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A role for metformin in the treatment of Dupuytren disease?

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ABSTRACT

Dupuytren disease (DD) is a hand-localized fibrotic disorder characterized by a scar-like, collagen-rich cord. Treatment usually comprises surgical removal of the cord, but is associated with a high relapse rate, in some cases requiring finger amputation. There is currently no consensual medical approach for treating DD. Numerous preclinical studies have highlighted antifibrotic properties of metformin, and the aim of this study was to assess a potential antifibrotic role of metformin in DD. Fibroblasts from DD cords (DF) and phenotypically normal palmar fascia (PF) were extracted from surgical specimens and cultured. The fibrotic status of DF and PF was compared at baseline, and under profibrotic (TGF-β stimulation) and antifibrotic (metformin stimulation) conditions, using quantitative RT-PCR, western blot, immunocytochemistry, and a functional fibroblast contraction assay. At baseline, DF showed higher levels of fibrotic markers and contraction capacity compared with PF. Both types of fibroblasts responded to TGF-β stimulation. Treatment of DF and PF with metformin did not affect basal levels of fibrotic markers and contraction but largely prevented their induction by TGF-β. In conclusion, our data show that metformin inhibits TGF-β-induced expression of fibrotic markers and contraction in hand-derived fibroblasts. This supports the case for a clinical trial to assess the repurposing of metformin as an adjuvant to surgery, to prevent, reduce, or delay recurrence in at-risk DD patients.

1. Introduction

Dupuytren disease (DD) is characterized by a progressive fibrosis of the palmar aponeurosis, caused by excess production and deposition of extracellular matrix proteins [1]. Phenotypically, DD presents as nodules and cords within the palmar aponeurosis that induce a flexion contracture of the metacarpophalangeal and interphalangeal joints. DD predominantly affects men of Northern European descent [2,3]. The prevalence of DD varies according to demographic, genetic, and medical risk factors, and has been estimated in the range of 10% in Northwestern Europe [4,5]. Treatments for DD include percutaneous fibrinolysis [1,6,7], but open surgical procedures are the most commonly used in clinical practice [8]. Although surgical management of DD is safe and effective, the recurrence rate is high, reported as ranging from 20% to 80% [9,10]. Unfortunately, morbidity associated with DD recurrence is high, with more than 40 DD-associated finger amputations per year reported in France [5]. In this context, there is a need for a treatment, adjuvant to the surgical procedure, that can help to reduce the DD recurrence rate.

At the cellular level, DD is a fibroproliferative disorder characterized by fibroblast differentiation and proliferation, leading to excessive secretion of extracellular matrix proteins and to the development of a scar-like, collagen-rich cord that affects the palmar fascia of the hand [11–13]. Activated profibrotic fibroblasts (myofibroblasts) are characterized by the cytosolic expression of the alpha-smooth muscle actin protein (α-SMA), encoded by the ACTA2 gene. Exposure of myofibroblasts to stress leads to the incorporation of α-SMA protein into stress fibers [14]. Many well-known factors have been reported to trigger myofibroblast differentiation, of which TGF-β is the most studied. In DD, the expression level of TGF-β and associated signaling molecules is high [15], and palmar cord DD fibroblasts (DF) have been reported to

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proliferate in response to TGF-β stimulation [16]. Fibroblasts derived from the transverse carpal ligament of patients unaffected by DD, as well as DD myofibroblasts, exhibited increased isometric contraction of collagen lattices when exposed to TGF-β [17]. TNF-α has also been shown to promote myofibroblast differentiation [18], although this effect was only observed at a 0.1 ng/mL test concentration, while lower and higher concentrations were ineffective.

Metformin is a first-line treatment for type II diabetes. Prescribed since 1957, this is a very well tolerated drug with few side-effects. Metformin exhibits pleiotropic cellular effects, including antifibrotic properties, in preclinical models of different types of fibrosis. It has been shown to reduce TGF-β-induced extracellular matrix protein production in human fibroblasts derived from nasal polyps [19]. Furthermore, in mouse models metformin decreases bleomycin- and gefitinib-induced lung fibrosis through regulation of TGF-β signaling [20–22]. Antifibrotic effects have also been demonstrated in other animal models of fibrosis [23,24]. Pharmacological action of metformin is mediated via the phosphorylation of AMP-activated protein kinase (AMPK) [25]. AMPK functions to regulate intracellular energy balance via lipid and glucose metabolism, and also a wide array of cell functions [26]. AMPK activation by metformin was shown to be responsible for inhibiting TGF-β-induced collagen production in mouse renal fibroblasts [27]. In the light of these preclinical data, we hypothesized that metformin may have a role to play in DD treatment, either as a first-line therapy to prevent surgery or as an adjuvant treatment immediately after surgery to prevent or reduce DD recurrence in at-risk patients.

2. Methods

2.1. Human tissues

DD cords and phenotypically normal palmar fascia tissue samples from patients undergoing carpal tunnel surgery were collected prospectively from April 2015 to April 2019. A written, informed consent form was obtained for all included patients. Permission for collection, storage and experimental manipulation of tissues was granted by the Direction Générale de la Recherche et l'Innovation (ID no.: DC-2015-2345).

2.2. Cell culture

Cells were extracted from surgical specimens using modifications of a previously published protocols [28,29]. Briefly, tissue samples were cut into small pieces and digested in DMEM (Gibco) containing 1% penicillin-streptomycin (Gibco), 10% (vol/vol) fetal bovine serum (FBS) (Gibco), and 5 mg/mL type I collagenase + DNase I (Roche Diagnostic), for up to 2 h at 37 °C. After filtration, cells were cultured in DMEM containing 10% (vol/vol) FBS and 1% penicillin-streptomycin. Cells up to passage 4 were used for experiments. TGF-β, metformin, and 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) were purchased from Peprotek, Sigma Aldrich, and Biotrend, respectively.

2.3. Western blotting and immunocytochemistry

Western blotting and immunocytochemistry were performed as previously described [30,31], respectively. F-actin was stained with Alexa Fluor 488 phalloidin (Fisher Scientific) or phalloidin-iFluor 594 (Abcam) reagent. Anti-FN1 and anti-HSP90 antibodies were purchased from Santa Cruz, and anti-α-SMA was purchased from Abcam.

2.4. RNA isolation, reverse transcription, and quantitative PCR

Total RNA, reverse transcription, and quantitative PCR were performed as previously described [31]. The primers used were: ACTA2: forward 5'-CTGTTCCAGCCATCCTTCAT-3'; reverse 5'-TCATGATGCTGTTGTAGGTGGT-3'; RPL32: forward 5'-CCTTGTGAAGCCCAAGATCG-3'; reverse 5'-TGCCGGATGAACTTCTTGGT-3'. Expression levels were evaluated using the comparative CT method. For normalization, transcripts of RPL32 were used as an endogenous control for gene expression.

2.5. Traction force microscopy

Cell contraction was assessed by traction force microscopy as previously described [32], using collagen-coated polyacrylamide hydrogels with a shear modulus of 4 kPa coated with red fluorescent beads (Soft-Trac; Cell Guidance Systems).

2.6. Statistics

Statistical analysis was performed using GraphPad Prism. An unpaired, two-tailed Student's t-test or unpaired two-tailed Mann-Whitney test was used for statistical comparison between two groups. For comparisons between multiple groups, one-way ANOVA followed by Bonferroni's post-hoc tests was used.

3. Results

3.1. Palmar and Dupuytren fibroblasts are phenotypically different at baseline

To compare normal palmar fibroblasts with Dupuytren fibroblasts (PF and DF, respectively), the level of expression of the gene encoding α-SMA, ACTA2, was measured by qRT-PCR. The relative expression level of α-SMA in PF was set to a value of one. In DF, this value was significantly higher (Fig. 1A), indicating a profibrotic phenotype of DF. This increase at the mRNA level was also observed at the protein level (Fig. 1B). Western blot analysis also showed that the expression of another fibrotic marker, fibronectin (FN1), was lower in PF than in DF. The expression of both α-SMA and FN1 was induced by TGF-β, and this induction was quantitatively greater in PF than in DF (Fig. 1B).

We next assessed the contractile properties of PF and DF (Fig. 2). The basal contractile activity of PF was systematically lower than that of DF. Stimulation of PF with TGF-β resulted in a large increase in contraction, while the same stimulation resulted in a statistically significant but modest increase in contraction in DF. Overall, these results confirm the fibrotic nature of DF.

3.2. Effect of metformin on palmar and Dupuytren fibroblasts

We next evaluated whether metformin could reduce ACTA2 expression. In basal conditions (Fig. 3A and B), metformin had little effect on ACTA2 expression in both PF and DF, and the AMPK activator, AICAR, produced similar effects. Stimulation of both PF and DF with TGF-β resulted in a dramatic increase in ACTA2 expression (varying from a 5- to 50-fold increase, depending on the patient-source of the sample tested). The magnitude of this induction was substantially reduced when cells were exposed concomitantly to TGF-β and metformin (Fig. 3 C and D). A similar reduction was observed when cells were exposed concomitantly to TGF-β and AICAR, suggesting that the effect of metformin was mediated by activation of AMPK. Western blot analysis showed that exposure of cells to either metformin or AICAR reduced the TGF-β-induced increase in α-SMA and FN1 levels, but did not alter α-SMA and FN1 protein levels in basal conditions, in both PF and DF (Fig. 4). Immunofluorescence analysis of α-SMA expression in PF (Fig. 5A) and DF (Fig. 5B) confirmed these observations.

Finally, we analyzed the contractile properties of PF and DF treated with metformin or AICAR. In basal conditions, metformin or AICAR did not consistently affect contraction in PF (Fig. 6A). Stimulation of PF with TGF-β resulted in marked increases in contraction, which were largely abolished by metformin or AICAR treatment. A similar phenomenon was observed with DF (Fig. 6B).

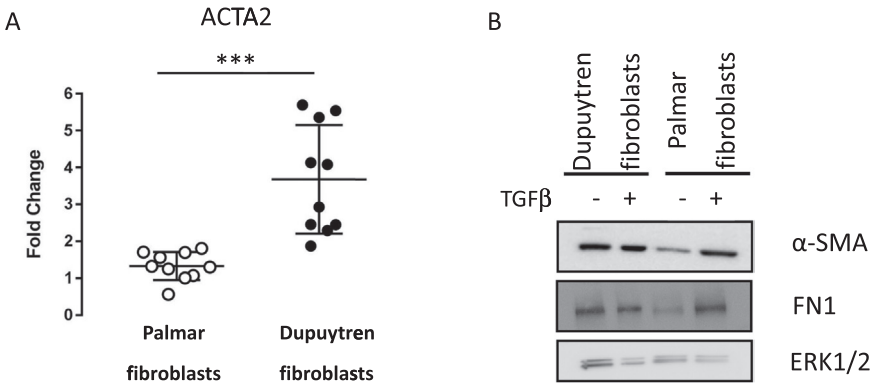

Fig. 1. Expression of fibrotic markers in palmar (PF) or Dupuytren (DF) fibroblasts. PF and DF were prepared and cultured, total RNA was prepared and extracted, and ACTA2 levels were determined by qRT-PCR (A). Each point corresponds to an individual tissue donor. The data present the relative expression of ACTA2 normalized to the expression of RPL32. ***: $p < 0.001$. Western blot analyses to detect α-SMA and FN1 (B) were performed on proteins extracted from PF and DF, treated or not with 1 ng/mL TGF-β. The blots presented are representative of two independent experiments performed on samples from different patients.

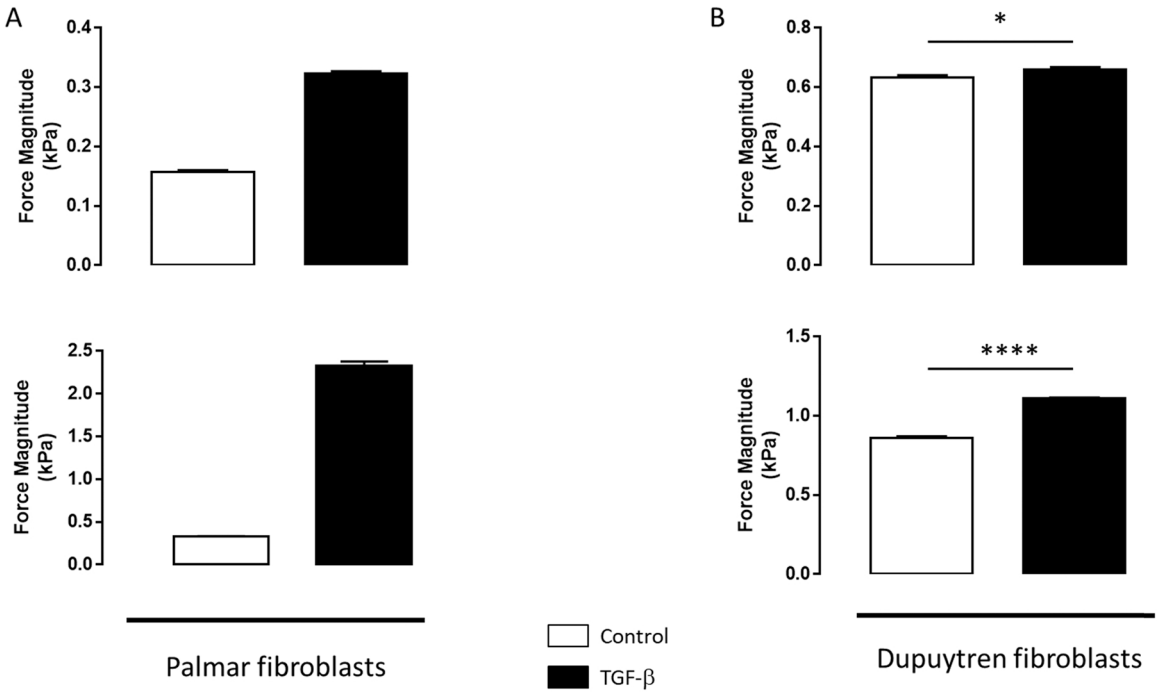

Fig. 2. Contractile properties of PF and DF, stimulated or not with TGF-β. PF (A) or DF (B) were cultured in the absence (white bars) or presence (black bars) of TGF-β (1 ng/mL) and contraction was assessed by traction force microscopy, using collagen-coated polyacrylamide hydrogels with shear modulus of 4 kPa coated with red fluorescent beads. The results presented are the mean contractions ± SEM of two PF and two DF obtained from different tissue donors. *: $p < 0.05$; ****: $p < 0.0001$.

4. Discussion

In recent years, new, nonsurgical experimental treatments for DD have emerged, and have been or are currently being assessed. For example, a procedure comprising local injection of *Clostridium histolytum* and manipulation of the digit has been tested on a large number of patients and was approved for use by the FDA in 2010 [33]. A limited level of efficacy of this procedure has been demonstrated [34].

Surgical management remains the mainstream treatment for DD. The main problem encountered is the high rate of recurrence after surgery, which has been reported to be around 20% [10], but could be as high as 80% [9]. Reducing this recurrence rate would likely lead to a significant improvement in life quality of DD patients.

The data presented in this report confirm the already-reported fibrotic nature of DF. High levels of expression of the ACTA2 gene, and of its encoded protein, α-SMA, are detected in DF, as compared with PF. FN1 is also more highly expressed in DF than in PF (Fig. 1). Stimulation with TGF-β led to a marked increase in these fibrotic markers in PF, but this increase was much lower in DF, suggesting that the expression of fibrotic markers in DF is near to maximal. A similar conclusion was reached using a functional contraction assay, which showed that PF contract less than DF in basal conditions and that TGF-β-stimulated contraction was stronger in PF than in DF (Fig. 2). Metformin treatment alone did not have a major impact on fibrotic marker expression or the contractile properties of PF and DF, but was associated with a consistent reduction in TGF-β-stimulation of these parameters in both PF and DF (Figs. 3–6). Treatment with AICAR produced similar effects to those observed with metformin, indicating that the metformin effects were mediated by AMPK.

Overall, these results suggest that metformin could have a role in reducing the level of TGF-β-induced myofibroblasts in DD. Considering that the surgical procedure removes DD fibroblasts present in the cord, it is tempting to hypothesize that disease recurrence is due to a stimulation of resident PF (by TGF-β and other cytokines) that triggers their differentiation into pathologic myofibroblasts, initiating a new flare of DD. These data thus support the case for a clinical trial to evaluate metformin as an adjuvant to surgery, to prevent, reduce, or delay recurrence in at-risk DD patients. The potential clinical effect of metformin to delay

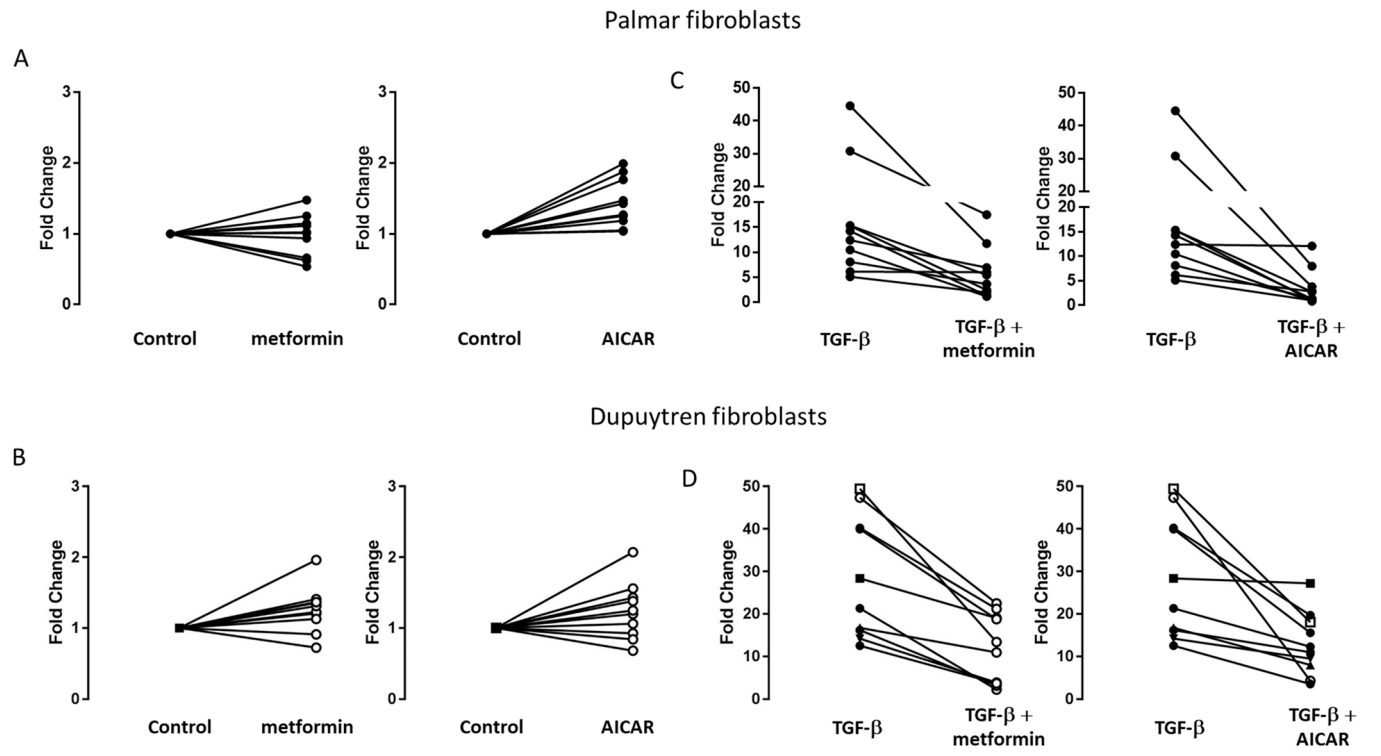

Fig. 3. ACTA2 expression in PF and DF treated with AICAR or metformin. PF (A, C) or DF (B, D) were cultured in the absence (A, B) or presence (C, D) of TGF-β (1 ng/mL) and AICAR (500 μM) or metformin (5 mM), as indicated. After 48 h, total RNA was extracted and ACTA2 levels were determined by qRT-PCR. Each doublet of points was obtained using cells from an individual tissue donor. The data presented are the relative expression of ACTA2 normalized to the expression of RPL32.

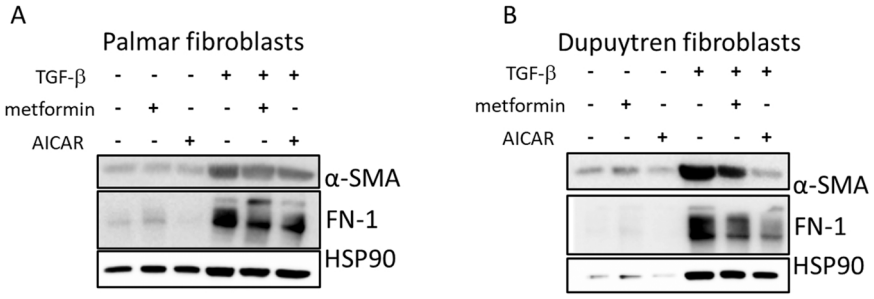

Fig. 4. Western blot analysis of the expression of fibrotic markers in response to AICAR or metformin. PF (A) or DF (B) were cultured in the absence or presence of TGF-β (1 ng/mL), AICAR (500 μM), and metformin (5 mM), as indicated. After 48 h, proteins were extracted and separated by electrophoresis. The blots presented are representative of two independent experiments performed on samples from different tissue donors.

disease progression could also be studied in the early stages of DD.

The production of profibrotic cytokines is thought to be the result of localized inflammation, which may play a significant role in the development and progression of DD [18,35]. For example, TNF-α has been reported to promote the conversion of fibroblasts to myofibroblasts [18] and a clinical trial based on this observation has been initiated. This trial is assessing the effects of TNF-α inhibitors on DD progression [36]. The data obtained to date are encouraging and form the basis of an ongoing phase 2b clinical trial [36]. Other cytokines induced by low-grade inflammation have been proposed as therapeutic targets in DD [35]. In this context, anti-inflammatory effects of metformin have been largely documented [37,38] and they could provide an additional therapeutic angle that would complement its direct action on fibroblasts reported in this study. Unfortunately, the lack of an animal model for DD prevents a validation of this double-edge mechanism.

5. Conclusions

In conclusion, we show that metformin can prevent TGF-β-induced myofibroblast differentiation, but is unable to revert the fibrotic phenotype of myofibroblasts in DD patients. Assuming that DD myofibroblasts are largely removed by the surgical procedure, our data suggest that metformin should be studied as an adjuvant treatment that could delay or reduce disease recurrence by preventing PF differentiation in myofibroblasts. This hypothesis cannot be tested in a preclinical model of DD due to the lack of an available model but, considering the good safety record of metformin, a clinical trial in which it is used as an adjuvant to surgery in patients at risk of DD recurrence could be envisaged to address this question.

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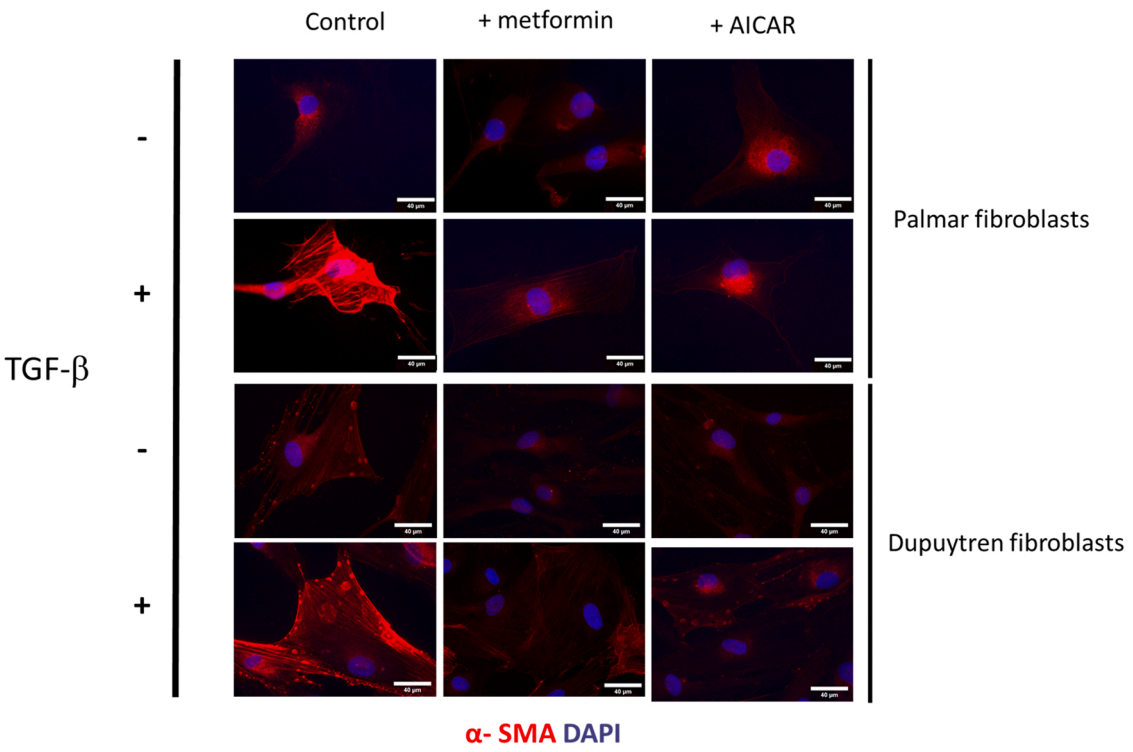

Fig. 5. Immunocytochemical detection of α-SMA in PF or DF, cultured in the absence or presence of TGF-β, AICAR, and metformin. The image presented are representative fields obtained using cell samples from two different tissue donors. Concentrations: TGF-β (1 ng/mL), AICAR (500 μM) or metformin (5 mM). Red: α-SMA, blue: DAPI staining of the nuclei.

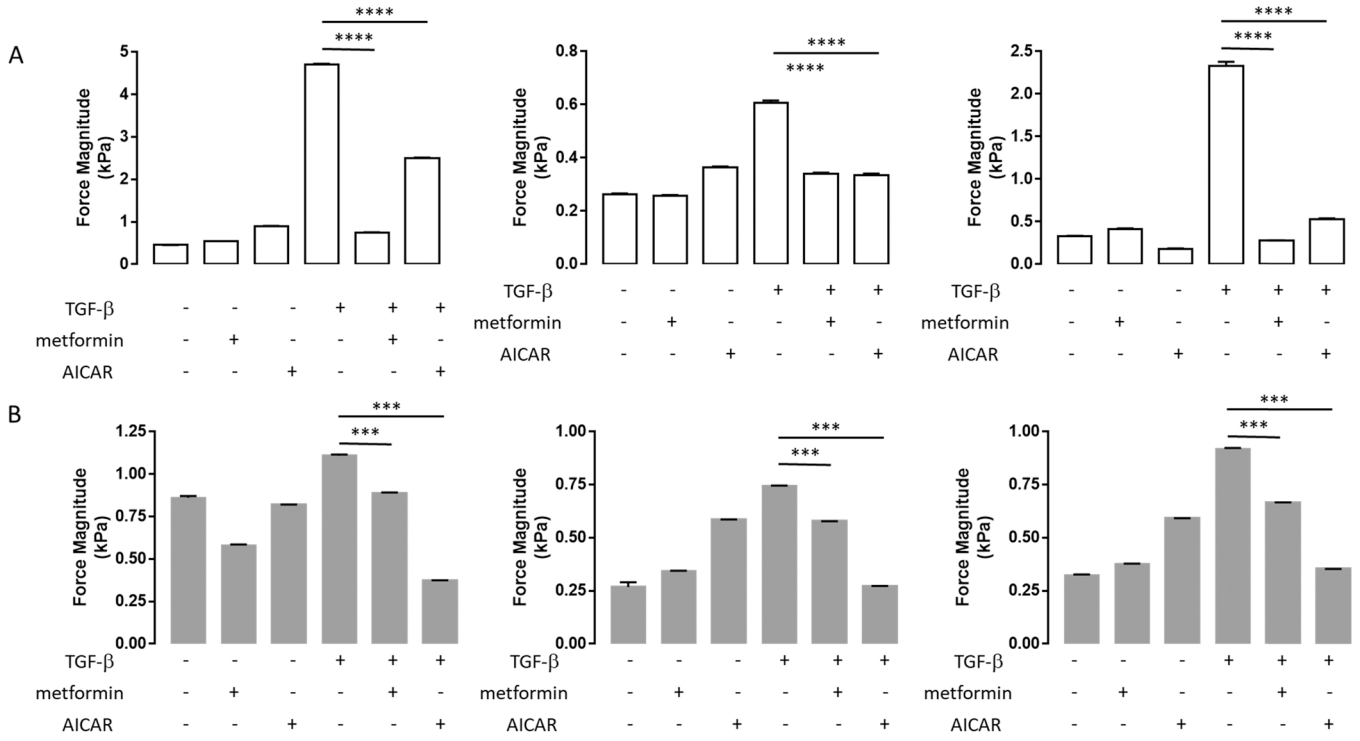

Fig. 6. Effects of AICAR and metformin on the contractile properties of PF and DF, stimulated or not with TGF-β. PF (white bars) or DF (gray bars) were cultured in the absence or presence of TGF-β (1 ng/mL), AICAR (500 μM), and metformin (5 mM), as indicated. Contractions were assessed by traction force microscopy using collagen-coated polyacrylamide hydrogels with shear modulus of 4 kPa coated with red fluorescent beads. The results presented are the mean contractions ± SEM of three PF and three DF obtained from different tissue donors. ***: $p < 0.001$; ****: $p < 0.0001$.

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CRediT authorship contribution statement

This manuscript has been approved by all authors.

Conceptualization: **SB, BM, GV**; Data Collection: **AB, mL, SD, JF, RR, GV**; Resources: **OC, MCE, MAP, TB**; Supervision: **GV**; Methodology: **AB, JF, BM, GV**; Writing – original draft, Writing – review & editing: **mL, OC, GV**.

Declarations of interest

None.

Data availability

Data will be made available on request.

References

[1] B. Shih, A. Bayat, Scientific understanding and clinical management of Dupuytren disease, Nat. Rev. Rheumatol. 6 (12) (2010) 715–726.
[2] F.Z. Hu, A. Nystrom, A. Ahmed, M. Palmquist, R. Dopico, I. Mossberg, J. Gladitz, M. Rayner, J.C. Post, G.D. Ehrlich, R.A. Preston, Mapping of an autosomal dominant gene for Dupuytren's contracture to chromosome 16q in a Swedish family, Clin. Genet. 68 (5) (2005) 424–429.
[3] S. Hindocha, S. John, J.K. Stanley, S.J. Watson, A. Bayat, The heritability of Dupuytren's disease: familial aggregation and its clinical significance, J. Hand Surg. Am. 31 (2) (2006) 204–210.
[4] N.S. Godtfredsen, H. Lucht, E. Prescott, T.I. Sorensen, M. Gronbaek, A prospective study linked both alcohol and tobacco to Dupuytren's disease, J. Clin. Epidemiol. 57 (8) (2004) 858–863.
[5] M. Maravic, S. Lasbleiz, E. Roulot, J. Beaudreuil, Hospitalization for Dupuytren's disease: a French national descriptive analysis, 2002–2009, Orthop. Trauma. Surg. Res. 100 (6) (2014) 589–592.
[6] J. Beaudreuil, H. Lellouche, P. Orcel, T. Bardin, Needle aponeurotomy in Dupuytren's disease, Jt. Bone Spine 79 (1) (2012) 13–16.
[7] L.C. Hurst, M.A. Badalamente, V.R. Hentz, R.N. Hotchkiss, F.T. Kaplan, R.A. Meals, T.M. Smith, J. Rodzvilla, C.I.S. Group, Injectable collagenase clostridium histolyticum for Dupuytren's contracture, N. Engl. J. Med. 361 (10) (2009) 968–979.
[8] K.E. Brandt, An evidence-based approach to Dupuytren's contracture, Plast. Reconstr. Surg. 126 (6) (2010) 2210–2215.
[9] L. Satish, W.A. LaFramboise, S. Johnson, L. Vi, A. Njarlangattil, C. Raykha, J. M. Krill-Burger, P.H. Gallo, D.B. O'Gorman, B.S. Gan, M.E. Baratz, G.D. Ehrlich, S. Kathju, Fibroblasts from phenotypically normal palmar fascia exhibit molecular profiles highly similar to fibroblasts from active disease in Dupuytren's Contracture, BMC Med. Genom. 5 (2012) 15.
[10] T. Balaguer, S. David, T. Ihrai, N. Cardot, G. Daideri, E. Lebreton, Histological staging and Dupuytren's disease recurrence or extension after surgical treatment: a retrospective study of 124 patients, J. Hand Surg. Eur. Vol. 34 (4) (2009) 493–496.
[11] G.H. Dolmans, P.M. Werker, H.C. Hennies, D. Furniss, E.A. Festen, L. Franke, K. Becker, P. van der Vlies, B.H. Wolffenbuttel, S. Tinschert, M.R. Toliat, M. Nothnagel, A. Franke, N. Klopp, H.E. Wichmann, P. Nurnberg, H. Giele, R. A. Ophoff, C. Wijmenga, G. Dutch Dupuytren Study, G. German Dupuytren Study, S. LifeLines Cohort, B.-G. Consortium, Wnt signaling and Dupuytren's disease, N. Engl. J. Med. 365 (4) (2011) 307–317.
[12] T.B. Layton, L. Williams, F. McCann, M. Zhang, M. Fritzsche, H. Colin-York, M. Cabrita, M.T.H. Ng, M. Feldmann, S.N. Sansom, D. Furniss, W. Xie, J. Nanchahal, Cellular census of human fibrosis defines functionally distinct stromal cell types and states, Nat. Commun. 11 (1) (2020) 2768.
[13] R. Dobie, C.C. West, B.E.P. Henderson, J.R. Wilson-Kanamori, D. Markose, L. J. Kitto, J.R. Portman, M. Beltran, S. Sohrabi, A.R. Akram, P. Ramachandran, L. Y. Yong, D. Davidson, N.C. Henderson, Deciphering mesenchymal drivers of human Dupuytren's disease at single-cell level, J. Invest. Dermatol. 142 (1) (2022) 114–123, e118.
[14] B. Hinz, Formation and function of the myofibroblast during tissue repair, J. Invest. Dermatol. 127 (3) (2007) 526–537.
[15] C. Krause, P. Kloen, Ten, P. Dijke, Elevated transforming growth factor beta and mitogen-activated protein kinase pathways mediate fibrotic traits of Dupuytren's disease fibroblasts, Fibrogenes. Tissue Repair 4 (1) (2011) 14.
[16] P. Kloen, C.L. Jennings, M.C. Gebhardt, D.S. Springfield, H.J. Mankin, Transforming growth factor-beta: possible roles in Dupuytren's contracture, J. Hand Surg. Am. 20 (1) (1995) 101–108.
[17] M. Wong, V. Mudera, Feedback inhibition of high TGF-beta1 concentrations on myofibroblast induction and contraction by Dupuytren's fibroblasts, J. Hand Surg. Br. 31 (5) (2006) 473–483.
[18] L.S. Verjee, J.S. Verhoekx, J.K. Chan, T. Krausgruber, V. Nicolaidou, D. Izadi, D. Davidson, M. Feldmann, K.S. Midwood, J. Nanchahal, Unraveling the signaling pathways promoting fibrosis in Dupuytren's disease reveals TNF as a therapeutic target, Proc. Natl. Acad. Sci. USA 110 (10) (2013) E928–E937.
[19] I.H. Park, J.Y. Um, S.M. Hong, J.S. Cho, S.H. Lee, S.H. Lee, H.M. Lee, Metformin reduces TGF-beta1-induced extracellular matrix production in nasal polyp-derived fibroblasts, Otolaryngol. Head Neck Surg. 150 (1) (2014) 148–153.
[20] N. Sato, N. Takasaka, M. Yoshida, K. Tsubouchi, S. Minagawa, J. Araya, N. Saito, Y. Fujita, Y. Kurita, K. Kobayashi, S. Ito, H. Hara, T. Kadota, H. Yanagisawa, M. Hashimoto, H. Utsumi, H. Wakui, J. Kojima, T. Numata, Y. Kaneko, M. Odaka, T. Morikawa, K. Nakayama, H. Kohrogi, K. Kuwano, Metformin attenuates lung fibrosis development via NOX4 suppression, Respir. Res. 17 (1) (2016) 107.
[21] S. Rangarajan, N.B. Bone, A.A. Zmijewska, S. Jiang, D.W. Park, K. Bernard, M. L. Locy, S. Ravi, J. Deshane, R.B. Mannon, E. Abraham, V. Darley-Usmar, V. J. Thannickal, J.W. Zmijewski, Metformin reverses established lung fibrosis in a bleomycin model, Nat. Med. 24 (8) (2018) 1121–1127.
[22] V. Kheirollahi, R.M. Wasnick, V. Biasin, A.I. Vazquez-Armendariz, X. Chu, A. Moiseenko, A. Weiss, J. Wilhelm, J.S. Zhang, G. Kwapiszewska, S. Herold, R. T. Schermuly, B. Mari, X. Li, W. Seeger, A. Gunther, S. Bellusci, E. El Agha, Metformin induces lipogenic differentiation in myofibroblasts to reverse lung fibrosis, Nat. Commun. 10 (1) (2019) 2987.
[23] C.X. Lin, Y. Li, S. Liang, J. Tao, L.S. Zhang, Y.F. Su, Y.X. Huang, Z.K. Zhao, S.Y. Liu, J.M. Zheng, Metformin attenuates cyclosporine A-induced renal fibrosis in rats, Transplantation 103 (10) (2019) e285–e296.
[24] M. Wu, H. Xu, J. Liu, X. Tan, S. Wan, M. Guo, Y. Long, Y. Xu, Metformin and fibrosis: a review of existing evidence and mechanisms, J. Diabetes Res. 2021 (2021) 6673525.
[25] A. Martin-Montalvo, E.M. Mercken, S.J. Mitchell, H.H. Palacios, P.L. Mote, M. Scheibye-Knudsen, A.P. Gomes, T.M. Ward, R.K. Minor, M.J. Blouin, M. Schwab, M. Pollak, Y. Zhang, Y. Yu, K.G. Becker, V.A. Bohr, D.K. Ingram, D. A. Sinclair, N.S. Wolf, S.R. Spindler, M. Bernier, R. de Cabo, Metformin improves healthspan and lifespan in mice, Nat. Commun. 4 (2013) 2192.
[26] N.B. Ruderman, D. Carling, M. Prentki, J.M. Cacicedo, AMPK, insulin resistance, and the metabolic syndrome, J. Clin. Invest. 123 (7) (2013) 2764–2772.
[27] J. Lu, J. Shi, M. Li, B. Gui, R. Fu, G. Yao, Z. Duan, Z. Lv, Y. Yang, Z. Chen, L. Jia, L. Tian, Activation of AMPK by metformin inhibits TGF-beta-induced collagen production in mouse renal fibroblasts, Life Sci. 127 (2015) 59–65.
[28] S.C. Martinico, S. Jezzard, N.J. Sturt, G. Michils, S. Tejpar, R.K. Phillips, G. Vassaux, Assessment of endostatin gene therapy for familial adenomatous polyposis-related desmoid tumors, Cancer Res. 66 (16) (2006) 8233–8240.
[29] I. Peerlinck, S. Amini-Nik, R.K. Phillips, R. Iggo, N.R. Lemoine, S. Tejpar, G. Vassaux, Therapeutic potential of replication-selective oncolytic adenoviruses on cells from familial and sporadic desmoid tumors, Clin. Cancer Res. 14 (19) (2008) 6187–6192.
[30] B. Cambien, K. Lebrigand, A. Baeri, N. Nottet, C. Compin, A. Lamit, O. Ferraris, C. N. Peyrefitte, V. Magnone, J. Henriques, L.E. Zaragosi, S. Giorgetti-Peraldi, F. Bost, M. Gautier-Isola, R. Rezzonico, P. Barbry, R. Barthel, B. Mari, G. Vassaux, Identification of oncolytic vaccinia restriction factors in canine high-grade mammary tumor cells using single-cell transcriptomics, PLoS Pathog. 16 (10) (2020), e1008660.
[31] G. Savary, E. Dewaeles, S. Diazzi, M. Buscot, N. Nottet, J. Fassy, E. Courcot, I. S. Henaoui, J. Lemaire, N. Martis, C. Van der Hauwaert, N. Pons, V. Magnone, S. Leroy, V. Hofman, L. Plantier, K. Lebrigand, A. Paquet, C.L. Lino Cardenas, G. Vassaux, P. Hofman, A. Gunther, B. Crestani, B. Wallaert, R. Rezzonico, T. Brousseau, F. Glowacki, S. Bellusci, M. Perrais, F. Broly, P. Barbry, C. H. Marquette, C. Cauffiez, B. Mari, N. Pottier, The long noncoding RNA DNM3OS is a reservoir of fibromirs with major functions in lung fibroblast response to TGF-beta and pulmonary fibrosis, Am. J. Respir. Crit. Care Med. 200 (2) (2019) 184–198.
[32] J.L. Martiel, A. Leal, L. Kurzawa, M. Balland, I. Wang, T. Vignaud, Q. Tseng, M. Thery, Measurement of cell traction forces with ImageJ, Methods Cell Biol. 125 (2015) 269–287.
[33] M.A. Badalamente, L.C. Hurst, P. Benhaim, B.M. Cohen, Efficacy and safety of collagenase clostridium histolyticum in the treatment of proximal interphalangeal joints in dupuytren contracture: combined analysis of 4 phase 3 clinical trials, J. Hand Surg. Am. 40 (5) (2015) 975–983.
[34] C. Boe, P. Blazar, N. Iannuzzi, Dupuytren contractures: an update of recent literature, J. Hand Surg. Am. 46 (10) (2021) 896–906.
[35] D. Izadi, T.B. Layton, L. Williams, F. McCann, M. Cabrita, A.I. Espirito Santo, W. Xie, M. Fritzsche, H. Colin-York, M. Feldmann, K.S. Midwood, J. Nanchahal, Identification of TNFR2 and IL-33 as therapeutic targets in localized fibrosis, Sci. Adv. 5 (12) (2019) eaay0370.
[36] J. Nanchahal, C. Ball, D. Davidson, L. Williams, W. Sones, F.E. McCann, M. Cabrita, J. Swettenham, N.J. Cahoon, B. Copsey, E. Anne Francis, P.C. Taylor, J. Black, V. S. Barber, S. Dutton, M. Feldmann, S.E. Lamb, Anti-tumour necrosis factor therapy for Dupuytren's disease: a randomised dose response proof of concept phase 2a clinical trial, EBioMedicine 33 (2018) 282–288.
[37] L.P. Bharath, B.S. Nikolajczyk, The intersection of metformin and inflammation, Am. J. Physiol. Cell Physiol. 320 (5) (2021) C873–C879.
[38] Y. Li, L. Yang, Y. Wang, Z. Deng, S. Xu, H. Xie, Y. Zhang, J. Li, Exploring metformin as a candidate drug for rosacea through network pharmacology and experimental validation, Pharm. Res. 174 (2021), 105971.