Objectives: We aimed to identify molecules that play a role in chronically activated fibroblasts.

Methods: To identify molecules specifically upregulated in human fibroblast-like cells, RNA-sequencing was performed. Identified adhesion proteins were further validated in skin biopsy samples from patients with limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc), and evaluated correlation between expression levels and clinical parameters. Overexpressed proteins were further addressed in vitro. Functional effects were assessed by qPCR, hydroxyproline, and migration assays. Mouse models of systemic sclerosis were used to functionally validate adhesion proteins in vivo.

Results: We identified adhesion proteins as significantly upregulated molecules in chronically active fibroblasts of skin biopsy samples from SSC patients compared to fibroblasts from healthy controls. Expression levels were correlated with the modified Rodnan skin score in the skin of SSC patients. We observed higher expression levels of class I molecules in the mouse model of topoisomerase I induced skin fibrosis. This result was also observed in bleomycin induced lung fibrosis model suggesting important functions of adhesion proteins during fibrotic tissue remodeling across different organs. Fibroblast-specific knockout resulted into significantly attenuated bleomycin-induced fibrosis. Upon bleomycin challenge, hydroxyproline content was diminished in mice with genetic deficiency of adhesion proteins. In addition, COL1A1, COL1A2 and LUM mRNAs and also the number of myofibroblasts were significantly lower in knockout mice compared to wild type mice. In vitro, knockout of adhesion proteins resulted into a significant alteration of the migratory potential of fibroblasts.

Conclusion: Our results demonstrate that adhesion proteins play an essential role in the pathogenesis of systemic sclerosis. Understanding the molecular mechanisms of adhesion proteins may lead to a novel therapeutic intervention in human SSC and related disorders.


PRELIMINARY RESULTS SHOW AN INCREASED EXPRESSION OF COINHIBITORY RECEPTORS IN SYSTEMIC SCLEROSIS

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Background: Recent studies suggest dysregulation in T cell activation in systemic sclerosis (SSc). Co-inhibitory-receptors (Co-IRs) such as TIM-3, PD-1 and LAG-3 play a crucial role in controlling excessive T cell activation and in maintaining immune homeostasis. Engagement of these receptors by their ligands limits cytokine production in response to TCR or activating NK receptor stimulation and hence limit tissue damage from excessive immune activation. However, chronically increased expression of multiple Co-IRs is a hallmark of immune exhaustion. We evaluate the role of these soluble Co-IRs in diffuse SSC (dcSSc).

Objectives: Establish the role of CoIR and their ligands in diffuse systemic sclerosis. Understand how immune regulatory mechanisms influence the development of fibrosis. Provide a better understanding of the disease and fibrosis in general.

Methods: PBMCs (Peripheral blood mononuclear cells) and dermal fibroblasts from SSc patients were isolated and investigated for markers of T cell inhibition.

Results: The proportion of CD4+ T cells expressing PD1 were markedly increased in SSC patients compared to healthy volunteers and Rheumatoid Arthritis patients.

There was increased expression of both TIM3 and PD1 in the CD4+ T cells. (Figure 1)

Similarly, the co-expression of these receptors on the CD4+ T cell population was elevated compared to healthy volunteers. (figure 2)

Conclusion: Soluble co-inhibitors are differentially expressed in early dcSSc compared to healthy volunteers and other autoimmune diseases. Our preliminary data indicates that these co-inhibitors could play an important role in unravelling the pathogenesis of systemic sclerosis. Inhibition or activation of these receptors through different treatment modalities can be utilized as a novel patient centric treatment strategy.

References:

Figure 1 CD3+CD4+ T cells Co inhibitor Receptor Expression

Figure 2 CD3+CD4+ T cells showing Co- expression of Co-inhibitor Receptors
Background: Upregulation of the innate immune response via the activity of Toll-like receptors and the NLRP3 inflammasome have been suggested as initiating events that can drive fibrosis in systemic sclerosis (SSc) (Pharmacol Ther. 2018;192:163). Lenabasum, a cannabinoid receptor type 2 agonist, is known to activate the resolution phase of acute human innate immune responses triggered through Toll-like receptor activation, favoring production of pro-resolving lipid mediators, reducing inflammatory infiltrates, and increases bacterial clearance (Clin Pharmacol Ther. 2018;104:675). Given the potential importance of inflammasome activation in the pathogenesis of SSc, the question remained whether lenabasum inhibits inflammasome activation.

Objectives: Assess effects of lenabasum on IL-1β and IL-18 production induced by inflammasome activation.

Methods: Primary human macrophages were derived from monocytes, stimulated with LPS and ATP to active inflammasomes and cultured with lenabasum. Levels of IL-1β and IL-18 were measured in cell supernatants by ELISA. Separately, human PBMCs were activated with 0.1 μg/mL LPS ± 10 μM lenabasum for 24 hours, and effects of lenabasum on the levels of IL-1β and other pro-inflammatory cytokines were measured.

Results: Lenabasum significantly inhibited IL-1β and IL-18 secretion by monocyte-derived macrophages, with IC50 = 66.73 ± 3.92 nM and 349.23 ± 21.27 nM, respectively. A control inflammasome activation inhibitor, MCC950, which showed IC50 = 18.33 ± 1.22 nM for IL-1β inhibition and IC50 = 21.43 ± 0.81 nM for IL-8 inhibition.

Conclusion: Lenabasum inhibits inflammasome activation, which could contribute to potential therapeutic efficacy in SSc and other autoimmune diseases.

References:

Figure


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AB0153

ADIPOSE-DERIVED STROMAL/STEM CELLS FROM SYSTEMIC SCLEROSIS PATIENTS SUCCESSFULLY EXERT A PARACRINE ANTI-FIBROTIC ACTIVITY AND INDUCE A PRO-ANGIOGENIC PHENOTYPE OF SCORERDERMA FIBROBLASTS IN VITRO

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Background: Adipose-derived stromal/stem cells (ADSCs) are multipotential non-hematopoietic progenitor cells with anti-inflammatory, immunomodulatory and regenerative effects. They have the advantage of accessibility and potent pro-angiogenic effects when compared with other stem cells, such as bone-marrow derived stem cells. Recent studies have shown that autologous fat grafting may be effective in the treatment of fibrotic and vascular complications in systemic sclerosis (SSc), despite a pro-fibrotic signature.

Objectives: Aim of the study was to better characterize the proliferative and secretory profile of ADSCs in normoxic and hypoxic conditions, and to evaluate the mechanisms that underlie observed clinical effects.

Methods: Adipose tissue samples were obtained by liposuction from 12 SSc patients and 10 healthy donors (HD). ADSCs were purified according to their adherence to the plastic and characterized to express specific MSC surface antigens by flow cytometry analysis. Proliferation of ADSCs from SSc patients and normal controls was evaluated in normoxic and hypoxic conditions. Fibroblasts derived from SSc patients and HD were co-cultured to observe their anti-fibrotic and pro-angiogenic effects on ADSCs.

Results: Normoxic and hypoxic culture conditions did not modify the proliferation rate of both normal and SSc ADSCs. Hypoxia significantly increased mRNA expression of VEGF by HD and SSc ADSCs but had no effect on the mRNA expression of pro-fibrotic mediators, i.e. TGFβ and TGFR. Normal and SSc fibroblast proliferation was significantly reduced in both co-culture systems (p < 0.001) and by treatment with normoxic and hypoxic conditioned medium (CM) (p=0.001 and p=0.002). In the same conditions, mRNA expression and protein secretion of TGF-β1, CTGF and Col I were significantly reduced (p = 0.003, p<0.02, p=0.04). These results were confirmed when normal and SSc fibroblasts were cultured in the presence of ADSCs normoxic and hypoxic CM (p=0.02 and p=0.01). Furthermore, a significant increase in mRNA expression and production of VEGF was observed in SSc fibroblasts cultured in the presence of normoxic and hypoxic CM (p = 0.002 and p< 0.0001), respectively.

Conclusion: We found that treatment with the medium from normoxic and hypoxic pre-conditioned ADSCs has an anti-fibrotic effect through both the inhibition of fibroblast proliferation and key mediators of fibrosis. The increased expression of VEGF by SSc fibroblasts in the presence of normoxic and, even more, hypoxic ADSCs CM, suggests that ADSCs can induce a paracrine pro-angiogenic phenotype, even in fibroblasts with a pro-fibrotic signature. Altogether, these results show that ADSCs may exert their anti-fibrotic and pro-angiogenic effects on SSc fibroblasts by the secretion of paracrine factors, partly explaining the mechanisms underlying beneficial clinical results of fat graft in SSc patients.

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AB0154

MERTK AND THE RESOLUTION OF INFLAMMATION IN IGG4-RELATED DISEASE

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Background: IgG4-Related Disease (IgG4-RD) is characterized by fibrotic lesions in serum IgG4 elevation, and prompt response to glucocorticoids. B and T lymphocytes are considered the initiators of tissue inflammation in IgG4-RD, but the prominent stromal reaction observed at disease sites suggest that a dysregulation of processes involved in the resolution of inflammation could be pathologically relevant as well. Mer receptor tyrosine kinase (MERTK) and its ligands protein S (ProS1) have a pivotal role in the resolution of inflammation through the activation of a well-characterized signaling pathway that ultimately dampens the immune response and promotes the recovery of tissue function.

Objectives: The aim of this study was to assess the role of MERTK in the resolution of IgG4-RD.

Methods: We analyzed the expression of MERTK and its ligand ProS1 in stromal cells of IgG4-RD lesions. Immunohistochemistry and single-cell sequencing were performed. MERTK-expressing cells were selected and their gene expression profile was compared with other cell types using single-cell RNA sequencing.

Results: MERTK was expressed in stromal cells of IgG4-RD lesions. ProS1 expression was higher in MERTK-expressing cells compared with other cell types. The gene expression profile of MERTK-expressing cells showed enrichment for genes involved in the resolution of inflammation, including those involved in phagocytosis and activation of macrophages.

Conclusion: These results suggest a potential role of MERTK in the resolution of inflammation in IgG4-RD. Further studies are needed to investigate the mechanisms underlying this role and its potential therapeutic implications.

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