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ABSTRACT BOOK

Scientific organizers
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SELECTED ORAL COMMUNICATIONS

Contribution of concurrent RB1 and p53 pathway disruption to the development of the Primitive Neuronal Component in Glioblastoma

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GBM-PNC is a rare histological variant of glioblastoma with nodules of poorly differentiated and highly proliferating primitive cells, expressing less GFAP glial marker and more primitive neuronal features, such as dissemination propensity. In our cohort, 18/24 GBM-PNCs harbor cell cycle control impairment with disrupted Retinoblastoma-associated protein 1 (RB1) tumour suppressor function and either TP53 mutation or Mdm2-4 amplification.
We established *in vitro* models to test our hypothesis that both p53 and Rb1 pathway signaling impairments are required to commit a conventional GBM toward the gain of a GBM-PNC phenotype.
We selected two RB1 wildtype conventional GBM stem cell populations (GSCs), one harboring a non-synonymous TP53 mutation, and designed a CRISPR/Cas9 lentiviral vector to target *RB1*. Mock and knockout neurospheres were tested for proliferation, invasion, apoptosis and cell cycle assay.
In vitro tumorigenesis was modelled with organoids, cultured in Matrigel, formalin-fixed and paraffin-embedded and stained for the phenotypic expression of the glial and neuronal markers.
Interestingly, all TP53mut RB1-/- organoids significantly downregulated GFAP expression and maintained high β-III-tubulin neuronal marker expression as compared to the mocks. As expected, no markers modulation was observed in the TP53wt RB1-/- controls.
Moreover, the cell cycle assay showed an increasing polyploidy in TP53mut RB1-/- clones, reminiscent of the mitotic aberrations typical of the GBM-PNC phenotype, characterized by multinucleated cells and apoptotic processes.
Our results confirm that RB1 loss alone is not enough to trigger the switching of a conventional GBM into a GBM-PNC biphasic phenotype. Rather, we demonstrate that the concomitant alterations of p53 pathway control and RB1 function, disrupting cell cycle control, could represent the predisposing feature for a conventional GBM to become a GBM-PNC, since the observed reduced glial phenotype and the aneuploidy in TP53mut RB1-/- GSCs clones could reflect the development of a primitive neuronal component and its typical chromosomal aberrations.

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Selected Topic: Neural stem cells and cancer

Involvement of DNA repair in high-grade glioma recurrence: mechanistic insights into the nucleotide excision repair pathway in glioma stem cells

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High-grade gliomas (HGGs) are the most common primary brain malignancies, accounting for about 50% of brain cancers. Grade 4 IDH wild-type glioblastoma (GBM) is the deadliest and most difficult-to-treat, since tumor recurrence and therapy resistance remains a significant challenge. GBM Stem Cells (GSCs) are a critical subpopulation responsible for tumor initiation, progression, and recurrence. Although GSCs exhibit enhanced double-strand break repair capabilities, their proficiency in repairing DNA double-helix distorting lesions from platinum-based chemotherapeutics or oxidative stress is poorly understood. The Nucleotide Excision Repair (NER) pathway repairs various DNA lesions, including those mentioned above. NER operates through two sub-pathways: Global Genome Repair (GGR) for non-transcribed DNA and Transcription-Coupled Repair (TCR) for transcribed strands. These sub-pathways differ in lesion recognition but converge to complete the repair process. The resumption of transcription post-repair is crucial in TCR. Our aim is thus to mechanistically characterize the NER pathway in GSCs compared to bulk Glioblastoma cells (GBM). To evaluate NER efficacy in GSCs and GBM cells, we conduct golden standard NER proficiency assays following UV lesion induction, analyzing key NER factors' repair process, timing, and damage accumulation rates. Patient-derived glioma stem cell lines (GSCs), and the differentiated counterpart into GBM bulk cells, are used in these experiments. Our initial findings reveal distinct responses to UV-induced DNA damage in GSCs and highlight significant differences in the transcription resumption step post-repair between GSCs and GBM cells. Furthermore, we evaluated the timing and efficiency of gap refilling by the DNA replicative machinery in both cell types. These promising results elucidate the contribution of NER efficacy to GSC drug resistance, providing insights that could inform novel therapeutic strategies to overcome chemoresistance in glioblastoma.

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Selected topic: CNS primary tumours

Investigating the role of PTX3 in the biology of glioblastoma

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Glioblastoma Multiforme (GBM) is the most common and aggressive brain tumor in adults, classified as grade IV tumor by WHO. Common therapies include surgical removal, chemotherapy and radiotherapy; however, relapses are inevitable. In addition, it is hypothesized that the relapses are mainly due to a subpopulation of stem cells with self-renewal properties, called glioblastoma stem cells (GSC) localized in specialized niches. These cells are resistant to conventional treatments thanks to their ability to escape apoptosis and activate DNA repair mechanisms. Pentraxin 3 (PTX3) is a soluble pattern recognition receptor belonging to the humoral arm of the innate immunity that is also involved in several aspects of tumor growth, angiogenesis, metastasis and cancer immune-regulation. To date, a correlation between PTX3 and tumor aggressiveness in GBM has been described, but studies regarding its possible implication in GSC stemness are still missing.
We used human GSC BT302 cells, derived from glioblastoma specimens diagnosed according to WHO criteria, to obtain PTX3 silenced cells. PTX3 presence and production was assessed by Western blot, qPCR, ELISA and immunostainings. Proliferation, invasion and angiogenic assays were performed to analyse the effects of PTX3 silencing. In addition, RNA microarray analysis was conducted to identify the up- and down-regulated pathways, in response to PTX3 modulation.
Preliminary observations revealed a wide expression of PTX3 in GSC and specific silencing in a GSC line revealed a significant reduction of cell growth, invasiveness and angiogenic capacity in GSC after PTX3 knock-down, that results in a decreased tumorigenic capacity *in vivo*. Furthermore, bioinformatic analysis revealed a possible implication even of apoptotic and hypoxia regulation pathways, whose activation seems to be altered after PTX3 silencing.
Our data suggest that PTX3 silencing may impair tumor features *in vitro* and *in vivo*. This sets the basis for further characterization of the pro-tumoral role of PTX3 in glioblastoma.

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Selected Topic: Glial cells in cancer

Study on the contribution of neuronal environment in glioblastoma malignancy

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Glioblastoma (GBM) interaction with the surrounding microenvironment and brain resident cells has gained recently increasing relevance. The communication between neurons and glioma cells plays a role in tumor growth and invasion. However, the specific mechanisms involved and how different cell states and mutational background impact on this interaction are still to be defined. To this end, we developed an *in vitro* model displaying the neuro-tumoral unit where primary human GBM Stem-like Cell Lines (hGSCs) derived from different patient samples were co-cultured with murine primary neurons. After 7 days of coculture, neurons boost glioblastoma cells proliferation and this supportive effect occurred even without physical contact suggesting a putative role of soluble factors. Our results also indicated that the enhanced proliferation was tightly dependent on neuronal activity, with higher and lower proliferative rates associated to enhanced or reduced firing, respectively. To address GBM heterogeneity, a panel of twelve cell lines heterogeneous in transcriptional subtype and in genetic aberrations, was evaluated. Results showed that the vast majority of the cell lines (75%) showed a higher proliferative rate when cultured with neurons ($p < 0.05$), with the exception of three cell lines that appears to be insensitive. Our functional data were confirmed also by bulk RNA-seq: Ingenuity Pathway analysis revealed the specific enrichment of proliferation and cell division related processes, while neuronal-insensitive lines resulted significantly upregulated in apoptosis and cell death pathways (Z score > 1.5).
These results pinpoint the central role of the neuro-tumoral unit in glioblastoma progression. Indeed, neuronal activity boost cancer cells proliferation through mechanisms that might require paracrine signaling. Further ongoing analysis of neuronal-induced pathways could elucidate the molecular mechanism underpinning neurons-to-glioblastoma communication.

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Selected Topic: Cancer-neuron synapses

Investigating the role of voltage-gated sodium channels in glioblastoma stem cells: implications for therapeutic targeting

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Glioblastoma (GBM) is the most aggressive adult glioma, with glioblastoma stem cells (GSCs) driving tumor recurrence and resistance to current therapies. Our study investigates the role of voltage-gated sodium channels (Na_v) in GSCs, focusing on their cell cycle-dependent functional expression. We employed molecular biology and electrophysiological techniques to examine Na_v activity in GSCs. Pharmacological manipulation of Na_v revealed its crucial role in maintaining the resting membrane potential (RMP) during the G0 phase, contributing to GSC stemness. Inhibiting Na_v increased GSC sensitivity to temozolomide (TMZ), inducing cell cycle re-entry and differentiation. Additionally, Na_v blockade diminished self-renewal and multipotency of GSCs by modulating the ERK signaling pathway. Our findings suggest that Na_v channels regulate GBM stemness by depolarizing the RMP.

The functional expression of Na_v in GSCs, predominantly during the G0 phase, indicates its pivotal role in sustaining their stemness profile. Pharmacological inhibition of Na_v channels using specific blockers enhanced the efficacy of TMZ, a standard chemotherapeutic agent for GBM, by driving GSCs out of the quiescent state into the proliferative cycle. This re-entry into the cell cycle increased the vulnerability of GSCs to TMZ-induced cytotoxicity. Furthermore, Na_v inhibition significantly reduced the self-renewal capacity and multipotency of GSCs, while promoting their differentiation. Mechanistic studies revealed that Na_v modulates the RMP and negatively regulates the ERK signaling pathway, which is crucial for maintaining the stemness and survival of GSCs. These insights highlight Na_v as a promising prognostic biomarker and therapeutic target for GBM treatment, potentially improving patient outcomes by targeting GSCs and overcoming therapy resistance. Our comprehensive study underscores the potential of targeting Na_v channels to disrupt the stemness and resistance mechanisms in GSCs, offering a novel approach to enhance the effectiveness of existing GBM therapies.

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Selected Topic: Neural stem cells and cancer

Exploiting metabolic vulnerabilities to sensitize resistant glioblastoma tumor initiating cells toward LSD1-directed therapy

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Glioblastoma (GBM), the most prevalent primary brain tumor in adults, remains incurable despite multimodal therapies. Adaptation to harsh microenvironmental conditions, such as nutrient deprivation, proteostasis disruption, hypoxia, and drug treatment, induces molecular and metabolic changes in GBM that result in stress tolerance and drug resistance.
We demonstrated that Lysine-specific histone demethylase 1 (LSD1) regulates the survival, adaptation, and recovery of tumor-initiating cells (TICs). LSD1 genetic and pharmacological targeting with a specific and brain-penetrant LSD1 inhibitor (LSD1i) disrupts TICs' ability to maintain homeostasis following endoplasmic reticulum (ER) stress or nutrient deprivation by impeding a proper Activating Transcription Factor 4 (ATF4)-dependent integrated stress response (ISR).
Our findings indicate that LSD1i treatment induces physical and functional rearrangements of mitochondria and ER, resulting in impaired bioenergetic activity and ATP production that predispose TICs to cell death. These samples are largely dependent on glycolysis and are unable to further enhance it when mitochondrial energy production is inhibited by LSD1i.
However, only a subset of patient-derived TICs benefits from LSD1i therapy, while other displayed resistance. LSD1i-resistant samples exhibit reduced susceptibility to stress from LSD1i and known ER stressors like Thapsigargin and Tunicamycin by efficiently restoring energy homeostasis and properly activating ATF4-dependent ISR. Remarkably, LSD1i-resistant samples demonstrate metabolic flexibility, switching between glycolytic and oxidative metabolism to ensure their survival.
Identifying specific metabolic vulnerabilities offers unique therapeutic opportunities: LSD1i selectively targets TICs dependent on glycolysis, while the metabolic plasticity of LSD1i-resistant TICs enables their adaptation to stressful microenvironments and therapeutic challenges.

Presenter cannot be present

STAT3 expression in brain metastases from breast cancer: correlations with different molecular subtypes and clinical outcome

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Background. STAT3 expression in peritumoral reactive astrocytes (RA) of brain metastases (BM) from breast cancer (BC) may favor a pro-metastatic environment.
Material and Methods. Eighty-five BM specimens from BC were identified from the biobank of Pathology Unit of University of Turin and Spanish national BrM network (RENACER). pSTAT3 expression was scored in RA of peritumoral tissue according to Priego et al. (Nat Med 2018). Clinical, molecular data, and intracranial progression (i-PFS) were retrospectively retrieved.
Results. Immunohistochemistry for GFAP and pSTAT3 was feasible in 68/85 (80%). 15/68 patients (21.1%) had luminal BM, 27/68 (39.7%) HER2-positive BM, and 26/68 (39.2%) triple negative BM. 56/68 (82.4%) showed positive staining of pSTAT3, of which 9/68 (13.3%) scored with 3, 26/68 (38.2%) with 2, 21/68 (30.9%%) with 1, and 12/68 (17.6%) with 0 (negative). High pSTAT3 expression (score 2-3) was observed in 17/27 (62.9%) BM from HER2-positive BC and in 15/26 (57.7%) BM from TNBC, while most of BM from luminal BC (12/15 – 80%) had low or absent pSTAT3 (score 0-1) (p=0.021). Overall i-PFS was 16 months (range 7-41): low pSTAT3 BM (score 0-1) had a median i-PFS of 21 months versus 12 months for high pSTAT3 BM (score 2-3). A shorter median i-PFS was observed in high pSTAT3 BM from TNBC (4 months) as compared with low pSTAT3 BM (11 months). Conversely, i-PFS of high pSTAT3 BM (7 months) was similar to low pSTAT3 BM (6 months) in HER2-positive BC.
Conclusion. pSTAT3 expression in RA is higher expressed in BM from TNBC and confers an early progression in comparison with those with low pSTAT3. Further investigation on BM from non-small cell lung cancer are ongoing. These data support the investigation of STAT3 inhibitor silibinin in preventing intracranial recurrence in a clinical trial (SILMET trial, NCT05689619).

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Selected Topic: brain metastases

Brain metastasis in a model of canine haemangiosarcoma: investigating the role of miRNAs

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Haemangiosarcoma is a highly metastatic and lethal cancer of blood vessel-forming cells that commonly spreads to the brain in both humans and dogs. MicroRNAs (miRNAs) are short noncoding single-stranded RNA molecules that play a crucial role in regulating the gene expression. Some miRNAs can function as oncogenes or tumour suppressors, influencing important processes in cancer, such as angiogenesis.
This preliminary study aimed to investigate the role of miRNAs in canine brain metastasis (BM) derived from haemangiosarcoma.
We first performed a discovery-based approach using miRNAseq in tissues derived from BM, peri-BM, and control brain healthy tissues (CB). We included in the analysis the heart primary tumor (PT) and the control heart healthy tissue (CH). Based on the miRNA-seq results we performed unsupervised and supervised analysis to clusterize the samples according to the expression of the whole miRNome and to detect the main differentially regulated miRNAs. We than added miRNA-qPCR experiments to finally identify key miRNAs in BM formation. Among the whole miRNome, the main dysregulated miRNAs in BM resulted: miRNA-208a, miRNA133a, miRNA10b, miRNA133c. Moreover, according to miRNome results combined with the known pathways regulating the haemangiosarcoma and related BM formation, we also selected miRNA-19b, miRNA-21, miRNA-141, and miRNA-494 as putative key players.
In PT, miRNA-10b showed a significant increase in expression, while miRNA-494 and miRNA-141 exhibited downregulation. Moreover, the overexpression of miRNA-10b was retained in metastatic brain lesions. Healthy tissues demonstrated significantly different expression patterns compared to cancerous tissues. In particular, the expression of miRNA-10b was nearly undetectable in both control brain tissue and peri-metastatic cerebral tissue. Preliminary immunohistochemistry-based analysis point to PTEN as a key player in the BM process, probably regulated by miRNA-10b, miRNA-141, miRNA-494.
These findings can provide a rationale for the development of miRNA-based therapeutic strategies, aimed at selectively treating haemangiosarcoma.

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Selected topic: Brain metastasis

Molecular mechanisms of perineural invasion in pancreatic adenocarcinoma

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Perineural invasion (PNI) is a key event at the basis of tumor dissemination in pancreatic ductal adenocarcinoma (PDAC). During PNI, cancer cells invade nerves and migrate along them, establishing a special microenvironment promoting cancer growth and neural remodeling. PNI has a 100% prevalence in PDAC, is associated with early recurrence and poor prognosis and there are no available therapies targeting it. To clarify the mechanisms governing PNI and to characterize the crosstalk between PDAC and nerve cells, we replicated PNI *in vitro*, exploiting primary Schwann cells – DRG cocultures and murine K8484 PDAC cells. Further, to evaluate more physiologically these interactions and the involvement of PNI in tumor formation, we developed K8484 spheroids, orthotopically transplanted them in mice and followed tumor progression.
Our *in vitro* results showed that K8484 cells affect myelin stability by both paracrine signaling and direct interactions. Interestingly, we identified a cancer-derived factor as one of the molecules responsible for myelin degeneration. Indeed, both the inhibition of its downstream signaling in myelinated cocultures and the ablation of its expression in cancer cells rescued myelin degeneration. Notably, human PDAC cells highly express this protein in invaded nerves. Thus, we characterized *in vivo* the role of this factor in tumor development, generating knocked-out K8484 spheroids. Unlike control spheroids, orthotopic transplantation of null spheroids in murine pancreata generated smaller tumors in absence of metastatic events, confirming a crucial role for this protein in PDAC growth and spreading.
In this study, we analyzed the interactions between PDAC cells and nerves and partially clarified the molecular mechanisms at the basis of PNI in PDAC. We also identified a specific molecule as a promoter of tumor development and progression, which could potentially become a new target for PDAC therapy.

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Selected Topic: Perineural invasion in peripheral tumours

Characterization of CD271^{+} Schwann Cells as *in vitro* model of schwannomatosis

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Schwannomatosis (SWN) is a rare genetic disorder characterized by the development of multiple schwannomas, one of the most common neoplasms of the peripheral nerve, originating from a clonal population of Schwann cells (SCs). Schwannomas are usually solitary, non-aggressive, slow-growing tumors that often affect the head and neck regions. Clinically, SWN presents with chronic pain, neurological deficits, and occasional subcutaneous masses. Unlike neurofibromatosis type 1 (NF1) and type 2 (NF2), SWN patients typically do not develop vestibular schwannomas, which are hallmarks of NF2. The condition is primarily linked to mutations in the SMARCB1 and LZTR1 genes. However, from a pathogenomic point of view, it is not excluded that mutations in other genes, not yet identified, may occur. The identification of these genes and/or cellular mechanisms underlying SWN development was limited by the lack of *in vitro* cell models. In this study, therefore, primary human SCs derived from schwannomas were isolated using a positive immunomagnetic cell isolation system based on their surface expression of NGF receptor p75 (CD271). These cells were characterized by flow cytometry analysis of different tumor cell suspensions: tumor digestion, pre- and post-purification specimens. Subsequently, SCs from peripheral schwannomas were immortalized using the LtAg-SV40 to obtain a continuous cell line for future studies aimed at better understanding schwannoma pathogenesis. Overall, the human SC cultures described herein represent an excellent *in vitro* model for wide genome screening and genomic editing studies. The characterization of cells from patients with different clinical presentations, through genotype-phenotype correlation studies, will make possible the identification of new molecular pathways and/or genes potentially linked to SWN.

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Selected topic: Glial cells in cancer

Calcitonin gene-related peptide (CGRP) as possible key factor for neuroinflammatory modulation of *in vitro* neuroblastoma growth and migration

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Crosstalk between nervous and immune systems has proven in many disorders. While solid evidences support the role of immune system in neuronal development and plasticity, neuroimmune axis in cancer development and progression is still to be defined. Recent experimental data suggest that calcitonin gene-related peptide (CGRP) modulation might improve tumor progression via CGRP/Ramp1 axis [1]. We studied if CGRP might have a role in growth and migration of human SH-SY5Y neuroblastoma cell line and growth and wound repair of HBEC-5i cerebral microvascular endothelial cell line (Incucyte analyses). We have demonstrated that CGRP concentration of 300 pg/ml induced a significant increase in SH-SY5Y proliferation, while a more physiological concentration like 30 pg/ml didn't influence proliferation. Similar high CGRP levels are found in plasma of patients with multiple sclerosis and neuroinflammatory diseases, including migraine, while no data are available on neuroblastoma. Preliminary data showed the involvement of CGRP/Ramp1 axis also in our cell model (qPCR). The receptor activity modifying protein 1 (Ramp1) facilitates the localization of calcitonin-like receptor (CLR) to the plasma membrane and high levels are related to metastatic phenotype and poor prognosis of tumors like prostate cancer, melanoma, and osteosarcoma [2]. Interestingly, only low CGRP doses had significant effects on HBEC-5i wound healing, while higher doses reversed this effect. We also evaluated if induced NETosis (neutrophil extracellular traps) could modulate CGRP effects and endothelium function. We demonstrated that NETs were able to significantly improve HBEC-5i wound healing and proliferation, while reducing SH-SY5Y proliferation in a dose and time dependent manner, and counteracting CGRP effects.
These data suggest a possible role for CGRP in a cancer cell-peptidergic neuronal circuit in tumor microenvironment and vascularization. Our findings, although preliminary, establish a likely functional connection between cancer and neurons, proposing CGRP/Ramp1 pathway as potential therapeutic target and possible repurposing of anti CGRP drugs.

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Selected topic: Drug repurposing based on brain/neurons-tumour interactions; Neuroimmune axis in cancer and cancer microenvironment

Deciphering the connection between neurodegeneration and cancer via lncRNAs: a role for *MINCR*

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Recent research has underscored the pivotal role of lncRNAs in both cancer and neurodegeneration, two significant health challenges worldwide. For instance, dysregulated expression of *MALAT1* and *HOTAIR* has been implicated in both conditions. Notably, the MYC-induced Long Non-Coding RNA (*MINCR)* was identified as upregulated in various cancers and conversely downregulated in Amyotrophic Lateral Sclerosis (ALS) patients, standing out as a promising candidate in elucidating the interconnectedness between these diseases. Here, we reannotated bona fide *MINCR* alternative isoforms by analyzing data from PolyA site database, FANTOM CAGE project, and long-read sequencing and then through PCR validation. Their localization was assessed in prostate cancer and neuroblastoma cell lines, revealing cell type-dependent and isoform-specific subcellular localization. *MINCR* functional characterization was carried out using transient and stable approaches. Modulation of *MINCR* RNA level was obtained through gapmer and mixmer Antisense Oligonucleotides (ASO) specifically designed on the reconstructed annotation. Stably overexpressing and CRISPR-Cas9-mediated knockout cell lines were created. RNA sequencing and phenotypic characterization (e.g. cellular growth for cancer, and spheroid formation and differentiation for neurodegeneration) of transiently and stably over/down-expressing cells were used to reveal the pathways governed by *MINCR*. In prostate cancer, amplification of chromosome 8q24, a region called “gene desert” because of the presence of almost only lncRNAs, is associated with disease severity. *MINCR*, among these lncRNAs, exhibits overexpression in prostate cancer, particularly in metastatic cases, and correlates positively with cell cycle-related genes, suggesting its involvement in disease progression. *MINCR* silencing by ASO impacts on cell cycle-related pathways leading to decreased cell proliferation of different prostate cancer cell lines. In contrast, altered expression of *MINCR* in ALS cellular model leads to modifications in pathways related to apoptosis and neuronal differentiation. Further exploration into *MINCR* isoforms and cellular functions holds promise for the development of novel therapeutic approaches for both cancer and neurodegenerative disorders.

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Selected Topic: Neurodegenerative disorders and cancer

The intricated cross-talk between the neuronal and vascular system in the control of tumor development: a reappraisal of published data to define novel pharmacological strategies

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The development of the neuronal and cardiovascular systems proceeds in a parallel spatiotemporal manner, with various common signalling molecules and pathways.
Well recognized proangiogenic factors, involved in the neovascular growth of aberrant vessels in tumor mass and metastasis diffusion and growth, exert trophic and prosurvival function on peripheral nerves. An example is vascular endothelial growth factor (VEGF) that when not appropriately available causes neuropathies. On the other end, nerve growth factor involved in axon development and neuronal differentiation shows its tyrosin kinase receptors on vascular endothelial cells, promoting angiogenesis.
Evidence from our group documented that various types of neuronal derived modulators or neurotransmitters exert proangiogenic properties and that their inhibition at receptor/post-receptor level or impairment of their synthesis blunts with their proangiogenic and protumoral features. Examples are membrane components as gangliosides, peptide molecules as the tachykinin substance P, the calcitonin gene related peptide, the neurokines midkine and pleiotrophin and the gaseous neuromodulator nitric oxide. Controversial data are instead reported for endocannabinoids on tumor angiogenesis and tumorigenesis and incomplete information is available for opioid peptides and cancer. Many of these molecules are typical transmitters of the peripheral sensory efferent systems, involved in neuroinflammation and with their receptors also expressed on immune cells, thus strengthening the intricated cross talk between nerves and capillaries also within the tumor microenvironment.
Due to the ability of tumor cell to manipulate the surrounding tissues and cells to their own advantage, it is hypothesized that tumors can redirect the nervous components for their growth, escape from immune cell attack and resistance to drug treatments.
To define target(s) common to vascular endothelial and neuronal signaling can be an innovative strategy to control the multiple escape facets of malignancy.

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Selected Topic: Drug repurposing based on brain/neurons-tumour interactions

Tumor-Associated Macrophages promote tumor innervation and neural regeneration

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Tumor nerve infiltration significantly influences cancer progression. Conversely, many cancers stimulate nerve infiltration, creating a favorable environment that further accelerates tumor progression via nerve growth. The tumor microenvironment also contributes to cancer growth by generating tumor-associated macrophages (TAMs) with roles in angiogenesis, extracellular matrix remodeling, and immunomodulation. However, TAMs' role in nerve growth remains underexplored. In this research, we reveal that both human and mouse TAMs have a distinct neurogenic profile and actively promote neurite extension and axonal regeneration. Our results show that TAMs facilitate nerve growth within tumors. Using *in vitro* and *in vivo* models, we pinpoint secreted phosphoprotein 1 (Spp1) as a key mediator of TAM-induced neurogenic activity, leading to the activation of neuronal mTORC2 signaling.

We also investigate the potential of TAMs for central nervous system (CNS) regeneration. By transplanting TAMs into a severe spinal cord injury model, we observe significant rewiring of the damaged neural parenchyma, resulting in enhanced nerve regeneration, cyst recovery, and improved motor functions. Proteomic and functional analyses confirm that activation of mTORC2 signaling in neural parenchyma is necessary for the TAM-induced spinal cord regeneration.

These findings uncover a new role for TAMs in tumor innervation, nerve growth, and neural repair. This opens new translational avenues, for using TAMs as a novel cell therapy approach for neural tissue regeneration.

Microglia-neuron crosstalk in the remodelling of peritumoral circuits

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Glioblastoma (GB) malignancy results from a crosstalk between tumor cells and their microenvironment, including neurons, glia cells and the immune system. GB-mediated neuronal overexcitation and excitotoxicity result in greater susceptibility to epilepsy and neurodegeneration, common comorbidities of glioma. Moreover, neuron-to-glioma synapses drive tumor progression through paracrine signalling. In addition, tumor-associated microglia contribute to a supportive microenvironment that facilitates tumor proliferation and migration as a function of their activation state. In physiological conditions microglia (MG) sculpt inhibitory cortical circuits during mouse postnatal development. Here, we investigate the role of microglia in the pathological remodeling of the peritumoral circuits during glioma growth. In mice bearing glioma in the motor cortex, the phasic and tonic inhibitory transmission in the peritumoral neurons is reduced, consistently with a significant reduction in density of VGAT+ boutons. Peritumoral neurons also exhibited a depolarized resting membrane potential (RMP), lower firing threshold, and hence increased firing frequency, possibly predisposing to seizures. Furthermore, grip strength test revealed motor impairment linked to the progression of the glioma. Depletion of MG cells in both control and glioma-bearing mice reduced to a similar extent the frequency of GABAergic post-synaptic currents, suggesting that elimination of microglia during glioma growth does not prevent the decrease in the inhibitory tone. Interestingly, in glioma-bearing mice depleted from microglia tumor proliferation index was reduced and peritumoral neurons showed RMP and firing frequency similar to control mice, suggesting a recovery from hyperexcitability. The expression of small-conductance Ca^{2+}-activated K^{+} (SK) channels, showed to be modulated by activated microglia and inversely related to firing rate, appeared to be reduced in glioma-bearing mice and increased upon microglia depletion. So far, our findings demonstrate that glioma alters synaptic transmission and excitability in the peritumoral area, and that microglia depletion can selectively counteract these alterations.

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Selected Topic: Brain cancer/metastasis remodelling of brain circuits and functions

Understanding the role of astrocyte-mediated phagocytosis in brain tumors

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In glioblastoma multiforme (GBM), by far the most aggressive brain tumor, the tumor-associated astrocytes play an important role in enhancing the tumor proliferation. However, the mechanisms regulating these events remain to be elucidated.
We recently showed that astrocytes are able to remove neuronal terminals through the atypical chemokine receptor 3 (ACKR3) and this process is overactivated during pathologies. Of note, the expression of ACKR3 is also enhanced in phagocytic astrocytes present in the tumor microenvironment, as observed in a mouse model of GBM. We therefore hypothesized that astrocytes eliminate neural synapses allowing the tumor to grow and proliferate into the surrounding space through ACKR3.
Here, we investigate the ability of tumor cells to modulate the phagocytic activity of astrocytes using GBM cells and human astrocytes. Using a molecular biology approach, such as qPCR, we analyzed the expression of ACKR3 gene, alongside other phagocytic receptors in astrocytes, upon exposure to GBM released molecules contained in the spent medium (conditioned medium, CM). In our preliminary results, we observed an increase of the gene encoding for ACKR3 as well as the GFAP marker of reactivity in astrocytes following the treatment. In addition to that, we are investigating how the exposure of GBM's factors is influencing the ability of astrocytes to internalize synapses using live imaging assays. Furthermore, given the importance of chemokines CXCL11 and CXCL12 as ligands of the ACKR3 receptors, the presence of these two factors will be evaluated in co-cultures of astrocytes and GBM. Future steps will include the analyses of potentially interesting biomolecules evaluated in the *in vitro* assays in serum of patients affected by glioblastoma.
Overall, this research will reveal a novel strategy by which astrocytes contribute to brain tumor progression offering a new window for therapeutic intervention.

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Selected Topic: Glial cells in cancer

Inflammatory dynamics in schwannomatosis: interactions between Schwann cells and monocytes

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Emerging research suggests that the cellular microenvironment is a complex process influencing cell physiology, with inflammation playing a critical role in cellular growth and neoplastic transformation. With particular emphasis on schwannomatosis (SWN), a rare tumoral disorder characterized by the development of multiple schwannomas of the peripheral nerve, in this study we focused on the physiopathological role of immune cells on Schwann cells (SCs). Herein we explored, *in vitro*, the molecular pathways and biomarkers involved in such inflammatory response, co-culturing monocytes with SCs, naive or obtained from a clonal population of tumor.

The physiological interaction between Schwann and immune cells is modified at both sides. First, we found that the supernatant harvested from monocytes and M0 macrophages increased the migration and chemotactic response of tumoral SCs and induced the expression of inflammatory genes in SCs (i.e. TLR-4 and CD68). Moreover, when monocytes were co-cultured with SCs, a significant increase in CD163 and IL-10 expression, together with a significant decrease in IL-12 expression, confirmed the M2 macrophages phenotype of co-cultured immune cells.

Overall, we found a significant crosstalk between tumoral SCs and monocytes, demonstrating that schwannoma cells can induce M2 macrophage polarization. Otherwise, SCs develop a pro-inflammatory phenotype and alter their chemotactic migration. Importantly, these evidences could play a crucial role in the development of future therapeutic strategies against neoplasm transformation of the PNS.

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Selected Topic: Neuroimmune axis in cancer and cancer microenvironment

Natural Killer cells modulate peri-tumoral neuron activity in Glioblastoma

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Glioblastoma (GBM) is the most aggressive primary brain tumor, representing a significant clinical challenge due to its infiltrative nature and resistance to therapy. GBM cells release several cytokines that silence the cytotoxic activity of infiltrated immune cells, creating an immunosuppressive/pro-tumoral microenvironment. In this scenario, the invasion of Natural Killer cells is weak, and tumor cells attenuate NK-mediated killing. Moreover, it was reported that peri-tumoral neuronal activity fosters malignant behavior of GBM, shaping the balance excitation/inhibition with effects on GBM-related epilepsy, and building chemical synapses between presynaptic neurons and postsynaptic tumor cells, supporting tumor invasion and growth. In this scenario, we investigated NK cells' impact on neuronal activity in murine GBM models. At first, we described that both *in vitro* then in GBM-bearing mice, NK cells contact peri-tumoral neurons at synaptic and soma levels. Moreover, qRT-PCR analysis unravelled that peri-tumoral neurons increase the expression of chemokines (such as cxcl10 and cxcl9) able to recruit immune cells, and express membrane proteins that trigger the cytotoxic activity of infiltrated NK cells, inducing neuronal death. We further described a neuromodulatory role of NK cells in peri-tumoral area, since whole-cell patch clamp recordings revealed heightened firing frequency of excitatory peri-tumoral neurons in the absence of NK cells, in NK cell-depleted GBM-bearing mice. This study sheds light on GBM pathophysiology, offering insights into potential therapeutic targets.

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Selected Topic: Neuroimmune axis in cancer and cancer microenvironment.

Effects of endocrine disruptors chemicals on miRNAs dysregulation and neuronal cells proliferation

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Endocrine disruptors chemicals (EDCs) are ubiquitous compounds that have received increasing attention for their ability to interfere with endogenous hormones through transport, metabolism, and receptor binding. Emerging evidence suggests that exposure to EDCs, may interfere with the finely tuned mechanisms governing neuronal cell proliferation, thereby impacting brain health and potentially predisposing individuals to neurodevelopmental disorders and neurodegenerative conditions.
The present study investigates the effect of subtoxic concentrations of diethyl phthalate (DEP), primarily utilized as plasticizers, and 17-alpha ethinyl estradiol (EE2), a synthetic estrogen commonly used in oral contraceptives and estrogen replacement therapy, in differentiated SHSY-5Y cells.
Cells were exposed to different concentrations of EDCs for 48h to identify the experimental conditions to which cytotoxicity and oxidative stress were not induced. Results obtained showed the ability of the EDCs under study to interfere with the epigenetic machinery. The deregulation of miRNAs implicated in neurotoxicity, such as hsa-miR-200a-3p, 18b-5p, 653-5p influenced the expression of numerous genes involved in the EGFR/Ras/p53 and PI3K/Akt/mTOR pathways. These pathways were also validated by Western Blotting analysis, that showed a significant shift of the cellular response toward a pro-survival fate. Although these analyses are preliminary, they allow to investigate the ability of some EDCs to modulate, with differential modes of action, pathways involved in neurodegeneration and tumour development. Understanding the intricate interplay between EDCs exposure, miRNA dysregulation, and proliferation deregulation in neuronal cells is crucial for assessing the neurotoxic potential of these compounds and developing targeted interventions and regulatory measures to mitigate its adverse effects on brain health.

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Selected Topic: Neurodevelopment disorders and cancer susceptibility

How to prevent "chemobrain": a systematic preclinical study to support predictive models for human patients

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Thanks to the great improvement of cancer therapies, the number of "long-term survivors" is progressively increasing, highlighting the impact of "chemobrain" on patients' and survivors' quality of life. The term "chemobrain" indicates a cognitive deterioration, occurring in most patients during and after chemotherapy, lasting for years after therapies cessation. We started a systematic, preclinical study to investigate the occurrence of altered cognitive performance in 8ws old female mice (C57BL6, N=15 for each treatment group) treated with some chemotherapeutics, alone and combinations, using a dosing schedule mimicking the clinical use. In details, the following drugs and the respective vehicle controls are included in the study: cyclophosphamide, doxorubicin, methotrexate, 5-fluorouracil, oxaliplatin, CMF schema (cyclophosphamide + methotrexate + 5-fluorouracil); AC schema (doxorubicin + cyclophosphamide). Mice were tested for Spatial Working and Reference Memory (Y-maze), associative memory (Contextual Fear Conditioning test), general cognition and executive functions (puzzle box), and spontaneous locomotion before and after treatments; feces and blood sample were collected before, along, and after treatments, for gut microbiota composition and inflammatory cytokines. Brain tissues collected at sacrifice will be analyzed for neurotransmitters, tissue, and single population transcriptomic, neuropathology. The data available so far, indicated that general cognition and executive functions are severely impaired in doxorubicin- or cyclophosphamide-treated mice (both drugs: $p<0.0001$) when compared to vehicle-treated mice; Spatial Working and Reference Memory are also impaired (both drugs: $p<0.05$), while associative memory and locomotion were not affected. Body weigh loss of mice is less than 10% along the experiments. The final aim of the project is to generate a predictive demonstrator for chemobrain onset driven by plasma cytokines and gut microbiota composition and based on a machine learning/artificial intelligence model trained and validated with preclinical data sets.

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Selected Topic: Cognitive effects of cancer and cancer treatments

Cancer-specific association between neurodegenerative-related genes and cellular pathways, clinical outcome, and drug response

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Epidemiological studies that examined the association between neurodegenerative disorders and cancer support a general inverse association; for instance, patients with Parkinson's Disease tend to have a lower risk for cancer in general, and cancer patients have a lower risk for Parkinson's Disease. Conversely, positive associations have been reported with specific cancers, suggesting that these specific associations could be explained by common genetic factors between the two diverse classes of conditions. However, the underlying mechanisms behind these specific associations are still unclear.
To address this, we selected fifty genes causally associated with neurodegenerative disorders, such as MAPT, alpha-synuclein, Pink1, etc., and performed an in-silico pan-cancer analysis of their transcriptomic profiles, survival correlations, and gene expression analyses. To provide new evidence for the relevant role of these genes in specific cancers, we analyzed transcriptomic profiles from over 10,000 clinical samples across 32 cancer types and over 1,300 pre-clinical samples across 28 cancer types provided by the TCGA and DEPMAP datasets, respectively.
We found that the expression of several neurodegenerative genes is associated with key cancer hallmarks, including inflammation, proliferation, and epithelial to mesenchymal transition, exhibiting cancer-specific patterns. In some cancer types, the functional networks of neurodegenerative genes were affected by the P53 mutational status. We also identified new associations of neurodegenerative genes with clinical outcomes and drug responses in a context-specific manner.
Overall, our findings indicate that neurodegenerative genes are potential major players in multiple types of cancer. Importantly, the impact of these genes on cancer appears to be heavily influenced by the specific cellular environment.

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The role of prokineticins and histone demethylase KDM6A in bortezomib-induced painful neuropathy and mood disorders

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Chemotherapy-induced peripheral neuropathy (CIPN) is a major adverse effect of many cancer therapeutic agents, including bortezomib (BTZ). The appearance of this neuropathy can lead to dose reduction or even to the suspension/cessation of chemotherapy, thus increasing cancer-related mortality. Several mechanisms including oxidative stress, mitochondrial damage, and ion channels dysfunction seem to be involved in CIPN development. However, in the last years particular attention has been devoted to neuroinflammation. In this frame, prokineticins (PKs), a new family of chemokines, has been proposed to participate in the development and maintenance of painful neuropathies as well as in driving the epigenetic control of genes involved in cellular differentiation. On these bases, the present study investigated the correlation between epigenetic mechanisms and PKs in the spinal cord of BTZ-induced neuropathy suffering mice. Moreover, given the strong relationship between prolonged pain and mood disorders, the presence of anxiety/depression as well as of supraspinal neuroinflammation has been also evaluated in BTZ-treated animals. Results showed that BTZ induced an early upregulation of the KDM6A, nuclear receptors PPARs, and IL-6, followed by a delayed increase in PK2 and IL-1β. Moreover, the administration of the antagonist of PK system PC1 attenuated mechanical allodynia and prevented the increase of PK2 and of IL-1β in BTZ neuropathic mice. Interestingly, the blockade of PKRs signaling counteracted the increase of KDM6A and induced an increase of PPARs gene expression in the spinal cord of painful mice. Furthermore, PC1 treatment prevented the development of supraspinal inflammation, and ameliorated depressive-like symptoms in BTZ-induced neuropathy suffering mice. These overall data highlighted the involvement of epigenetic modulatory enzymes in spinal tissue phenomena associated to BTZ painful neuropathy, and emphasize the role of the PKs in promoting and sustaining, at spinal and supraspinal levels, the increase in proinflammatory cytokines which participate to the development of BTZ-induced painful neuropathy and mood disorders.

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Selected Topic: Cancer and pain

Vulnerability of white matter to chemotherapy drugs: focus on oligodendrocytes and oligodendrocyte precursor cells

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The number of cancer patients who survive the pathology is constantly increasing, but this is accompanied by growing evidence of severe side effects. More than 60% of patients treated with systemic chemotherapy show neurological disabilities and altered brain imaging involving the white matter. Oligodendrocytes (OLs) and their precursors (OPCs) appear to be particularly sensitive to chemotherapeutic drugs.
To investigate the role of OPC vulnerability in chemotherapy-mediated neurotoxicity, we used primary OPCs (rats, P10) isolated by immunomagnetic separation (O4), from cerebral cortex (ctx) and optic nerve (opn), cultured as OPCs (bFGF/PDGF) or OLs (triiodothyronine, 3 days). The components of the chemotherapeutic mix FOLFIRINOX (folic acid, FA; 5-fluorouracile, 5-FU; irinotecan, IN; oxaliplatin, OX) and dexamethasone (DEX), have been selected as test drugs.
Using a colorimetric assay (MTT) and a cell-based high-content screening (HCS), we performed a dose-response curve of each molecule on ctx-OPCs, finding that IN and OX show marked toxicity at low doses (2 μM). DEX co-treatment enhanced the toxicity in ctx-OPCs, with no effect on differentiation and mature ctx-OLs. The opn-OPCs resulted also sensitive to IN and OX treatment, while the toxicity enhancement of DEX co-exposure was more evident, acting on 5-FU, IN, and OX.
To evaluate lineage selectivity of these effects, we also included primary cortical neurons, which resulted less sensitive, with a clear toxic effect only for OX.
We demonstrated that undifferentiated OPCs are highly sensitive compared to post-mitotic OLs and neurons, and opn-OPC results the most sensitive cell. This highlights a key role of OPCs in the neurotoxic side effect chemotherapies, indicating possible novel prevention target to guarantee a better quality of life of cancer patients.

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Selected Topic: glial cells in cancer

The role of oncological infrastructures on the mood disorders experienced by cancer patients

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Research on the relationship between the Built Environment (BE) and cancer has increased in recent years. However, it has primarily focused on analyzing the impact of the BE on patients' exposure to carcinogens, leaving the BE's impact on the cancer course and outcomes still underexplored. For cancer patients, oncological infrastructures (OI) represent the main point of contact with the BE, for some of them: the last, and while their exposure to carcinogens is controlled through construction standards written in manuals, the spaces inside the OI are designed to meet the needs of the procedures associated with cancer treatment rather than the treatment of patients themselves. To fully understand this dimension, we must map the journey of cancer patients inside the OI, understand their experiences and go beyond the medical procedures. Reflecting on the analysis of 50 structured questionnaires from breast cancer patients undergoing chemotherapy treatment in the Maria Sklodowska-Curie National Oncology Center in Poland. This research suggests ways in which indoor spaces can enhance mood disorders, particularly the depressive episodes. The question this research is trying to answer are: who is the focus of the treatment? Are OIs designed to treat cancer or are they designed to treat people with cancer? Are spaces inside an OI capable of stimulating our nervous system to improve our well-being and maybe the response to the treatment? Are there maybe spaces missing inside an OI that prevent it from that? Is there really a need to focus on architectural spaces of an OI when there is so much technology available?

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Targeting Citron Kinase catalytic activity for high grade brain tumors treatment

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Medulloblastoma (MB) and glioblastoma (GBM) are the most frequent high-grade brain tumors (HGBT) in children and adulthood, respectively. Despite the improvements in patient survival, many patients still die and those who survive suffer from neurological and endocrine disorders. Therefore, more effective therapies are needed. Citron Kinase (CITK), is required during neurodevelopment for correct cytokinesis and genomic stability of normal neural progenitor cells. CITK is validated as target for MB treatment as its depletion induces apoptosis and reduces tumor growth *in vivo*. Moreover, loss of CITK leads to cytokinesis failure and DNA double strand breaks (DSBs) accumulation in MB cells. On this basis, we are working on developing CITK inhibitors as a possible strategy for HGBT treatment. Stemming from published binding data between kinase inhibitors and the kinome, we identified Lestaurtinib as a promising CITK inhibitor, able to inhibit CITK catalytic activity at nanomolar concentrations. We found that Lestaurntib impairs MB and GBM cell proliferation, leading to accumulation of DNA double strand breaks and cell death, recapitulating CITK knockdown effects. Moreover, Lestaurtinib is effective *in vivo* in reducing the progression of MBs arising in SmoA1 immunocompetent transgenic mice and increasing their survival. Our findings strongly suggest that Lestaurtinib induces phenotypes in MB and GBM cells that closely resemble the effects of CITK knockdown. The observed reduction in cell proliferation and enhanced mice survival underscore the potential of Lestaurtinib, as well as more specific CITK inhibitors, as promising candidates for HGBT treatment. These results warrant further in-depth investigation to harness their therapeutic potential for high-grade brain tumors.

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Selected Topic: Drug repurposing based on brain/neurons-tumour interactions

Antitumor potential of targeting glutamatergic signalling in patient-derived glioblastoma stem cells

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Glioblastomas (GBMs) are diffuse adult IDH-wt astrocytic gliomas, characterized by poor prognosis. GBM cellular heterogeneity and the presence of GBM stem cells (GSCs), are responsible for tumor growth, relapse, and therapy resistance, being unaffected by current treatment. Epilepsy, a frequent comorbidity in GBM patients, shares common pathophysiological mechanisms. GBM microenvironment, characterized by active glioma-neural crosstalk, plays a key role in driving GBM growth and invasiveness, and in particular, high glutamate levels and signaling contribute to both GBM growth and generation of seizures. In a drug repurposing approach, the noncompetitive antagonist of AMPA glutamate receptor perampanel, approved as antiepileptic drug, was studied to determine its potential antitumor activity in GBM.
The effects of perampanel were assessed in 2D and 3D cultures of 5 patient-derived GSCs, expressing AMPAR. Perampanel showed a concentration-dependent antiproliferative, mainly cytostatic, activity in 2D GSCs and differentiated GBM cell cultures, with a potency within the micromolar range (mean IC50≈100μM), while lower efficacy was demonstrated in an immortalized human oligodendrocyte cell line. Perampanel (100μM) significantly inhibits active proliferating cells, impairs self-renewal (-50%) and invasive capacity (-30%) of GSCs, as measured by EdU-fluorescent staining, spherogenesis, limiting dilution (ELDA) and 3D invasion assay, respectively. Perampanel enhances temozolomide and radiation cytotoxic effects and elicits a significant reduction of the growth of fluorescently-labelled GSCs orthotopically injected into zebrafish embryos.
In conclusion, perampanel shows *in vitro* and *in vivo* antitumor efficacy, reducing GSC stemness and invasiveness, and non-stem GBM cell survival, providing the basis for targeting glutamate-mediate signaling involved in GBM–neuron network hyperexcitability, to control tumor growth and seizures.

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Ketogenic diet induces an inflammatory reactive astrocytes phenotype reducing glioma growth

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In recent years, the use of the ketogenic diet (KD) has proven beneficial as a complementary approach to standard glioma therapy. The metabolic shift induced by the KD leads to the generation of ketone bodies, particularly β-hydroxybutyrate (β-HB), that can be used as an alternative energy source. Studies have highlighted the interplay between metabolic reprogramming in tumor cells and the surrounding microenvironment, suggesting that metabolic therapies such as the KD may also modulate the activity of microglia and astrocytes, potentially influencing GBM progression, but the mechanisms have not been yet clarified.

Here we investigated the effect of the KD in glioma-bearing mice showing a reduction in tumor growth and an increased median survival rate respect to the mice fed with a matched control diet.

To explore a possible mechanism by which KD might exert its function, we proposed glial cells as mediators of the KD effect on tumor growth. Specifically, we describe that KD *in vivo*, and β-HB *in vitro* can induce a pro-inflammatory phenotype in astrocytes and that pro-inflammatory astrocytes isolated from the glioma-bearing mice or induced by the β-HB treatment, exhibit increased levels of glutamate transporters which are functionally active in reducing the extracellular glutamate. Moreover, we described increased intracellular basal Ca^{2+} levels in GL261 treated with β-HB or co-cultured with astrocytes, this condition is paralleled by a reduction in tumor microtubes structures among GL261 cells. All these data suggest that β-HB, triggering a pro-inflammatory astrocytes phenotype, can reduce glioma proliferation, excitotoxicity, and glioma connectivity thus proving a beneficial effect on brain parenchyma.

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Using rational approach for drugs and diagnostics tools development to selective targeting human aldehyde dehydrogenase 1A3 in gliomas

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Elevated aldehyde dehydrogenase (ALDH) activity correlates with poor outcome for many solid tumours as ALDHs may regulate cell proliferation and chemoresistance of cancer stem cells (CSCs). Glioblastoma (GBM) is the most aggressive primary brain tumour for which both effective treatments and efficient tools for an early-stage diagnosis are lacking. Of the many ALDH isoforms, several studies have implicated the elevated expression of Aldehyde dehydrogenase 1A3 (ALDH1A3) as a target for the development of novel therapeutics. In particular ALDH1A3 belongs to an enzymatic superfamily composed by 19 different isoforms, with a scavenger role, involved in the oxidation of a plethora of aldehydes to the respective carboxylic acids, through a $NAD(P)^+$-dependent reaction. Thanks to the structural analysis of our human ALDH1A3 model, combined with *in-silico* studies, we were able to identified specific inhibitors of ALDH1A3[1-3]. Indeed, we isolated inhibitors selective and competitive only versus human ALDH1A3. Our results reveal that ALDH1A3 is the primary binding protein for these molecules in MES GSC lysates and that their inhibitory effect on retinoic acid biosynthesis is comparable with that of ALDH1A3 knockout. Our compounds show anti-metastatic activity in wound healing and invasion assays and induces the downregulation of cancer stem cell markers[4-6]. In addition, using the same rational approach, we synthetized a curcumin-based fluorescent probe that is able to bind to ALDH1A3 without showing any appreciable interaction with other ALDH1A isoenzymes. Indeed, its fluorescent signal is detectable only in our positive controls *in vitro* and absent in cells that lack ALDH1A3. Remarkably, *in vivo*, our probe selectively accumulates in glioblastoma cells, allowing the identification of the growing tumour mass. The significant specificity of our compounds is the necessary premise for development glioblastoma cells detecting probes to be possibly used during neurosurgical operations[7].

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Blood-Brain Barrier Penetrating And Promising Drug Delivery Systems In Glioblastoma Therapy: Exosome-Nanoliposome Hybrid

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Gliomas constitute nearly 80% of lethal brain tumors and are classified based on the cells they originate from. Glioblastoma is an astrocytic tumor with a still poor prognosis, despite recent advances in important therapeutic modalities. The most important factor to overcome in its treatment is the presence of the Blood-Brain Barrier (BBB). Therefore, new invasive and non-invasive drug delivery strategies for Glioblastoma are needed to overcome both the intact blood-brain barrier and to develop new systems for effective treatment. Exosomes are bioactive molecules released from cells, carrying characteristics of their originating cell and playing a role in cellular communication. In recent years, there have been studies focusing on the development of exosomes as non-invasive drug delivery systems, which have garnered significant interest due to their size and ability to traverse biological barriers. However, there are many challenges that need to be overcome in exosome research. Delivering drug-loaded exosomes effectively to the brain without degradation is particularly crucial, and the use of appropriate nanocarriers plays a significant role in this process. This study hybridized exosomes derived from Wharton's Jelly Mesenchymal Stem Cells (WJ-MSCs) with nano-liposomes through membrane reinforcement, loading them with teozolomide anticancer drug. As a result, the obtained exosomes and nano-liposomes maintained their characteristics, demonstrating successful fusion of two different nanoparticles and the development of a new generation carrier system. Additionally, it is anticipated that drug-loaded exosome-liposome nanovesicles, with their prolonged release capability and ability to cross the blood-brain barrier (BBB), could be utilized as a promising method for the treatment of Glioma tumors.

Keywords: Glioma tumors, Exosome, Nano-Liposome, Hybrid Exosome, Hybrid Nanovesicles

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POSTER PRESENTATIONS

The extracellular Nicotinamide Phosphoribosyltransferase favors the brain invasion of mammary carcinoma cells

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The extracellular Nicotinamide Phosphoribosyltransferase (eNAMPT) has been reported to act as a cytokine and its serum concentration in breast cancer patients correlates with TNM staging, tumor size and lymph node metastasis.
To explore the role of eNAMPT in tumor microenvironment, 4T1 triple negative breast cancer cells engineered to release 15 times the amount of eNAMPT (SP-NAMPT) and SCR control cells were injected into Balb/c mice to obtain an orthotopic model of mammary carcinoma.
Although there are no significant changes in the size of the primary tumor, SP-NAMPT mice reveal more leaky vessels and elevated presence of circulating tumour cells at 28 days, suggesting that higher eNAMPT levels may facilitate the migration of tumour cells from the primary mass to the metastatic site. SP-NAMPT mice, in fact, reveal increased numbers of metastasis in several distant organs, i.e. lung, liver and brain. To have a confirmation of this hypothesis and to investigate the effect of eNAMPT directly on tumor cells, a wound healing assay was performed using tumour cells taken from the primary mass and lung metastasis and it was observed that SP-NAMPT cells deriving from both sites are more prone to migrate compared to SCR cells. Additionally, to specifically explore the effect of eNAMPT in the brain during the metastasis formation, the rate of permeation through the barrier in presence of eNAMPT was analyzed *in vitro* using a BBB model. The barrier permeability is increased after 48 hours and 72 hours treatment with rNAMPT 1mg/ml and 500ng/ml, respectively. Moreover, Western Blot analysis and immunofluorescence revealed that a shift of claudin from the membrane to the cytosol occurs after rNAMPT treatment.
Our results suggest that eNAMPT, beside a direct effect on tumor cells in promoting cell migration, could act by creating a favorable environment to the establishment of metastases.

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Selected Topic: brain metastasis

TEAD1 epigenetic silencer curbs glioblastoma malignancy by silencing the YAP/TAZ oncogenic transcriptional cascade

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The molecular implications underlying the relapses of glioblastoma multiforme (GBM), that represent the major challenges to counteract GBM, are not yet well characterized nowadays. Indeed, GBM recurrence is inevitable. Despite current treatments, virtually all patients relapse due to the presence of cancer stem cells (CSCs) that are able to self-renew and regenerate the tumor, remaining quiescent or exhibiting very low proliferative activity.
Considered the urgent medical need to counteract GBM recurrence, we generated a synthetic epigenetic silencing factor called TES (TEAD1 epigenetic silencer) in order to stably switch off the YAP/TAZ oncogenic transcriptional cascade which drives tumor malignancy. TEAD1, in association with YAP and TAZ coactivators, plays a key role in GBM pathogenesis and progression since their interaction initiates the expression of a wide set of genes associated with epithelial-to-mesenchymal transition, cell migration, proliferation, and invasion. Notably, TEAD1 binding domain is fused with the KRAB repressor domain and DNMA methyltransferase 3A/L catalytic domains. In this way, TES silences the transcriptional network controlled by TEAD1, the nuclear transducer of the YAP/TAZ signalling pathway, thus curbing GBM malignancy. TES anti-tumoral activity was tested both *in vitro* and *in vivo* GBM models, and its transcriptional output and epigenetic activity was assessed.
TES induced a robust transcriptional silencing of a large group of YAP/TAZ targets genes by the induction of stable epigenetic modifications as assessed by RNA-sequencing. Moreover, TES promoted a strong proliferative loss, reduction of migration and high cell death in glioma cell lines and in patient-derived GBM CSCs *in vitro*. In accordance, local viral delivery of TES in orthotopic intracranial mouse xenograft repressed the development of the human GBM-like masses.
Collectively, the strategy exploited by TES consists in silencing the entire transcriptional network controlled by TEAD1, thus achieving high levels of efficacy against GBM recurrences and malignancy.

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Selected Topic: CNS primary tumor

The neuroprotective effects of sodium valproate on DNA damage and oxidative stress in neuroblastoma cells

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Aim: Neuroblastoma (NB) is the most common childhood tumor originating from neural precursor cells. DNA damage and oxidative stress are important findings in NB to develop novel therapies. Sodium valproate or valproic acid is a simple branched-chain carboxylic acid and is primarily used for epileptic seizures and bipolar disorder treatment. It has been shown to induce differentiation, cell death and neurite enhancement. This study aims to investigate the potential therapeutic effect of valproate on SH-SY5Y neuroblastoma cells.
Methods: SH-SY5Y NB-cells were seeded into 96-well plates at $5x10^3$ cells/well and incubated with different doses of valproate (1, 10, 125, 250, 500 mM) for 24h. 1 mM H_2O_2 was used to obtain maximal toxicity as a positive control. The cell viability was evaluated by MMT assay. Time-lapse imaging of cell orientations was captured for 16 hours. Then, DNA damage and cell viability results were compared by using the comet method. Additionally, the total oxidant/antioxidant levels of the cells were measured.
Results: The protective dose of VPA was statistically determined as 10 mM in the MTT assay results ($p< 0.05$). A significant correlation was observed between MTT and comet results. Total oxidant levels were significantly decreased in valproate-treated cells, especially in 10 mM while the higher doses of valproate caused increased oxidative stress ($p<0.05$).
Conclusion: This study showed the effects of various doses of valproate on DNA damage and oxidant/antioxidant balance. These results suggested that valproate may have dose-dependent beneficial effects against neuroblastoma and higher doses may be detrimental to DNA and cell survival. Therefore, this preliminary study helps to understand the effects of valproate in neuroblastoma to investigate synaptic transmission in cancer further.

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Reciprocal interaction between cancer cells and adult neural progenitor cells: any relevance in tumour progression?

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Adult Neural Progenitor Cells (aNPC) are self-renewing and multipotent cells in the central nervous system, responsible for generating neurons and glial cells but also endowed with non neurogenic properties that may be relevant under both physiological and pathological conditions.
Recent research suggested that aNPC can influence the behavior of cancer cells, particularly in brain metastasis. The interaction between aNPC and cancer cells is likely a dynamic and multifaceted process whose implications in oncology are currently underinvestigated. This interaction can occur through the secretion of extracellular vesicles (EV), signaling molecules, and direct cell-cell contact.
To better understand the crosstalk between aNPC and cancer cells, we extracted and characterized Evs derived from murine aNPC and tested their effects on 4T1 breast cancer cells. Simultaneously, we investigated the impact of the secretome from 4T1 cells on differentiating murine aNPC from two adult neurogenic regions, hippocampus and hypothalamus.
Our preliminary *in vitro* data demonstrate that EV derived from aNPC enhance the migration of 4T1 breast tumor cells without altering their cell cycle progression. Moreover, 4T1-derived conditional medium stimulates NPC differentiation towards the astrocytic lineage at the expenses of their neuronal differentiation, suggesting a bidirectional crosstalk between aNPCs and breast cancer cells.
Our future plans aim at further understanding the functional and reciprocal interplay among aNPCs, Anpc-derived astrocytes, and 4T1 cells *in vitro*, and in the long term, *in vivo* in the syngeneic 4T1 model of breast cancer brain metastasis. Additionally, we plan to investigate the role of Anpc-Evs in brain metastasis and how aNPCs and their progeny from different neurogenic niches may potentially contribute to this process.

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Selected Topic: Neural stem cells and cancer

mir21 carried in plasma derived EVs of glioma-bearing mice is a promising biomarker to easily anticipate the tumor diagnosis

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In the contest of Glioblastoma multiforme (GBM), the most aggressive and deadly brain tumor, an early detection is a challenge and relevant to increase the survival of patients. In the last decades, the analysis of liquid biopsies (plasma, saliva, urine, CSF) represented a way useful for the diagnosis and the cure of different diseases, including tumors. The collection of liquid biopsies is a non-invasive technique, permit to collect serial samples and monitor changes in patients instead of current invasive techniques, such as histological and neurological examination, used to diagnose GBM.
Both in physiological and pathological processes, neural cells communicate each other and with periphery releasing growth factors and extracellular vesicles (EVs). EVs are composed of bilayer membranes, distinguished on biogenesis, size and contain specific lipids, proteins and nucleic acids that can change the functions of recipient cell.
In this study, we focused the attention on the analysis of brain and plasma EVs collected one week after glioma inoculation, in an *in vivo* model to find a biomarker to anticipate the diagnosis of GBM.
Thanks to a miRnomic analysis of EVs, in our syngeneic model (GL261 cells inoculated in C57bl6N mice) we found that miR21 is overexpressed earlier during glioma growth. We confirmed these data through qRT-PCR on miRNA expressed in brain and plasma EVs in glioma-bearing mice.
We found a progressive volume tumor growth associated with a progressive miR21 expression, in brain EVs. Otherwise, in plasma EVs, we observed a peak of miR21 expression, one week after inoculation, but this effect doesn't persist later. We also used a xenogeneic model (U87-MG cells inoculated in SCID mice) and we found the same result.
In conclusion, miR21 carried in EVs isolated from plasma of glioma-bearing mice represents a promising biomarker in GBM patients to easily anticipate the diagnosis.

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Extracellular vesicles isolated from patients as a drug delivery system for glioblastoma

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Glioblastoma is the most common primary brain tumour, known for its extreme aggressiveness and difficulty of complete eradication, therefore the risk of recurrence is high. Moreover, the effectiveness of current therapies is limited by the difficulty of delivering drugs across the blood-brain barrier. In this context, extracellular vesicles represent a potential delivery system for therapeutic and diagnostic agents targeted at glioblastoma. It has recently been demonstrated (1) that autologous extracellular vesicles, when loaded with ICG—a fluorescent diagnostic approved for clinical use—and reintroduced into circulation, recognize the tumour from which they originated. The aim of the project is to provide proof of principle that vesicles isolated from the plasma of glioblastoma patients are able to cross the blood-brain barrier and recognize glioblastoma. This could favor the future clinical application of extracellular vesicles, enhancing the effectiveness of current therapies and promoting the complete resection of the tumour through intraoperative imaging techniques. Extracellular vesicles were isolated through ultracentrifugation, characterized with Nanoparticles Tracking Analysis and loaded with diagnostic agents. Biodistribution studies were carried out in healthy mice, while homing studies were conducted in orthotopic glioma mouse models. Biodistribution studies showed that extracellular vesicles isolated from the plasma of glioblastoma patients, accumulate in the brain when administered in healthy mice, a unique behaviour of glioblastoma vesicles. Subsequently, homing studies in orthotopic glioma models demonstrated that vesicles loaded with contrast agents accumulate at the glioblastoma site, as shown by the imaging techniques used and confirmed by the histopathological analysis of brain tissue. The results suggest that extracellular vesicles isolated from glioblastoma are potentially able to cross the blood-brain barrier and accumulate at the glioblastoma site if present. This lays the foundation for a possible clinical application of autologous glioblastoma extracellular vesicles to deliver drugs targeted at the tumour in the CNS.

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Oleoylethanolamide and palmitoylethanolamide enhance IFNβ-induce apoptosis in human neuroblastoma SH-SY5Y cells

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Background: Oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) are classified as bioactive lipids with high affinity for the nuclear PPARα receptor. Recently, there has been an increased interest in the impact of these lipids on malignant tumors. Nonetheless, their effects on human neuroblastoma cells have not been reported yet. Type I interferons (IFNs) are immunomodulatory cytokines known for their antiviral and anti-proliferative properties in different pathologies, including melanoma, leukemia, hepatitis B and C. The present study aims to investigate the effects of combining OEA or PEA with IFNβ in the human neuroblastoma SH-SY5Y cells. We mainly focus on elucidating any possible overlapping signaling pathways that may be activated by these two distinct sets of compounds when present simultaneously in a pharmacological combination, thereby suggesting a promising role of OEA and PEA in shaping multimodal and alternative therapeutic strategies.

Results: This study examined the impact of OEA and PEA on human neuroblastoma SH-SY5Y cells treated with IFNβ. We assessed cell viability, proliferation, and signaling. Co-exposure to OEA or PEA with IFNβ causes enhanced apoptotic cell death, including caspase 3 and PARP cleavage, as well as a drop in survivin and IKBα levels. Furthermore, OEA and PEA did not affect IFNβ signaling via the JAK-STAT pathway or the STAT1-inducible protein kinase R (PKR). OEA and PEA significantly boosted p38 MAP kinase phosphorylation and programmed death-ligand 1 (PD-L1) levels in total cell lysate and surface membranes. GW6471, a PPARα inhibitor, and genetic silencing of the receptor were found to reduce PD-L1 and PARP activation.

Conclusions: OEA and PEA inhibit cell survival, proliferation, and clonogenicity by modifying and amplifying the intrinsic apoptotic pathway in human SH-SY5Y cells through PPARα activation. This mechanism is independent of the IFNβ-induced route.

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Validation of SOX2 Epigenetic Silencer to reduce GBM in immunocompetent mouse model

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Current therapies are still unsatisfactory in preventing recurrences of glioblastoma multiforme (GBM). Tumor-initiating cancer cells infiltrate the parenchymal tissue and become resistant to adjuvant treatments, thereby supporting relapses. By rational engineering of SOX2 transcription factor (a key promoter of GBM malignancy) through the assembling with domains from epigenetic repressors (the Kruppel-associated box and DNA methyltransferase3A/L catalytic domains), a new synthetic factor called SES (Sox2-Epigenetic-Silencer) has been generated. It induces the silencing of the entire pathway regulated by SOX2 itself.
SES inhibits both glioma cell lines and patient-derived cancer stem cells *in vitro* and *in vivo*. Indeed, its expression, through local viral delivery in mouse xenografts, induces strong regression of human tumors and survival rescue. SES induces gene deregulation in GBM cell lines, as assessed by RNA sequencing (RNA-seq). Genomic investigations confirmed the expected epigenetic editing on molecular pathways which are SOX2 targets, justifying the high levels of efficacy in repressing aggressive brain tumors.
However, in order to move to a translational approach, SES efficacy and safety have to be confirmed in immunocompetent mouse models of GBM. To do so, we are exploring the syngeneic model, trough the injection of murine GBM cells in C57Bl6 mice and evaluating the immune response after SES treatment and tumor burden reduction.

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Etoposide as a new pharmacological tool to induce a pro-inflammatory senescent phenotype in human microglial HMC3 cells

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Cellular senescence in peripheral tissues is linked to aging and aging-related disorders, but its involvement in brain aging has been only recently explored. Senescent cells undergo cell cycle arrest and strengthen their secretory function, acquiring the so-called senescence-associated secretory phenotype (SASP). Proliferation-competent glial cells can undergo senescence both *in vitro* and *in vivo*, and they are likely to participate in neuroinflammation, a hallmark of the aging brain.
Etoposide is a topoisomerase II inhibitor widely used as anti-cancer reagent that induces DNA stress and proliferation arrest of neoplastic cells, as well as neuro- and glial-toxicity. Recently, etoposide has been demonstrated to induce cellular senescence of glial cells *in vitro*, an event that can promote neuroinflammation and cognitive decline in cancer patients. However, a detailed characterization of its effects on human microglial cells is missing. Here we investigated the effects of etoposide exposure on human microglial HMC3 cells.
HMC3 cells were treated either with 10μM etoposide (ETO) for 24h or 48h, or only for 24h and collected after 24h or 48h of washout (WO). Viability, cell cycle as well as morphological and senescence evaluations were performed through imaging flow cytometry. Expression of the senescence markers β-galactosidase, γH2A.X and laminB1 were performed by immunofluorescence or Western Blot (WB). Gene expression analyses of pro-inflammatory cytokines associated with the senescence-associated secretory phenotype (SASP) was performed by Real Time PCR.
ETO treatment, despite not significantly affecting cell viability, resulted in the time-dependent increase of the fraction of cells in G2 phase. Along with cell cycle blockade, nuclear area, whole cell dimension and autofluorescence increased, resulting in an enhancement of the senescence index (SI) both after 48h of ETO and at 24h ETO+ 24h WO, compared to control and to 24h exposure. Accordingly, the number of β-galactosidase positive cells and of γH2A.X foci within cell nuclei, increased over time after ETO treatment, even after WO, while laminB1 levels decreased. Over-expression of the SASP-related genes *tnf1*, *il1b* and *il6* was detected upon ETO exposure, and, interestingly, treatment with ETO for 24h followed by 24h WO, showed in the highest increase of *il1b* and *il6*, compared to the other conditions.
Microglial cells showed a relative resistance to ETO treatment in terms of viability, but rapidly acquire a senescent pro-inflammatory phenotype, even after short exposure and washout, that may result in central nervous system toxicity. Etoposide-induced microglial senescence can also represent a useful experimental model to identify new pharmacological targets to treat cognitive decline associated with microglial activation in aging-related disorders.

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Selected Topic: glial cells in cancer

A patient-derived Glioblastoma organoid model to ensure 3R principle in pre-clinical research

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Glioblastoma multiforme (GBM) is the most prevalent primary malignant brain tumor in adults characterized by high invasiveness, heterogeneity and recurrence rate. GBM poor prognosis requires to target biomedical research towards the discovery of effective treatment strategies. In this scenario, 3D cultures are emerging as representative *in vitro* models to study tumor biology. In this work, we aimed to describe a robust and reproducible protocol for the generation and the maintenance of a patient-derived GBM organoid model, also defined tumoroid, that could accurately recapitulate original tumor phenotypical characteristics and marker expression. Tumoroids were responsive to temozolomide (TMZ) treatment, a chemotherapeutic drug used as standard of care therapy, proving to be suitable models to study personalized therapies. As a part of our effort, we focused on developing animal-free protocols for 2D and 3D cell cultures in order to further ensure 3R Principle in preclinical research. Given this, our model allows us to investigate pathophysiological mechanisms underlying GBM: in particular, we are focusing on the role of autophagy, a lysosomal-mediated degradation pathway regulating cellular homeostasis and associated with human cancer, in GBM. To date, autophagy involvement in GBM is still controversial but recent studies suggest an impairment of autophagy efficiency, mostly involving the autophagy initiator ULK1. We will hopefully evaluate the involvement of autophagy actors in GBM using a reliable patient-derived GBM organoid model.

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Zinc-loaded Exosome Drug Delivery System Activity on SH SY5y Cells

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Aim: It is known that exosomes, which are therapeutic agents and biomarkers of interest about diseases, serve as carrier systems for biomolecules such as DNA, RNA and proteins, contribute positively to signal transduction, cell migration, proliferation and differentiation and increase tissue regeneration. Exosomes obtained from mesenchymal stem cells found in the human umbilical cord have been observed to have a healing effect on neurological diseases; It has been observed that it reduces cerebral edema, relieves brain lesions after traumatic brain injury, reduces subsequent cell death and suppresses apoptosis, pyroptosis and ferroptosis. The purpose of this study is the combination of exosome structure with zinc, which is important for brain functions, and its use as a drug transport system. For this purpose, the activities of zinc-loaded exosomes on SHSY5y neuroblastoma cells were examined.
Methods: Exosomes were obtained from Wharton gel mesenchymal stem cells using the sucrose method. NTA, which is a gold standard in the characterization phase, was determined by the particle/ml ratio. SEM was also imaged. It has been determined that over 2 billion particles of 98-110 nm in size have been obtained with this method. Loading processes were carried out by incubating these exosomes with zinc at determined doses. Analysis of zinc-loaded exosomes was measured by both HPLC and Zeta sizer. Additionally, NTA analysis was performed after loading. The effectiveness of the exosome carrier system, whose loading potential was determined, was examined on SHSY5y neuroblastoma cells planted in 96 wells and compared with exosome alone, zinc alone and negative control with the MTT method.
Results: During the characterization phase, it was determined that more than 2 billion exosomes with dimensions of 98-110 nm were obtained by NTA. In NTA analysis after elevation, it was observed that exosome sizes increased. This shows that zinc particles were successfully loaded. Its effectiveness on neuroblastoma cells was measured by one-way ANOVA Tukey test in the results obtained by comparing it with the MTT method at the rates of 10,20,50,75,100%.
Conclusion: Disorders caused by zinc deficiency have become a public problem. There are studies arguing that this deficiency has an effect on depression, schizophrenia, stroke, seizures, neurodegenerative disorders, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and prion disease, and traumatic brain injury. Since exosomes are neuroprotective nanostructures that can pass through the blood-brain barrier, they are very effective in drug transport systems. In this study, the effectiveness of zinc-loaded exosomes, especially on cancerous neuroblastoma cells, was comparatively examined.

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The role of β-Hydroxy-β-methyl butyrate (HMB) in glioma growth suppression

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Glioblastoma (GBM) represents one of the most aggressive malignancies of the central nervous system (CNS), characterized by limited therapeutic options and high recurrence rate. One of the factors contributing to the poor prognosis of glioblastoma is its highly invasive nature, which enables glioblastoma cells to migrate and infiltrate adjacent healthy brain tissues. This diffuse infiltration complicates efforts to achieve complete surgical resection, thereby limiting the effectiveness of surgical interventions and contributing to the tumor's recurrence and resistance to treatment. This invasive behavior is driven by a complex interplay of genetic, molecular, and environmental factors that regulate cytoskeletal dynamics, cell adhesion, and extracellular matrix degradation. GBM cells secrete proteolytic enzymes, including serine proteases, cathepsins, and matrix metalloproteinases (MMPs), which degrade ECM proteins. Previous studies reveal that the gut microbiota plays a significant role in glioblastoma progression through the modulation of neurotransmitters and immune responses, and also gut microbiome can influence the growth of gliomas by altering the composition of microbes and metabolites, impacting tumor development. Previously we have shown that gut microbiota plays an important role in the gut-brain axis both in physiological and glioma conditions. Here we show that in the presence of glioma, fecal metabolite β-hydroxy-β-methylbutyrate (HMB) was significantly reduced compared to the same mice before tumor cell inoculation. Of note, HMB administration reduces tumor growth and proliferation in carcinoma cells. To understand whether HMB could be effective against glioma cells, we first demonstrated *in vitro* that HMB directly inhibits proliferation in both mouse and human glioblastoma cell lines and inhibits glioma cell basal cell migration. In *in vivo* studies, oral gavage of HMB to glioma-bearing mice resulted in a significant reduction in tumour volume compared to control mice, suggesting that this leucine metabolite, which is produced in the gut, may have an anti-tumour role in glioma.

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Selected topic: Brain metastasis

STAG2 role in brain tumors: _in vitro_ and _in vivo_ models

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Approximately 90000 people in the world are diagnosed with a primary brain tumor (pBT) each year, with a 5-year survival rate of 12% in adults. pBTs consist of a set of neoplasms differing in cellular origin, presentation, treatment, and cause; however, the genetics and predisposing factors underlying pBT are still largely unknown. The involvement of cohesin complex in brain development and its role in gene expression regulation and DNA signaling repair, makes this conserved structure an interesting candidate in pBT onset. Among cohesins, *Stromalin 2* (*STAG2*), known as onco-suppressor gene (COSMIC database), is the most frequently mutated subunit in cancers, and it is supposed to promote tumorigenesis by altering cohesin complex functions. Thus, we hypothesized a possible STAG2 involvement in pBT pathogenesis.
We studied the effect of *STAG2* deficiency in cellular models by *shRNA* infection and the results of its silencing on viability, proliferation and DNA damage, evaluated through MTT assay and specific marker expression by flowcytometry. Moreover, cell dissemination by cell spheroids, using commercially available matrices simulating cell-matrix interaction has been investigated. We then implemented 3D brain/cerebellum organoids and, by *shRNA* electroporation, we enabled the transient silencing of *STAG2*, to study the effect of its downregulation on brain and cerebellum development-related genes.
In addition, the *in vivo* fly model, obtained by silencing *Stromalin* (*SA)*, the ortholog of *STAG2*, showed increased proliferation and defective differentiation of neuroblasts, , with a lowered life expectancy and masses development within fly brains, consistent with the tumor phenotype and suggesting a cohesin involvement in malignant transformation.
These models, besides the comprehension of *STAG2* role in tumorigenesis, will represent a basis for testing possible targeted therapies, such as PARP inhibitors, recently implemented in trials for glioblastoma carrying cohesin variants and showed to be more effective in tumors with altered DNA signaling repair.

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Selected Topic: CNS primary tumours

microRNA and proteomic profiling of cerebrospinal fluid in glioma patients

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High-grade gliomas are the most aggressive brain tumors in adult, characterized by a poor prognosis and a very high mortality.
Tissue biopsy currently represents the "gold standard" material to achieve a molecular characterization of the tumor.
Recently, great attention is being addressed to liquid biopsy to reduce the use of invasive diagnostic tests.
In this perspective, the cerebrospinal fluid (CSF), which surrounds the central nervous system and is in direct contact with every possible pathological component, is considered the ideal source to investigate consistent biomarkers in brain tumors.
We performed a proteomic and miRNA analysis on CSF samples of 12 glioma patients and 12 normal pressure hydrocephalus, matched for age and sex, as non-tumoral controls.
miRNA profiling showed 12 miRNAs dysregulated in the CSF of glioma patients compared to controls, 8 of which resulted significantly downregulated in the glioma samples.
Proteomic analysis identified a total of 1859 proteins; 197 and 23 of which were upregulated or downregulated respectively in glioma compared to control samples.
Moreover, through the integration of various algorithms, a central panel comprising 11 upregulated proteins exhibited the ability to optimize the differentiation between glioma and control CSF samples.
Notably, these 11 proteins were also predicted targets of at least one of the downregulated miRNAs, suggesting a possible miRNA-protein regulatory network in glioma patients.
In conclusion, these initial findings propose that the synergistic utilization of miRNA and protein expression profiling in CSF could facilitate the discovery of a distinct signature for glioma patients, warranting further validation and investigation.

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Selected Topic: CNS primary tumours

Enriched environment cues suggest a new strategy to counteract glioma: engineered rAAV2-IL-15 microglia modulate the tumor microenvironment

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Several types of cancer grow differently depending on the environmental stimuli they receive. In glioma, exposure to an enriched environment (EE) increases the overall survival rate of tumor-bearing mice, acting on the cells that participate to define the tumor microenvironment. In particular, environmental cues increase the microglial production of interleukin (IL)-15 which promotes a pro-inflammatory (antitumor) phenotype of microglia and the cytotoxic activity of natural killer (NK) cells, counteracting glioma growth, thus representing a virtuous mechanism of interaction between NK cells and microglia. To mimic the effect of EE on glioma, we investigated the potential of creating engineered microglia as the source of IL-15 in glioma. We demonstrated that microglia modified with recombinant adeno-associated virus serotype 2 (rAAV2) carrying IL-15 (rAAV2-IL- 15), to force the production of IL-15, are able to increase the NK cells viability in coculture. Furthermore, the intranasal delivery of rAAV2-IL-15 microglia triggered the interplay with NK cells *in vivo*, enhancing NK cell recruitment and pro-inflammatory microglial phenotype in tumor mass of glioma-bearing mice, and ultimately counteracted tumor growth. This approach has a high potential for clinical translatability, highlighting the therapeutic efficacy of forced IL-15 production in microglia: the delivery of engineered rAAV2-IL-15 microglia to boost the immune response paves the way to design a new perspective therapy for glioma patients.

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Selected Topic: Glial cells in cancer

A new pericyte face: from the neurovascular unit to tumor reprogramming

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The microvasculature of the central nervous system (CNS) has the higher endothelial cell (EC)-pericyte ratio, due to the presence of blood brain barrier (BBB).
This is the reason why the physiology of pericytes, mural cells enwrapping ECs, is mostly described in tissues belonging to the CNS. Emerging roles of pericytes in cancer progression have increased the attention on these cells in the recent years.
Using an immortalized cell line of brain pericytes, we explored the role of the cytokine nicotinamide phosphoribosyltransferase (eNAMPT), demonstrating its abilities as a chemotactic and pro-angiogenic agent for pericytes. NAMPT is a pleiotropic protein existing as an intracellular and a released form. The extracellular form has cytokine-like properties, and it is secreted by different cell types, including cancer cells. Although eNAMPT is a pro-angiogenic factor for ECs, the link between eNAMPT and tumour angiogenesis is wanting, and no data are available on pericytes.
Interestingly, RNA sequencing on eNAMPT-treated pericytes reveals an activation of these cells toward a pro-inflammatory phenotype via NF-kB pathway.
Given our evidence on the role of eNAMPT on these cells we took advantage of a murine model of mammary carcinoma showing that a tumor microenvironment (TME)-enriched in eNAMPT exhibits a massive vascularization mainly promoting pericyte recruitment and facilitates metastasis in lungs, liver and brain. Specifically, the eNAMPT-mediated vessels were leaky and permeable, sustaining a favourable setting for tumor cell intravasation. Nonetheless, western blot analysis of tumoral tissues revealed the presence of the glial fibrillary acidic protein (GFAP) modulated by the eNAMPT-enriched TME, suggesting a strong remodelling of tumor stroma.
Moreover, using an *in vitro* model of BBB we showed an increased permeability mediated by eNAMPT.
In conclusion, eNAMPT-mediated tumoral vessels are abundant and leaky despite the higher pericyte coverage sustaining the ability of mammary carcinoma cells to invade new tissues, including brain.

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Selected Topic: Neuroimmune axis in cancer and cancer microenvironment

Development of new antibodies against glioblastoma

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Glioblastoma (GBM) is the most diffuse and aggressive neoplasm of the nervous system. It is characterized by aggressive growth and high rates of recurrence. Despite the advancements in conventional therapies, the prognosis for GBM patients remains poor. ErbB receptor tyrosine kinases are involved in several cellular processes, such as proliferation, differentiation, cell survival, migration, and invasion. Among them, ErbB3 is overexpressed in GBM tissue and, after binding with its specific ligand, Neuregulin-1 (NRG1), phosphatidylinositol 3-kinase (PI3K)/AKT pathway is activated. That's why a promising new GBM therapeutic approach has considered ErbB3 as a target. In fact, recent studies show how the use of anti-ErbB3 monoclonal antibodies can have positive effects in fighting tumors. There is a consideration to be made regarding the complicated physiological characteristics of intracranial tumors. These include the presence of the blood-brain barrier (BBB), which leads to insufficient penetration of therapies. For these reasons the aim of this project is to evaluate the effective BBB crossing of new anti-ErbB3 monoclonal antibodies, using different approaches. First, we use an *in vitro* model of the BBB composed of murine bEnd.3 endothelial cells and primary murine astrocytes. This co-culture system creates a barrier that reaches a trans-endothelial electrical resistance (TEER) measured in the absence or presence of murine GBM cells GL261. For *in vivo* experiments we will test the best administration route of new anti-ErbB3 monoclonal antibodies to reach the brain in GBM-bearing, by labeling and visualizing anti-ErbB3 and by evaluating its efficacy on tumor volume and mice survival.

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Selected Topic: BBB and cancer

Defining the role of SEMA6A as a vascular barrier modulator in glioblastoma multiforme

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The blood-brain barrier (BBB) is a key structure that regulates substance influx and efflux to maintain a homeostatic environment for several brain functions. BBB dysregulation is a hallmark of brain disorders, including glioblastoma multiforme (GBM), the most frequent and aggressive adult brain tumor. BBB disruption in GBM is heterogeneous, occurring mainly in tumor core and correlating with higher grade of malignancy. Thus, a better comprehension of mechanisms underlying BBB dysregulation in GBM-bearing brain is pivotal to provide insights into GBM progression.
In our recently published work, we found that semaphorin 6A (SEMA6A) is a potent regulator of BBB permeability both *in vivo* and *in vitro*. Further, SEMA6A inhibition has been associated to decreased glioma cell proliferation and tumorigenicity. Based on these premises, we hypothesize that SEMA6A could be a key factor implicated in the regulation of BBB within tumor mass.
Here, we showed that SEMA6A is expressed by GBM neoplastic cells, with higher levels in GBM stem-like subpopulations located in tumor core, by mining publicly available transcriptomic dataset at single cell level from mouse and human brains bearing GBM.
Then, leveraging patient-derived GBM cell lines, we confirmed SEMA6A expression by GBM cells at both transcript and protein levels, particularly by cells belonging to the most aggressive proneural GBM subtype.
Finally, we showed that GBM-derived SEMA6A can increase BBB permeability by measuring trans-endothelial electric resistance across HUVEC monolayer, a simplified *in vitro* model of BBB, upon exposure to conditioned media obtained from GBM or control cells.
Overall, our data will highlight for the first time the effects of GBM-derived SEMA6A on vascular permeability. This knowledge will help to better understand the role of SEMA6A as well as BBB dysregulation on GBM progression, thus providing, in the long-run, possible new pharmacological targets for GBM treatment.

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Exploring the role of Rhotekin in cortical development and glioblastoma susceptibility

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Glioblastoma (GBM), a grade IV glioma, is the most common and aggressive malignant brain tumor in adults. GBM is thought to result from the accumulation of somatic mutations in neural stem cells (NSCs) and glial precursor cells, which confer selective growth advantages and lead to uncontrolled proliferation. GBM treatment has had very limited success due to the complex biology, highlighting the need for a better understanding of pathogenetic mechanisms to develop new therapeutic approaches.
Rhotekin (RTKN), a downstream effector of Rho GTPases, is essential for the survival of neurons by regulating NSCs differentiation. RTKN is overexpressed in various types of cancer, including gliomas. However, only one study has investigated RTKN in brain tumors, finding that aberrant RTKN localization correlated with impaired motility of GBM cells.
Herein, we found that RTKN is expressed in radial glial cells (RGCs) of ventricular and subventricular zones of the mouse neocortex. Using an acute knock-down strategy on mouse embryos, we observed that *Rtkn*-knocked down cells accumulated in lower layers, suggesting that RTKN could alter cortical migration.
Further experiments in a mouse model of immature neurons revealed that RTKN modulation induces morphological changes such as reduced cell complexity upon RTKN deletion. Additionally, RTKN appears to regulate cytoskeletal dynamics by promoting actin polymerization and stabilization, as shown by pCofilin/Cofilin ratio in depleted immature neurons.
Analyzing a mouse GBM single-cell transcriptome, we found *Rtkn* expression in GBM and ependymal cells derived from RGCs. Notably, *Rtkn* is specifically expressed in late-stage tumor cells within the stem-like GBM cell group, suggesting a potential positive selection of RTKN expression in glioma progression.
This evidence suggests that RTKN might play a crucial role during cortical development and might represent a novel potential gene to be investigated during glioma progression.

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Selected Topic: neurodevelopment disorders and cancer susceptibility

Tumor-Associated Macrophages (TAM): a novel therapeutic strategy for spinal cord injury

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Spinal cord injury (SCI) is a severe condition resulting from physical trauma leading to the permanent loss of sensory and motor functions. Following SCI, the injury site undergoes neuron and oligodendrocyte loss, along with hemorrhage that disrupts the supply of oxygen and nutrients. Additionally, SCI microenvironment is characterized by extensive inflammation, resulting in cyst and scar formation which interrupt signal transmission. Despite significant medical advances, aimed at minimizing spinal cord damage and enhancing spared neural connection functionality, currently, no effective therapy for SCI exists.
Interestingly, SCI hostile microenvironment shares similarities with tumors, including hypoxia, reduced vascularization, immune cell infiltration, and increased extracellular matrix deposition. In tumors, Tumor-Associated Macrophages (TAMs) play a crucial role in promoting growth, progression, and metastasis releasing pro-angiogenic signals, remodeling the extracellular matrix, and suppressing the immune response.
These properties suggest a regenerative potential of TAMs applicable to the SCI hostile microenvironment. To this aim, we performed severe contusive SCI at T11 vertebra level on C57BL/6 male mice followed by repeated TAMs/Vehicle intraparenchymal administrations. At 3-, 10- and 17-days post injury injured mice received $2*10^6$ TAMs or saline solution close to the lesion site. At the end of the experiment, animals were sacrificed and perfused for further analysis. We found that TAM treatment significantly improved motor functionality, neural survival, axonal regeneration, cyst remodeling, immunomodulation and vascularization.
In conclusion, our findings indicate that TAMs exhibit neuro-regenerative properties and therapeutic potential for the treatment of CNS injuries including SCI.

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Deciphering Schwann cell-tumor cell crosstalk in hepatocellular carcinoma

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In the last few decades, numerous studies have demonstrated the multifaceted role of the peripheral nervous system (PNS) during the development, expansion, and invasion of solid tumors [1]. Schwann cells (SC), which constitute the main glial component of peripheral nerves, make a significant contribution to the progression of various types of cancer, through a direct interplay with cancer cells and by actively regulating the tumor microenvironment (TME) [2]. Hepatocellular carcinoma (HCC) is the most common primary liver tumor and one of the leading causes of cancer-related death worldwide [3]. Several studies have highlighted the potential implication of autonomic innervation in HCC onset and progression [4], but the contribution of SC has not yet been documented. Therefore, this study aims to characterize SC distribution and degree of activation in the TME of HCC, and to investigate the molecular mechanisms and biological effects involved in SC-HCC cells crosstalk. Using an immunohistochemical approach on human healthy liver and HCC tissue sections, we observed a considerable presence of SC in the tumor capsule and infiltrating cancerous parenchyma. Moreover, *in vitro* experiments revealed that paracrine signals released by SC promote the aggressive transformation of HCC cells. Indeed, when treated with SC-conditioned medium, the human hepatoma Hep3B cell line underwent epithelial-mesenchymal transition and showed stronger migratory abilities and morphological alterations. 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.

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Selected Topic: Glial cells in cancer

The role of hydrogen sulfide on glioblastoma growth: a gut-brain approach

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Glioblastoma multiforme (GBM) is the most common and deadly malignant brain tumor, with a low life expectancy and poor efficacy of standard therapies, often leading to relapse. However, it is already known that lifestyle changes, such as restricted diets and fasting, have an impact on GBM growth. Less clear is the effect of red meat intake on the tumor. One observational study found that people who consume a diet high in animal protein and fat show changes in the microbiota composition, particularly an increase in common hydrogen sulfide (H_2S) producing bacteria. This metabolite appears to counteract GBM growth. Recent studies highlight the role of the gut-brain axis in altering the GBM microenvironment and growth. Our aims are to investigate: 1) the effect of a standard animal-protein diet on tumor growth; 2) diet-induced gut microbiota modification; 3) the possible involvement of H_2S in tumor progress. To assess such hypothesis, we fed mice isocaloric diets with standard content of proteins derived from red meat (protein diets) or animal-derived proteins (control) and we orally administered amino-oxyacetic acid (AOAA), an inhibitor of H_2S biosynthesis. After two weeks on the diets, we orthotopically injected murine glioma cells (GL261). Three weeks after the injection, we collected stool for each group to assess H_2S concentration and brain for tumor volume. Our results show an increased concentration of H_2S in the faeces and a decreased tumor volume in mice fed the animal-protein diet compared to controls, this effect disappeared in mice treated with AOAA. These results are consistent with our *in vitro* data showing a reduction in GL261 viability following treatment with sodium hydrosulfide (NaHS), an H_2S donor. These results suggest that a standard red meat intake may have an antitumor effect on GBM compared to a standard animal-derived protein diet and that this effect is H_2S-dependent.

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Bioinformatic analysis identifies a novel neurogenic signature of Tumor-Associated Macrophages

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Nerve growth within tumors influences cancer development and progression. Likewise, many cancers promote nerve infiltration that enhances tumor growth. Tumor-Associated Macrophages (TAMs) promote cancer growth and are endowed with pro-tumoral functions, including angiogenesis, extracellular matrix remodeling, and immunomodulation. However, the role of TAMs in nerve growth is still unexplored. In this work, we define a novel neurogenic signature of TAMs, and we validate their direct role in promoting neural growth. We first performed single-cell RNA sequencing (scRNA) analysis of human samples from astrocytoma, glioblastoma, and healthy brain tissues. We identified seven different clusters, including TAMs, microglia, NK cells, endothelial cells, and T lymphocytes. TAM cluster was characterized by the expression of the TAM hallmarks like MIF, EEF1A1, RPL39, LDHA, PLTP, and ADAM8 and was clearly separated from the microglia clusters identified by TMEM119. We found that enrichment of the 694 DEGs of TAMs showed an upregulation of GOs related to neurogenesis, axonal growth, and neural regeneration compared to the other no-myeloid clusters. We defined the neurogenic signature of TAM by the 154 DEGs upregulated in the selected neurogenic GOs. We then validated TAM neurogenic signature on different datasets of bulk-RNA sequencing isolated from human breast and endometrial cancer and mouse glioblastoma. In all the datasets tested, TAMs significantly upregulated the neurogenic signature compared to M0 or M2 macrophages. To assess the capability of TAMs to promote neural growth, we cocultured TAMs with human motor neurons derived from pluripotent stem cells, mouse neural stem cells, and dorsal root ganglions. We evaluated the expression of mature neuronal marker β3-Tubulin with or without TAM presence. The co-culture with TAMs increased the expression of β3-Tubulin compared to controls in all the tested *in vitro* models. Overall results suggested that TAMs, compared to other macrophages, showed a unique neuro-regenerative signature and promoted neuronal differentiation.

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Selected Topic: Neurogenesis and axonogenesis in cancer

Effects mediated by β-Arrestin1 activation by M2 muscarinic receptor in human Glioblastoma cells

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Glioblastoma (GB) is the most aggressive primary brain tumor in humans, characterized by a strong drug resistance and recurrence. The identification of a new therapeutic target for GB may be of great clinical relevance. Our previous data demonstrated that the activation of M2 receptor (M2R) by orthosteric agonist Arecaidine-Propargyl-Ester (APE) and dualsteric agonist N8-Iper (N8) decreased cell proliferation, survival and migration in GB cells. The interaction among M2R and β-Arrestin1 was previously described by Lefkowitz's group. Albeit canonical interaction between β-Arr1 with GPCR promotes receptor desensitization and internalization, however, recently new roles for β-Arr1 are emerging. In the present work we investigated the involvement of β-Arrestin1 in mediating the effects of M2R upon M2 agonist activation.
By cell transfection of M2-Flag and β-Arr1-EGFP costructs, we observed that after activation of the receptor by both selective agonists, the β-Arr1 appears expressed in the cytoplasm and plasma-membrane upon acute treatment and present in vescicles in colocalization with M2R, upon chronic treatment. These first results confirm the canonical role of β-Arr1 in M2R internalization. The effects on M2 receptor internalization appears more evident at high concentration of the agonsits and at chonic time of treatment. To evaluate whether the other effects M2-mediated was dependent on β-Arr1, we produced U251 stable cell line in which the β-Arr1 was silenced by shRNA. A faint change in cell morphology and a slowdown of cell proliferation rate was observed in β-Arr1 silenced cells compared with scramble cell line and untrasfected cells. Moreover in β-Arr1 silenced cells, the stimulation of M2 receptor by the two agonists did not cause the same effects observed in untransfected cells both in terms of cell proliferation and migration, confirming the strategic role of β-Arr1 as modulator of signaling transduction pathways downstream M2Rs activation.

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Role of ER-mitochondria interaction in the mechanism of chemotherapy-induced peripheral neuropathy

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Chemotherapy (CT)-induced peripheral neuropathy (CIPN), causing painful paresthesia and numbness, is a common adverse effect of the treatment with different classes of chemotherapeutic agents, known to exert neurotoxic effects over sensory neurons and glial cells. Molecular mechanisms of CIPN remain poorly understood. Noteworthy, mitochondria have been reported as common targets of several CT-based regimens, both in terms of their morphology/motility and of their specific functionality, which is of paramount importance for the maintenance of cellular homeostasis. In the context of mitochondrial function, growing body of evidence highlights the relevance of mitochondria-ER contact sites (MERCS), highly-organized morpho-functional units involved in response to stress, calcium homeostasis, bioenergetics, autophagy, protein homeostasis and apoptosis. We used cellular models of Schwann cells (MSC80) and sensory neurons (F11) to investigate if mitochondrial dysfunction in CIPN induced by bortezomib (BTZ), a 1st generation proteasome inhibitor approved as first-line therapy for multiple myeloma, could be linked to alterations of MERCS. To assess MERCS alterations, we exploited state-of-the-art split-GFP contact site sensors (SPLICS). Intracellular Ca^{2+} handling and mitochondrial membrane potential ($\Delta\Psi m$) were also assessed, using both dedicated dyes and genetically-encoded probes. We found that BTZ induced profound changes in mitochondrial network, which was more prominent in MSC80 Schwann cells. Strikingly, BTZ also drastically reduced the amount ER-mitochondria contact sites. In MSC80 Schwann cells, these alterations were accompanied by a strong BTZ-induced depolarization of the mitochondrial membrane. Of note, this depolarization, as well as mitochondrial morphology, was partially reverted when the cells were co-treated with isoallopregnanolone and allopregnanolone in addition to BTZ. These compounds belong to the class of neuroactive steroids, that, intriguingly, have already been reported to exert neuroprotective effects over peripheral nerves in different experimental models of peripheral neuropathy, including docetaxel-induced peripheral neuropathy.

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Selected Topic: Cancer and Pain

Interactions between cancer and nervous system in a mouse model of breast cancer

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The interaction between the central and peripheral nervous system and tumor microenvironment (TME) has recently garnered interest. Tumor innervation is a relevant clinical parameter in cancer development and prognosis, with higher nerve density correlated with worst prognosis. Neural invasion and proliferation enable tumor growth through a self-sustaining cycle; cancer cells interact with neurons promoting neurites growth, and nerves sustain and spread cancer cells, facilitating metastatization.
In the context of breast cancer, the development of brain metastases occurs in a high percentage of patients, with a significant impact in terms of survival and quality of life. Breast cancer is highly innervated, and the type of innervation can be a significant indicator for tumor progression: sympathetic innervation positively regulates cancer growth and spread, as well as sensory innervation; on the contrary, the presence of parasympathetic nerves is related to a reduced tumor development.
Based on these premises, our aim is to understand the crosstalk between TME innervation and the formation of brain metastases. The first step of our study was the histological characterization of tumor innervation in a syngeneic orthotopic mammary carcinoma mouse model, BALB/C mice injected with 4T1 cells. In the primary tumor we observed the presence of innervation markers such as the pan-neuronal marker PGP9.5 and neurofilament heavy (NF-200). Furthermore, in areas of the tumor surrounded by adipocytes NF-200 colocalized with tyrosine hydroxylase expression, suggestive of sympathetic innervation. At present, studies are ongoing in the primary tumor to characterize the different nerve fibers within the TME, their origin (PNS/CNS), and the signalling pathways. In parallel, the crosstalk between cancer and neural cells, including neural stem/progenitor cells, in breast cancer brain metastasis is also under investigation. A better understanding of breast cancer innervation, and how nerves in TME and the CNS/PNS dialogue can be a promising strategy to expand the repertoire of cancer therapies.

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Modulating the Gut-Brain Axis: The Impact of Fecal Material Transplantation on Glioblastoma

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Glioblastoma multiforme (GBM) has been identified as the most lethal type of brain tumor, characterized by a low lifespan of 12.1 to 14.6 months after diagnosis, and a 5-year survival rate as low as 5%. It has been observed that glioblastoma bearing patients often host gut microbiota of different richness and diversity compared with healthy individuals. Furthermore, the important role of gut-brain axis in shaping the glioblastoma development has made it a popular target to modulate. Among several approaches, fecal material transplantation (FMT) is one of the most direct ways to modulate the gut microbiota composition. FMT is a procedure of transplanting fecal material from a healthy donor to a diseased individual, after being pre-treated with broad spectrum antibiotics (ABX) to ensure that new microbiota get established. To assess if FMT from healthy mice to glioblastoma bearing mice can eventually influence the tumor development, we treated mice orthotopically injected with murine glioma cells GL261 with terminal stool of healthy age-matched mice or vehicle through oral gavage. Treatments were performed three times a week for two weeks in total with or without ABX pre-treatment. Stool collections were performed along the entire experimental plan to perform metagenomic analysis on gut microbiota. Preliminary data showed that FMT treatment reduced tumor growth with respect to the mice receiving only PBS, particularly in the presence of ABX pre-treatment. In these same mice the $CD8^+$ T cells were significantly higher in the circulation than the other groups and circulating NK cells, showed higher cytotoxic activity compared to controls. These preliminary results suggested that a higher immune activation was possibly induced in mice treated with healthy FMT previously depleted with ABX.

Towards liquid biopsy for glioblastoma: relying on plasma extracellular vesicles for tumor diagnosis and monitoring and biomarker identification

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Glioblastoma (GBM) is the most aggressive adult brain tumor. Current diagnostic methods lack sensitivity and specificity, and the invasiveness of tissue biopsy and the lack of reliable GBM biomarkers hamper its prompt diagnosis and monitoring. Our objective is to identify GBM biomarkers to assess via liquid biopsy. GBM sheds Extracellular Vesicles (EVs) that cross the blood brain barrier and shuttle tumor-derived information. The stability and complex molecular cargo of EVs stamps them as potential GBM biomarkers.

We isolated EVs by Size Exclusion Chromatography (SEC) from 2mL of plasma, and measured their concentration and size by Tunable Resistive Pulse Sensing (TRPS). EVs are more abundant and larger in primary GBM plasma with respect to healthy subjects and patients with brain malignancies that mimic GBM at neuroimaging. EVs abundance and diameter drop 72h post-surgery, confirming that EVs are indicative of tumor presence.

We extract EV-DNA and analyze it by droplet-digital PCR (ddPCR), but did not retrieve parental alterations, due to its scarcity and to the dilution of tumor-EVs in plasma. Indeed, multiplex flow cytometry revealed similar expression profiles of a panel of surface proteins on EVs from GBM and controls, which confirms the prevalence of non-tumor EVs in circulation. Still, we found the EV markers CD63 and CD81 and the T-cell markers CD8 and HLA-DRDPDQ enriched in GBM.

Profiling the EV proteome by mass spectrometry in healthy individuals, and in matched pre- and 72h post-operative GBM, we identified 73 proteins upregulated in pre-operative GBM samples, including proteins associated with GBM pathology and tumor microenvironment.

These results benchmark the significance of assessing EVs to enhance diagnostic accuracy and allow longitudinal monitoring of GBM. Repeated profiling of the EV proteome could guide personalized treatment choices based on the molecular evolution of the tumor.

The identification of GBM EV surface protein biomarkers will permit the isolation of tumor-EVs to implement the analysis of EV-DNA for non-invasive tumor profiling.

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Examining the Effectiveness of Neuroblastoma Exosomes on DNA Damage in 2D and 3D Neural Stem Cell Model

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Aim: According to the stem cell hypothesis, cells are under the influence of the microenvironment. The maintenance and differentiation of these cells is unthinkable without the presence of this microenvironment. Cells carry necessary information to other cells through nanocargo-like structures called exosomes. Cancer cells also spread exosomes, which specifically support cancer transformation and metastasis, into their niche. The aim of this study is to examine the cancerization potential of exosomes obtained from SH-SY5Y neuroblastoma childhood cancer cells on both 2D and 3D neuronal stem cells.
Methods: After collecting approximately 200 ml supernatants of SH-SY5Y neuroblastoma cells, exosomes were obtained by ultracentrifuge method. The size and quantity of the obtained exosomes were determined and compared by measuring them with NTA and Zeta sizer. It was measured that approximately 10^9/ml particles, especially those of 100 nm and around, were collected. The IC50 value of the determined amounts of exosomes on L929 cells was measured by the MTT method. Then, the selected dose particle/ml exosome ratio was examined both on 2D neural stem cell IPSCs and in the 3D hydrogel neurosphere niches created from the same IPSCs. The cells were followed for approximately the 7th day and time-lapse imaging was performed. After the 7th day, DNA damage in the 2B and 3B groups, to which cancer exosome was applied, was measured by the comet method.
Results: In this research, genotoxic efect of cancer exosomes on neural IPSCs were evaluated with comet assay. The amount of DNA breaks was measured under a fluorescent microscope. It was determined DNA damage as percentage of tail intensity. It has been observed that the activities of cancer exosomes cause different extents of damage to neural IPSCs in both 2D and 3D environments.
Conclusion: This highlights the importance of the cancer niche and exosomes of cancer cells in cancer development. In addition, exosomes in the niche can be considered as precursors of cancer stem cell formation.

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