Background: Systemic sclerosis (SSc) is a chronic autoimmune disease characterized by microangiopathy and fibrosis. In physiological wound healing, fibroblasts are transiently activated for tissue repair. In contrast, fibroblasts are persistently activated during fibrosis and thus resulted in progressive matrix deposition and tissue remodeling. However, the pathogenesis of the fibrotic process is not fully understood.

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

Methods: To identify molecules significantly upregulated in human 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 also 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 adaptor 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 adaptor 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, knockdown of adaptor proteins resulted into a significant alteration of the migratory potential of fibroblasts.

Results: We identified adaptor 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 also 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 adaptor 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 adaptor 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, knockdown of adaptor proteins resulted into a significant alteration of the migratory potential of fibroblasts.

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

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References:
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) (Pharma-
col 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 poten-
tial 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, stimu-
lated 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. Separa-
tively, 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-inflam-
atory cytokines were measured.

Results: Lenabasum significantly inhibited IL-1β and IL-18 secretion by mono-
cyte-derived macrophages, with IC₅₀ = 66.7 ± 3.92nM and 349.23 ± 21.27nM, respectively. A control inflammasome activation inhibitor, MCC950, which showed IC₅₀ = 18.33 ± 1.22nM for IL-1β inhibition and IC₅₀ = 21.43 ± 0.81nM for IL-8 inhibition.

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

References:

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aceuticals, Inc., Employee of: Corbus Pharmaceuticals, Inc., Ping Zang Employee of: Corbus Pharmaceuticals, Inc., Barbara White Shareholder of: Corbus Pharmace-

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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 SCLERODERMA 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 mar-
row derived stem cells. Recent studies have shown that autologous fat grafting may be effective in the treatment of fibrotic and vascular complications in sys-
temic 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 behind ADSCs observed clinical effects.

Methods: Adipose tissue samples were obtained by liposuction from 12 SSc pa-
tients 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. Fibro-
blasts and ADSCs derived from SSc patients were co-cultured in direct and indirect culture systems and compared to HD. Fibroblasts proliferation, mRNA expression and protein secretion of VEGF and known fibrotic mediators includ-
ing TGF-β1, TGFR, CTGF. Collagen type I (Coll I) were analyzed in the same condi-
tions.

Results: Normoxic and hypoxic culture conditions did not modify the prolif-
eration 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, ie 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 expres-
sion 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 preconditioned ADSCs has an anti-fibrotic effect through both the inhi-
bition of fibroblast proliferation and key mediators of the 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. Alto-
gether these data show that ADSCs may exert their anti-fibrotic and pro-angi-
ogenetic 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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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, 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 dysreg-
ulation of processes involved in the resolution of inflammation could be patho-
logically 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. MerTK and the processes involved in the resolution of inflammation have never been addressed in IgG4-RD.

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