aug;71(8):1669-1683.

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RXRA signaling induces terminal differentiation and apoptosis in Acute Myeloid Leukemia

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Acute Myeloid Leukemia (AML) is the deadliest form of leukemia both in adults and children. This dismal survival rate depends mostly on the high rate of chemoresistance. Therefore, less toxic and more efficient therapies are needed.

Retinoids are bioactive metabolites of vitamin A that bind to their cognate nuclear receptors to engage transcriptional programs that influence cell growth, differentiation, and death. All-trans-retinoic acid (ATRA) has been a transformative therapy in acute promyelocytic leukemia (APL), improving patient survival from ~10% to greater than 90%. On the contrary, ATRA treatment in non-APL forms of AML has shown highly variable results. However, multiple groups have hypothesized that molecular phenotypes may correlate with responses.

Our previous studies have revived interest in RXRA signaling in AML. We demonstrated that, in AML driven by KMT2A-MLLT3, rexinoids partially suppressed AML growth and triggered differentiation. Moreover, genetic ablation of RXR accelerated AML growth, while concomitant activation of both RXRA and RARA precipitated differentiation or apoptosis. In addition, we have identified an activating mutation in RXRA ($RXRA^{DT448/9PP}$), which potently activates rexinoid/retinoid downstream signaling and suffices to induce terminal differentiation of KMT2A-MLLT3-transformed cells.

Here we demonstrate that only $RXRA^{WT}$ but not our $RXRA^{DT448/99PP}$ mutant gives rise to a 36kDa truncated form of RXRA (tRXRA), which is known to have unique pro-tumorigenic molecular properties. We observed that tRXRA expression varies throughout the different AML subtypes and negatively correlates with retinoid-induced differentiation.

These intriguing observations imply that even though rexinoids and retinoids synergize for myeloid differentiation of AMLs, more profound "unconventional" activation of RXRA can initiate terminal differentiation, and tRXRA can be a new valuable target to enhance retinoid response in specific AML subtypes.

This study gives a deeper and revolutionary look at the mechanisms that govern ATRA-effectiveness (or lack thereof) in AML and smooths the path to developing novel anti-leukemia therapeutic strategies.

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Morphological modifications of portal tracts and hepatic sinusoids in people with porto-sinusoidal vascular disorder

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Porto-sinusoidal vascular disorder (PSVD) is a liver condition characterized by the development of lesions involving portal veins and sinusoids in absence of cirrhosis. This term comprises a spectra of liver alterations and clinical/pathophysiological entities, and was introduced in order to improve histological and clinical diagnosis. We performed an evaluation of the morphological alterations affecting the livers of people with PSVD and compared those observed in patients with or without portal hypertension (PH).

Blank slides of liver biopsies from N=30 subjects (N=13 with and N=17 people without portal hypertension, respectively) were obtained from Policlinico Umberto I, San Camillo Hospital and Humanitas University. Sections were stained with hematoxylin/eosin and Sirius red to assess histology and fibrosis. Immunohistochemistry for CD34, and CD42b was performed. Normal liver samples (N=6) from healthy organ donors were used as controls.

Signs of obliterative portal venopathy (OPV) were observed in all PSVD patients. When the percentage of affected portal tracts was evaluated, patients with PH showed significantly more frequent OPV and portal inflammation compared to those without PH (p=0.011 and p=0.001, respectively). Septal fibrosis was less frequently observed in PSVD patients without PH compared to those with PH (p=0.019). Interestingly, sinusoid dilation and perisinusoidal fibrosis were comparable between the two groups. In keeping with this, sinusoid capillarization (i.e. CD34+ endothelial cells in sinusoids) was not different between PSVD patients with and without PH. Finally, the number of platelets within liver sinusoids was slightly higher in PSVD patients without PH compared to those with PH.

Our results show how, in PSVD, hepatic sinusoids alterations develop independently from OPV. The clarification of pathophysiological events leading to the development of portal tract injury in patients with PSVD can improve the understanding of this recently-classified condition, and can help stratify the risk of complications in these patients.

References

1) De Gottardi A.; Sempoux C.; Berzigotti A. Porto-sinusoidal vascular disorder. J Hepatol 2022; 77(4):1124-1135.
2) Gioia S.; Carnevale R.; Tavano D.; Overi D.; Ridola L.; Nardelli S.; et al. Association between gut-derived endotoxins and porto-sinusoidal vascular disorder with portal hypertension. Aliment Pharmacol Ther 2023;58(11-12):1205-1216.

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Morpho-functional changes of nuclear envelope in osteosarcoma

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Osteosarcoma (OS) cells exhibit a variety of features indicative of dysmorphic nuclei[1]. Although nuclear alterations have been served as diagnostic parameters for years, recent advances highlighted that complex regulatory mechanisms lie behind the alterations of nuclear envelope (NE) in cancer, thus opening the field to novel investigations. The linker of nucleoskeleton and cytoskeleton (LINC) complex, which consists mainly of SUN and nesprin proteins, mediates the interactions between lamins and cytoskeletal filaments, thus regulating not only the size and shape of the nucleus, but also mechanosignaling transduction, thereby influencing cytoskeleton dynamics and cell migration, two major dysregulated mechanisms in cancer[2].

We firstly performed an *in silico* analysis exploiting available datasets, showing a significant lower expression of *LMNA* and *EMD* genes, and alterations of NE components SUNs in osteosarcoma patients. These alterations in gene expression prompted us to perform deeper *in vitro* analysis employing a panel of OS cell lines and normal human osteoblasts (NHOst). Western blotting analysis confirmed reduced protein levels of lamin A/C and increased levels of SUN1 in OS cells compared to NHOst, while SUN2 protein amount was generally low. Immunofluorescence analyses demonstrated that lamin A/C, emerin and SUN proteins missed their typical localization and were localized also in the cytoplasm, although nuclear rim localization was partially maintained.

Being LINC complex proteins involved also in regulating mesenchymal stem cell commitment, we evaluated their expression and localization during normal and pathological differentiation. Our results showed a strong increase of lamin A, SUN1, Nesprin 2, and emerin in both settings during differentiation, accompanied by a partial re-localization of NE proteins in OS cells.

Our data demonstrate that NE proteins alterations have functional implications in tumor progression, and these results warrant further investigation to better characterize how NE alterations are related to cancer progression.

Keywords: nuclear envelope, osteosarcoma, nuclear alterations, cell differentiation

References

1) Evangelisti C, Paganelli F, Giuntini G, Mattioli E, Cappellini A, Ramazzotti G, et al. Lamin A and Prelamin A Counteract Migration of Osteosarcoma Cells. Cells. 2020;9:774.
2) Bell ES, Lammerding J. Causes and consequences of nuclear envelope alterations in tumour progression. Eur J Cell Biol. 2016;95:449–64

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Ultrastructural study on the effects of Menstrual Blood-Derived Stem Cells and Exosomes on ovarian folliculogenesis in PCOS rats

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Polycystic ovary syndrome (PCOS) is a metabolic disorder responsible for irregular folliculogenesis and associated with infertility. Stem cell therapy is a promising approach for treating the syndrome and restoring damaged tissues. Specifically, the use of menstrual blood-derived stem cells (MenSCs) in xenogeneic animal models is becoming an innovative method for evaluating their efficacy in restoring ovarian function. This approach provides crucial preclinical data needed for designing clinical trials for PCOS patients [1]. In this study, to induce PCOS in female rats, 1 mg/kg letrozole dissolved in 1% carboxymethylcellulose (CMC), was orally administrated for 21 consecutive days. Animals were then divided into six groups: 1) Control (without intervention); 2) Sham (receiving CMC orally); 3) PCOS (received letrozole 1 mg/kg); 4) PCOS-Sham (PCOS ovaries were punctured without any administration); 5) PCOS-MenSCs (PCOS ovaries were injected with 2×106 MenSCs) and 6) PCOS-MenSCs-Exo (PCOS ovaries injected with MenSCs-derived exosomes) groups [2]. After collection, ovaries were fixed and subjected to the standard preparative for Light and Transmission Electron Microscopy [3]. Ovaries from the Control and Sham groups showed normal morphology, with numerous growing follicles and corpora lutea in the cortex and a rich vascularization. The PCOS and PCOS-Sham groups showed also follicular cysts; follicular cells were rich in lysosomes, multivesicular bodies, autophagic bodies, and vacuolated mitochondria. Differently, in the PCOS-MenSCs and PCOS-MenSCs-Exo groups, the ovarian structure appeared more similar to controls. Follicular cells were rich in organelles, including well-preserved mitochondria. Ultrastructural alterations, such as enlarged cisternae of the endoplasmic reticulum or vacuolization, were occasionally observed. In conclusion, these preliminary results suggest that the administration of MenSCs and MenSC-derived exosomes could have a positive impact on restoring ovarian function.

References

1) Prockop, D.J.; Oh, J.Y.; Lee, R.H. Data against a common assumption: xenogeneic mouse models can be used to assay suppression of immunity by human MSCs. Mol Ther. 2017, 25, 1748–56.
2) Roodbari, A.S.; Solhjoo, S.; Palmerini, M.G.; Mansouri, M.; Ezzatabadipour, M. The effect of human menstrual blood-derived stem cells on ovarian folliculogenesis, angiogenesis and collagen volume in female rats affected by the polycystic ovary syndrome. J Ovarian Res. 2023, 22, 16–170.
3) Belli, M.; Zhang, L.; Liu, X.; Donjacour, A.; Ruggeri, E.; Palmerini, M.G.; Nottola, S.A.; Macchiarelli, G.; Rinaudo, P. Oxygen concentration alters mitochondrial structure and function in in vitro fertilized preimplantation mouse embryos. Hum. Reprod. 2019, 34, 601–611.

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Red photobiomodulation promotes skeletal myoblast differentiation and counteracts TGF-β1' anti-myogenic effects *in vitro*: evidence from morpho-functional analyses

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Adult skeletal muscle regenerates lost damaged tissue mainly thanks to the activity of a population of resident stem cells namely satellite cells (SCs). In the case of chronic or severe damage, SCs' functionality may be compromised by the occurrence of an aberrant fibrotic reparative response[1]. Strategies aimed to improve the muscle intrinsic regenerative capacity while limiting the excessive deposition of fibrotic tissue may be promising. In this perspective, photobiomodulation (PBM) (i.e. application of light with 400-1100 nm wavelength using different laser or LED devices, power density less than 100 mW/cm^2 and energy density less than 10 J/cm^2 at target) may represent a valid option based on its well-known pro-regenerative effects and increasing evidence of its antifibrotic potential. However, PBM's effects on skeletal muscle are controversial and there are no univocal guidelines for its use[2,3]. To this aim we evaluated the effects of different treatments of red PBM (laser diode 635±5 nm, energy density: 0.4, 4 and 8 J/cm^2, single exposure) on murine C2C12 myoblasts undergoing differentiation in the presence or absence of TGF-β1 (2ng/ml) for 24, 48 and 72h and on differentiated myotubes (72h). Morphological analyses revealed that red PBM with 4 J/cm^2 energy density improved myoblast differentiation and did not affect viability and features of differentiated myotube. Red PBM with 4 J/cm^2 energy density was able to counteract the anti-myogenic action of transforming growth factor (TGF)-β1. These results were corroborated by electrophysiological recordings of membrane passive properties and ion currents.

This study provides experimental evidence for the pro-myogenic effects of red PBM and the essential groundwork for further investigation.

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References

1) Guilhot, C.; Catenacci, M.; Lofaro, S.; Rudnicki, M.A. The satellite cell in skeletal muscle: A story of heterogeneity. Curr Top Dev Biol. 2024,158:15-51.
2) Fisher, S.R.; Rigby, J.H.; Mettler, J.A.; McCurdy, K.W. The Effectiveness of Photobiomodulation Therapy Versus Cryotherapy for Skeletal Muscle Recovery: A Critically Appraised Topic. J Sport Rehabil. 2019 Jul 1;28(5):526-531.
3) Shepherd, D.W.; Norris, J.M.; Simpson, B.S.; Player, D.J.; Whitaker, H.C. Effects of photobiomodulation therapy on regulation of myogenic regulatory factor mRNA expression in vivo: A systematic review. J Biophotonics. 2022 Feb; 15(2):e202100219.

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The influence of antiretroviral therapy on adipocyte differentiation

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The aim of this study was to investigate how antiretroviral drugs influence adipocyte differentiation using an in vitro adipogenesis model. We focused on some integrase inhibitors (CAB, DOR, RPV), administered individually or in combination, as well as two nucleoside reverse transcriptase inhibitors (NRTIs) (TAF and TDF) in combination with specific integrase inhibitors. For the experiments, we used 3T3-L1 cells, a commonly utilized cell line for studying adipocyte differentiation (1). The cells were cultured in differentiation medium for eight days (2, 3). Antiretroviral drugs were added to the differentiation medium at a concentration of 30 µg/ml on the first day and administered daily until the fourth day of differentiation (4). Red Oil O Staining evaluated intracellular lipid accumulation, while western blotting measured the expression levels of specific differentiation markers (PPAR and C/EBPα). Each integrase inhibitor induced adipocyte differentiation, evidenced by increased lipid droplet formation and up-regulation of PPAR and C/EBPα compared to the control group. Combination treatments of integrase inhibitors showed a synergistic effect, resulting in enhanced adipocyte differentiation and morphological changes indicative of a shift towards the adipocyte phenotype. Inhibition of adipocyte differentiation was observed with the TAF+RPV combination, marked by a reduction in lipid droplets and down-regulation of PPAR and C/EBPα compared to the control group. A similar but less pronounced inhibition was noted with the TDF+DOR combination. The study demonstrated that both integrase inhibitors and nucleotide analogues significantly influence adipocyte differentiation in 3T3-L1 cells. Combination treatments had more pronounced effects than single drug treatments, indicating a synergistic effect. Notably, TAF acted as an antagonist when combined with RPV and DOR, inhibiting adipocyte differentiation. These findings underscore the complex effects of antiretroviral drugs on adipose tissue biology and highlight the need for further research to elucidate the underlying mechanisms of these interactions.

References

1) Morrison, S. and S.L. McGee, 3T3-L1 adipocytes display phenotypic characteristics of multiple adipocyte lineages. Adipocyte, 2015. 4(4): p. 295-302.
2) Esposito, V., et al., Role of FAP48 in HIV-associated lipodystrophy. J Cell Biochem, 2012. 113(11): p. 3446-54.
3) Esposito, V., et al., Role of NEDD8 in HIV-associated lipodystrophy. Differentiation, 2009. 77(2): p. 148-53.
4) Perna, A., et al., Adipocyte differentiation of 3T3-L1 cells under tenofovir alafenamide, tenofovir disoproxil fumarate, and integrase strand transfer inhibitors selective challenge: an in-vitro model. Aids, 2023. 37(4): p. 561-570.

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Unique morphological, clinical, and molecular profile of blast phase occurring directly from polycythemia vera

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Polycythemia vera (PV) is a *JAK2*-mutated myeloproliferative neoplasm (MPN) characterized by clonal erythrocytosis and an intrinsic risk of transformation into acute myeloid leukemia (AML), termed blast-phase (BP). BP generally occurs as a secondary event following the fibrotic progression of PV, known as the post-PV myelofibrotic (MF) stage. However, in around one-half of patients, BP occurs directly from the florid PV stage. In this study, we describe the morphological, clinical, and molecular characteristics that typify BP resulting directly from PV (post-PV-BP) compared to BP evolving from post-PV myelofibrosis (post-PV-MF-BP), retrospectively analyzing histopathological, laboratory, and molecular data of a cohort of post-PV-BP (n=5) and post-PV-MF-BP (n=5).

The most significant differences between these two groups were revealed through analysis of bone marrow (BM) morphology: all post-PV-BP displayed significantly higher cellularity (median 70%, range: 60%–98% vs. 28%, range: 2%–41%, *P*=0.0245), and lower degree of fibrosis (*P*=0.008). Interestingly, dysplastic features involving all three myeloid lineages, most prominently the erythroid and megakaryocytic compartments, were observed in all post-PV-BP patients. By contrast, no sign of dysplasia was detectable in the post-PV-MF-BP (*P*=0.008).

Additionally, post-PV-BP showed significantly lower leukocyte count (*P*=0.03), and spleen diameter (*P*=0.03) compared to post-PV-MF-BP. Data from next-generation sequencing (NGS) analysis revealed that mutations located in genes involved in DNA methylation, including *DNMT3A*, *IDH1/ 2*, and *TET2*, were significantly more frequent in post-PV-BP (45% vs. 15%, *P*=0.038). Overall, our data suggest that BPs arising directly from PV show a peculiar bone marrow morphology and a dysplastic phenotype, consistent with the molecular signature of the disease, characterized by mutations of genes occurring with a high frequency in MDS and MDS/MPN. Further studies in larger cohorts are needed to confirm these findings and provide reliable guidance for treatment decisions.

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Effects of fossil-based and bio-based micro/nano-plastics on zebrafish histogenesis

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Plastic contamination (PLASTAMINATION) by non-biodegradable and biodegradable waste represents the main anthropogenic change in the biosphere. Microplastics (MPs) and nanoplastics (NPs) have become ubiquitous in the environment, contaminating ecosystems and reaching the human body through inhalation, ingestion, and dermal contact representing a potential risk for human and animal health. Recently, bio-based plastics such as polylactic acid (PLA) have been introduced as an 'eco-friendly' alterative to the fossil-based ones. However, their biosafety cannot be guaranteed due to the poor research in this field [1]. Thus, in the present study we investigated the potential harmful impact of PLA NPs on developing zebrafish, comparing it with the well-known harmful effects of their exposure to polystyrene (PS) MPs [2]. For this purpose, zebrafish embryos have been exposed to two different concentrations (0,1 and 1 mg/L) of both PS-MPs and PLA-NPs up to 120hpf. During this period, Zebrafish Embryo Acute Toxicity Test (ZFET) was performed to evaluate developmental alterations. Heart beat rate has been measured at 96 and 120hpf to assess physiological response. The distribution and accumulation of PLA-NPs and PS-MPs have been assessed by *in vivo* observation under fluorescence microscope. The morphological alterations have been evaluated through histological analysis. Finally, the inflammatory and oxidative stress marker expression has been assessed by RT-PCR analysis. The results of the study report preliminary data on toxicological potential of PLA, suggesting that the ecological risk related to its use requires further investigation.

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References

1) Lamparelli, E.P.; Marino, M.; Szychlinska, M.A.; Della Rocca, N.; Ciardulli, M.C.; Scala, P.; D'Auria, R.; Testa, A.; Viggiano, A.; Cappello, F.; Meccariello, R.; Della Porta, G.; Santoro, A. The Other Side of Plastics: Bioplastic-Based Nanoparticles for Drug Delivery Systems in the Brain. Pharmaceutics. 2023, 15, 2549.
2) Santoro, A.; Marino, M.; Vandenberg, L.N.; Szychlinska, M.A.; Lamparelli, E.P.; Scalia, F.; Rocca, N.D.; D'Auria, R.; Giovanna Pastorino, G.M.; Porta, G.D.; Operto, F.F.; Viggiano, A.; Cappello, F.; Meccariello, R. PLASTAMINATION: Outcomes on the Central Nervous System and Reproduction. Curr Neuropharmacol. 2024 Epub ahead of print.

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Unmasking a cholestatic signature: autophagy features and bile efflux genes could predict MASLD outcomes

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Metabolic dysfunction-associated Steatotic Liver Disease (MASLD) is characterized by excessive lipid accumulation within hepatocytes, often accompanied by liver inflammation and fibrosis, and may potentially lead to the development of hepatocellular carcinoma. Emerging evidence suggests that cholestasis in MASLD patients is a strong predictor of poor prognosis, indicating a potential role for impaired bile secretion in disease pathogenesis and progression. Autophagy, a cellular degradation pathway responsible for removing damaged organelles and protein aggregates, plays a crucial role in maintaining liver homeostasis. Within this process, lipophagy, the selective degradation of lipid droplets by lysosomes, and cholestophagy, the autophagic degradation of cholesterol, are essential for regulating lipid and bile acid levels, respectively. This study investigated the molecular and histological features of altered autophagy in MASLD patients without severe liver fibrosis. Blood samples were collected while liver biopsies underwent histological examination and gene expression analysis. Impaired autophagy was associated with cholestasis-related alterations in the biliary compartment, specifically ductular reaction and intermediate hepatobiliary cell expansion. Furthermore, an association between impaired autophagy and increased expression of bile efflux system-related genes, NR1H3 and NR1H4, was observed. Interestingly, in patients with a blood cholestatic profile, alkaline phosphatase levels did not correlate with ballooning, a key feature of MASLD-related hepatocyte damage. Instead, ballooning was associated with decreased expression of the bile efflux pumps genes, ABCG8 and ABCG5. These findings reveal a critical interplay between impaired autophagy, bile efflux system dysregulation, and morphological changes in cholestatic MASLD. Targeting these pathways holds promise for identifying patients at risk of disease progression and developing novel therapeutic strategies before severe fibrosis occurs.

References

1) Carotti S, Aquilano K, Valentini F, Ruggiero S, Alletto F, Morini S, Picardi A, Antonelli-Incalzi R, Lettieri-Barbato D, Vespasiani-Gentilucci U. An overview of deregulated lipid metabolism in nonalcoholic fatty liver disease with special focus on lysosomal acid lipase. Am J Physiol Gastrointest Liver Physiol. 2020 Oct 1;319(4): G469-G480.
2) Pennisi G, Pipitone RM, Cabibi D, Enea M, Romero-Gomez M, Viganò M, Bugianesi E, Wong VW, Fracanzani AL, Sebastiani G, Berzigotti A, Di Salvo F, Giannone AG, La Mantia C, Lupo G, Porcasi R, Vernuccio F, Zito R, Di Marco V, Cammà C, Craxì A, de Ledinghen V, Grimaudo S, Petta S. A cholestatic pattern predicts major liver-related outcomes in patients with non-alcoholic fatty liver disease. Liver Int. 2022 May;42(5):1037-1048.
3) Carotti S, Lettieri-Barbato D, Piemonte F, Ruggiero S, Rosina M, Zalfa F, Zingariello M, Arciprete F, Valentini F, Francesconi M, D'Amico J, De Vincentis A, Baiocchini A, Perrone G, Antonelli-Incalzi R, Morini S, Picardi A, Aquilano K, Vespasiani-Gentilucci U. Molecular and histological traits of reduced lysosomal acid lipase activity in the fatty liver. Cell Death Dis. 2021 Nov 18;12(12):1092.

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Mesenchymal Stromal Cell secretome and its evaluation in the treatment of Polycystic Ovary Syndrome

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Polycystic ovary syndrome (PCOS) affects worldwide about 10% of women of reproductive age and is characterized by hyperandrogenism, abdominal obesity, insulin resistance, type II diabetes, LH/FSH imbalance, and oligo-anovulation. Since its pathophysiology is very complex, no specific drugs have been approved so far. Any treatment should be chronic and adapted to the changing circumstances of each patient. However, accumulating evidence suggest that PCOS is a chronic low-grade inflammatory disease and mesenchymal stromal cells (MSCs) are known to counteract an inflammatory microenvironment. Moreover, the main abnormalities of PCOS are primarily ovarian. The present study aims to verify whether, in a mouse model of PCOS, the *in vivo* administration of culture media conditioned (CMs) by human MSCs derived from discarded tissues as adipose (ADSC) or dental pulp (DPSC) can counteract the hallmark symptoms of such disease. The final aim is to find specific molecules, mainly miRNAs and soluble factors, to which this recover capacity can be ascribed. In more detail, to test the therapeutic efficacy of such CMs on PCOS parameters *in vivo*, they were injected every other day for two weeks intravenously in prepuberal female mouse treated subcutaneously with the steroid hormone dehydroepiandrosterone (DHEA) for twenty-eight consecutive days. Histological and molecular analyses showed the efficacy of DHEA in inducing the PCOS phenotype. Moreover, a preliminary evaluation of the therapeutic effect of both CMs indicates a significant recovery by DPSC-CM of parameters such as body weight, estrous cycle, ovary morphology, follicular activation, insulin resistance, and AMH serum levels. Then, the DPSC-CM injection appears to mitigate the pathological alterations associated with PCOS. The analysis is now focused on the miRNome produced by both cell populations in order to evaluate the differential expression of specific miRNAs already known to be involved in the recovery of ovarian dysfunctions by counteracting oxidative stress and inflammation.

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Protective role of β3-adrenergic receptor agonist in hyperoxia-induced ileal injury: an in *vivo* model

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Fetuses develop in a hypoxic intrauterine environment (4%), and after birth, in ambient oxygen levels of 21%, the complete maturation of most organs occurs. The gut, being highly sensitive to oxygen, is particularly prone to developmental alterations if it is prematurely exposed to higher oxygen levels than those in utero [1]. One of the primary adverse consequences of hyperoxia is the uncontrolled production of reactive oxygen species, which severely damage the integrity of the intestinal epithelium [2]. The known antioxidant effect of β3-adrenergic receptors (β3-AR), its expression in relation to oxygen levels, and the established connection between β3-adrenergic activation and the prevention of hyperoxia-induced colon damage [3] serve as the basis for evaluating the potential of BRL37344, a selective β3-AR agonist, in protecting ileal development from the adverse effects of hyperoxia. To test this hypothesis, neonatal Sprague-Dawley rat pups were exposed to either 21% or 85% oxygen for 14 days and treated with the β3 agonist at doses 1, 3 or 6 mg/kg/day. Analyses revealed that the treatment with BRL37344 at 3 mg/kg completely prevented the increase of plasma 5-epi-5-F21-IsoP levels, a marker of oxidative stress, and counteracted the decrease in ileal cells expressing the β3 receptor. The latter effect was also observed with the lowest 1 mg/kg dose. Exposure to hyperoxia damaged the ileal mucosa and submucosa, leading to edema, leukocyte infiltration, disorganization of the brush border, loss of adherent junctions, increased mucus production, and compromised vascularization. The treatment of BRL37344 at 3mg/kg was able to protect against these alterations, indicating its potential clinical use in preventing ileal mucosal damage, the primary cause of necrotizing enterocolitis.

References

1. Chen CM, Chou HC. Hyperoxia disrupts the intestinal barrier in newborn rats. Exp Mol Pathol. 2016 Aug;101(1):44-9.
2. Liu DY, Lou WJ, Zhang DY, Sun SY. ROS Plays a Role in the Neonatal Rat Intestinal Barrier Damages Induced by Hyperoxia. Biomed Res Int. 2020 Dec 26; 2020:8819195
3. Filippi L, Nardini P, Zizi V, Molino M, Fazi C, Calvani M, Carrozzo F, Cavallaro G, Giuseppetti G, Calosi L, Crociani O, Pini A. β3 Adrenoceptor Agonism Prevents Hyperoxia-Induced Colonic Alterations. Biomolecules. 2023 Dec 6;13(12):1755.

Connective tissues

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Collagen fibrils in human tendons, is everything already said? A qualitative ultrastructural assessment

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Improving structural knowledge of human tendons is fundamental for a better understanding of pathological and regenerative processes occurring in these key structures. Recent advances in tendon biology indicate cellular heterogeneity between tendons of different muscles. In contrast, very little is known about potential differences in extracellular matrix in tendons across anatomy.

In the present work, we aimed to assess collagen fibril organisation ultrastructurally in human supraspinatus, semitendinosus, and quadriceps tendons, as well as in the anterior cruciate and patellar ligaments. Samples were obtained with appropriate permissions from seven different donors belonging to the Anatomy Gift Programme of the Royal College of Surgeons in Ireland in Dublin, that had been previously embalmed for routine anatomical examination.

Samples from the tendon mid-body were harvested and processed for Transmission Electron Microscopy (TEM).

We examined a total of 34 tendons (n=2 samples of each). Collagen fibrils were well recognisable in 32 tendons.

Overall, marked heterogeneity between samples from the same tendon type of different donors, or between the various tendons of the same donor, was observed in terms of collagen fibril shape (rounded vs more irregular-polygonal), size distribution, and width. The greatest similarity in fibril organisation was observed between patellar ligament and quadriceps tendon. The anterior cruciate ligament showed smaller, less rounded fibrils, which were more consistent in size. Supraspinatus also showed bundles perpendicular to the longitudinal tendon axis. Consistency in fibril organisation between both samples of the same individual tendon was observed in 26 cases.

This study demonstrates that ultrastructural analysis of collagen fibril is feasible in tissue from anatomical donors despite the embalming process not being a standard procedure of fixation for TEM examination. This first description also offers a basis for a more detailed and quantitative assessment of the collagen fibrils in human tendons.

References

1) Franchi, Marco, Trirè, Alessandra, Quaranta, Marilisa, Orsini, Ester, Ottani, Victoria. Collagen Structure of Tendon Relates to Function. *The Scientific World Journal*, 2007, 7, 132725.
2) Christopher K. Revell, Oliver E. Jensen, Tom Shearer, Yinhui Lu, David F. Holmes, Karl E. Kadler. Collagen fibril assembly: New approaches to unanswered questions. *Matrix Biology Plus*, 2021, 12, 100079.
3) Baldwin M, Buckley CD, Guilak F, Hulley P, Cribbs AP, Snelling S. A roadmap for delivering a human musculoskeletal cell atlas. *Nat Rev Rheumatol*. 2023 19, 738-752.

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Anti-CD94-induced expansion of highly functional adaptive NKG2C+ NKG2A– CD57+ self KIR+ NK cells from CMV-seropositive healthy donors

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Human Natural Killer (NK) cells are innate lymphocytes, short-living and providing rapid responses against viral infections and tumors. Adaptive NK cells are an NK cell subpopulation arising upon cytomegalovirus (CMV) infection, characterized by the expression of the CD94/NKG2C heterodimer (a HLA-E-specific activating receptor), by a highly mature $CD57^{+}KIR^{+}NKG2A^{-}$ phenotype and endowed with marked antitumor functions and prolonged lifespan[1]. In view of these traits, adaptive NK cells could be exploited in cell-based immunotherapies aimed at enhancing NK cell cytotoxicity against specific targets (e.g. through Chimeric Antigen Receptors (CAR)-engineering or in combination with cell engagers).

To this end, we developed a method to efficiently and specifically expand adaptive NK cells starting from NK-enriched cell preparations, obtained from the peripheral blood of selected CMV-seropositive healthy donors. Our system is based on the use of specific cytokines combined with an anti-CD94 monoclonal antibody that induces the selective expansion of adaptive CD94/NKG2C+ NK cells lacking the inhibitory heterodimer CD94/NKG2A.

The resulting expanded adaptive NK population consists in $NKG2C^{+}NKG2A^{-}$ NK cells, mainly co-expressing CD57 and a single self-KIR, already characterizing adaptive NK cells before the expansion. Moreover, expanded NK cells do not upregulate PD-1 and other immune checkpoints, but maintain the molecular signature of CMV-induced adaptive NK cells and their high ADCC abilities.

This protocol improves previous expansion methods, by eliminating genetically modified cells in the culture system as stimulus and avoiding unwanted upregulation of inhibitory receptors[2,3]. Thus, it provides a technically and economically advantageous method suitable for large-scale production of adaptive NK cells for immunotherapeutic purposes.

References

1) Miguel López-Botet, Andrea De Maria, Aura Muntasell, Mariella Della Chiesa, Carlos Vilches, Adaptive NK cell response to human cytomegalovirus: Facts and open issues, Seminars in Immunology, Volume 65, 2023, 101706, ISSN 1044-5323, https://doi.org/10.1016/j.smim.2022.101706.
2) Lisa L. Liu, Vivien Béziat, Vincent Y.S. Oei, Aline Pfefferle, Marie Schaffer, Sören Lehmann, Eva Hellström-Lindberg, Stefan Söderhäll, Mats Heyman, Dan Grandér, Karl-Johan Malmberg; *Ex Vivo* Expanded Adaptive NK Cells Effectively Kill Primary Acute Lymphoblastic Leukemia Cells. *Cancer Immunol Res* 1 August 2017; 5 (8): 654–665. https://doi.org/10.1158/2326-6066.CIR-16-0296
3) Haroun-Izquierdo A, Vincenti M, Netskar H, van Ooijen H, Zhang B, Bendzick L, Kanaya M, Momayyezi P, Li S, Wiiger MT, Hoel HJ, Krokeide SZ, Kremer V, Tjonnfjord G, Berggren S, Wikström K, Blomberg P, Alici E, Felices M, Önfelt B, Höglund P, Valamehr B, Ljunggren HG, Björklund A, Hammer Q, Kveberg L, Cichocki F, Miller JS, Malmberg KJ, Sohlberg E. Adaptive single-$KIR^{+}NKG2C^{+}$ NK cells expanded from select superdonors show potent missing-self reactivity and efficiently control HLA-mismatched acute myeloid leukemia. J Immunother Cancer. 2022 Nov;10(11): e005577. doi: 10.1136/jitc-2022-005577. Erratum in: J Immunother Cancer. 2023 Jan;11(1): PMID: 36319065; PMCID: PMC9628692.

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Alpha-synuclein induces subcellular and morpho-functional alterations in human monocyte-derived macrophages

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Spreading of excess alpha-synuclein (a-syn), a hallmark of Parkinson's disease, is shown to promote systemic inflammation by acting on peripheral blood cells, including monocytes and macrophages. However, the related intracellular mechanisms remain poorly explored. Autophagy is key to governing a-syn proteostasis, and it plays a functional role in monocytes and macrophages. Yet, if a-syn affects autophagy in these cells specifically, remains unknown. Here we investigate the subcellular, molecular, and morpho-functional effects of a-syn in human monocytes and macrophages with a focus on the autophagy-lysosomal pathway. Human THP-1 monocytic cells (TMs), derived macrophages (TDMs), and primary monocyte-derived macrophages (MDMs) were cultured w/wo recombinant a-syn (1uM) for 4 and 24h. By Confocal microscopy, Western Blot, qRT-PCR, and Elisa we assessed i) a-syn internalization; ii) inflammatory profile; iii) autophagy (LC3II/I, LAMP1/LysoTracker, p62, pS6/S6 ratio); iv) actin cytoskeleton (Phalloidin staining); v) Oil-red-O-stained lipid droplets (LDs); vi) FITC-IgG phagocytosis, and the fate of phagocytosed cargo. Extracellular a-syn was internalized by TMs, MDMs and TDMs, where it induced, time-dependently, the intracellular accumulation and release of pro-inflammatory mediators. In TDMs specifically, this was accompanied by mild toxicity, increased p62 protein, decreased LC3II/I ratio, and decreased LAMP1 at both protein and mRNA levels. This was independent of the mTOR activity index pS6/S6, but was associated with F-actin clumping, and a reduction of intracellular LDs, and their co-localization with LC3 and LAMP1. Finally, a-syn reduced both phagocytosis, and the clearance of phagocytosed cargo, which markedly filled LysoTracker-stained organelles resulting in a co-localization pattern reminiscent of engulfed/stagnant lysosomes. Cytoskeleton alterations, and LAMP1 reduction were recapitulated in MDMs. Our results suggest that contrarily to monocytic cells, which well-tolerate excess a-syn, macrophages undergo an exhaustion status resembling hypophagia, with autophagy-lysosome impairment potentially bridging hyper-inflammation, cytoskeleton abnormalities, cell toxicity, and lipid dyshomeostasis.

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Evaluation of EVs from peri-implant tissues at 24h from the surgery: a proof-of-concept study of the early response to different materials

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Background and aim: Early healing of the oral mucosa around dental implants is a complex dynamic process triggered by biological and molecular events, crucial for the long-term success of dental rehabilitation.

Previously, we described the early gene expression profile (24h after surgery) of the peri-implant mucosa (PM) around different abutment materials: Peek acted as a pro-inflammatory and fibrotic stimulator while Zirconia and Titanium seemed to be inert[1]. Extracellular Vesicles (EVs) are key players in information transfer within tissues and seem to be involved in wound healing[2]. The present study aimed to evaluate EVs obtained from PM 24h after implant placement and in contact with different abutment materials to describe the resulting fibroblast phenotype.

Methods: A total of 9 dental implants were placed in 4 patients and connected with abutments of different materials: (A) titanium, (B) zirconia and (C) PEEK. After 24 hours, PM biopsies were obtained. Two biopsies of gingiva were used as control (CTR). Human Gingival Fibroblasts were obtained from the biopsies and after the third passage cultured in an exosome-free medium. After starvation for 72h, the culture medium was collected and EVs were isolated by tangential flow filtration. EVs were then analysed through Nanoparticle Tracking Analysis and Surface Plasmon Resonance Imaging[3]. The following markers were finally examined: CD51, TGFβ R2 and CD9.

Results: EVs were successfully isolated. Among the different groups (A, B and C) no differences were shown concerning the concentration and mean size of EVs. Group A showed the highest expression of TGFβ R2, B responded with a low expression of CD51 and TGFβ R2, similar to CTR, while C showed a high expression of both markers, confirming our previous findings.

Conclusions: EVs evaluation, combined with molecular analysis, could be considered a novel option for describing PM's early response to different materials.

References

1. Pilloni A, Marini L, Gagliano N, et al. Molecular Analysis of Peri-implant Soft Tissue Response to Different Abutment Materials in Humans. A Pilot Study. Int J Oral Maxillofac Implants. Published online May 24, 2024. doi:10.11607/jomi.10910
2. Petit I, Levy A, Aberdam D. Purification of Extracellular Microvesicles Secreted by Dermal Fibroblasts. Methods Mol Biol. 2020;2154:63-72. doi:10.1007/978-1-0716-0648-3_6
3. Picciolini S, Gualerzi A, Vanna R, et al. Detection and Characterization of Different Brain-Derived Subpopulations of Plasma Exosomes by Surface Plasmon Resonance Imaging. Anal Chem. 2018;90(15):8873-8880. doi:10.1021/acs.analchem.8b00941

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Comparative evaluation of marginal adaptation and dimensional stability of three bioceramic root repair materials: a VP-SEM analysis

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Edge Bioceramic Retrofill, Endocem MTA, and One-Fil PT are three bioceramic root repair materials that have garnered attention in contemporary endodontics. Edge Bioceramic Retrofill is recognized for its bioactive properties and excellent sealing ability, making it suitable for retrograde fillings and root repairs. Endocem MTA, on the other hand, is valued for its biocompatibility and ability to form hydroxyapatite when in contact with tissue fluids, promoting tissue healing and reducing inflammation. One-Fil PT is notable for its ease of handling and delivery due to its premixed, injectable form, offering convenience during clinical applications. These materials share common advantages such as dimensional stability and good marginal adaptation to dentin walls, as evidenced by recent studies employing advanced analytical techniques like VP-SEM. Their development represents advancements in dental materials technology, aiming to enhance treatment outcomes and patient care in endodontic proceduresThis study investigated the marginal adaptation of three recently introduced bioceramic root repair materials Edge Bioceramic Retrofill, Endocem MTA, One-Fil PT using VP-SEM analysis. Extracted single-rooted lower incisors were used to simulate retrograde fillings. The results showed no statistically significant differences in the marginal gap between the materials and the dentin walls. All three materials exhibited good dimensional stability, with gap sizes comparable to previously published research on similar materials (1). This study employed VP-SEM, a valuable tool for analyzing bioceramic materials without altering their properties. The findings suggest that these bioceramics may be suitable for clinical applications in retrograde fillings and perforation repairs (2). However, further in vivo studies are needed to confirm long-term stability and assess the influence of sample preparation methods.

References

1) Zanza, A.; Reda, R.; Vannettelli, E.; Donfrancesco, O.; Relucenti, M.; Bhandi, S.; Patil, S.; Mehta, D.; Krithikadatta, J.; Testarelli, L. The Influence of Thermomechanical Compaction on the Marginal Adaptation of 4 Different Hydraulic Sealers: A Comparative Ex Vivo Study. *Journal of Composites Science* 2023, *7*, 10, doi:10.3390/jcs7010010
2) Donfrancesco, O.; Del Giudice, A.; Zanza, A.; Relucenti, M.; Petracchiola, S.; Gambarini, G.; Testarelli, L.; Seracchiani, M. SEM Evaluation of Endosequence BC Sealer Hiflow in Different Environmental Conditions. J. Compos. Sci. 2021, 5, 99. https://doi.org/10.3390/jcs5040099.

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A new player in the mechanobiology of deep fascia: Yes-Associated Protein (YAP)

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Recent studies have shown that fascial fibroblasts respond to mechanical stimuli, which leads to the remodeling of the extracellular matrix (ECM). Research on Yes-associated protein (YAP) has revealed its significant role in cell mechanics, influencing cellular properties such as shape, adhesion, and gene expression. This study aimed to explore the presence and activation of YAP in deep fascia following mechanical stimulation through focal extracorporeal shockwave (fESW) treatment.

Thoracolumbar fascia (TLF) samples were obtained from eight patients (ages 30–70, balanced by gender) undergoing elective spine surgeries at the University of Padova's Orthopedic Clinic. YAP levels in tissue and TLF-derived fibroblasts were analyzed using immunoblotting. Additionally, the expression of COL1A1 and HABP2 genes was measured in fibroblasts at 2, 24, and 48 hours post-fESW treatment.

The results indicated that YAP was present in all tested tissues. The ratio of active to inactive YAP (YAP/p-YAP) significantly increased in fascial fibroblasts after mechanical stimulation compared to untreated cells ($p = 0.0022$). Additionally, COL1A1 and HABP2 gene expression levels rose following treatment. These findings suggest that YAP is involved in fascial mechanotransduction, remodeling, regeneration, and fibrogenesis, positioning YAP as a critical factor in the mechanobiology of deep fascia.

References

1. Pirri, C.; Fede, C.; Petrelli, L.; De Rose, E.; Biz, C.; Guidolin, D.; De Caro, R.; Stecco, C. Immediate Effects of Extracorporeal Shock Wave Therapy in Fascial Fibroblasts: An In Vitro Study. Biomedicines 2022, 10, 1732.
2. Wang, C.; Zhu, X.; Feng,W.; Yu, Y.; Jeong, K.; Guo,W.; Lu, Y.; Mills, G.B. Verteporfin Inhibits YAP Function through Up-Regulating 14-3-3_ Sequestering YAP in the Cytoplasm. Am. J. Cancer Res. 2016, 6, 27–37.

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Blockade of sialylation with decrease in polysialic acid levels counteracts transforming growth factor β1-induced skin fibroblast-to-myofibroblast transition

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Aberrant sialylation with overexpression of the homopolymeric glycan polysialic acid (polySia) was recently reported in fibroblasts from fibrotic skin lesions. Yet, whether such a raise in polySia levels or sialylation in general may be functionally implicated in profibrotic activation of fibroblasts and their transition to myofibroblasts remains unknown. Therefore, we herein explored whether inhibition of sialylation could interfere with the process of skin fibroblast-to-myofibroblast transition induced by the master profibrotic mediator transforming growth factor β1 (TGFβ1). Adult human skin fibroblasts were pretreated with the competitive pan-sialyltransferase inhibitor 3-Fax-peracetyl-Neu5Ac (3-Fax) before stimulation with recombinant human TGFβ1, and then assayed for polySia expression, cell viability, proliferation, wound healing ability, and acquisition of myofibroblast-like morphofunctional features. Skin fibroblast stimulation with TGFβ1 resulted in overexpression of polySia, which was effectively blunted by 3-Fax preadministration. Pretreatment with 3-Fax efficiently lessened TGFβ1-induced skin fibroblast proliferation, wound healing ability, changes in cell morphology, and phenotypic and functional differentiation into myofibroblasts, as testified by a significant reduction in *FAP*, *ACTA2*, *COL1A1*, *COL1A2*, and *FN1* gene expression, and α-smooth muscle actin, N-cadherin, COL1A1, and FN-EDA protein levels, as well as a reduced contractile capability. Moreover, skin fibroblasts preadministered with 3-Fax displayed a significant decrease in Smad3-dependent TGFβ1 canonical signaling. Collectively, our in vitro findings demonstrate for the first time that aberrant sialylation with increased polySia levels has a functional role in skin fibroblast-to-myofibroblast transition and suggest that competitive sialyltransferase inhibition might offer new therapeutic opportunities against skin fibrosis.

Keywords: skin fibroblasts; myofibroblasts; skin fibrosis; fibroblast-to-myofibroblast transition; TGFβ1; sialylation; polysialic acid; sialyltransferase inhibitor

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Sarcoglycan sub-complex in MC3T3 cells seeded onto Titanium substrates

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Sarcoglycan sub-complex are members of the dystrophin-glycoprotein complex and plays a key role in cell-extracellular matrix interactions in muscle tissues. Literature has shown that this complex is expressed in different type of tissue such as epithelial tissue where they probably play a role as cell-cell interactors. Although these interesting data about the role of these proteins in non-muscle tissues, few data about their expression in connective tissue or non-muscle in vitro model exist. On this basis we decide to carry out an in vitro study to evaluate the expression of these glycoproteins in osteogenic cells seeded onto titanium (Ti) discs implants to verify if sarcoglycans are also expressed in this type of cells and if they could be involved in cell-substrate interactions. We investigated the expression of these proteins in MC3T3 cells seeded on Titanium implant discs, exposed or not to serum-derived proteins during the initial phase of cell culture. The cells, then, have been processed for immunofluorescence techniques; anti-sarcoglycans antibodies and anti-focal-adhesion antibodies, such as anti-vinculin, have been used. Our preliminary results show that the sarcoglycan sub-complex is expressed in 3T3L1 cells and that they colocalize with vinculin. These data strongly support that in osteogenic cells sarcoglycans could act as protein system involved in cell-substrate interactions.

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Role of period circadian protein 1 (PER1) in the determination of gender differences during cholestatic diseases

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Clock genes regulate circadian rhythms, which maintain homeostasis and regulate patho and physiological responses (1). Period circadian protein 1 (PER-1) is a transcriptional repressor, which maintains circadian rhythms in cells. Abnormal expression of clock genes is associated with circadian rhythm disturbances and carcinogenesis. No information exists regarding the role of PER-1 on biliary phenotype during cholestasis. We have shown that: (i) BDL rats have elevated expression of PER-1, CLOCK, ARNTL and CRY1 in cholangiocytes and (ii) overexpression of PER-1 inhibits the malignant-transformation of cholangiocytes via mir-34a in cholangiocarcinoma cell lines (2). We aimed to evaluate the effect of PER-1 on biliary proliferation, liver fibrosis and apoptosis and clock genes expression during cholestasis. *In vivo*, female and male wildtype and PER-1 knockout (*PER-1*$^{-/-}$) mice underwent BDL or sham for 7 days. We performed H&E, Sirius Red and Masson's trichrome staining for liver damage and liver fibrosis, respectively. Biliary proliferation, Intrahepatic Bile Duct Mass (IBDM) and apoptosis were evaluated by IHC for PCNA, CK-19 and ApoTag, respectively. The expression of clock genes was evaluated by IHC and by IF in liver sections. We observed sex differences between BDL and *PER-1*$^{-/-}$ BDL groups. Female *PER-1*$^{-/-}$ BDL showed: (i) decrease of liver damage, IBDM and liver fibrosis compared to female BDL groups; (ii) not change in PCNA-positive cholangiocytes, but elevated apotag-positive cholangiocytes, and (iii) decrease in ARNTL expression in bile duct compared to female BDL groups. We did not observe any change in liver damage, IBDM, liver fibrosis, apoptosis as well as clock genes expression in male BDL and *PER-1*$^{-/-}$ BDL. We have demonstrated that there is a gender difference in the phenotypic response to circadian rhythm alterations in cholestatic murine model. Local modulation of biliary circadian rhythm could be an innovative therapeutic approach in the recovery of biliary homeostasis during cholestatic liver diseases.

References

1) Xiao Y, Yuan Y, et al. Clock proteins regulate spatiotemporal organization of clock genes to control circadian rhythms. Proc Natl Acad Sci U S A. 2021.
2) Han Y, Meng F, et al. miR-34a-dependent overexpression of Per1 decreases cholangiocarcinoma growth. J Hepatol. 2016 Jun;64(6):1295-304.

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DNA damage and cell death in human oral squamous cell carcinoma cells: the potential biological effects of cannabidiol

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The most common oral cancer is squamous cell carcinoma (SCC) and conventional treatments, such as surgery, chemotherapy and radiotherapy, result in a 5-year survival of less than 50%, with physiological adverse effects affecting patient's quality of life1. The main chemical component of Cannabis, cannabidiol (CBD), has been shown to have antitumor properties and high therapeutic index2. Currently, no sufficient studies have been conducted to investigate a possible suppressive effect of CBD on oral cancer.

The aim of the present study is to investigate the in vitro biological effects of CBD on oral squamous cell carcinoma (HSC-3) cells. In particular, cell viability was assessed by MTT assay, morphology by SEM, apoptosis and cell cycle by flow cytometry, and DNA damage by phospho-γ-H2AX immunofluorescence detection. Cytotoxicity was evaluated using concentrations between 100μM and 1μM but only concentrations of 25μM and 6.25μM were selected for subsequent analysis as toxic and non-toxic dose, respectively.

CBD caused a dose- and time-dependent reduction in cell viability, cell cycle arrest in the G0/G1 phase associated with increased apoptosis and significant alterations in cell morphology (changes in cell shape, cell edges raised, disappearance of filopodia) especially with 25μM CBD, but still present even with 6.25μM CBD. In addition, CBD significantly increased DNA damage, as demonstrated by phosphorylation of H2AX.

In summary, the study shows -for the first time- that CBD inhibits oral cancer growth by causing DNA damage. In general, CBD induced cytotoxicity appears to be dose- and time-related. Doses of CBD ≥25μM showed a high reduction in viability. CBD-induced morphological changes could reduce the motility of HSC-3 cells. CBD could represent a new molecule to be tested for therapeutic purposes for its cytotoxic effects against oral squamous cell carcinoma. The mechanism involved in the suppressive effect caused by CBD, and a potential health risk-free therapeutic dose need further investigation.

References

1) Furness, S.; Glenny, A.M.; Worthington, H.V.; Pavitt, S.; Oliver, R.; Clarkson, J.E.; Macluskey, M.; Chan, K.K.; Conway, D.I. CSROC Expert Panel. Interventions for the Treatment of Oral Cavity and Oropharyngeal Cancer: Chemotherapy. Cochrane Database Syst. Rev. 2010,9, CD006386. Update in: Cochrane Database Syst. Rev. 2011,4, CD006386.
2) Valenti, C.; Billi, M.; Pancrazi, G.L.; Calabria, E.; Armogida, N.G.; Tortora, G.; Pagano, S.; Barnaba, P.; Marinucci, L. Biological Effects of Cannabidiol on Human Cancer Cells: Systematic Review of the Literature. Pharmacol. Res. 2022,181,106267.

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Evaluation of G protein-coupled receptor (GPR-120) agonist effect in intestinal and hepatic alterations induced in mice after administration of a high-fat and high-carbohydrate diet (WSD)

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Obesity and related metabolic diseases (type II diabetes, dyslipidemia, cardiovascular diseases, steatosis and non-alcoholic steatohepatitis and intestinal disfunction) represent a problem of global importance (1). Increasingly evidences demonstrates that the type of diet and the quantity of fatty acids (FFAs) play a key role in the onset and progression of these unresolved pathologies. FFAs represent the ligands of the G protein-coupled receptors (GPCRs). Among the GPCRs, the G protein-coupled receptor (GPR)-120 is involved in the appetite control, body weight regulation, adipogenesis, maintenance of energy homeostasis, chronic inflammation, and insulin resistance and fibrosis (2). The aim of the project is to evaluate the potential beneficial effect of a new synthetic agonist of GPR-120 (GprA), in the progression of the diet-induced alterations in the intestinal wall and the liver parenchyma. We monitored the progression of liver and intestinal damages in mice fed for 26 and 30 weeks with a "Western style" diet (WSD) and treated daily by oral gavage with 3 doses (30-60-90 mg/Kg) of GprA. The analyses in the liver highlighted that GprA at 90mg/Kg can attenuate the progression of steatosis both at the histological and molecular levels. Furthermore, at 30w, GprA is also effective in the reduction of collagen maturation and deposition, and expression of connective tissue growth factor (CTGF). Regarding the intestine, evaluations on both small and large intestine has been conducted. Macroscopic and histological features (villi length, crypt depth and collagen deposition) and gene expression (perilipin 2, mucin 2, zonulin 1 and CD68) did not highlighted clear effects of GprA on small intestine, as well as on colon.

Altogether, our data confirm the role of FFAR4 in liver alterations driven by a high fat diet, revealing that GprA represents a candidate for the development of a new therapeutic approach at least in the steatohepatitis.

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References

1) Boden, G. Obesity and free fatty acids. Endocrinol Metab Clin North Am 2008, 37, 635–46.
2) Ulven, T; Christiansen, E. Dietary Fatty Acids and Their Potential for Controlling Metabolic Diseases Through Activation of FFA4/GPR120. Annu Rev Nutr 2015, 35, 239-63.

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Ageing of facial fat, the role of extracellular collagen from an Electron microscopy point of view

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Fine anatomy and ultrastructural composition of adipose tissue is well known nowadays, On the other hand better understanding of adipose tissue ageing, especially in key district as face is a fundamental step for regenerative medicine and translational research in field such as plastic surgery. A lot of scientific studies have been published on the molecular and biochemical side of this matter, but less work has been performed on Extra cellular matrix collagen fibrils of face adipose tissue pads.

This work aims to describe collagen organization, through different ages, in samples harvested from two fat areas of the face using Scanning and Transmission Electron Microscopy techniques and Light microscopy.

During mayor maxillofacial surgery samples have been harvested from different patients aged 18 to 83 years old specifically Buccal Fat pad and deep malar fat pad. Fat samples have been fixed in Karnovsky. Later on, standard SEM, TEM and light Microscopy preparation was performed.

Collagen fibrils organization in face adipose tissue can be described mainly by 2 patterns; The first is a thick mash of small collagen fibrils, known as a pericellular basket like-structure that we found in all the samples with low variability. The second pattern is represented by bundles of intercellular fibrils with same diameter. Collagen bundles are the structural element giving adipose tissue the specific mechanical and histological properties.

Observation of Electron microscopy, both SEM and TEM, suggested that the quantity of intercellular collagen fibrils and bundles increase with growing age in both the fat-pads type analyzed. However, the organization and the cohesion of the collagen bundles gets lost as the age of the person increases. Taking into consideration lower magnification SEM pictures it is clear how the quantity of collagen increases whereas the bond between adipocytes decreases, therefore also the septa arrangement blends and gets less defined.

References

1) Dario Bertossi, Giamaica Conti, Paolo Bernardi, Donatella Benati, Mariele Ruffoli, Andrea Sbarbati, Pierfrancesco Nocini. Classification of fat pad of the third medium of the face. Aesthetic Medicine, 2015, 1, 103-109.
2) Kruglikov I, Trujillo O, Kristen Q, Isac K, Zorko J, Fam M, Okonkwo K, Mian A, Thanh H, Koban K, Sclafani AP, Steinke H, Cotofana S. The Facial Adipose Tissue: A Revision. Facial Plast Surg. 2016, 32, 671-682.
3) Wollina U, Wetzker R, Abdel-Naser MB, Kruglikov IL. Role of adipose tissue in facial aging. Clin Interv Aging, 2017, 12, 2069-2076.

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The cartilage-bone interplay: structural biology of the temporo-mandibular joint

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It is generally known that the cartilage/bone junction is mediated by the interposition of a calcified cartilage layer. This latter has been the subject of extensive research but its structure and functions are still unclear: in particular it is debated whether oxygen, nutrients and signaling molecules from the subchondral bone may cross this layer (1, 2) and contribute to the metabolism of the uncalcified cartilage or if, on the contrary, this latter is fed only by the perichondrium and/or the synovial fluid. A previous research on the growth plate has already shown that a single cartilage can have two completely different interfaces with the adjoining bones (3). In the present study the opposing surfaces of the temporo-mandibular joint have been investigated by light microscopy, by scanning electron microscopy and by high resolution micro-CT, the two latter being the only techniques allowing an unrestricted, face-on view of the mineralization front.

Our first results seem to contradict the common knowledge on these tissues.

The condylar surface is covered by a thin layer of fibrous tissue which become more cartilaginous with depth; this tissue appears to blend with the subchondral bone with no definite boundary. Small cartilage islets remain enclosed within the trabeculae of the underlying bone, where they are readily evidenced by all the techniques used.

The mandibular fossa is covered by a similar thin fibrous layer but the underlying surface is represented by a solid thick plate of bone, followed by a few bony planes parallel to the articular surface, and finally by the cancellous bone. There is no trace of cartilage on this side of the joint, and no visible communication between the soft tissue of the articular surface and the underlying vascular spaces. The research is still underway.

References

(1) Arkill, KP, and Winlove, CP. Solute transport in the deep and calcified zones of articular cartilage. Osteoarthritis Cartilage 2008, 16, 708–714.
(2) Oliveira Silva, M; Gregory, JL; Ansari, N; Stok, KS. Molecular Signaling Interactions and Transport at the Osteochondral Interface: A Review. Front Cell Dev Biol, 2020, 8, 750.
(3) Zecca, PA; Reguzzoni, M; Borgese, M; Protasoni, M; Filibian, M; Raspanti, M. Investigating the Interfaces of the Epiphyseal Plate: An Integrated Approach of Histochemistry, Microtomography and SEM. J Anat, 243, 870-877

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Skin telocyte secretome as conditioned medium prevents profibrotic differentiation of skin fibroblasts into myofibroblasts

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Telocytes (TCs) are a distinct type of stromal cells found in different organs of the human body, including the skin. By means of their characteristic prolongations (telopodes), skin TCs are arranged in networks intermingled with a multitude of neighboring cells and, hence, they are thought to contribute to skin homeostasis through both intercellular contacts and release of extracellular vesicles. A disruption of the dermal network of TCs was reported in both human fibrotic skin lesions and mouse model of bleomycin-induced skin fibrosis [1,2], but whether TC damage/disappearance may be a mere fibrosis consequence or contribute to fibrogenesis remains unclear. Hence, we examined the in vitro effects of skin TC secretome as conditioned medium (TC-CM) on skin fibroblast-to-myofibroblast transition induced by the key profibrotic mediator transforming growth factor β1 (TGFβ1). Primary cultures of adult human skin TCs and fibroblasts were established through immunomagnetic microbead-based cell separation [3]. Extracellular vesicle measurements in TC-CM were performed with nanoparticle tracking analysis (NanoSight). By combining morphological, gene/protein expression, and functional analyses we demonstrated the capability of TC-CM to significantly prevent TGFβ1-induced fibroblast activation and transition to myofibroblasts. TC-CM did not affect fibroblast viability, while it was able to inhibit TGFβ1-induced proliferation, wound healing capacity, and changes in cell morphology. TC-CM was effective in attenuating TGFβ1-induced skin fibroblast phenotypic and functional differentiation into myofibroblasts, as demonstrated by a significant reduction in *FAP*, *ACTA2*, *COL1A1*, *COL1A2*, and *FN1* gene expression, α-smooth muscle actin, N-cadherin, COL1A1, and FN-EDA protein levels, and collagen gel matrix contraction. Moreover, TC-CM significantly decreased TGFβ1-mediated activation of ERK1/2 signaling. This study demonstrates for the first time that TCs may contribute to skin homeostasis by preventing profibrotic activation of fibroblasts and provides the necessary groundwork for further investigation of the use of TC secretome as potential antifibrotic therapeutic intervention.

Keywords: telocytes; secretome; fibroblasts; myofibroblasts; skin fibrosis; fibroblast-to-myofibroblast transition; TGFβ1

References

1) Ibba-Manneschi, L.; Rosa, I.; Manetti, M. Telocyte implications in human pathology: an overview. Semin. Cell Dev. Biol. 2016, 55, 62–69.
2) Rosa, I.; Romano, E.; Fioretto, B.S.; Guasti, D.; Ibba-Manneschi, L.; Matucci-Cerinic, M.; Manetti, M. Scleroderma-like impairment in the network of telocytes/CD34+ stromal cells in the experimental mouse model of bleomycin-induced dermal fibrosis. Int. J. Mol. Sci. 2021, 22, 12407.
3) Romano, E.; Rosa, I.; Fioretto, B.S.; Lucattelli, E.; Innocenti, M.; Ibba-Manneschi, L.; Matucci-Cerinic, M.; Manetti, M. A two-step immunomagnetic microbead-based method for the isolation of human primary skin telocytes/CD34+ stromal cells. Int. J. Mol. Sci. 2020, 21, 5877.

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Cancer-associated microbiota contributes to tumor fibrosis by modulating fibroblast activity

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We recently reported that *in vivo* depletion of mammary cancer-associated microbiota promoted immune cell infiltration and activation in the tumor microenvironment, resulting in a shrinkage of the neoplastic mass. This effect was associated to the disappearance of *Staphylococcus epidermidis*, a microbe with potent immunomodulatory features[1]. While tumor-associated microbiota is documented to negatively affect the local immune response[2], the impact of such microbial community on tumor stroma is largely unknown. Here, we evaluated the ability of *Staphylococcus epidermidis* to modulate the activity of stromal cells, in particular fibroblasts. Mice were injected with 4T1 breast cancer cells in the mammary fat-pad, treated with oral ampicillin for 10 days and then peritumorally administered with saline or 10^5 Colony Forming Unit *Staphylococcus epidermidis* for 7 days. We observed an accelerated growth of *Staphylococcus epidermidis*-treated tumors compared to controls, paralleled by an increased collagen deposition, as revealed by Picosirius Red- and Masson Trichrome-stained nodules. *In vitro* exposure of *Staphylococcus epidermidis* conditioned medium (SECM) to NIH-3T3 mouse fibroblast cell line and primary murine fibroblasts resulted in augmented secretion of Collagen Type I and MMP-1, indicating an increased collagen deposition and remodeling. The mRNA levels for LH2b, TIMP-1 and LOX, involved in collagen turnover, did not show any statistical differences between the two experimental groups, while genes related to fibroblast activation, such as TGF- 1, ACTA1 and VIM, were up-regulated by SECM. Finally, stromal score, inferred by deconvolution analysis on gene profiling data of 45 breast cancer patient tumors classified as positive or negative for Staphylococcus genus by Real-time PCR, showed that Staphylococcus-positive patients were characterized by a higher stromal score compared to negative patients. Our results show that tumor-associated microbiota can exert pro-fibrotic effect in the tumor microenvironment by modulating fibroblast activity, highlighting a novel and deleterious function of bacteria colonizing the tumor mass.

References

1) Bernardo, G.; Le Noci, V.; Ottaviano, E.; De Cecco, L.; Camisaschi, C.; Guglielmetti, S.; Di Modica, M.; Gargari, G.; Bianchi, F.; Indino, S.; Sartori, P.; Borghi, E.; Sommariva, M.; Tagliabue, E.; Triulzi, T.; Sfondrini, L. Reduction of Staphylococcus epidermidis in the mammary tumor microbiota induces antitumor immunity and decreases breast cancer aggressiveness. Cancer Lett. 2023, 555, 216041.
2) Bernardo, G.; Le Noci, V.; Di Modica, M.; Montanari, E.; Triulzi, T.; Pupa, SM.; Tagliabue, E.; Sommariva, M.; Sfondrini, L. The Emerging Role of the Microbiota in Breast Cancer Progression. Cells. 2023, 12(15), 1945.

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Superficial fascia alterations in lipedema

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The pathophysiology of lipedema remains unclear: the putative causes include altered adipogenesis, microangiopathy, and disturbed lymphatic microcirculation, but no specific biomarkers has yet been found, and the diagnosis is currently made on clinical grounds alone. Usually the focus in lipedema is the adipose tissue, but our hypothesis is that the superficial fascia (SF) and the subcutaneous connective tissue can play a key role, supporting the adipose tissue and lymphatic vessels. Therefore, we analyze 12 inferior limbs for each condition (healthy/lipedema) by ultrasound imaging, immunohistochemistry, Total Collagen assay kit, ELISA immunoassays and immunoblotting, to analyze the changes associated with the disease in the organization of the tissue (thickness of the superficial fascia, orientation of the fibrous ligaments), and in vascularization, amount and distribution of collagen and inflammation.

The results showed in lipedema patients an alteration of thickness, echogenicity and texture at the level of the SF, SAT, and retinacula cutis based on ultrasound imaging. The histology confirmed the disarranged organization of both SF and retinacula cutis, with fat lobules collapsed and disrupted areas. The immunoblotting and the collagen kit demonstrated the increase of collagen deposition in the SF of lipedema patients (3 times more than ctrl: from ~8 to 24 μg/gr of tissue), confirming the increase of rigidity of the connective tissue. Lastly, the SF showed higher levels of the inflammatory marker TNF-α factor with respect to controls (from ~600 to 1100 pg/mg).

These analyses revealed a disarrangement, inflammation and fibrosis of the connective tissue in lipedema, demonstrating its role in organizing and supporting the adipose tissue. In this way, the SF can become a good diagnostic marker for diagnosis of lipedema.

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Snail slime obtained through Cherasco method as a promising strategy to counteract inflammation occurrence and to promote viability in human gingival fibroblasts

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Snail slime (SS) is a viscous secretion with a very low pH, obtained from different gastropod species. Recently SS has gained popularity for its cosmetic and skincare properties, such as antiaging, skin regeneration, acne control and hydrating effect due to the huge variety of its components. Several papers focused on this topic, even if the molecular mechanisms at the base of SS biological effect remain still unexplored. With this background this work is designed to test the SS, extracted with the cruelty-free Cherasco method on the most abundant cell populations of the skin to demonstrate the beneficial effect of SS. SS was administered to keratinocytes and fibroblasts, then, cell viability through MTT test, cytotoxicity by LDH assay, morphology by haematoxylin-eosin staining, gene and protein expression through Real-Time PCR and western blot, cell cycle phases by flow cytometry and collagen secretion trough ELISA test, were measured. Our results evidence SS capability to promote fibroblasts viability and to trigger recovery mechanisms by activating Erk protein. Moreover, an appreciable anti-inflammatory effect, due to the significant reduction of cyclooxygenase-2 expression, a positive modulation of blood vessels formation, demonstrated by an increased Angiopoietin 1 gene expression and a higher matrix deposition, evidenced by the augmented amount of released collagen I, can be identified.

This study focuses on an alternative SS extraction procedure able to preserve the animal life and to obtain a rich, natural and beneficial product which can be safely included in skin care formulations.

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Neuro-muscular-skeletal system in motor and sports activities

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Peri-partum respiratory management of pregnant women with neuromuscular disorders: a prospective observational study (IT-NEUMA-Pregn study)

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Pregnant women with neuromuscular diseases (NMDs) often display respiratory muscle impairment which increases the risk for pulmonary complications (PCs). The aim of this study was to identify pregnant NMDs patients with pulmonary risk factors and to apply in these women non-invasive ventilation (NIV) combined with mechanical insufflation-exsufflation (MI-E) in the peri-partum period.

We conducted a multicenter observational study on women with NMDs undergoing cesarean section or spontaneous labor in a network of 7 national hospitals. In these subjects we applied a protocol for screening and preventing PCs, and we evaluated PCs rate, maternal and neonatal outcome.

Twenty-four patients out of the 94 enrolled pregnant women were at risk for PCs and were trained or retrained to use NIV and/or MI-E before delivery. After delivery, 17 patients required NIV with or without MI-E. Despite nine out of the 24 women at pulmonary risk developed postpartum PCs, none of them needed reintubation nor tracheostomy. In addition, the average birth weight and Apgar score were normal. Only one patient without pulmonary risk factors developed postpartum PCs.

This study showed the feasibility of applying a protocol for screening and treating pregnant NMDs women with pulmonary risk. Despite a PCs rate of 37% was observed in these patients, maternal and neonatal outcome were favorable.

References

1) Longhitano Y, Zanza C, Romenskaya T, Saviano A, Persiano T, Leo M, Piccioni A, Betti M, Maconi A, Pindinello I, Boverio R, Rello J, Franceschi F, Racca F. Single-Breath Counting Test Predicts Non-Invasive Respiratory Support Requirements in Patients with COVID-19 Pneumonia. J Clin Med. 2021;11(1):179.
2) Hull J, Aniapravan R, Chan E, et al. British Thoracic Society guideline for respiratory management of children with neuromuscular weakness. Thorax. 2012;67(Suppl 1):1–40. doi: 10.1136/thoraxjnl-2012-201964.

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The impact of physical activity on re-entraining core body temperature rhythms following light/dark inversion in mice

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The suprachiasmatic nucleus in the hypothalamus regulates circadian rhythms influencing various physiological processes. Physical activity (PA) is known to modulate these rhythms by affecting factors like core body temperature (CBT) and metabolism. The aim of this study is to investigate the role of PA in readjusting CBT circadian rhythm after a light/dark (LD) inversion in mice.

Fifteen C57BL/6J mice were intraperitoneally implanted with a pill set to record CBT every 5 minutes. Subsequently, the LD schedule of the mice was reversed. At that point, eight mice were transferred to cages equipped with a running wheel (RW group), while seven mice remained in their regular cages (CTRL group).

Before the LD inversion, both groups exhibited the same Percentage Rhythm (PR), representing the strength of the rhythm, and amplitude, i.e. the width of the sinusoid that interpolates the rhythm. Following the LD inversion, both groups experienced a significant decrease ($p < .001$) in both PR and amplitude, with RW mice displaying an even more disrupted CBT rhythm ($p = .01$). Four days after the LD inversion, both groups returned to normal PR and amplitude values, but RW mice exhibited higher PR and amplitude than CTRL ($p < .001$).

Understanding the CBT regulation is fundamental to comprehending the physiological responses of organisms to environmental stimuli. From this data, it appears that PA plays a significant role in synchronizing an organism to the zeitgebers but not in resynchronizing it after a significant disruption.

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Vision and posture: effects of visual target distance on postural stability and plantar pressure distribution

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The visual system is composed by the Central Visual System (fovea) and by the Peripheral Visual System (peripheral vision). The vision is one of the most important peripheral receptors of Postural Control System (PCS) [1], including other peripheral receptors (stomatognathic, vestibular, and somatosensory) and anatomical structures of Central Nervous System (brainstem, cerebellum, basal ganglia, thalamus, and several cortical regions) [2]. PCS ensures postural stability [3] maintaining whole-body balance by modulating the myofascial chains against gravity.

Previous studies analyzed the changes of body sway during viewing nearby (VNT) and distant (VDT) targets in healthy population without considering the feet adaptations related to the body weight distribution. Aim of this study was to investigate how different distances of viewing target affect the variability of postural stability and plantar pressure parameters in healthy subjects.

31 stabilometric and plantar pressure parameters were acquired in 20 young healthy subjects by baropodometry performed in bipedal standing during VNT and VDT (0.70 m and 3 m from the heels, respectively). Variability and statistical differences between VDT and VNT were implemented.

Results showed the highest repeatability for plantar pressure parameters, a slightly high for CP speed and a lowest for CoPsa and Length Surface Function during both VNT and VDT. Moreover, a significant increase of load percentage on left-foot and right-Forefoot, of mean and maximum pressures on left-Forefoot, of CoPsa and CPspeed and a decrease of LSF were found during VDT.

This study revealed how a greater distance of viewing target affects body-weight distribution and increases body oscillations. These findings highlighted the importance of visual target distance for the correct interpretation of postural stability and plantar pressure parameters, and the need to standardize target distance during stabilometric exam.

References

1) Lee, D.N.; Lishmann, J.R. Vision-The most efficient source of propriocetive information for balance control. Agressologie 1976, 18A, 83–94.
2) Carini F., Mazzola M., Fici C., Palmeri S., Messina M., Damiani P. et al. Posture and posturology, anatomical and physiological profiles: overview and current state of art. Acta Biomed. 2017 Apr 28;88(1):11-16. PMID: 28467328; PMCID: PMC6166197.
3) Horak, F., & Macpherson, J. Postural Orientation and Equilibrium. Comprehensive Physiology, 2011. 255-292.

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Vertical Jump performance influenced by auditory and visual system

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Vertical jump (VJ) is considered a fundamental element for successful athletic performance [1]. A few studies analyzed the effects of visual and acoustic stimuli on VJ performance. Anatomically, visual stimuli are processed in the visual cortex, located in occipital lobe; while acoustic stimuli involve temporal lobe regions called auditory cortices, which receive direct afferents from the auditory thalamus [2, 3].

To the best of our knowledge, no study quantitatively assessed the variations of spatial-temporal and kinetic parameters during the execution of Countermovement Jump-Free Arms (CMJ-FA) with the administration of different acoustic and visual stimuli.

The aim of this study is to investigate the effects of visual and acoustic stimuli, in incentive and disincentive conditions, on CMJ-FA performance using an Inertial Measurement Unit.

Twenty male volleyball athletes were evaluated using "Baiobit" sensor. Five sessions, each with 3 CMJ-FA trials, were performed without sensory stimulus (NS), with incentive (IAS) and disincentive (DAS) acoustic stimulus, and with incentive (IVS) and disincentive (DVS) visual stimulus. Eight spatial-temporal and four kinetic parameters were evaluated.

The results indicated that DVS condition significantly decreased the Mean Time of Flight Phase, Mean and Peak Jump Height, and significantly increased the Impact Index with respect to NS. Additionally, DAS condition also showed a significant decrease in Mean Time of Flight Phase, Mean and Peak Jump Height compared to NS.

These findings underline the negative impact of disincentive conditions, particularly for visual stimuli, on jump performance. Moreover, an increase of force, found during landing phase in DVS, may predispose to muscle-skeletal lower limb injuries. For these reasons, results revealed that visual stimuli most affect jump performance.

Evidence of present study could be useful for sports trainers in improving athletes' control, desensitizing them to disincentive conditions, maintaining good performance, and reducing the risk of injuries during competitions.

References

1) Markovic G. Does plyometric training improve vertical jump height? A meta-analytical review. Br J Sports Med. 2007 Jun;41(6):349-55; discussion 355. doi: 10.1136/bjsm.2007.035113. Epub 2007 Mar 8. PMID: 17347316; PMCID: PMC2465309.
2) Celesia GG, DeMarco PJ Jr. Anatomy and physiology of the visual system. J Clin Neurophysiol. 1994 Sep;11(5):482-92. doi: 10.1097/00004691-199409000-00003. PMID: 7844239.
3) Angulo-Perkins A, Concha L. Music perception: information flow within the human auditory cortices. Adv Exp Med Biol. 2014; 829:293-303. doi: 10.1007/978-1-4939-1782-2_15. PMID: 25358716.

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Ultrasound imaging in football players with previous multiple ankle sprains: keeping a close eye on Superior Ankle Retinaculum

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The superior extensor ankle retinaculum (SEAR), a transverse fascial thickening located above the tibiatalar joint, plays a vital role in ankle stability. This study aimed to measure and compare the bilateral thickness and echogenicity of SEAR using ultrasound imaging in two groups: football players with multiple previous ankle sprains (group 1) and healthy volunteers (group 2).

In this cross-sectional study, ultrasound imaging measured the longitudinal and transversal axes of SEAR in 50 subjects (25 football players with past ankle sprains and 25 healthy subjects) using a new protocol. The results showed significant differences in SEAR thickness for both axes between the healthy and previously sprained sides in group 1 ($p = 0.0011$ and $p = 0.0032$) and compared to the corresponding sides in group 2 ($p = 0.003$ and $p = 0.004$). Additionally, group 1 exhibited significant differences in echogenicity between sides ($p = 0.0378$).

These findings suggest that football players with previous ankle sprains have a thicker and more inhomogeneous SEAR on the sprain side, indicating structural remodeling compared to the other side and healthy volunteers. During ultrasound examinations of these athletes, it's crucial to compare SEAR between sides for accurate assessments.

References

1. Gurau, T.V.; Gurau, G.; Voinescu, D.C.; Anghel, L.; Onose, G.; Iordan, D.A.; Munteanu, C.; Onu, I.; Musat, C.L. Epidemiology of Injuries in Men's Professional and Amateur Football (Part I). J. Clin. Med. 2023, 12, 5569.
2. Pirri, C.; Stecco, A.; Stecco, C.; Ozcakar, L. Ultrasound imaging and Fascial Manipulation for rigid retinacula in two cases of complex regional pain syndrome. Med. Ultrason. 2022, 24, 372–374. [

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In vivo identification of novel inflammation-related biomarkers on human tendons

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Rotator cuff tears (RCTs) are the most common upper extremity condition seen by orthopaedic surgeons, with a spectrum ranging from tendinopathy to full-thickness tears with arthritic change. The diagnosis of RCTs continues to be widely discussed by the scientific community and their management is still challenging due to the lack of specific molecular biomarkers [1]. Thus gaining a deeper insight into the nature and function of tendon-resident cells in tissue homeostasis and disease is imperative for developing new treatment strategies for tendinopathies.

Studies suggest that the early inflammatory response during the first stage of tendon healing plays a crucial role in the onset and progression of RCTs [2]. In this scenario, our group has previously demonstrated that, rotator cuff tendon-derived stem cell/progenitor cells (TSPCs) from human healthy donors stimulated with TNFα alone or in combination with IFNγ for 72 h *in vitro*, display a significant decrease of the $CD146^+CD49d^+$ and $CD146^+CD49f^+$ subpopulations, displaying stemness loss. In parallel, the same pro-inflammatory cytokines upregulate the expression of CD200 in the $CD146^+$ TSPCs population, a marker typically expressed on cells of the immune compartment [3].

With this background, ten human healthy donors and ten ones affected by RCTs were recruited and inflamed tendon samples and native ones from rotator cuff were analyzed in terms of functional and morphological parameters. The present study aims therefore at investigating the role of the CD200/CD200R molecular axis in RCTs *in vivo*. Morphological analyses were performed by hematoxylin/eosin staining and samples were further processed for immunohistochemical analyses of markers related to tendon inflammation and immunomodulation, such as CD200, CD200R, EpCAM, fibromodulin and E-cadherin. Our data confirm that tendon-resident cells own immunomodulatory properties by expressing markers related to the recruitment of immune cells, laying the grounds for uncover novel and alternative therapeutic target for the treatment of tendinopathies.

References

1) Bedi, A.; Bishop, J.; Keener, J.; Lansdown, D.A.; Levy, O.; MacDonald, P.; Maffulli, N.; Oh, J.H.; Sabesan, V.J.; Sanchez-Sotelo, J.; Williams, R.J. 3rd; Feeley, B.T. Rotator cuff tears. Nat Rev Dis Primers 2024, 10, 8.
2) Chisari, E.; Rehak, L.; Khan, W.S.; Maffulli, N. Tendon healing is adversely affected by low-grade inflammation. J. Orthop. Surg. Res. 2021, 16, 700.
3) Giancola, R.; Oliva, F.; Gallorini, M.; Michetti, N.; Gissi, C.; Moussa, F.; Antonetti Lamorgese Passeri, C.; Colosimo, A.; Berardi, A,C. CD200 as a Potential New Player in Inflammation during Rotator Cuff Tendon Injury/Repair: An In Vitro Model. Int J Mol Sci, 2022, 23, 15165.

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Acute effects of anodal cerebellar transcranial direct current stimulation (tDCS) on the cognitive parameters in healthy young adults

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The effects of cerebellar tDCS on cognitive processes are still unknown. This study aimed to assess the effects of anodal cerebellar tDCS on executive attention, divided attention, working memory, and cognitive flexibility in healthy young adults, using the Trail Making Test (TMT-A-B) and verbal fluency test. Twenty participants (10 women, 23±3 years, 22.8±2.6 kg/m^2; 10 men, 24±2 years, 24.8±5.8 kg/m^2), visited the lab on two separate days, one week apart, and received anodal and sham stimulation in a randomized order. The anodal electrode was placed 1cm below the inion, and the cathode was on the dominant supraorbital area. Pre-tests were conducted before and post-tests five minutes after a 20-minute tDCS session (2mA; Microstim). TMT-A measured sustained attention, speed, and motor function, while TMT-B added alternation, flexibility, inhibitory control, working memory, and attention. Phonological verbal fluency tests required participants to generate as many words as possible, starting with specific letters within 60s. Our results showed no significant differences in TMT-A and TMT-B between groups or pre- and post-intervention ($p>0.05$). Pre-intervention values were 21.8±5.6s (TMT-A) and 37.5±11.7s (TMT-B) for the anodal group; 22.3±7.6s (TMT-A) and 40.5±15.8s (TMT-B) for the sham group. Post-intervention values were 19.7±4.3s and 32.5±7.5s for the anodal group; 20.7±6.0s and 38.1±17.2s for the sham group. In the verbal fluency test, the total number of hits and words with 1, 2, and ≥4 syllables were similar ($p>0.05$). However, tDCS impaired the number of words with 3-syllable for anodal group ($p=0.002$; Pre 7.15±2.7 vs. Post 3.8±2.6). Conclusion: anodal cerebellar tDCS did not affect executive attention, divided attention, working memory, or cognitive flexibility in young adults. However, tDCS had a specific negative impact on generating 3-syllable words in the verbal fluency for the anodal group post-intervention. This suggests that while tDCS did not influence cognitive functions assessed, it may impair certain specific verbal fluency abilities.

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Changes in bioelectrical impedance parameters and salivary cortisol responses in semi-professional soccer players during the pre-season training period

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In the current sport context, monitoring training load and recovery is crucial to optimize performance and prevent injury, especially in soccer. Semi-professional teams, even if limited in resources compared to professional ones, face the same competitive and performance challenges, making effective monitoring of load and recovery essential.

This study focused on the analysis of the body's internal response to the training stimulus during the pre-season training period. Bioelectrical impedance vector analysis (BIVA) (to assess hydration status and body composition) and salivary cortisol tests (to assess stress and recovery) were performed on 15 players from an Italian team of the semi-professional soccer D league.

Salivary cortisol changes were assessed in the morning and in the afternoon of two different training days: T1 day, during the first week of the training period, and T2, 2 weeks after. T1 results showed a significant difference between pre- and post-training values in the afternoon. Conversely, T2 results showed no significance either in morning or afternoon.

The pre-training BIVA analysis performed in the same training sessions showed a significant difference between T1 morning and afternoon reactance (Xc) and phase angle (PA) parameters of the upper hemisoma; in contrast, T2 results showed no significance, as salivary cortisol levels.

These results confirm that at the beginning of the pre-season training period, cortisol production is higher after training, while it remains unchanged thereafter due to the principles of supercompensation and adaptation to training stress.

Similarly, the alterations of Xc and PA of the upper hemisoma in the afternoon of T1 are an indication of a greater difficulty in restoring the basal situation; the lower hemisoma, on the contrary, does not show similar alterations, highlighting a more effective recovery due to the fact that it is the component most stimulated by the specific training performed. Again, these alterations disappear as with salivary cortisol, as there is no significant difference in T2 results.

This study contributes to the existing literature on the topic by providing insights into relevant correlations between training stimuli and physiological responses.

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Polycystic ovary syndrome (PCOS) and physical activity as tertiary prevention: proposal of a physical protocol

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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, characterized by changes in hormone levels, associated with severe clinical problems such as: infertility, insulin resistance, hypertension, obesity, diabetes mellitus, dyslipidemia, depression, anxiety and increased cardiovascular risk.

It is estimated that 5-10% of the global female population is affected, with a higher prevalence in subjects with obesity, virilizing disorders, premature adrenarche and in association with family history.

The diagnosis of the pathology often occurs through the use of the Rotterdam criteria, which include: the size of the ovary, the morphology of the ovary, which appears multicystic, oligoamenorrhea or amenorrhea and hyperandrogenism.

The treatment modalities vary due to the heterogeneity of the patients, and include either the pharmacological approach, used to improve insulin resistance and to normalize menstrual cycles, hirsutism and l 'acne, which, more recently, a nutraceutical treatment. Surgery and additional specialized treatments are used to treat hirsutism and acne.

However, the main way in which PCOS is addressed is lifestyle modification, with the introduction of a healthy diet, low in fat and sugar, combined with regular physical exercise.

Motor activity, in addition to the fundamental weight loss, allows you to improve the manifestations of the disorder.

It positively influences steroidogenesis, and therefore indirectly fertility and menstrual anomalies, and improves hyperinsulinemia, insulin resistance, cardiometabolic risk levels and mental health, of women with PCOS.

A motor protocol that combines moderate and high intensity aerobic training programs with strength and resistance training programs leads to extremely positive results, especially regarding the improvement of androgen levels.

Based on the results of several studies, women with PCOS should engage in at least 90 minutes of moderate-intensity physical activity per week to achieve improvements in reproductive, cardiometabolic, and psychosocial domains.

References

1) Sadeghi, H. M.; Adeli, I.; Calina, D.; Docea, A. O.; Mousavi, T.; Daniali, M.; Nikfar, S.; Tsatsakis, A.; Abdollahi, M. Polycystic Ovary Syndrome: A Comprehensive Review of Pathogenesis, Management, and Drug Repurposing. International journal of molecular sciences, 2022 23(2), 583.
2) Cowan S.; Lim S.; Alycia C.; Pirotta S.; Thomson R.; Gibson-Helm M.; Blackmore R.; Naderpoor N.; Bennett C.; Ee C.; Rao V.; Mousa A.; Alesi S.; Moran L. Lifestyle management in polycystic ovary syndrome - beyond diet and physical activity. BMC Endocr Disord. 2023 Jan 16; 23(1):14.

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Analyzing anatomical differences in human posture using machine learning: applicability and reliability

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Posture has always been a crucial area of study in human anatomy, reflecting potential musculoskeletal imbalances. Florence Kendall's foundational work in 1952, "Posture and Pain," marked the beginning of modern postural assessment. Since then, various methods have been developed, each with its own advantages and limitations. Recently, machine learning (ML) models have emerged, identifying anatomical landmarks without skin markers. Google's MediaPipe is a sophisticated ML algorithm that precisely tracks 33 landmarks across the human body. This study aims to demonstrate the applicability and reliability of an ML method for posture analysis, provide normative data on the posture of healthy men and women, and explore new posture patterns using principal component (PCA) and cluster analyses (CA).

Photographs of 200 healthy participants (mean age 25.9 ± 5.2 years) were taken from the front, back, and sides. Statistical differences in postural parameters were evaluated using Student's t-test, Cohen's effect size, and the Intraclass Correlation Coefficient. Principal component and cluster analyses identified alternative patterns within the sample.

The analysis revealed anatomical sex differences in shoulder adduction angle (men: 16.12° ± 1.92°, women: 14.13° ± 1.53°, d = 1.14) and hip adduction angle (men: 9.90° ± 2.22°, women: 6.71° ± 1.53°, d = 1.67). No statistical difference was found in asymmetry between left and right anatomical landmarks. ICC results (0.67 to 0.95) confirmed measurement reliability. PCA and CA grouped the sample, identifying significant differences in anatomical shapes between two groups (CG1, CG2). The shoulder-hip distance for CG2 was 86.58 ± 10.73 compared to 57.51 ± 7.02 for CG1, with an effect size of d = 3.21.

This study introduces a machine learning approach for postural analysis, demonstrating the application of emerging technologies in human anatomy. This non-invasive method has the potential to revolutionize posture analysis and enhance its applications in physical therapy, ergonomics, and sports.

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Effects of high-intensity exercise training on mitochondrial adaptations and remodeling: a preliminary ultrastructural study

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Mitochondrial health is an important mediator of cellular function across a wide range of tissues, as it contributes to whole-body vitality in health and disease. The fine-tuning of several dynamic processes is imperative for organelle function and essential for meeting the metabolic demands of cells. Noteworthy, mitochondria feature a remarkable plasticity that allows them to adjust their size, structure, and capacity under different conditions including exercise. It is well established that different types of exercise can provide a powerful stimulus for mitochondrial biogenesis, morphological changes, and an increase in respiratory supercomplex formation as mechanisms triggered by exercise that may increase skeletal muscle function. However, there are conflicting findings in the literature regarding the efficacy and extent of mitochondrial adaptations induced by high-intensity interval training (HIIT). In a previous study, we demonstrated that HIIT produced a significant increase in the expression of mitochondrial complex enzymes (significant for enzymes corresponding to the Complex IV, II, and I of the mitochondrial chain) both in gastrocnemius and quadriceps muscle of trained mice, compared with sedentary controls. However, investigations to correlate biochemical data and changes in both mitochondrial network and individual mitochondrial morphology were not performed. Therefore, the present study aimed to investigate, through an ultrastructural approach, the mitochondrial adaptations and remodeling in mouse skeletal muscles following HIIT. In particular, we used a model of prolonged exercise training consisting of brief sequences of intense exercise (running at 90% of the maximal intensity) interspersed with recovery periods. The exercise was repeated 5 days per week, for 8 weeks. As exercise remains the most potent behavioral therapeutic approach for improving mitochondrial health, not only in muscles but potentially in other tissues, a deeper understanding of the mechanisms of mitochondrial adaptations would be of great interest to clinicians across many healthcare disciplines.

References

1) Memme, J.M.; Erlich, A.T.; Phukan, G.; Hood, D.A. Exercise and mitochondrial health. J Physiol. 2021, 599, 803–817.
2) Chen, W.; Zhao, H.; Li, Y. Mitochondrial dynamics in health and disease: mechanisms and potential targets. Sig Transduct Target Ther. 2023, 8, 333.
3) Bishop, D.J.; Botella, J.; Genders, A.J.; Lee, M.J.; Saner, N.J.; Kuang, J.; Yan, X.; Granata, C. High-Intensity Exercise and Mitochondrial Biogenesis: Current Controversies and Future Research Directions. Physiology (Bethesda). 2019, 34, 56–70.

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Kinematic differences in élite and non-élite karateka: a link between biomechanics and subjective evaluation

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The main aspect of karate is performing defensive and offensive techniques at the maximum explosiveness. A karateka's performance is evaluated subjectively by judges and coaches, defining athletes' placement in competitions, but objective evaluation of technical elements is still lacking. This study aimed to investigate biomechanical differences in a fundamental technique between non-élite and élite karateka (former, current or potential national team members). The goal was to determine whether subjective evaluations align with objective kinematic analysis.

Thirteen élite and 13 non-élite black-belt karateka (comparable by age, height, weight, and number of weekly workouts) performed a *mawashigeri* (roundhouse kick) with dominant limb at maximal explosiveness. Kinematic variables were acquired by a 9-camera motion capture system and consisted in: range of motion (ROM) of hip flexion (HF_{ROM}), hip abduction (HA_{ROM}), and knee flexion (KF_{ROM}), from the beginning to the maximal kick height; maximal kick height (KH), initial and final center of mass (COM) position, normalized by the karateka's height; execution time. The between-groups comparison for each variable was conducted using an independent t-test (α=0.05).

Results showed that élite karateka had a greater HA_{ROM} (p=0.014), KF_{ROM} (p=0.001) and KH (p=0.015) than non-élite karateka.

A greater KF_{ROM} could indicate a more complete and precise technique, as the knee should be almost fully flexed in the first part of *mawashigeri*, fully extended at the peak KH. Higher HA could explain the consequent higher KH, indicating a greater flexibility and joint mobility of the élite group.

These results showed how some objective parameters were better in élite karateka, aligning with judges and coach subjective opinions on movement quality, which have been used to distinguish the skill levels of the athletes. However, further research should be conducted to provide quantitative analysis of technique execution, both for training improvements and as additional evaluation for athlete selection.

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Anatomical and morphological changes of the spine induced by core exercises

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Core exercises, when performed correctly, are essential for stabilizing the morphological structures of the spine to maintain correct posture, and tailoring training programs is considered crucial. This study aims to investigate the acute anatomical, morphological, and physiological effects of core exercises on the spine in healthy subjects without musculoskeletal disorders. Forty participants were analyzed with infrared thermography (IRT) and rasterstereography (RS) to analyze abdominal muscles activation and changes of the spine. Four groups were formed: Crunch Exercise (CE), Plank Exercise (PE), Russian twist exercise (RE), and Control (CG). RS and IRT were performed before and after the exercises to observe anatomical and morphological changes. For sagittal imbalance, the pre/post differences observed were 6.8 mm for the CE group, 0.1 mm for the PE group, -5.1 mm for the RE group, and -0.5 mm for the CG, with statistical significance (< 0.001). Regarding cervical depth, the differences were 6.2 mm for CE, 2 mm for PE, -4.4 mm for RE, and 0.4 mm for CG ($p < 0.001$). For lumbar depth, the differences observed were 0 mm for CE, -0.2 mm for PE, -0.1 mm for RE, and -0.5 mm for CG ($p = 0.893$). For coronal imbalance, the changes were -3.4 mm for CE, 4.2 mm for PE, 2 mm for RE, and -0.1 mm for CG ($p < 0.001$). The IRT indicated no muscle asymmetry post-exercise and highlighted different activations of the rectus abdominis and internal oblique muscles depending on the exercises performed. This study emphasizes the importance of individualized exercise programs and how abdominal exercises can impact the spine. By understanding the effects of various exercises on spine posture, fitness experts can design routines promoting spine's health and preventing back problems.

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Biomechanical alteration of gait and temperature evaluation of the knee in people with osteoarthritis: a pilot study

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Osteoarthritis (OA) is a common musculoskeletal disorder with more than 500 million people affected worldwide (1) according to the World Health Organization. Finding new ways to analyse people with OA is fundamental to improving disease identification and treatment. In this field, infrared thermography (IRT) is gaining interest considering its ease of use, non-harmfulness and non-invasiveness (2). This study aims to evaluate the biomechanical alterations of gait and temperature (Tsk) in people with knee OA, compared to healthy control, using two markerless systems: IRT and walker view. We recruited 10 males with knee OA (age 66.6 ±4.8, height 169±8,7, weight 76.9±10.1), and a Kellgren/Lawrence score of at least 2, and 10 healthy males (age 62.4±6.7, height 174±4.9, weight 75.1±5.86) with no musculoskeletal disorders. Participants were evaluated with the Flir E54 thermal camera to measure the average Tsk of the knee, and the Walker view treadmill to analyse the gait. Six regions of interest (ROIs) were selected on the thermograms (3): patellar, medial patella, and lateral patella, suprapatellar, medial and lateral areas. The gait parameters considered were: knee flexion, extension, total range of motion and step length. Shapiro-wilk test assessed the normality of data, and an independent t-test was used for significant differences between groups (α=0.05). The t-test resulted significant for the Tsk of all the ROIs, namely, patellar (0.005), medial patella (0.004), lateral patella (0.015), medial (0.004), lateral (0.024) and suprapatellar (0.016). Significant differences were detected for the knee flexion (0.007), total range of motion (0.021) and step length (<0.001). We found that these markerless systems were able to detect alterations in the biomechanics of gait and in Tsk of different ROIs of the knee, suggesting the feasibility of this approach for analyzing OA.

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References

1) Long H, Liu Q, Yin H, Wang K, Diao N, Zhang Y, Lin J, Guo A. Prevalence Trends of Site-Specific Osteoarthritis From 1990 to 2019: Findings From the Global Burden of Disease Study 2019. Arthritis Rheumatol. 2022 Jul;74(7):1172-1183. doi: 10.1002/art.42089. Epub 2022 Jun 2. PMID: 35233975; PMCID: PMC9543105.
2) Schiavon G, Capone G, Frize M, Zaffagnini S, Candrian C, Filardo G. Infrared Thermography for the Evaluation of Inflammatory and Degenerative Joint Diseases: A Systematic Review. Cartilage. 2021 Dec;13(2_suppl):1790S-1801S. doi: 10.1177/19476035211063862. PMID: 34933442; PMCID: PMC8804782.
3) De Marziani L, Boffa A, Angelelli L, Andriolo L, Di Martino A, Zaffagnini S, Filardo G. Infrared Thermography in Symptomatic Knee Osteoarthritis: Joint Temperature Differs Based on Patient and Pain Characteristics. J Clin Med. 2023 Mar 16;12(6):2319. doi: 10.3390/jcm12062319. PMID: 36983319; PMCID: PMC10055129.

Clinical and forensic anatomy, promotion of sectorial activity and its role in education

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The relevant role of scanning electron microscopy in the study of endometrial pinopodes in Medically Assisted Reproduction (MAR)

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The Medically Assisted Reproduction (MAR) in recent times has allowed couples previously unable to conceive to achieve viable pregnancy. Although MAR has improved outcomes for struggling couples, a new challenge has emerged: recurrent implantation failure (RIF).

Embryo implantation is a complex process involving fine tuning synchronization between trophoblast cells and uterine epithelium. Multiple endocrine-driven factors play a key role in uterine receptivity, and this complexity could be considered one of the major reasons for the implantation failure thus leading to RIF.

The pinopode - a hormonally regulated, large cellular protrusion on the uterine epithelial surface - represents a peculiar cellular structure that is believed to reflect receptivity.

Pinopodes are transient 5 to 10 μm cytoplasmic protrusions of the luminal plasma membrane of uterine epithelium. They are larger when compared to other epithelial plasma membrane protrusions, including blebs, cilia and microvilli, and occur during the window of implantation (~4 days in humans).

They appear and disappear within seconds to minutes on the surface of the cells.

Favorable fertility outcomes in women have been recently reported in association with the presence of mature, fully developed, pinopodes since they are directly linked to progesterone increase. Therefore the potential utility of these hormone-regulated cell structures to predict implantation in a clinical setting is object of increasing studies.

In this work we aim to evaluate the ultrastructural morphology of developing pinopodes by scanning electron microscopy to elucidate the different frames of the evolution and involution of these specific endometrial structures: Developing Pinopode (DP), Fully-Developed Pinopode (FDP), Regressing Pinopode (RP). We studied two cases of endometrial biopsies obtained after informed consensus. This ultrastructural study showed that it is possible to identify the different developmental steps which can be useful to improve a more personalized and successful therapeutic approach in the field of MAR.

References

1) Quinn K.E. et al. Pinopodes: Recent Advancements, Current Perspectives, and Future Directions. Mol. Cell Endocrinol. (2019) 501: 110644. doi:10.1016/j.mce.2019.110644
2) Bashiri A et al. Recurrent Implantation Failure-update overview on etiology, diagnosis, treatment and future directions. Reproductive Biology and Endocrinology (2018) 16:121. doi.org/10.1186/s12958-018-0414-2
3) Busnelli A. et al. Efficacy of therapies and interventions for repeated embryo implantation failure: a systematic review and meta-analysis. Scientific Reports (2021) 11:1747. doi.org/10.1038/s41598-021-81439-6

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A novel instrument for examining the vertebral artery: the transversoclasiotome

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To access the vertebral vessels, it is necessary to open the foramen transversarium of the cervical vertebrae. Currently, no tools are devoted to cutting the anterior lamina of the transverse processes, and alternative methods have produced uncertain results. This report describes and tests a new tool called the transversoclasiotome. After conducting a thorough review of the literature and patent databases, a blueprint for the transversoclasiotome was developed. A prototype was then tested on ten fresh-frozen cadavers within the Body Donation Program at the University of Padova, which serves as both the Regional and National Reference Center for the Preservation and Use of Gifted Bodies.

The transversoclasiotome is comprised of two delicate branches arranged like a pair of scissors. One branch acts as a cutting jaw, while the other serves as a knocker with a rounded tip. Both branches are angled at 30° to the principal axis and close parallel to each other. The cutting jaw aligns with a slit on the knocker profile without protruding beyond it, even when fully closed. It functions by cutting and wedging. Testing on fresh bodies has shown that it is suitable for its intended purpose, with an appropriate response to the pressure exerted on the bone lamina. The tool cleanly cuts the section without sliding off when closing on the bone.

The vertebral vessels were not damaged during instrument insertion or cutting, and their morphological characteristics are detailed. The transversoclasiotome has been demonstrated to be effective in sectioning the anterior lamina of the transverse processes of the cervical vertebrae. It meets the criteria of clinical anatomy for educating and training clinicians or surgeons, for forensic clinical anatomy during medico-legal investigations, and for research purposes.

Keywords: Cadaver lab, Body donation, Surgical simulation, Education

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Life stories: anamnestic form of the corpse/patient compiled by medical students during the anatomical-clinical simulation laboratory

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Simulation is taking on a rapidly growing role even if translational evidence supporting simulation for procedural learning is certainly not overwhelmingly positive; however, its role is indisputable where traditional experiences are either inadequate or unavailable. For high-quality, low opportunity procedural scenarios, simulation learning should be mandatory to assure high competence bar. In the current academic year the Institute of Anatomy in collaboration with the surgical departments has proposed a clinical/anatomical training model through a simulation of semiotics and maneuvers basic interventions on the cadaver to allow the student to acquire knowledge integrated sectoral and surgical anatomy of the body as well as the semiology and fundamental interventional manoeuvres. Approaching a corpse is obviously very different from learning simulation and to underline the ethical aspect we proposed to the students to compile a medical history sheet of their patient-cadavers. Each group was instructed to report their observations about their patient-cadavers, including a general description, evidence of disease, surgery, abnormalities, results of imaging and clinical maneuvers. This integrated approach allowed the students to formulate their observations into plausible hypotheses. Moreover, focusing on the donor as a whole and not only the clinical simulation manoeuvres helped the student to create respect and awareness of the gesture made by the donor, who donates for a community of students.

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"Live Cadaver" model for internal carotid artery injury simulation in endoscopic endonasal skull base surgery

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Introduction: Intraoperative injury of the internal carotid artery (ICA) is the most dreaded complication in endoscopic endonasal surgery (EES) of skull base. Training for ICA injury is practically impossible in live operative settings. In this study, we evaluate a pulsatile perfusion-based live cadaveric model for ICA injury simulation in a laboratory setting. The major emphasis of the study was to evaluate various means of controlling acute bleeding and evaluating the practical utility of this model for training purposes.

Methods: Five embalmed uninjected cadaveric heads were prepared for study by connecting to a pulsatile perfusion pump system filled with artificial blood solution. EES approaches were used to evaluate different types of ICA injuries similar to operative scenarios. Various methods of managing ICA injuries such as packing, clipping, trapping, were evaluated. The educational advantages of the live cadaver model were assessed using questionnaires given to participants in a hands-on dissection course.

Results: The trainee was faced with several scenarios similar to those encountered during an actual intraoperative ICA injury. Packing, clipping and trapping of the ICA injury was successfully achieved in all segments of the ICA. Clip-based reconstruction techniques were successfully developed. All trainees reported gaining new knowledge, learning new techniques. The responses to the questionnaire confirmed the significant educational value of this model.

Conclusions: The live cadaver model presented here provides real life experience with major vessel injury during EES in a laboratory setting. This model could significantly improve current training for management of intraoperative vascular injuries during EES.

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Environmental care in medical education and scientific research thanks to body donation

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A deep understanding of human anatomy is essential for medical professionals and is a critical milestone both in pre-graduate and post-graduate programs.

From ancient times, the exploration of the human body has involved the practice of dissection, which still represents the gold standard in the study of anatomy. Especially in the development of new surgical devices and techniques, it can be likened to the patient zero, thereby reducing potential risks in living subjects.

In the present days, many Universities, especially in Italy, face a systematic shortage of donated bodies, so artificial models, made of plastic materials, are employed in broad courses with numerous students. In this first-phase learning, the synthetic model could represent a viable option, being preparatory to a more complex and realistic anatomy. Their application, however, is often confined to a narrow and simplistic view. Furthermore, the utilization of increasingly complex artificial models often entails substantial environmental resources expenditure. Nowadays, there is a heightened awareness of the environment and impact on the Earth, leading to the introduction of the concept of the "three Rs". These principles "Reduce, Reuse, and Recycle" serve as foundational guidelines for promoting sustainability and environmentally conscious behaviours in daily lives. Aligning with the principles of the "three Rs", the utilization of donated bodies can represent a sustainable approach, eliminating the resource-intensive processes involved with artificial model and contributing to a circular and environmentally conscious practice. The versatility of donated bodies for reuse and their organic, biodegradable nature further supports their ecological sustainability.

In conclusion, the environmentally friendly attributes of donated body use, coupled with its educational effectiveness, highlight its value as a preferred choice over synthetic models. However, it is important to say that artificial models serve a complementary function in medical education, bridging the gap where donated bodies are unavailable.

References

1) Orsini, E.; Quaranta, M.; Ratti, S.; Mariani, G.A.; Mongiorgi, S.; Billi, A.M.; Manzoli L. The whole body donation program at the University of Bologna: A report based on the experience of one of the oldest university in Western world. Ann Anat. 2021, 234.
2) Orsini, E.; Quaranta, M.; Mariani, G.A.; Mongiorgi, S.; Cocco, L.; Billi, A.M.; Manzoli, L.; Ratti, S. Near-Peer Teaching in Human Anatomy from a Tutors' Perspective: An Eighteen-Year-Old Experience at the University of Bologna. Int J Environ Res Public Health. 2021, 398.

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Clinical anatomy of maxillary sinus variations

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The study of clinical anatomy of maxillary sinus variations is relevant to understand the related pathogenesis of inflammatory and oncologic diseases and for surgical planning, in cases of functional endoscopy surgeries, sinus floor lift and bone volume augmentation [1]. The study aimed to assess the variability of the intraosseous arterial vascular supply, the presence of the Underwood septa and the morphometry of the maxillary sinus volume, using cone beam computed tomography (CBCT) images and related imaging softwares (InVivo® and InVesalius®) for measurements.

100 CBCTs were considered for arterial vascular supply assessment; 100 CBCTs were considered to study the features of Underwood septa; 18 CBCTs were selected to assess the maxillary sinus volume variability.

The presence of the intraosseous arterial supply was 100% in the considered sample, with caliber ranging from 0.4 mm to 1.8 mm. The presence of septa within the maxillary sinus was 19%, and the mean measure of the maxillary sinus volume was 14.3 mm^3, in the related examined samples.

The results of the morphometric analysis showed how the maxillary sinus, described through centuries by several anatomists [2] shows variations clinically relevant regarding the vascular supply, the presence of bony septa and consequently the sinus volume, and to be aware in surgical planning stage and during the procedures.

The innovation technology in computed tomography imaging allows clinicians to search and spot these variations and prevent eventual intra-operative risks.

References

1) Bernardi, S.; Bianchi, S.; Gerardi, D.; Petrelli, P.; Rinaldi, F.; Piattelli, M.; Macchiarelli, G.; Varvara, G. Anatomy of Maxillary Sinus: Focus on Vascularization and Underwood Septa via 3D Imaging. Tomography 2024, 10, 444-458. https://doi.org/10.3390/tomography10040034

2) Bernardi, S., Angelone, A. M., & Macchiarelli, G. Anatomy in dentistry: From the beginnings to contemporary reality. Clin. Anat. 2022 35, 711– 722. https://doi.org/10.1002/ca.23869

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The probative uniqueness value played by facial scars for the personal identification

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Personal identification refers to the matching of antemortem and postmortem data to ascertain/exclude the identity of a person/cadaver. In some humanitarian contexts, photos are the only available antemortem material provided by families or retrieved from social-networks[1,2]. The face, commonly exposed in photos, may express 'personal descriptors' that give important insights for personal identification if their uniqueness is quantified with probabilistic approaches[2,3].

This study aims at collecting systematic data on facial scars frequency and verifying their potential in personal identification through a probabilistic methodological approach previously presented for facial nevi[3]. A retrospective analysis of 3D facial scans of 1039 Italian subjects was performed to attest the incidence of scars discriminated according to size, morphology and position in 12 facial areas. From the gathered data a probabilistic approach based on descriptive facial scars codes was implemented, providing likelihood ratios for all patterns.

Facial scars proved uncommon if compared to facial nevi as only 366 out of 1039 subjects (35%) own at least one (vs 91% owned at least a nevus). Among the subjects presenting facial scars, only 26.3% owned codes representing a unique 'identifying' pattern if dimension and morphology are considered in addition to number and position. Although facial scars proved infrequent (with limited uniqueness) and commonly placed in the cheeks and forehead, their identifying potential is indisputable when their number increases in other facial areas, reaching strong probative values of uniqueness. Moreover, their anatomical parameters can be inherently 'identifying', adding a further probative value to that provided by this study based uniquely on number, position, and dimensional and morphological gross discrimination. Finally, although in the general population facial scars are less frequent and theoretically with a minor identifying potential than facial nevi, if used in combination with other personal anatomical descriptors and/or skin marks might play as essential clues for the identification.

References

1) Olivieri, L.; Mazzarelli, D.; Bertoglio, B.; De Angelis, D.; Previderè, C.; Grignani, P.; Cappella, A.; Presciuttini, S.; Bertuglia, C.; Di Simone, P.; Polizzi, N.; Iadicicco, A.; Piscitelli, V.; Cattaneo, C. Challenges in the identification of dead migrants in the Mediterranean: The case study of the Lampedusa shipwreck of October 3rd 2013. *Forensic Sci Int* 285, 121-128 (2018).
2) Cappella, A.; De Angelis, D.; Mazzarelli, D.; Vitale, A.; Caccia, G.; Fracasso, T.; Cattaneo, C. Rediscovering the value of images in supporting personal identification of missing migrants, *Legal Medicine* 54, 101985 (2022)
3) Cappella, A.; Solazzo, R.; Mazzarelli, D.; Gibelli, D.; Dolci, C.; Sforza, C.; Cattaneo, C. The potential of facial nevi in personal identification. *Sci Rep* 14, 6206 (2024).

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Detection of early signs of vital response in human bone fractures by histological analysis on non-decalcified samples

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While it is known that healing processes start almost immediately after traumatic injuries, the early changes occurring in the bone tissue in response to the damage have yet to be deepened. Such information would represent a crucial guide to analyze the viability of a lesion, thus allowing optimization of forensic diagnostics to assess whether a fracture occurred before or after death. To date, non-decalcified histological sections have proven to be a useful and high-performance technique in many fields of biomedical research, providing more accurate data on the bone tissue structure than traditional decalcified sections, but they are still seldom used in forensic science. In this study, early vital reactions occurring in bone tissue following a traumatic event within 24 hours from death were evaluated by non-decalcified histological analysis, with the objective of identifying potentially useful markers in the diagnosis of the vitality of bone injuries. Samples of human ribs characterized by fractures occurred before death with different survival times (between 0 and 22 hours) and a negative control (an unaffected rib) were processed to obtain ground sections, stained with toluidine blue and pyronine yellow for light microscopic observation. In all samples, fracture extremities appeared to be non-linear, irregular, and characterized by a marked staining along the whole fracture rim, differently from what observed in the control specimen. Signs of active bleeding and blood clots were detected. In addition, newly deposited reparative tissue was observed in the sample after 22 hours of survival. In conclusion, the current preliminary results seem to support the validity of this method in the assessment of early tissue reactions after bone fractures. Further investigations, including immunohistochemical analyses on ground sections would be useful to complete the comprehension of such biological phenomena.

References

Pechnikova, M; Porta, D; Cattaneo, C. Distinguishing between perimortem and postmortem fractures: are osteons of any help? Int. J. Legal Med. 2011, 125: 591-595.

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Altered miRNome impacts on immune responses, inflammatory processes and tissue repair in children affected by Long Covid

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The emerging research on the involvement of microRNAs (miRNAs) in paediatric Long Covid, drawing insights from studies on adults. Various miRNAs (are implicated in COVID-19 pathology, including antiviral responses and interaction with the SARS-CoV-2 genome. In adult Long Covid patients, distinct miRNAs involved in immune responses and inflammation are dysregulated and the ones especially regulating ACE2 expression, could be responsible for organ-specific complications (1). In our study we intended to dissect the potential

implications of miRNAs in paediatric Long Covid, to better understand their role and potential clinical applications (2-3).

Children were evaluated at least 8 weeks after their initial infection and classified as "fully recovered" (no persisting symptoms and return to pre-Covid activity levels) or as having "Long Covid" (persistent symptoms impacting daily life for at least eight weeks, with other diagnoses excluded). Through epigenetic/transcriptomic analysis we compared miRNome of different patients (n=34) e once revealed potential microRNA, we studied their pathways. We found miRNAs able to characterize the disease when compared to healthy children; moreover, we found 4 miRNAs (hsa-miR-26a-5p, hsa-miR-423-3p, hsa-miR-29a-3p, hsa-miR-424-5p) able to significantly distinguish Long-Covid and recovered cohorts. We found that these miRNAs are all involved in the regulation of similar gene pathways. Among them we predicted that the 4 miRNAs characterizing the disease state were able to modulate AKT expression and consequently different functions of many immune cells. In particular, AKT represents one of the main triggers of immune response and is responsible for cell activation and proliferation and antibody production. These data are in line with the long-lasting persistency of Long-Covid.

The identification of specific miRNA signatures could help in diagnosing, prognosing, and monitoring paediatric Long Covid, and miRNAs may serve as potential therapeutic targets. Moreover, targeting the gene(s) modulated by dysregulated miRNAs could represent al alternative strategy to counteract this disease.

References

1 Widiasta, A., et al. Potential Role of ACE2-Related microRNAs in COVID-19-Associated Nephropathy. Non-coding RNA Research 2020, 5, 153–166, doi:10.1016/j.ncrna.2020.09.001.
2 Di Sante, G., et al. Immunopathology of SARS-CoV-2 Infection: A Focus on T Regulatory and B Cell Responses in Children Compared with Adults. Children 2022, 9, 681, doi:10.3390/children9050681.
3 Di Sante, G., et al. Immune Profile of Children with Post-Acute Sequelae of SARS-CoV-2 Infection (Long Covid); Pediatrics, 2021

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Anatomical features and variations of the vertebrobasilar system

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The posterior circulation of the brain constitutes the vertebrobasilar system and its branches, which are responsible for about 20% of the brain blood supply. The aim of this study was to describe the morphological characteristics of the vertebrobasilar system. We examined radiographs of 103 patients, 58 male and 45 females, age range from 25 - 82, mean age 58.4 years who had CT angiography undertaken for a variety of clinical reasons, performed as a part of their medical treatment at the University Institute for Radiology in Skopje, Macedonia. The left vertebral artery arose from the left subclavian artery in 94.17% and the right vertebral artery had origin from the right subclavian artery in 99.02%. Variable origin of the left vertebral artery from the aortic arch was noticed in 5.82% and in one patient (0.97%) we found atypical arisen of the right vertebral artery from the right common carotid artery. The diameter of the vertebral artery was 3.20 ± 0.74 mm on the right side and 3.33 ± 0.76 mm on the left side. The mean length of the basilar artery was 31.60 ± 5.1 mm (from 21.4 mm to 44.1 mm). The mean diameter of the basilar artery was 3.27 ± 0.52 mm (from 2.22 to 4.87 mm). Most of the SCA arise from the basilar artery as a single vessel. The most common variations of the SCA were duplication (frequency 1.94% on right and 0.97% on left) and origin from PCA (frequency 1.94% bilateral). In four patients (3.88%) we found fenestrations of posterior brain circulation, three fenestrations (2.91%) was on the basilar artery and one fenestration (0.97%) was on the vertebral artery. In one patient persistent trigeminal artery was found. A sound knowledge of vertebrobasilar system anatomy and variations is important during diagnostic, operative and endovascular procedures.

References

1) Wang, S.; Ren, WJ.; Zheng, L.; Sun, ST.; Zhang, BH.; Chen, Y.; Xiang, LB. Anatomical Variations of the Vertebral Artery: Analysis by Three-Dimensional Compjuted Tomography Angiography in Chinese Population. Orthop Surg. 2021;13(5):1556-1562.
2) Tsantili, AR.; Karampelias, V.; Samoilis, A.; Chrysikos, D.; Antonopoulos, I.; Spanidis, Y.; Protogerou, V.; Troupis, T. Anatomical Variations of Human Vertebral and Basilar Arteries: A Current Review of the Literature. Morphologie. 2023;107(357)169-175.

Keywords: vertebral artery; basilar artery; anatomy; variations

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Facial features in Cri-du-Chat syndrome: 3D morphometric analysis and possible genotype-phenotype correlations

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Cri-du-Chat syndrome (CdCs) is a rare genetic condition due to deletions of the short arm of chromosome 5 (5p). The manifestations of the disorder include a typical cry at birth, distinctive facial appearance and intellectual disability, with phenotypic variability. They have been related to the size and position of 5p deletion, with increasing knowledge (1). Specifically, facial dysmorphisms have been associated with deletions in the region 5p15.2. The study aimed to quantify facial dysmorphisms in CdCs and explore possible genotype-phenotype correlations with regard to specific facial features.

Seven subjects with cytogenetically confirmed diagnosis of CdCs (5 males, 2 females; age range 18-44 yrs) were included in the study. Array-CGH detected 5p deletions ranging from 18.4 to 31.3 Mb, all of them encompassing the region 5p15.2. From facial stereophotogrammetric images, linear distances, angles, facial shape and asymmetry between homologous areas of trigeminal innervation (2,3) were quantified and compared with data from 201 control subjects, matched for sex and age (Mann-Whitney test, Bonferroni correction for multiple testing). Genotype-phenotype correlations were investigated by an exploratory Spearman's analysis.

Patients showed a significant decrease in facial width, middle and lower facial depths, palpebral fissure length and philtrum length, thus corroborating the typical facial appearance described in CdCs. Facial asymmetry was also confirmed, being the patients significantly more asymmetric than control subjects in the middle and lower third of the face. Despite the complete loss of the region 5p15.2 in all subjects, Spearman's analysis detected a significant relationship between the 5p deletion size and the philtrum length, palpebral fissure length, and facial width. The results of the study suggest that facial dysmorphisms in CdCs could be influenced by the activity of genes located in additional regions of the chromosome. The promising findings need however to be confirmed extending the study on a larger sample of patients.

References

1) Villa, R.; Fergnani, V.G.C.; Silipigni, R.; Guerneri, S.; Cinnante, C.; Guala, A.; Danesino, C.; Scola, E.; Conte, G.; Fumagalli, M.; Gangi, S.; Colombo, L.; Picciolini, O.; Ajmone, P.F.; Accogli, A.; Madia, F.; Tassano, E.; Scala, M.; Capra, V.; Srour, M.; Spaccini, L.; Righini, A.; Greco, D.; Castiglia, L.; Romano, C.; Bedeschi, M.F. Structural brain anomalies in Cri-du-Chat syndrome: MRI findings in 14 patients and possible genotype-phenotype correlations. Eur J Paediatr Neurol. 2020, 28,110-119.
2) Dolci, C.; Rigamonti, A.E.; Cappella, A.; Gibelli, D.M.; Grugni, G.; Caroli, D.; Sforza, C.; Sartorio, A. Robustness of distinctive facial features in Prader-Willi syndrome: a stereophotogrammetric analysis and association with clinical and biochemical markers in adult individuals. Biology 2022, 11, 1148.
3) Cappella, A.; Solazzo, R.; Yang, J.; Hassan, N.M.; Dolci, C.; Gibelli, D.; Tartaglia, G.; Sforza, C. Facial asymmetry of Italian children: a cross-sectional analysis of three-dimensional stereophotogrammetric reference values. Symmetry 2023, 15, 792.

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Importance of training for emergency medicine physician and critical care nurses in life-saving procedures using donor labs: the experience of the high-tech anatomical center at the University of Bologna

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The proficiency of emergency medicine professionals and critical care nurses in performing life-saving procedures, particularly those that are time-dependent, is paramount to patient outcomes. Training in donor labs offers a unique and superior educational experience compared to other modalities. This abstract aims to highlight the scientific importance of donor-based training for these healthcare providers, drawing on recent studies and educational protocols, and to share the experience of the new high-tech anatomical center at the University of Bologna.

Background. Effective management of critical situations in emergency and critical care settings hinges on the ability of healthcare providers to perform advanced and timely interventions. Procedures such as mini-thoracotomy, difficult intubations, and intraosseous access are crucial, yet they are performed infrequently enough to necessitate regular, rigorous training. Donor labs provide an unparalleled realistic training environment that enhances the learning experience and improves procedural competence.

Methods. This presentation highlights the innovative approach of the new high-tech anatomical center at the University of Bologna, which incorporates advanced simulation technologies and donor-based training. The focus is on hands-on practice with donor models and the integration of continuous medical education. Additionally, findings from recent studies and educational interventions that assess the impact of donor-based training programs on the competence and confidence of emergency medicine professionals and critical care nurses are synthesized.

Studies indicate that donor-based training programs significantly enhance both theoretical knowledge and practical skills of healthcare providers. For example, these programs improve understanding and comfort in performing critical procedures and increase confidence and proficiency in emergency interventions such as airway management and ultrasound diagnostics.

Conclusion. Donor labs are invaluable for high-level training of emergency medicine professionals and critical care nurses, providing realistic, hands-on experience that significantly enhances procedural skills, confidence, and clinical knowledge. Integrating donor-based training into medical education, as exemplified by the high-tech anatomical center at the University of Bologna, is recommended to maintain high standards of care and improve patient outcomes in critical, time-dependent situations.

Keywords: Anatomy, emergency medicine, critical care, life-saving procedures, donor lab training.

References

Tabas, J., Rosenson, J., Price, D., Rohde, D., Baird, C. H., & Dhillon, N. (2005). A comprehensive, unembalmed donor-based course in advanced emergency procedures for medical students. Academic emergency medicine.

Takayesu, J., Peak, D., & Stearns, D. (2017). Donor-based training is superior to simulation training for cricothyrotomy and tube thoracostomy. Internal and Emergency Medicine.

Zaia, B., Briese, B., Williams, S. R., & Gharahbaghian, L. (2012). Use of donor models in point-of-care emergency ultrasound education for diagnostic applications. The Journal of emergency medicine.

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The antiquity of spina bifida: morphological and historical aspects of an evolution-related congenital anomaly

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Spina bifida (SB) represents a congenital anomaly which arises from incomplete development of the neural tube and can be subdivided into SB occulta and aperta. The former is a milder and often asymptomatic condition, while the latter is incompatible with life. Traditionally a topic of neurosurgical interest, recent studies have highlighted the fact that the global prevalence of SB is increasing hence suggesting a micro-evolutionary process under way. Moreover, palaeopathological and historico-medical research on this condition can yield additional light on the antiquity and evolution of this anomaly through time. Here we recapitulate the history of its scientific discovery and descriptions of this condition with a focus on the seminal study by Nicolaes Tulp (1595-1674) until present-day clinical paediatric analysis. Furthermore, we also deal with the palaeopathological record of this condition in ancient populations and present morpho-radiological data from some ancient cases including one retrieved from the excavations of the Greek-era necropolis of Leontinoi (Sicily). Future research perspectives are offered.

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Can frequencies of cranial non-metric traits be used for personal identification? Application to a real case of humanitarian forensic anthropology

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Personal identification represents one of the most critical procedure in forensic anthropology, and is acquiring more and more importance in humanitarian forensic contexts where victims of migration are often unknown and require a precise description of anatomical characteristic useful to reach a positive identification. A possible help to personal identification may derive from the assessment of non-metric traits. i.e. morphological anatomical variants, reported in general population with different frequencies, which may allow to provide a potentially individualizing profile based on compound frequencies, similarly to DNA and fingerprint approaches.

This presentation aims at assessing the frequencies of different cranial non-metric traits in crania recovered from the shipwreck occurred in the Mediterranean Sea in April the 18th 2015, as part of the attempt at identifying the victims.

The study sample includes 119 crania of male individuals and 35 non-metric traits (24 pair and symmetric, 11 unpaired) were assessed on each cranium. The frequencies of each variant were used to calculate the compound frequency of all the variants in single individuals (i.e., the product of the frequencies), which represents the possibility to find an individual with the same combination of cranial features within the present sample.

Overall, 99.2% of individual showed a compound frequency inferior to 1 out of 1 billion: in other words less than a single person out of 1 billion people would show the same combination of traits. Moreover, in 91.6% of cases the compound frequency is lower than 1 out of 10 billion, demonstrating that some mix of non-metric traits may have a potential for personal identification, similarly to alleles in genetics and minutiae in fingerprint analysis.

In conclusion, the use of cranial non-metric traits seems promising for improving the chances of personal identification in humanitarian forensic anthropological contexts.

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References

1) Cattaneo, C.; De Angelis, D.; Mazzarelli, D.; Porta, D.; Poppa, P.; Caccia, G.; D'Amico, M.E.; Siccardi, C.,; Previderè, C.; Bertoglio, B.; Tidball-Binz, M.; Ubelaker, D.; Piscitelli, V.; Riccio, S. The rights of migrants to the identification of their dead: an attempt at an identification strategy from Italy. Int. J. Legal Med. 2023,137,145-156.
2) Palamenghi, A.; Borlando, A.; De Angelis, D.; Sforza, C.; Cattaneo, C.; Gibelli, D.Exploring the potential of cranial non-metric traits as a tool for personal identification: the never-ending dilemma. Int. J. Legal Med. 2021,135,2509-2518

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From skull to story: reconstructing lives of 19th century patients from Ospedale de' Pazzi

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Prior to the XVIII century, Bologna lacked a dedicated asylum, and the "pazzi" were treated alongside prostitutes, criminals, and miserables. The Ospedale de' Pazzi was established in 1710 within the Sant'Orsola complex through a donation from an Anonymous Benefactor and was closed in 1867 due to complaints of inadequacy, following the opening of the "Francesco Roncati" Provincial Psychiatric Hospital.

At least 38 skulls derived from patients who died in the first half of XIX century at the Ospedale de' Pazzi are preserved inside the Anatomical Centre of the University of Bologna. These were likely provided to Professor Luigi Calori in the mid-19th century for craniometric and anthropological studies. This project aimed to reconstruct the lives of these patients through anthropological and archival analyses. Biological profiles were constructed, revealing that most individuals were adults (over 20 years), classified into young adults and adults. Sex estimation indicated approximately three times more males than females. Geographic origin analysis suggested that more than 70% of individuals exhibited typical European characteristics.

Dental pathology observations provided insights into the patients' hygienic and nutritional conditions. Over 50% showed caries, abscesses, tartar, *ante-mortem* tooth loss, and notably, enamel hypoplasia, corroborating historical complaints about poor sanitary conditions and vitamins and calcium deficiency.

Detailed patient lives were further investigated through clinical records from the Minguzzi-Gentili Library Archive. Precise demographic information (names, dates of death, pathologies, etc.) on the skulls enabled the reconstruction of patients' lives and comparison with previously obtained anthropological and dental data.

This interdisciplinary study successfully combined biological, dental, and archival evidence to provide a comprehensive reconstruction of the lives of patients from the Ospedale de' Pazzi, highlighting the historical context and living conditions of these individuals. However, further analysis, using virtual anthropology methods, such as geometric morphometrics, could provide further information about the topic.

References

1) A. Alvisi. L'antico Ospedale dei Pazzi in Bologna, Fava e Garagnani, 1881
2) D. Gualandi. Della costruzione di un manicomio pubblico: dissertazione, Istituto delle Scienze, 1850
3) T.D. White, M.T. Black, P.A. Folkens, Human Osteology, Academic Pr, 2011

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Improved aesthetic physicians knowledge of face anatomy through cadaver dissection

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An adequate knowledge of face anatomy is an essential prerequisite for a safe aesthetic medicine practice. Cadaveric dissection is an effective teaching method of anatomy in medical training. In cadaveric dissection, the learner plays the central role of the teaching process and can observe structures in their natural localization.

The Camerino and Turin Universities have been organizing for several years an international Master in Aesthetic Medicine and Therapeutics. This Master in the last 3 years has enrolled 1,000 attendants of different nationalities and consisted of two programs, one for national users, which did not include a cadaver lab and one for international users that included a cadaver lab. Attendants to the national course were 250 (145 females and 105 males), whereas those to the international course were 750 (400 females and 350 males).

Each attendant received a multiple-choice questionnaire with 25 questions to ascertain knowledge of face anatomy. One week after, the attendants of the national program followed a series of video face dissection of 4 (first day) + 8 (second day) hours. The attendants to the international program followed face-to-face a dissection course with one session of 4 hours with the demonstration made by the teacher (first day). A second session lasting 8 hours in which each attendant (4 per one anatomical sample) did directly the dissection after a training session supervised by a teacher (second day). The day after the second day of training, attendants of the two groups received another multiple-choice questionnaire. A comparison between the pre-course and post-course test results between the two groups was therefore done.

A statistical difference in pre-course and post-course test evaluation was noticeable between the two groups of attendants. Comparatively, the scores obtained by attendants who made directly dissections were significantly higher than those of attendants following dissection with videos.

Our data suggest that cadaver anatomy training improves facial anatomy knowledge. The best scores obtained by those doing directly the dissection indicate that cadaver dissection may enhance confidence in performing facial aesthetic procedures. These findings suggest the need to introduce anatomy cadaver dissection programs in courses for aesthetic physicians training.

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Tattooed human skin flaps: a multidisciplinary case study

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The "Luigi Cattaneo" Anatomical Wax Collection is part of the University Museum Network and is located at the Anatomical Institute of Bologna University. It displays a rich collection of anatomical preparations and wax models dating back to the 19th century, most of them concerning both teratology and anatomical freaks, according to the spirit of the museum collections of that period. Two of these displayed "odds" consisted of tattooed human skin flaps with different subjects (sacred/religious and erotic/amorous) placed on wooden platforms. The Museum of Cultures of Milan (MUDEC) requested them for a loan for the exhibition "Tattoo. Tales from the Mediterranean" and this was the opportunity to conduct multidisciplinary research. The skin flaps underwent a restoration process according to a slightly modified protocol used for parchment and some interesting details were revealed. Four samples (two from each wooden plate) were selected to be investigated before restoration. An X-ray fluorescence (XRF) analysis by means of a Macro XRF (MA-XRF) scanner prototype was performed to do a non-destructive analysis of the elemental composition and distribution of different pigments in the historical manufacts [1]. On the same samples an ATR-FTIR spectroscopic analysis by means of Bruker VERTEX 70V interferometer coupled with the Hyperion 3000 Vis/IR microscope was later performed [2]. The non-destructive nature of the radiation used and the non-invasiveness of the investigation method when carried out in ATR mode aimed at analyzing the preservation state of the collagen within the flaps and providing chemical information on the outermost layer of the tattooed skin. Few drawbacks emerged (surface not perfectly flat, protruding hairs detected as "obstacles" by the scanner) and raw data analysis is still ongoing but deepening knowledge of the history of the samples will be useful to establish proper restoration and conservation protocols and to develop cultural heritage analysis instruments and procedures.

References

1. Mazzinghi A, Ruberto C, Castelli L, Czelusniak C, Giuntini L, Mandò PA, Taccetti F. MA-XRF for the Characterisation of the Painting Materials and Technique of the Entombment of Christ by Rogier van der Weyden. Applied Sciences. 2021; 11(13):6151.
2. LIU, Guan-Lin; KAZARIAN, Sergei G. Recent advances and applications to cultural heritage using ATR-FTIR spectroscopy and ATR-FTIR spectroscopic imaging. Analyst, 2022, 147.9: 1777-1797.

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Pulmonary veins variation number and its clinical implication: a new case report

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The lung veins belong to the pulmonary circulation and are blood vessels that drain freshly oxygenated blood from the lungs to the heart's left atrium, by their correspondent and independent ostia. Focusing on the "normal" gross anatomy, the human body has four pulmonary veins, transferring blood by two left and two right veins [1]. However, since the second half of the 20th century, it has been reported different branching patterns in the number of pulmonary veins as well as the number of left atrium ostium sometimes might not correspond in quantity [2]. Furthermore, it has been suggested that such anatomical variation may influence and initiate atrial fibrillation, and therefore attention to this variability is necessary [3].

The purpose of this clinical case study is to describe a rare anatomical variation in the number of pulmonary veins that were found during cadaver dissection classes with medical students performed at the ICLO Teaching and Research Center (Verona, Italy). After a detailed examination and dissection of the thorax portion of the body, the present case report, belonging to a woman 75 years old, reported a total of five pulmonary veins. Specifically, it was found that the examined right lung had 3 instead of 2 pulmonary veins that drain into the left atrium of the heart. The left lung was "normally" drained with 2 pulmonary veins.

The observed anatomical variation in the number of pulmonary veins is of clinical significance to radiologists, electrophysiologists, and cardio-thoracic surgeons while performing surgical procedures such as segmentectomy but also on the heart.

References

1) Shukla, L.; Gaur, N.; Soni, G.; Dhall, U. Variation in number and drainage pattern of pulmonary veins draining into the left atrium. J. Anat. Soc. India 2012, 61(1), 5-8.
2) Prasanna, L.C.; Praveena, R.; D'Souza, A.S.; Bhat, K.M.R. Variations in the Pulmonary Venous Ostium in the Left Atrium and Its Clinical Importance. J. Clin. Diagn. Res. 2014, 8(2).10-11.
3) Skowerski, M.; Wozniak-Skowerska, I.; Hoffmann, A.; Nowak S.; Skowerski T.; Sosnowski M.; Wnuk-Wojnar A.M.; Mizia-Stec K. Pulmonary vein anatomy variants as a biomarker of atrial fibrillation – CT angiography evaluation. BMC Cardiovasc. Disord. 2018, 18(1), 146.

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AI-driven predictive models for determining age and sex from cranial measurements

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The ability to accurately determine the age and sex of individuals from cranial measurements has significant implications in forensic anthropology and bioarchaeology. Leveraging artificial intelligence (AI) advancements, we have developed predictive models that utilize cranial measurements to estimate these demographic variables. Additionally, these models have been adapted to predict cephalometric measurements from non-radiological data, enhancing their utility in clinical settings. This study utilized a dataset comprising cranial measurements from diverse populations. Advanced AI algorithms, including Support Vector Machines (SVM), Artificial Neural Networks (ANN), and Deep Neural Networks (DNN), were employed to develop predictive models. The dataset was split into training and validation sets, and cross-validation techniques were applied to ensure model robustness and prevent overfitting. Feature selection and hyperparameter tuning were conducted to optimize model performance. The predictive models demonstrated high accuracy in estimating sex, with the SVM and ANN models achieving accuracies between 86% and 91%. The DNN model further improved accuracy, reaching over 93%. For age prediction, models treated age as a continuous and categorical variable, with the constant model achieving a mean absolute error (MAE) of less than 2 years and the categorical model achieving an accuracy of approximately 85%. The cephalometric prediction models showed promising results, with significant reductions in mean squared error (MSE) compared to actual clinical measurements. Integrating AI algorithms in the analysis of cranial measurements significantly enhances the accuracy of age and sex estimation. Furthermore, extending these models to predict cephalometric measurements from non-radiological data offers a promising tool for clinical applications, potentially reducing the need for radiographic imaging. Future research will expand the dataset and refine the models for broader applicability and improved accuracy.

References

1. Del Bove, A.; Veneziano, A. A Generalised Neural Network Model to Estimate Sex from Cranial Metric Traits: A Robust Training and Testing Approach. Appl. Sci. 2022, 12, 9285.
2. Foreman, A.; Lazenby, R.A. Use of Craniometric Data in a Generalized Procrustes Analysis to Establish Human Biological Affinities. Forensic Sci. Int. 1996, 82, 39-53.
3. GADNN: A Revolutionary Hybrid Deep Learning Neural Network for Age and Sex Determination Utilizing Cone Beam Computed Tomography Images of Maxillary and Frontal Sinuses. BMC Med. Res. Methodol. 2023.

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Forensic clinical anatomy of pediatric abusive head trauma

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Forensic Clinical Anatomy is "the practical application of anatomical knowledge and methods (from ultrastructural to macroscopic aspects), endowed with substantial clinical/surgical implications, to the ascertainment and evaluation of medico-legal problems" (1-2). Pediatric abusive head trauma is an injury to the intracranial contents or skull of an infant or child younger than 5 years old due to violent shaking (shaken baby syndrome) and/or blunt impact. The outcome ranges from complete recovery to significant brain damage or to death. Abusive head trauma with shaking mechanism is frequently characterized by the so-called triad: encephalopathy, intracranial subdural haemorrhage, and retinal haemorrhages. Other pathological findings include bruises/ecchymoses, fractures, lesions of the spinal ligaments, spinal subdural haemorrhages (3), hypoxic-ischaemic/haemorrhagic injuries of the spinal cord, focal haemorrhages of intraorbital adipose tissue or in meningeal spaces of optic nerves. The different aspects of pediatric abusive head trauma are strictly anatomical in nature, with regards to their pathophysiologic mechanisms and correlated methods of investigation in a forensic context.

In the present work, we addressed Forensic Clinical Anatomy issues with reference to our forensic casistics of six cases (one with recovery, one with significant brain damage, four autoptic cases) of pediatric abusive head trauma.

In our experience, from a dissective methodological point of view, judicial autopsies in these cases must be integrated with anatomical dissections to specifically evaluate the different anatomical structures 'layer-by-layer'. Post mortem imaging of sampled anatomical structures (long bones, spinal structures) may be useful to better define in vivo imaging. Sampling for histopathological studies must be performed through strictly anatomical methods, in order to permit the identification of the injured structures (nerves, vessels, muscles). An integrated forensic-anatomical approach in pediatric abusive head trauma is not only useful for better definition of the single case but may also widen the knowledge about the anatomical bases of some findings.

References

1) Porzionato, A.; Macchi, V.; Stecco, C.; Loukas, M.; Tubbs, R.S.; De Caro, R. Forensic clinical anatomy: A new field of study with application to medico-legal issues. Clin. Anat. 2017, 30, 2-5.
2) Porzionato, A.; Macchi, V.; Loukas, M.; De Caro, R. Forensic clinical anatomy – Definitions, Methods and Fields. In: Ferrara SD, editor. P5 Medicine and Justice. Innovation, Unitariness and Evidence. Berlin: Springer; 2017, 377-394.
3) Porzionato, A.; Macchi, V.; De Caro, R. Physical Abuse of Children. N. Engl. J. Med. 2017, 377, 398-399.

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Guidelines for the donation and acceptance of recognizable anatomical parts after amputation to CIR-COSCIENZA for study, training and research purposes

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Body donation to science plays a role in significantly improving medical-surgical training and biomedical knowledge. Despite technological advances in medicine, the community of medical professionals and researchers still need to rely on anatomical dissection and biological material of human origin to improve their skills and contribute to the advancement of healthcare. In this context, the ability to use not only cadavers but also recognizable human body parts, such as amputated limbs, is valuable. In Italy, however, such pivotal resources are hardly available, in part due to the lack of a legal environment that encourages body donation to science. Indeed, the implementation of Law 10/2020 on the post mortem disposition of corpses and tissues for study, training, and scientific research purposes is a major challenge. In addition, no specific regulation governs the living donation of recognizable anatomical parts. Consequently, they are always disposed of by the healthcare facility treating the amputated person, in line with Article 3 of the Regulation governing the management of healthcare waste (Presidential Decree of July 15, 2003, n° 254) requiring their cremation or burial at the patient's request.

Acknowledging the value of recognizable anatomical parts, the Interdepartmental Research Center for the Valorization of Bodies Donated to Science (CIR-COSCIENZA) of the University of Palermo has taken an unpreceded and original initiative. Since its foundation in 2018, CIR-COSCIENZA has been committed to ensuring a dignified and respectful treatment of human bodies donated to science from a multidisciplinary approach and has formulated some guidelines for the living donation and acceptance of recognizable anatomical parts after amputation for study, training, and research purposes. In line with the legal and ethical standards and norms regulating body donation at the national and international levels, these Guidelines aim to promote medical progress and research, respecting the patients' self-determination, human dignity, and the right to physical and mental integrity as primary interests.

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Advancing anatomical-medical training: implementing reventilation and revascularization technologies in body donor simulations

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Medical training utilizing body donors is pivotal in the evolution of medical education, providing a critical, hands-on component that complements theoretical learning. Such training offers a unique and realistic approach to mastering intricate medical procedures, essential for improving surgical accuracy and patient outcomes. This investigation, conducted at the University of Bologna's Anatomy Center, explores the integration of reventilation and revascularization in body donors through the use of SimLife® technology [1]. Utilizing the well-established body donation program and dissection rooms of the Center, the presented study conducted extensive simulations to assess the efficacy of SimLife® technology. These simulations were designed not only to test the adaptability of such technologies in the Italian medical training context, but also to evaluate their impact across a broad spectrum of medical and surgical specialties. By engaging 62 participants from 13 different specialties, this study systematically collected qualitative feedback on the simulation experience. Results indicated a significant appreciation for the realism and educational value provided by SimLife® technology, underscoring its potential to revolutionize medical training methods. Under this light, this study reinforces the indispensable role of body donation in medical education and training, and highlights the transformative potential of SimLife® technology to further enhance and revolutionize this field.

The presented research received financial support from the Italian Ministry of Health through CENTRO_RIF_DONAZ_SALME_2023 to Lucia Manzoli, for the Reference Center for the research use and conservation of body donors at the University of Bologna and from the European Union – NextGenerationEU through the Italian Ministry of University and Research under PNRR – M4C2-I1.3 Project PE_00000019 "HEAL ITALIA" to Elisa Boschetti and Alessandra Ruggeri CUP J33C22002920006 of the University of Bologna.

References

1) Delpech, P.O.; Danion, J.; Oriot, D.; Richer, J.P.; Breque, C.; Faure, J.P. SimLife a new model of simulation using a pulsated revascularized and reventilated cadaver for surgical education. J Visc Surg 2017, 154, 15-20.

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Attitudes and willingness towards body donation: a survey from the University of Sassari, Italy

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Post-mortem body donation plays a central role in medical education, with important implications for training and advances in scientific research. The experience of the last decades showed that a lack of donation programs leads to a shortage of bodies for medical education. In Italy, a recent law, entered into force in 2020, clearly details mandatory criteria for body donation: informed consent of living donors, management of donors' bodies, and identification of

national reference centres. The present study aimed to assess attitudes and willingness to donate their body of academics and medical students at an Italian university. A cross-sectional study was conducted between February – March 2023. An ad hoc questionnaire was administered online to collect data on attitudes about body donation, cadaveric body dissection, and sociodemographic and academic information. A multivariate logistic regression model was implemented to evaluate the factors influencing body donation. 434 subjects completed the questionnaire (70%

females; 88 % of students). Overall, 72.8% were willing to body donation donate their own body.

Our multivariate analysis revealed that knowing organ donors and religion was significantly associated with body donation. Surprisingly, only 32% of the participants knew the national law regulating body donation. These findings emphasize the significance of comprehending the factors that affect body donation, especially in relation to socio-cultural and religious influences. This understanding, combined with knowledge of the protocols governing body donation, is crucial as it could potentially enhance the willingness to donate.

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Anthropometric indices: waist circumference and waist-to-hip ratio cut-off percentiles to identify abdominal obesity in children from North Macedonia

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Abdominal obesity (AO) has been associated with children's risk of metabolic and cardiovascular disease. For this reason, this study aimed to provide gender-specific cut-off percentiles of anthropometric indices WC and WHR to identify AO in children aged 9 from North Macedonia. In this study, a total of 320 children aged 9 (160 boys and 160 girls) were investigated. We selected four parameters to measure (weight and height) and two circumferences (waist and hip) using a standard protocol. The following indices are taken into consideration Body-Mass Index (BMI), Waist Circumference (WC), and Waist-to-Hip Ratio (WHR). The percentile distribution of the tested parameters was done by gender. General obesity based on the BMI cut-off occurs at 5.63% in boys and 6.88 % in girls. Abdominal obesity across cut-off points WHR and WC-for age>=90th percentile occur at 11.88% and 6.26% in boys and 12.5 and 11.25% in girls respectively. Both the WHR and WC identify more children with abdominal obesity, but we note that more girls were classified as obese than boys. However, the anthropometric indices of WC and WHR, complement nutritional evaluation and are of great importance for the early detection of AO in our 9-year-old children. These findings support the need to use WC and WHR as strong predictors for AO in routine clinical practice.

References

1) Schroder, H.; Ribas, L.; Koebnick, C.; Funtikova, A.; Gomez, SF.; Fito, M.; Perez-Rodrigo C, Serra-Majem, L. Prevalence of abdominal obesity in Spanish children and adolescents. Do we need waist circumference measurements in pediatric practice? Plos One. 2014;9(1):e87549.
2) Asif, M.; Aslam, M.; Altaf, S.; Mustafa, S. Developing waist circumference, waist-to-height ratio percentile curves for Pakistani children and adolescents aged 2-28 years using Lambda-Mu-Sigma (LMS) method. J Pediatr Endocrinol Metab. 2020;33(8):983-993.

Keywords: children; BMI; WC; obesity

VERBALE DELLA SEDUTA AMMINISTRATIVA E DELL'ASSEMBLEA GENERALE DEI SOCI SIAI, 2023

SIAI Società Italiana di Anatomia e Istologia

VERBALE DELLA SEDUTA AMMINISTRATIVA E DELL'ASSEMBLEA GENERALE DEI SOCI DELLA SOCIETÀ ITALIANA DI ANATOMIA E ISTOLOGIA (SIAI) TENUTASI PRESSO L'AULA MAGNA DEL CENTRO SERVIZI DIDATTICI, FACOLTA' DI MEDICINA E CHIRURGIA, UNIVERSITA' DEGLI STUDI DI MODENA E REGGIO EMILIA.

In data 12 Settembre 2023, alle ore 17:30, in seconda convocazione, ha avuto luogo, presso l'Aula Magna del Centro Servizi Didattici, Facoltà di Medicina e Chirurgia dell'Università degli Studi di Modena e Reggio Emilia, l'Assemblea Generale dei Soci della Società Italiana di Anatomia e Istologia per discutere il seguente Ordine del Giorno:

1) Comunicazioni del Presidente.
2) Relazione del Tesoriere sul rendiconto finanziario dell'anno 2022 e sulla previsione finanziaria per l'anno 2024. Relazione dei Revisori dei Conti.
3) Attività dei Collegi di Anatomia Umana e di Istologia ed Embriologia Umana. Relazioni dei Presidenti o dei loro Delegati.
4) Aggiornamento sull'Italian Journal of Anatomy and Embryology.
5) Aggiornamento sul sito web della SIAI.
6) Assegnazione Premio alla Carriera.
7) Assegnazione Premi Ricercatori under 40.
8) Assegnazione Premio Migliore Comunicazione Orale.
9) Assegnazione Premi Poster.
10) Prossimi Congressi Nazionali SIAI: proposte temi di relazione.
11) Borsa di Studio SIAI intitolata al Prof. Giovanni Orlandini.
12) Proposte di ammissione nuovi Soci e proposte per Soci Emeriti ed Onorari.
13) Commemorazione Soci Scomparsi.
14) Varie ed eventuali.

Presiede la riunione il Presidente della SIAI, Prof. Lucio Ildebrando Maria Cocco; funge da Segretario Verbalizzante la Prof. Gigliola Sica, collegata online.
Il Presidente dichiara aperta l'Assemblea e procede alla discussione dell'Ordine del Giorno.

1. **Comunicazioni del Presidente.**

Il Presidente, Prof. Lucio I. M. Cocco, dà il benvenuto ai convenuti.

In primis comunica che il Ministro dell'Università e Ricerca, Anna Maria Bernini, sta rivedendo i SSD BIO/16 e BIO/17 e che Ella avrà un incontro entro la fine del mese di settembre con il Prof. Paolo Vincenzo Pedone, Presidente del CUN.

Poi aggiorna i Soci sull'iscrizione della SIAI nell'elenco delle Società Scientifiche accreditate presso il Ministero della Salute.

2. **Relazione del Tesoriere sul rendiconto finanziario del 2022 e sulla previsione finanziaria per il 2024. Relazione dei Revisori dei Conti.**

Il Presidente dà la parola al Prof. Gianpaolo Papaccio, Tesoriere della SIAI.

Il Prof. Papaccio illustra nei dettagli il rendiconto finanziario dell'anno 2022 e la previsione finanziaria dell'anno 2024, già inviati in tempo utile a tutti i Soci **(Allegati N.1 e 2)** ed informa l'Assemblea che i Revisori dei Conti hanno depositato la loro Relazione **(Allegato N.3)** da cui si evince che hanno approvato senza riserve i documenti suddetti.

In particolare, il Tesoriere evidenzia che nel 2022 solo 216 Soci avevano provveduto a pagare la quota annuale e che vi erano state spese aggiuntive dovute sia al rifacimento del sito WEB sia alla erogazione di una Borsa di Studio.

I pagamenti sono stati tutti possibili, anche se con un disavanzo di bilancio, grazie agli accantonamenti degli anni precedenti.

Da ciò il Tesoriere evidenzia e fa presente che l'attuale ammontare delle quote sociali, ferme a soli 60,00 EURO da moltissimi anni, non potrà più permettere se non a malapena le spese correnti.

Sulla previsione finanziaria per il 2024 ribadisce che si potrà dare corso a quanto già preventivato ma, senza un aumento delle quote sociali, non si potrà prevedere l'erogazione della Borsa di Studio a meno che non si riduca l'importo del 50% o si apporti una riduzione di altre spese.

Infine, il Prof. Papaccio comunica che, per le suddette motivazioni, il Consiglio Direttivo ha approvato l'aumento dell'importo della quota sociale a 80,00 EURO.

Quindi, passa alla esposizione dell'elenco dei Soci decaduti **(Allegato N.4)** perché non in regola con il pagamento delle quote associative o su loro richiesta. Il numero di questi Soci quest'anno ammonta a 12; altri 61, se non si metteranno in regola entro il prossimo anno, decadranno.

3. **Attività dei Collegi di Anatomia Umana e di Istologia ed Embriologia Umana. Relazioni dei Presidenti o dei loro Delegati.**

Il Prof. G. Papaccio illustra l'attività del Collegio dei Docenti di Istologia ed Embriologia Umana nel periodo Settembre 2022/Settembre 2023,

In particolare, informa che il Collegio nell' Assemblea del mese di Febbraio 2023 ha provveduto a:

- rivedere integralmente lo Statuto, poi depositato dal notaio;

- rivedere integralmente il Regolamento;
- nominare il Comitato Scientifico;
- effettuare una giornata Scientifica a seguire l'Assemblea Amministrativa del giorno precedente;
- assegnare il premio Monesi-Rizzoli, terza edizione, con nuovo Regolamento;
- nominare una Commissione didattica relativamente alla percezione della importanza della disciplina negli Studenti degli anni seguenti;
- indicare nella data del 7 Novembre quella per i Seminari dei nuovi PO in servizio dal 1° Novembre 2023.

Inoltre, il Presidente illustra le altre attività anche di natura scientifica di cui si sono occupati la Giunta o lui personalmente ed in particolare sottolinea la numerosità dei PO di Istologia che da 30 quest'anno raggiungeranno al 1° Novembre il numero di 43 e dal 2024 il numero di 49, al netto dei pensionamenti.

Prende, poi, la parola il Prof. A. Montella che riassume l'attività del Collegio dei Docenti di Anatomia Umana nel corso dell'ultimo anno come segue:

- Numerose riunioni del Consiglio Direttivo.
- Riunioni plenarie dei Soci in numero di tre: a Ravenna, a Roma e a Modena in occasione di questo congresso SIAI.
- Definizione collegiale della Declaratoria del GSD già recepita dal CUN come segue:

Nuova Declaratoria per il Gruppo Scientifico-Disciplinare
GSD "ANATOMIA UMANA" *(SC 05/H1 e SSD BIO/16)*

Il Gruppo Scientifico-Disciplinare (GSD) ***ANATOMIA UMANA*** *Si interessa dell'attività scientifica e didattico-formativa nel campo della conformazione, dell'organizzazione e della struttura del corpo umano, dei sistemi e organi che lo costituiscono, nei loro aspetti macroscopici, dissettori, microscopici, ultramicroscopici e molecolari con i relativi aspetti funzionali e ricadute cliniche, nei vari periodi della vita, con specifico riferimento anche al singolo individuo e in un appropriato contesto di sesso-genere. Analizza, inoltre, l'organogenesi e le varie fasi dello sviluppo, identificando le diversità individuali, le varianti congenite, le cause primarie degli eventi morfogenetici e le loro principali alterazioni di interesse clinico. Si avvale di metodiche d'indagine macroscopiche, dissettorie, microscopiche sino al livello molecolare, topografiche, strumentali e applicative sperimentali per l'acquisizione di immagini e dati informativi ai diversi livelli di risoluzione. Studia conoscenze anatomiche applicate alle problematiche di interesse clinico, chirurgico, anatomo-radiologico e delle scienze sportive, del movimento e del mantenimento dello stato di salute. Il GSD Anatomia Umana rappresenta il presupposto dottrinale indispensabile per la comprensione degli aspetti fisiopatologici, semeiologici, anatomopatologici e della medicina clinica di precisione in tutte le sue declinazioni.*

- Con riferimento alla Legge 10 Febbraio 2020, n.10, recante «Norme in materia di disposizione del proprio corpo e dei tessuti post mortem a fini di studio, di formazione e di ricerca scientifica»: con decreto del Direttore della Direzione Generale Della Prevenzione Sanitaria del Ministero della Salute del 21 Novembre 2022, sono stati elencati i centri di riferimento per la conservazione e l'utilizzazione dei corpi dei defunti ai fini del trasferimento delle risorse di cui all'art.1, comma 499, della legge 30 Dicembre 2020 n. 178, per l'anno 2021:
    - I.R.C.C.S. Multimedica;
    - Alma Mater Studiorum – Università di Bologna;
    - Università degli studi di Padova;
    - Università degli studi di Brescia;
    - I.R.C.C.S. Ospedale San Raffaele – Gruppo San Donato;
    - I.R.C.C.S. Istituto neurologico mediterraneo Neuromed;
    - Azienda ospedaliero universitaria di Sassari.
- In data 28.04.2023 è stato pubblicato sulla Gazzetta Ufficiale DECRETO DEL PRESIDENTE DELLA REPUBBLICA 10 Febbraio 2023 n. 47 il Regolamento recante norme in materia di disposizione del proprio corpo e dei tessuti post mortem a fini di studio, di formazione e di ricerca scientifica.
- Sono stati avviate le attività di vari gruppi di lavoro allo scopo di approfondire, in riunioni specifiche con un numero ristretto di colleghi, le problematiche relative alla organizzazione didattica e ad altri aspetti di interesse del Collegio.
- Il 24 giugno 2023 a Roma si è tenuta la seguente giornata di studio:

- Sono state deliberate le variazioni dello Statuto indispensabili per poter essere in regola con l'Agenzia delle Entrate.

4. Aggiornamento sull'Italian Journal of Anatomy and Embryology.

Il Presidente dà la parola al Prof. Domenico Ribatti, Direttore Scientifico dell'Italian Journal of Anatomy and Embryology (IJAE). Il Prof. Ribatti aggiorna i Soci sullo stato delle pubblicazioni dell'IJAE. Sono disponibili online il fascicolo 127/1 e 127/supplemento che contiene gli Abstract del Congresso di Modena e Reggio Emilia e sono già stati raccolti diversi contributi per il Fascicolo 127/2 che sarà disponibile entro il mese di Dicembre 2023.

5. Aggiornamento sul sito web della SIAI.

La Prof. G. Sica aggiorna i convenuti sullo stato del Sito web della Società.

Riferisce che la sezione "News" viene costantemente aggiornata con l'inserimento dei nuovi eventi. Sono stati inseriti i Bandi della Borsa di Studio SIAI e i criteri di valutazione, il nuovo Statuto ed il nuovo Regolamento del Collegio dei Docenti di Istologia ed Embriologia Umana.

6. **Assegnazione Premio alla Carriera.**

Il Presidente legge il verbale della Commissione per l'assegnazione del Premio alla Carriera **(Allegato N.5).** La Commissione nominata dal Consiglio Direttivo del 24 Giugno 2023 e composta dai Proff. Michelangelo Cordenonsi, Antonio Filippini e Alessandro Vercelli, dopo aver valutato i curricula dei due candidati proposti in base al Regolamento del Premio alla Carriera, approvato dal Consiglio Direttivo del 24 Giugno 2023, e vista l'elevata statura accademica di entrambi, popone in via eccezionale il conferimento di due premi: al Prof. Giuseppe Anastasi, per il consistente e fondamentale contributo alle attività organizzative della SIAI, e al Prof. Gastone Marotti per l'assoluto valore scientifico di alcune pubblicazioni che hanno dato lustro alla Società.

7. **Assegnazione Premi Ricercatori under 40.**

Il Presidente procede alla lettura del verbale del Comitato Scientifico **(Allegato N.6)** per l'attribuzione di numero 2 Premi a Ricercatori under 40. Il Comitato Scientifico, nelle persone dei Proff. Emanuela Marcenaro (Presidente), Guido Carpino (Segretario), Antonio De Luca (Membro), Matilde Yung Follo (Membro) e Bianca Maria Scicchitano (Membro), dopo aver attentamente valutato in base al Regolamento approvato dal Consiglio Direttivo i curricula dei Candidati, propone di assegnare i due premi a: Dott. Giovanni Cirillo e Dott. Matteo Giovarelli.

8. **Assegnazione Premio Migliore Comunicazione Orale.**

La Commissione, per l'Assegnazione del Premio alla Migliore Comunicazione Orale, ovvero i Moderatori delle Sessioni "Dalla Morfologia alla Patologia Molecolare", Commissione formata dai Proff. Cutroneo, De Caro, Gaudio, Follo, Marcenaro e Ribatti, si riunirà il 13/09/2024, appena terminata l'ultima comunicazione della Sessione per l'assegnazione del premio, assegnazione che sarà comunicata nella seduta terminale del congresso.

9. **Assegnazione Premi Poster.**

La Commissione, designata alla valutazione dei Poster e composta dai Proff. Artico, Boido e Gagliano, dopo aver visionato i lavori di entrambe le Sessioni, ha proposto di attribuire i premi alle Dott.sse Maria Gemma Nasoni e Angelica Perna.

10. **Prossimi Congressi Nazionali SIAI: Proposte temi di relazione.**

Viene confermata dalla Prof. Simona Sivori dell'Università degli Studi di Genova, la disponibilità ad organizzare, insieme ai Colleghi delle Sezioni di Anatomia e Istologia, il prossimo Congresso Nazionale. Come tema preferenziale, vista l'attività scientifica relativa al sistema immunitario, viene proposta la seguente tematica: "Sistema Immunitario e sue interconnessioni morfo-funzionali: dai meccanismi molecolari alle applicazioni terapeutiche".

11. Borsa di Studio SIAI, intitolata al Prof. Giovanni Orlandini.

Il Presidente dà lettura del bando per la Borsa di Studio SIAI (**Allegato N.7**), intitolata al Prof. Giovanni Orlandini, già inserito sul sito web della Società. Quindi invita la Dott.ssa Assunta Virtuoso, vincitrice della Borsa di Studio SIAI intitolata al Prof. Francesco Antonio Manzoli a fare una breve relazione sulla sua esperienza.

12. Proposte di ammissione Nuovi Soci e proposte per Soci Emeriti ed Onorari.

La Prof. Sica presenta l'elenco dei nominativi di coloro che hanno presentato domanda quali Soci Ordinari:

1. Alviano Francesco
2. Barone Virginia
3. Bertani Nicole
4. Bonetti Antonella
5. Bonomo Roberta
6. Calderan Laura
7. Cappariello Alfredo
8. Carriero Ilenia
9. Carton Flavia
10. Casalin Irene
11. Cianciulli Antonia
12. Colleluori Georgia
13. Contessotto Paolo
14. Cortese Katia
15. Cossu Vanessa
16. Della Rocca Ylenia
17. Donfrancesco Orlando
18. Fantone Sonia
19. Forte Flavio
20. Galassi Francesco Maria
21. Galasso Letizia
22. Galgani Alessandro
23. Gatti Marta
24. Koufi Foteini Dionysia
25. Monti Manuela
26. Orofino Francesca
27. Panciera Tito

28. Parigi Martina
29. Pellegrini Carolina
30. Pirri Carmelo
31. Sicurella Mariaconcetta
32. Stanga Serena
33. Ucci Maria Assunta
34. Vaccarezza Mauro
35. Ventura Alessia

13. Commemorazione dei Soci scomparsi.

Il Prof. Antonio Paparelli viene commemorato dal Prof. M. Gesi. Il Prof. Dario Cantino viene commemorato dal Prof. Alessandro Vercelli e il Presidente comunica di aver da poco ricevuto dal Prof. Fumagalli la notizia della scomparsa della Prof. Elena Pompili, che sarà commemorata dal Prof. Fumagalli nel prossimo congresso.

Nulla al punto 14) all'OdG.

Essendo esaurito l'OdG, il Presidente dichiara chiusa la seduta alle ore 19:15. Il presente verbale viene letto, approvato e sottoscritto seduta stante.

Il Presidente	Il Segretario Verbalizzante	Il Tesoriere
Lucio I. M. Cocco	Gigliola Sica	Gianpaolo Papaccio

Firmato digitalmente da Gianpaolo Papaccio
Data: 31.07.2024 13:09:36 CEST

SIAI Società Italiana di Anatomia e Istologia

Bilancio consuntivo anno 2022 ALLEGATO N. 1

CAUSALE DELLE ENTRATE	ENTRATE Euro	CAUSALE DELLE USCITE	USCITE Euro
Quote sociali incassate nel corso dell'anno 2022 (n°216) incluse le quote arretrate, le quote incassate non al netto e in attesa di integrazioni e le quote non riconducibili allo stato di alcun Socio	**€ 12.960,00**	**Elenco spese per attività statutarie**	
Incasso Contributi per stampa articoli su IJAE	**€ 3.658,50**		
		Quota di Iscrizione al Congresso SIAI 2022 di n.1 Socio vincitore del premio poster	€ 415,00
		Premio (2) Ricercatore under 40, anno 2022	€ 4.000,00
		Premio "Migliore comunicazione orale", anno 2022	€ 1.000,00
		Contributo per l'organizzazione del Convegno G.I.S.N. anno 2022	€ 500,00
		Spese varie (mantenimento conto corrente bancario, spese bollo e commissioni bancarie ecc.), anno 2022	€ 930,62
		Pagamento F24 R.A. Notaio anno 2022	€ 343,00
		Pagamento F24 R.A Commmercialista anni 2020 - 2021	€ 513,88
		Contributo Congresso Padova, anno 2022	€ 5.000,00
		Quota associativa EFEM, anno 2022	€ 419,00
		Spese sito web (FILARETE)	€ 4.055,00
		Borsa di Studio Dr. A. Virtuoso	€ 10.000,00
		Elenco spese di funzionamento	
		Compenso per consulenza commercialista relativo alla stesura dei bilanci consuntivi anni 2020 – 2021 e previsionali 2022 - 2023	€ 2.224,90
		Spese per il funzionamento del Consiglio Direttivo, anno 2022	€ 1.730,00
		Spese notarili	€ 2.100,00
		Spese Pubblicazioni IJAE	€ 2.592,50
TOTALE DELLE ENTRATE	**€ 16.618,50**	**TOTALE DELLE USCITE**	**€ 35.823,90**

DISAVANZO DELL'ESERCIZIO FINANZIARIO 2022	**€ 19.205.40**		

CAUSALE DELLE ENTRATE	ENTRATE Euro	CAUSALE DELLE USCITE	USCITE Euro
Saldo Conto Corrente Bancario al 31/12/2021	**€ 58.590,44**		
TOTALE SALDO FINANZIARIO AL 31/12/2021	**€ 58.590,44**		
DISAVANZO DELL'ESERCIZIO FINANZIARIO 2022	**€ 19.205,40**		
SALDO FINANZIARIO AL 31/12/2022	**€ 39.385,04**		
STANZIAMENTI IMPEGNATI AL 31/12/2022			**Euro**
Spese per ECM, anno 2022			€ 500,00
Spese per il funzionamento della Segreteria, Tesoreria e Presidenza, anno 2022			€ 4.000,00
TOTALE IMPEGNO DI SPESA			**4.500,00**
SALDO DISPONIBILE al 31.12.2022	**€ 34.885,04**		

SIAI Società Italiana di Anatomia e Istologia

Relazione di accompagnamento al rendiconto economico e finanziario per l'anno 2022

Come risulta dal bilancio consuntivo, il saldo finanziario al 31/12/ 2022 è pari ad **€ 39.385,04**
A tale importo devono essere sottratti **€ 4.500,00** impegnati nel bilancio previsionale del 2022, ma non ancora effettivamente utilizzati alla data del 31/12/2022, per le seguenti voci di spesa:

- **Spese per ECM, anno 2022: € 500,00**
- **Spese per il funzionamento della Segreteria, Tesoreria e Presidenza, anno 2022: € 4.000,00;**

Pertanto l'anno 2022 si chiude con un **saldo disponibile** di **€ 34.885,04**

Durante il 2022, le quote associative incassate sono state soltanto 216 comprese alcune quote arretrate ed integrazioni di versamenti di quote non corretti, per un totale di **€ 12.960,00**, che sommate sia all'avanzo (incasso) delle quote liberali (dei contributi) per la stampa degli articoli pubblicati sull'IJAE pari ad **€ 3.658,50** sia al saldo finanziario al 31/12/2021 pari ad **€ 58.590,44,** hanno dato la disponibilità di **€ 75.208,94.**
Le entrate hanno permesso di coprire le spese previste e non previste, includendo i fondi impegnati e non erogati.
La rispondenza dei Soci ai solleciti da parte del Tesoriere in merito alla regolarizzazione dei pagamenti delle quote associative, si è rivelata inferiore rispetto all'anno precedente sia in quanto alcuni non hanno risposto (circa 100 Soci), sia in quanto tutti gli altri erano già in regola con i versamenti delle quote arretrate tutte raccolte durante l'anno 2021. Rimane ancora un piccolo numero di Soci che debbono regolarizzare la loro posizione, che è molto inferiore rispetto agli anni trascorsi grazie ad un'opera capillare di rientro nella regolarizzazione delle quote. Il Tesoriere sottolinea che il pagamento delle quote da parte dei Soci deve essere puntuale, ad inizio di ciascun anno solare, tale da consentire alla SIAI di effettuare una adeguata programmazione delle attività statutarie e di intraprendere nuove iniziative. Il disavanzo che si è registrato è dovuto solo alla mancata corresponsione in tempo (nell'anno solare) da parte di un buon numero di soci.
L'attuale condizione se da una parte è ancora florida, dall'altra porta alla luce che, ove mai si dovessero verificare spese ulteriori, le quote sociali di soli 60,00 euro non basterebbero al normale funzionamento. Pertanto si invita il Direttivo ad iniziare a prendere in considerazione un adeguamento delle quote, tenuto conto anche dell'impennata dei prezzi.

Il Tesoriere
Prof. Gianpaolo Papaccio

Firmato digitalmente da Gianpaolo Papaccio
Data: 06.06.2024 12:35:30 CEST

Previsione finanziaria 2024 ALLEGATO N. 2

SOCI NEL 2022:	**424**
SOCI NEL 2023:	**441**
SOCI ORDINARI 2023:	**423***
***compresi n.** 44 nuovi soci ratificati all' Assemblea di Settembre 2023	
SOCI DIMISSIONARI/CANCELLATI/DECEDUTI 2023:	**27**
SOCI ORDINARI DIVENUTI EMERITI 2023	**1**
SOCI RIAMMESSI 2023:	**1**
SOCI EMERITI/ONORARI:	**18**

Quote Sociali anno 2024	423	€	25.380,00
Quote Sociali arretrate 2020 – 2023		€	8.620,00
Contributi liberali per pubblicazione lavori scientifici su Italian Journal of Anatomy and Embryology		€	7.000,00
Totale Entrate		€	**41.000,00**

USCITE

Contributo al 77° Convegno Nazionale 2024, atti di convegni, altri contributi a convegni, partecipazione a convegni, organizzazione eventi scientifici, borse di studio, etc.	€	15.000,00
Accantonamento per premi poster dell'anno 2024 e per premio alla migliore comunicazione orale assegnati nell'anno 2024	€	3.000,00
Accantonamento per premi SIAI (Premio alla Carriera e n. 2 Premi Ricercatori under 40), anno 2024	€	4.200,00
Contributo alla Firenze University Press per pubblicazione lavori scientifici su Italian Journal of Anatomy and Embryology, anno 2024	€	5.000,00
Spese per sito web della Società, anno 2024	€	3.000,00
Spese per la partecipazione Meeting Comitato Internazionale per la Terminologia Anatomica e Istologica, FICAT, anno 2024	€	1.000,00

Quota adesione all'European Federation for Experimental Morphology, EFEM, anno 2024	€	400,00
Quota adesione all'International Federation of Anatomical Associations, IFAA, anno 2024	€	400,00
Spese varie (bancarie, necrologi, etc.), anno 2024	€	1.000,00
Spese impreviste, anno 2024	€	2.000,00
Totale spese per attività statutarie	***€***	***35.000,00***
Spese per il funzionamento della Segreteria, Tesoreria, Presidenza e Consiglio Direttivo	€	6.000,00
Totale spese di funzionamento	***€***	***6.000,00***
Totale Uscite	€	**41.000,00**

Relazione di accompagnamento alla previsione finanziaria per l'Anno 2024

La chiusura del bilancio consuntivo del 2022 con un saldo disponibile di **€ 36.583,90** ha permesso al Tesoriere di sostenere alcune spese indicate nella Previsione Finanziaria del 2023.

Al 31 agosto 2023, sono state incassate 497 quote sociali comprensive di quelle relative all'anno in corso e arretrate (dal 1° settembre 2022 al 31 agosto 2023).

Al 31 agosto 2023, il totale delle entrate è pari a **€ 65.681,53** e comprende le quote riscosse finora.

Il piano previsionale del 2023 prevedeva entrate pari a **€ 42.700,00**, dovute alla riscossione delle quote dell'anno in corso, più una cifra forfettaria concernente il recupero delle quote arretrate. In particolare, in tale previsione, come in quelle degli anni precedenti, è stata indicata questa cifra forfettaria sulla base dell'esperienza relativa alle difficoltà di ottenere il pagamento degli arretrati da tutti i Soci non in regola.

La Società conta attualmente 441 Soci, di cui 423 Soci Ordinari e 18 Soci Emeriti o Onorari (esonerati dal pagamento della quota Sociale). Nel corso del 2022 ad oggi (31 agosto 2023), n. 27 Soci sono stati cancellati poiché decaduti o hanno espresso la volontà di rassegnare le dimissioni dalla Società, n.1 Socio ordinario è divenuto Socio emerito e n.1 Socio è stato riammesso.

Allo stato attuale, dei 423 Soci Ordinari che sono tenuti a pagare la quota associativa:

- 1 Socio è in regola fino al 2025;
- 10 Soci sono in regola fino al 2024
- 296 Soci sono in regola fino al 2023;
- 44 Soci sono in regola fino al 2022, devono la quota 2023;
- 61 Soci sono in regola fino al 2021, devono la quota 2022 e 2023;
- 3 Soci sono in regola fino al 2020, devono le quote 2021, 2022 e 2023;
- 8 Soci sono in regola fino al 2019, devono le quote del 2020, 2021, 2022 e 2023.

Il Tesoriere fa presente che cercherà di raggiungere la parità di bilancio e di fare previsioni finanziarie quanto più possibile aderenti alla realtà. Riferisce inoltre che nel corso del 2023, un discreto numero di Soci ha risposto positivamente all'azione di richiamo per il recupero delle quote arretrate. Rimane un piccolo numero di Soci che non ha mai risposto ai solleciti di pagamento; pertanto, in base a quanto stabilito nello Statuto (Art. 15) ed al parere del Direttivo SIAI, si è già provveduto alla revisione dell' Elenco dei Soci che sarà ancora revisionato ove mai tali Soci non provvedessero, secondo le norme statutarie, al pagamento delle quote arretrate

Il Tesoriere fa presente che, essendo di molto diminuiti i soci morosi e che l'ammontare delle quote arretrate si è considerevolmente ristretto, la Società, d'ora in poi, ha la contezza su quanto

effettivamente essa possa contare sulle proprie risorse finanziarie, che, in rapporto al numero elevato di costi, è molto esigua se non vi saranno finanziamenti esterni onde poter raggiungere gli scopi sociali ed aumentare le liberalità in favore dei giovani.

Il Tesoriere

Prof. Gianpaolo Papaccio

Firmato digitalmente da Gianpaolo Papaccio
Data: 20.06.2024 12:59:43 CEST

ALLEGATO N.3

Modena, 12.09.2023

Il giorno 12.09.2023 alle ore 13.30, presso il Centro Didattico della Facoltà di Medicina e Chirurgia, area Policlinico, Largo del Pozzo 71, Modena, i Revisori dei Conti dei Bilanci della SIAI, nelle persone dei proff. Guido Carpino, Matilde Y. Follo e Stefania Montagnani, si sono riuniti per esaminare i documenti ricevuti in data 30.08.2023 e 06.09.2023 via mail. Dopo attento esame, i proff. Carpino, Follo e Montagnani hanno ritenuto approvare senza riserve i documenti così come inviati.

La riunione termina alle ore 14.

Prof. Guido Carpino _____

Prof.ssa Matilde Y. Follo _____

Prof.ssa Stefania Montagnani _____

ALLEGATO N.4

SOCI DECADUTI

BODA ENRICA

CENTURIONE LUCIA

GOBBI PIETRO

ICARO CORNAGLIA ANTONIA

IPPOLITO CHIARA

MIGLIACCIO ANNA RITA

MORESI VIVIANA

NARO FABIO

NORI STEFANIA

PAROLINI ORNELLA

PERRA DOLCI M. TERESA

SARCHIELLI ERICA

ALLEGATO N.5

8 settembre 2023

A chi di pertinenza

In data 16 agosto 2023 alle ore 830, per via telematica, si è riunita la commissione istituita dal Consiglio Direttivo della SIAI per l'attribuzione del premio alla carriera, secondo il regolamento vigente (approvato dal CD del 24 giugno 2023).

La Commissione è composta dai professori Michelangelo Cordenonsi, Antonio Filippini e Alessandro Vercelli. I membri della Commissione hanno ricevuto la documentazione in data 1 agosto dalla prof.ssa Gigliola Sica, segretario della Società. Tale documentazione consiste in 4 documenti:

a) Proposta di conferimento al prof. Marotti da parte dell'allieva Carla Palumbo;
b) CV del prof. Marotti;
c) CV del prof. Anastasi;
d) Elenco pubblicazioni del prof. Anastasi.

La Commissione esamina i CV dei due candidati, giudicandoli entrambi degni del titolo. In particolare, si mette in luce l'assoluto valore scientifico di alcune pubblicazioni del prof. Marotti, che hanno dato lustro alla Società, e il consistente e fondamentale contributo alle attività organizzative della SIAI da parte del prof. Anastasi.

La Commissione, pertanto, vista l'elevata statura accademica di entrambi i candidati, propone in via eccezionale il conferimento di due premi alla carriera (come già successo in passato, quandanche in seguito alla interruzione di un anno dovuta alla pandemia), cosa che potrebbe costituire un precedente per il futuro, quando si abbiano due candidati, uno anatomico e uno istologo.

Michelangelo Cordenonsi

Antonio Filippini

Alessandro Vercelli

VERBALE DELLE RIUNIONI DEL COMITATO SCIENTIFICO PER L'ATTRIBUZIONE DI NUMERO 2 PREMI AI RICERCATORI "UNDER 40"

Il Comitato Scientifico, nelle persone di:

Prof.ssa Emanuela Marcenaro (in qualità di Presidente)
Prof.ssa Bianca Maria Scicchitano (in qualità di Componente)
Prof.ssa Matilde Yung Follo (in qualità di Componente)
Prof. Antonio De Luca (in qualità di Componente)
Prof. Guido Carpino (in qualità di Segretario)

si è riunito telematicamente (via TEAMS) giovedì 3 agosto e mercoledì 30 agosto 2023 per valutare le Candidature per l'assegnazione dei 2 Premi ai ricercatori "under 40".

Sono pervenute n. 3 Candidature nelle persone di:

Dott. Giovanni Cirillo (presentato dal Prof. Michele Papa)
Dott. Matteo Giovarelli (presentato dalla Prof.ssa Chiarella Sforza)
Dott.ssa Cristina Meregalli (presentata dal Prof. Guido Cavaletti)

Il Comitato ha dapprima valutato la posizione dei Candidati rispetto al pagamento delle quote di iscrizione alla SIAI e ha rilevato che tutti i Candidati sono in regola.

Successivamente, il Comitato ha valutato attentamente i curricula dei Candidati, la loro produzione scientifica, il contributo personale nelle pubblicazioni, la congruenza delle tematiche di ricerca con gli SSD di riferimento, la continuità temporale e i loro indici bibliometrici con particolare riferimento agli ultimi anni.

Alla fine di tale valutazione, il Comitato propone di assegnare all'unanimità i 2 Premi a:

Dott. Giovanni Cirillo, la cui attività di ricerca si concentra nel campo delle neuroscienze e in particolare si focalizza sullo sviluppo di nuove tecniche di imaging per l'analisi della plasticità del SNC sia in modelli cellulari che animali.

Dott. Matteo Giovarelli, la cui attività di ricerca è principalmente focalizzata nell'ambito dello studio del muscolo striato scheletrico e si sviluppa utilizzando modelli sia cellulari sia in vivo, in cui vengono analizzati gli aspetti morfologici strutturali e ultrastrutturali.

Addì, 30 agosto 2023

Prof.ssa Emanuela Marcenaro

Prof.ssa Bianca Maria Scicchitano

Prof.ssa Matilde Yung Follo

Prof. Antonio De Luca

Prof. Guido Carpino

BANDO PER BORSA DI STUDIO DELLA SOCIETA' ITALIANA DI ANATOMIA E ISTOLOGIA (SIAI)

Articolo 1

Tema dell'iniziativa

La **Società Italiana di Anatomia ed Istologia (SIAI)** ha istituito **nel 2022** una **Borsa di Studio** del **valore di Euro 10.000,00 di durata semestrale, da svolgersi presso prestigiose Istituzioni estere. La SIAI garantirà inoltre una indennità di viaggio di importo non superiore ad Euro 1.000,00 a fronte della esibizione dei titoli di viaggio. La prima edizione della Borsa è stata intitolata al Prof. Francesco Antonio Manzoli. La seconda, a far data dal 1° Febbraio 2024, sarà intitolata al Prof. Giovanni Orlandini.**

Possono partecipare candidati che posseggano i requisiti esposti nell'**Articolo 2**.

Le tematiche di ricerca, che faranno parte del progetto presentato da ciascun candidato, dovranno essere coerenti con quelle delle discipline che costituiscono la Società Scientifica (Anatomia ed Istologia). **Qualora vengano prodotte pubblicazioni scientifiche nell'ambito del progetto, sarà obbligatorio citare la SIAI nei ringraziamenti.**

Articolo 2

Requisiti per la partecipazione

Possono partecipare alla selezione:
- i dottorandi di ricerca;
-gli assegnisti di ricerca;
-i borsisti;
-i ricercatori di tipologia RTD-A

I candidati debbono inoltre possedere i seguenti requisiti:
-essere iscritti alla SIAI ed essere in regola con i pagamenti della quota sociale sino all'anno di emissione del bando;
-avere una età inferiore ai 35 anni;
-prestare servizio in una struttura **universitaria** ed **afferente** alla Anatomia o alla Istologia.

Ciascun candidato deve inoltrare formale lettera di richiesta, con allegato CV in formato europeo, corredato **dalle pubblicazioni.**

La **richiesta** deve contenere le seguenti informazioni:

- cognome e nome del candidato;
- paese di residenza;
- cittadinanza;
- data di nascita;
- indirizzo e-mail;
- numero di telefono;
- un dettagliato programma di ricerca che si intende effettuare utilizzando anche (e non soltanto) le risorse di cui alla Borsa;
- **lettera formale di accettazione** da parte della Istituzione **ospitante.**

Articolo 3

Esclusione

-Non possono partecipare **al bando** familiari entro il IV grado dei membri del **Consiglio Direttivo** della SIAI;
-I candidati che non posseggano i requisiti esposti nell'Articolo 2.

Articolo 4
Assegnazione della Borsa di Studio
La Borsa di Studio sarà assegnata a seguito di un **procedimento** di selezione effettuato dal **Comitato Scientifico della SIAI**.
Il Comitato selezionerà e classificherà le candidature pervenute via mail all'indirizzo segreteria.siai@unicatt.it indicando nell'oggetto "Candidatura Borsa Studio" e allegando tutta la documentazione richiesta entro il **30 Ottobre 2023.**
Saranno accettate unicamente le candidature inviate alla suddetta mail ed entro la data suindicata.

Articolo 5
Tempistiche di assegnazione della Borsa di Studio e modalità di comunicazione
Il Comitato Scientifico si impegna a comunicare il nominativo del candidato selezionato entro e non oltre il 31.12.2023 sul sito www.siaionline.it e tramite indirizzo di posta elettronica (indicato nella candidatura) alla persona selezionata.
Qualora il vincitore risultasse impossibilitato ad accettare la Borsa di Studio, essa verrà assegnata ad altro candidato secondo l'ordine di classifica stabilito dal **Comitato Scientifico**.

Articolo 6
Relazione finale sull'attività svolta
Il vincitore della Borsa di Studio è tenuto a presentare entro e non oltre tre mesi dal termine della missione di ricerca, sia una dettagliata relazione delle attività svolte sia un consuntivo delle spese sostenute ed effettuate con i proventi della Borsa ottenuta.

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