THU0340

IMPACT OF LANIFIBRANOR, A PAN-PPAR AGONIST, ON THE PULMONARY VASCULAR TONE

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Background: Pulmonary hypertension (PH) is common in patients with fibrosing interstitial lung diseases (ILDs) such as systemic sclerosis (scleroderma) and has a significant impact on survival. The pan-peroxisome proliferator-activated receptor (PPAR) agonist lanifibranor is currently being tested in a phase II clinical trial in scleroderma patients. Although, the antifibrotic effects of lanifibranor have been previously shown in preclinical mouse models of scleroderma and related PH (1), its effects in modulating the pulmonary vascular tone are largely unknown.

Objectives: To evaluate the acute effect of lanifibranor (pan-PPAR agonist) and selective PPAR agonists on the pulmonary vascular tone.

Methods: Freshly isolated intra-pulmonary arteries from explanted human and rat lungs were used in wire myography experiments to assess the acute effect on pulmonary vascular tone of pan-PPAR agonist -lanifibranor and the selective PPAR agonists PPAR α -fenofibrate, PPAR β/δ -GW50156 and PPAR γ -troglitazone (0.01 μ M-30 μ M) versus vehicle (DMSO). The intra-pulmonary arteries were pre-constricted with 300nM of the thromboxane A2 receptor agonist U46619. The contribution of endothelium and nitric oxide was investigated using both native and endothelium-denuded vessels, with and without the nitric oxide synthase inhibitor L-NAME.

Results: Isometric tension measurements in rat pulmonary arteries showed that lanifibranor and the selective PPAR agonists PPAR β/δ -GW50156 and PPAR γ -troglitazone, induced a significant relaxation compared to their vehicle controls, whereas the PPAR α agonist fenofibrate did not show any vasoactive effect on the pre-constricted arteries. Lanifibranor showed a significant vasodilatory effect already at 1 μ M whereas GW50156 and troglitazone showed significant vasodilatory effects starting from 10 μ M as compared to their vehicle controls. At the maximum concentration used (30 μ M), lanifibranor caused a relaxation response of -85% whereas GW50156 and troglitazone caused just -45% and -50% vasorelaxation (n=6), respectively. The vasodilation induced by pan-PPAR and selective PPAR agonists remained unaffected by pre-treatment of L-NAME or denuded endothelium. Lanifibranor had vasodilatory effects that were strongly superior to any of the selective PPAR agonists in the isolated pulmonary artery from the explanted human lungs (n=3).

Conclusion: The current study revealed that the pan-PPAR agonist lanifibranor caused a rapid dose-dependent relaxation in both rat and human intra pulmonary arteries which were superior to any other PPAR agonists suggesting additive effects of the pan-PPAR agonism. The observed vasoactive effects of pan-PPAR are independent of nitric oxide and endothelium, delineating the specific effect of PPAR agonism on the pulmonary artery smooth muscle cells. These results suggest that lanifibranor could be a promising therapy for vasculopathies such as PH observed in scleroderma patients.

REFERENCE:

- [1] Avouac J et al. Pan-PPAR agonist IVA337 is effective in experimental lung fibrosis and pulmonary hypertension. *Ann Rheum Dis.* 2017 Nov;76(11):1931-1940.

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THU0341

MICRORNA27A3P REGULATES WNT SIGNALLING BY TARGETING SFRP-1 IN SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc) is an autoimmune idiopathic connective tissue disease that results in fibrosis of the skin and lungs. Wnt signaling is a highly conserved signaling pathways that is involved in organogenesis and tissue homeostasis. In recent years it has emerged that aberrant Wnt signaling is key in SSc and animal models of the disease. The Wnt pathway comprises of secreted glycoproteins that bind to specific receptors that initiate the pathway and result in beta catenin stabilization. However, secreted antagonists are secreted to block and negatively regulate Wnt signaling. We and others have found reduced sFRP-1 in SSc, a key Wnt antagonist. Although lower in SSc skin and isolated fibroblast from patients the mechanism governing this is unknown. This work seeks to identify epigenetic mechanisms.

Objectives: Determine the epigenetic regulation of sFRP-1 in SSc.

Methods: Serum sFRP-1 was measured in healthy and diffuse SSc patients to ascertain the levels of sFRP-1. MiR27a was identified as a possible microRNA that targets sFRP1 using in silico modeling. miR27a was transfected into healthy dermal fibroblasts using transfection methods and sFRP-1 was measured. Collagen was also measured by western blotting after transfection. MiR27a was also measured in SSc dermal fibroblasts using specific Taqman probes and normalized to RNU44 miR. Cloning of the 3'UTR of sFRP1 into a luciferase reporter vector was performed and then transfected into HEK293 cells with miR27a mimics or with concentration matched scramble and after 24 hours luciferase was monitored.

Results: Significantly reduced expression of sFRP-1 was found in SSc serum compared to controls (n=10). SSc dermal fibroblasts had 3 fold higher levels of miR27a compared to healthy fibroblasts. Increased miR27a in healthy dermal fibroblasts resulted in significantly less sFRP-1 secreted into conditioned media and this was co-incident with an increase of collagen1 levels. Finally cloning of the 3'UTR binding site in a luciferase plasmid and overexpression of miR27a led to reduced levels of luciferase in HEK293 cells in vitro.

Conclusion: Reduced sFRP-1 in systemic sclerosis is mediated by miR27a and this leads to fibrosis via enhanced Wnt signaling. Targeting the epigenetic changes that mediate this may be a therapy in SSc.

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THU0342

SYSTEMIC SCLEROSIS IS A DISEASE OF A PREMATURELY SENESCENT, INFLAMMATORY AND ACTIVATED IMMUNOME

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Background: Systemic sclerosis (SSc) is an autoimmune disease characterized by excessive fibrosis of skin and internal organs, and vascular dysfunction (1). Association of T and B cell subsets have been reported in SSc, however there is lack of systematic studies of functional relations between immune cell subsets in this disease (2,3,4). This lack of mechanistic knowledge hampers targeted intervention.

Objectives: In the current study we ought to determine differential immune cell composition and their interactions in peripheral blood of SSc patients and its impact on disease severity and progression.

Methods: Mononuclear cells from blood of SSc patients (n=20) and healthy controls (n=10) were analysed by mass cytometry using a 36 marker (cell-surface and intracellular) panel to aid in identification of major PBMC lineages including T cells, B cells, monocytes and NK cells and their subsets. Transcriptome analysis (m-RNA sequencing) was performed on sorted T and B cell subsets. Unsupervised clustering of mass cytometry data was performed using in-house developed analysis software MARVIS. This software combines dimension reduction and clustering steps to identify all possible cellular subsets. Further, custom R scripts helped in identifying nodes that were differentially expressed between the study groups and also phenotype of these nodes.

Results: Unsupervised clustering analysis revealed significant differences in the frequencies of T and B cell subsets in patients. Correlation network analysis highlighted an overall dysregulated immune architecture coupled with domination of inflammatory senescent T cell modules in SSc patients. Transcriptome analysis of sorted immune cells revealed an activated phenotype of CD4 and MAIT cells in patients, accompanied with increased expression of inhibitory molecules, reminiscent of

phenotype exhibited by functionally adapted, exhausted T cells in response to chronic stimulation.

Conclusion: Overall this study provides an in depth analysis of systemic immunome in SSC, highlighting role of inflammation and chronic stimulation mediated "premature senescence" of immune cells, with implications to delineate mechanism of pathogenesis and identify diagnostic/therapeutic targets.

REFERENCES:

- [1] Denton, C. P. and D. Khanna (2017). "Systemic sclerosis." *Lancet* 390 (10103): 1685-1699.
- [2] Fuschioti, P. (2017). "Current perspectives on the role of CD8+ T cells in systemic sclerosis." *Immunol Lett*. In Press
- [3] Liu, M., et al. (2016). "New insights into CD4(+) T cell abnormalities in systemic sclerosis." *Cytokine Growth Factor Rev* 28: 31-36.
- [4] Sanges, S., et al. (2017). "Role of B cells in the pathogenesis of systemic sclerosis." *Rev Med Interne* 38(2): 113-124.

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THU0343

PU.1 INHIBITOR DB1976 CONTROLS FIBROBLAST POLARIZATION IN SYSTEMIC SCLEROSIS AND LEADS TO REGRESSION OF FIBROSIS IN DIFFERENT MODELS OF ORGAN FIBROSIS

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Background: Persistent activation of fibroblast with excessive release of extracellular matrix is a hallmark of systemic sclerosis (SSc). Fibroblasts can either acquire a "pro-fibrotic" phenotype with excessive matrix production or a "pro-inflammatory" phenotype with releasing of matrix-degrading enzymes and subsequent tissue destruction. Despite these well-characterized phenotypic differences, the molecular mechanisms that drive polarization of fibroblasts into these two functionally opposing phenotypes remain enigmatic.

Objectives: We aimed to evaluate the transcriptional network that promotes the extracellular matrix-producing fibrotic fibroblast fate.

Methods: We investigated the transcriptional network that induces the pro-fibrotic phenotype of fibroblasts by in silico and immunofluorescence analyses of human fibrotic skin, lung, liver and kidney, and performed functional assays to address the fibrogenic potential of fibroblasts in vitro and in several mouse models of systemic sclerosis. The heterocyclic diamidine DB1976 was used as new therapeutic compound to induce regression of fibrosis.

Results: We identified the ETS transcription factor PU.1 as molecular checkpoint for acquisition of a "pro-fibrotic" phenotype of fibroblasts. Our data demonstrate that expression of PU.1 is effectively silenced in fibroblasts during tissue homeostasis. When the epigenetic control of PU.1 is lost and PU.1 expression is induced, fibroblasts differentiate into a fibrotic phenotype that includes the transcription of numerous pro-fibrotic mediators. PU.1 polarized resting fibroblasts and even repolarized extracellular matrix-degrading inflammatory fibroblasts to an extracellular matrix-producing fibrotic phenotype. PU.1 is associated with a network of pro-fibrotic factors including members of the TEAD-HIPPO, canonical TGF- β -SMAD and AP1 signaling pathways. Other transcription factors with fibrotic abilities, such as SNAI2 and myocyte enhancer factor (MEF) 2, bind in close vicinity to PU.1-binding sites within the genome and contribute to the recruitment of the transcription machinery that drives the switch towards the fibrotic phenotype. PU.1 has a major coordinating role within this complex network of transcription factors in fibroblasts, as the inactivation of PU.1 alone is sufficient to prevent fibrotic polarization in vitro and in vivo. Finally, we investigated pharmacological targeting of PU.1 as a

potential strategy to prevent uncontrolled fibrotic tissue remodelling. DB1976 showed anti-fibrotic effects in vivo in various fibrosis models and across several organs. Treatment with DB1976 not only prevented bleomycin-mediated skin fibrosis, but also induced regression of pre-established fibrosis, and was well tolerated.

Conclusion: These findings suggest that PU.1 inhibition may represent a novel and effective therapeutic approach to treat a wide range of fibrotic diseases. Inactivation of PU.1 effectively reverted the fibrotic phenotype of fibroblasts to a resting state and induced the regression of tissue fibrosis.

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THU0344

A PILOT STUDY ASSESSING THE EFFECT OF AIR POLLUTION ON EXTRACELLULAR VESICLES IN SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc) is a severe autoimmune disease characterized by a progressive multi-organ fibrosis. The identification of specific diagnostic and prognostic biomarkers remains an unmet need. Over the past few years, it has been suggested that extracellular vesicles (EVs) and environmental toxicants, such as particulate matter (PM), may have an important role in the pathogenesis of autoimmune diseases. At present, no data are available on the impact of PM exposure on EVs from patients with SSc.

Objectives: Our aim was to evaluate the effects of PM with aerodynamic diameter $\leq 10 \mu\text{m}$ (PM₁₀) and $\leq 2.5 \mu\text{m}$ (PM_{2.5}) on EVs in SSc and osteoarthritis (OA) as control.

Methods: Plasma EVs were analyzed by Nanosight and flow cytometry after labeling with the following markers: CD14 (monocyte), CD61 (platelet), CD25 (T-reg), human endogenous retrovirus w (HERV-w), human leukocyte antigen G (HLA-G). Demographic and clinical data were collected for each patient. Plasma EV concentrations were measured in SSc and OA patients and were analyzed by generalized linear regression models. Daily PM concentrations, estimated by Regional Environmental Protection Agency at municipality resolution, were used to assign short-term exposure (mean of the 7 days preceding the evaluation) to each study subjects.

Results: 12 consecutive patients with limited cutaneous SSc (11 female, median age 66.8, median disease duration 12.3, 7 anti-centromere positive, median mRSS 3.5) and 12 patients with OA (median age 67.1, median disease duration 9.3, 8 female) were enrolled. The increase of PM_{2.5} led to a decrease of HERV-w+ microvesicles (MV) in both SSc ($\beta = -0.10$; $p = 0.01$) and OA ($\beta = -0.09$; $p = 0.01$) and of HLA-G+ ($\beta = -0.11$; $p < 0.01$) only in SSc. Similar results were observed analyzing PM₁₀ exposure. Analysis of EVs concentration according to their dimensions showed a negative association in the size range of exosomes (63-