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The Nrf2-Antioxidant Response Element Signalling Pathway Controls Fibrosis and Autoimmunity in Scleroderma

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Dysregulations in the oxidant/antioxidant balance are known to be major factors in the pathogenesis of systemic sclerosis (SSc). NRF2 acts as a key player in the antioxidant defense by controlling the transcription of antioxidant and cytoprotective genes. In this article, the authors explored the involvement of a down-expression of NRF2 in the pathogenesis of SSc and the relevance of a NRF2 agonist, as a potent therapeutic option in SSc.

The authors firstly demonstrated that NRF2 and its key target anti-oxidant genes (Heme oxygenase-1 (HO-1), Glutamate-cystein ligase (GCL) and thioredoxin) were indeed downregulated in skin fibroblasts from SSc patients. They also confirmed that such reduction was also observed in the murine models of HOCL-induced SSc and bleomycin-induced SSc. Secondly, they highlighted the potential protective role of NRF2 in SSc, showing that NRF2-/- mice exposed to HOCL presented higher collagen content in skin and lung when compared to wild type HOCL-exposed mice. This exacerbated phenotype in NRF2-/-mice was also associated with a higher production of H2O2, higher levels of anti-DNA topoisomerase 1 antibodies and higher percentages of spleen M2 macrophages.

In a therapeutic perspective, the authors tested the antioxidant properties of Dimethyl fumarate (DMF), a NRF2 agonist, on human fibroblasts and endothelial cell lines and also on murine primary skin fibroblasts in vitro. DMF significantly enhanced Glutathione content in these three cell types, with subsequent reduction of H2O2 production. The same results were observed in vitro, in primary skin fibroblasts from SSc patients, in a dose-dependent manner. Secondly, the authors evaluated the effects of a treatment with DMF in vivo on the severity of HOCL-induced SSc in mice. DMF prevented the development of SSc in this mouse-model with significant reduction of collagen content in skin and lungs, reduced levels of anti-DNA topoisomerase 1 antibodies and a reduced IL-13 mRNA expression, a well-known pro-fibrotic M2 cytokine.

These protective effects of DMF were associated with an enhanced expression of NRF2 target anti-oxidant genes (HO-1 and GCL) in skin fibroblasts, suggesting that NRF2 was a key mediator of the effects of DMF.
In demonstrating the key role of NRF2 as a protective factor from fibrosis in SSc, the authors support the hypothesis that NRF2 agonists, such as DMF, may represent promising therapeutic options for SSc in the future.

October 2018

Therapeutic interleukin-6 blockade reverses transforming growth factor-beta pathway activation in dermal fibroblasts: insights from the faSScinate clinical trial in systemic sclerosis

Christopher P denton, Voon H Ong, Shiwen Xu, Haiyin Chen-Harris, Zora Modrusan, Robert Lafyatis, Dinesh Khanna, Angelika Jahreis, Jeffrey Siegel, Thierry Sornasse. Ann Rheum Dis. 2018 Sep;77(9):1362-1371.

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The faSScinate study was a randomised, double-blind, placebo (PBO)-con- trolled phase 2 study of Tocilizumab (TCZ) in adult SSc patients with <5 years' disease duration, a modified Rodnan skin score (mRSS) between 15 and 40 units and active progressive disease according to specified clinical or laboratory features. As previously reported, primary clinical data from this trial showed a trend of benefit in favour of TCZ for the primary endpoint (mRSS) and a strong trend at 48 weeks in exploratory endpoints including lung function. In this ancillary study, authors aimed to define the molecular and functional basis of the mechanism of IL6 blockage using explant dermal fibroblasts from a representative subset of patients with SSc enrolled into faSScinate before and after treatment with TCZ for 24 weeks compared with controls from the PBO arm of the trial.

They first confirmed that SSc explant dermal fibroblasts presented hallmark functional properties of activated fibrotic cells (production of extracellular matrix, migratory capacity…). Using RNA-sequencing, they found that those dermal fibroblasts collected at baseline presented typical profibrotic signature dominated by genes regulated by TGFβ. These functional properties were attenuated by TCZ treatment for 24 weeks in SSc explants with a decrease in protein production, in migration, and in contractility of fibroblasts. In the same line, profibrotic expression profile of SSc dermal fibroblasts was normalised in TCZ-treated patients and stable in PBO-treated patients.

Gene expression data confirmed that therapeutic treatment with TCZ for 24 weeks profoundly alters the SSc-associated molecular phenotype dermal fibroblasts. Exploring the correlation between gene expression and mRSS across all patients and time points, only one, PRKCE, had a nominal correlation p≤0.05 that remained significant after correction for multiple testing. Treatment with TCZ resulted in a significant upregulation of PRKCE expression that was underexpressed in SSc dermal fibroblasts at baseline, as previously reported in SSc lung fibroblasts

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Using the Ingenuity Pathway Analysis, authors observed that treatment with TCZ for 24 weeks reversed the activation status of key pathological pathways associated with SSc such as fibrotic, contractility or migration.

The interest of studying the effect of IL6 track blockage has recently been limited by the discontinuation of TCZ development in scleroderma for the current period. This decision is justified by insufficient clinical efficacy and despite the demonstration of an undeniable biological effect; the argument is major in the therapeutic decision.

However, this study is extremely interesting if only for the precise and modern longitudinal phenotypic and molecular description of fibroblasts in scleroderma patients. These are fundamentally different from healthy fibroblasts. Cause or consequence of the SSc phenotype, the question is not resolved here.

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Visualisation of interstitial lung disease by molecular imaging of integrin αvβ3 and somatostatin receptor 2

Janine Schniering, Martina Benešová, Matthias Brunner, Stephanie Haller, Susan Cohrs, Thomas Frauenfelder, Bart Vrugt, Carol A Feghali-Bostwick, Roger Schibli, Oliver Distler, Cristina Mueller, Britta Maurer. Ann Rheum Dis. 2018 Nov.

The aim of this article was to evaluate two nuclear imaging markers that are specific for different stages of fibrosis in interstitial lung diseases (ILD). It is well known that HRCT and 18F-FDG-PET/CT show only morphological changes, they do not allow the discrimination of different pathophysiological stages of ILD (inflammation, active fibrotic remodelling or established fibrosis). So far, there is a lack of markers for disease staging, thus prediction of disease progression and drug response is poor. The most common pattern of SSc related ILD is non-specific interstitial pneumonia (NSIP), but author also described changes in idiopathic pulmonary fibrosis (IPF), which usually presents as usual interstitial pneumonia (UIP).

Two molecules of interest were integrin alpha-v-beta-3 (αvβ3) and somatostatin receptor 2 (SSTR2). Alpha v integrins are key molecules in the pathogenesis of fibrosis in ability to activate matrix bound latent transforming growth factor beta (TGF-β), the prototypical profibrotic cytokine in tissue fibrosis. αvβ3 activates TGF-β and establishes an autocrine-signalling loop in fibroblasts, thus driving myofibroblast differentiation.

Targeted imaging of integrin αvβ3 can be realised with arginine-glycine-aspartic acid (RGD) tripeptide-based radiotracers, which have already been validated. SSTR2 is a G-protein-coupled receptor, which is expressed on various cellular key players of lung remodelling, for example, epithelial cells, inflammatory cells and potentially fibroblasts.

SSTR2 can be targeted with a series of peptides, which are already part of the routine management of neuroendocrine tumours and have recently been proposed for the visualisation of fibrotic changes in experimental and human ILD. In this research authors used well-defined model of bleomycin (BLM)-induced lung fibrosis in mice and lung sections from patients with different types of ILD (IPF, SSc-ILD and other types of CTD-ILD).

In mice BLM-induced lung fibrosis (n= 10) inflammation was at the highest levels 7 days after intratracheal instillation of BLM, and collagen deposition reached its maximum at day 14. Compared with saline-treated controls (n=6), the lungs of BLM-challenged mice showed significantly increased expression of integrin αvβ3 and SSTR2 at all time points. αvβ3 was most abundant on day 7, compared to SSTR2, which gradually increased with the degree of lung remodelling and peaked on day 14.

In highly inflamed and fibrotic human lungs (n=39 for IPF; n=11 for SSc-ILD; n=9 for CTD-ILD), expression of integrin αvβ3 and SSTR2 was 3 to 4 fold increased ($p<0.05$) compared with lungs from healthy subjects (n=26). Expression was independent of the underlying etiological subtype of ILD. A significantly higher expression of SSTR2 was found in the lungs with UIP pattern compared with those with NSIP pattern ($p<0.01$). In contrast, the expression of integrin αvβ3 did not differ between both histological subtypes. Authors examined the expression of these molecules on different inflammatory cell types, including leucocytes, macrophages and T cells. Substantial expression of SSTR2 was found on pulmonary bronchial and alveolar epithelial cells, whereas expression of integrin αvβ3 was only rarely observed. While integrin αvβ3 was strongly expressed on the pulmonary vasculature, including endothelial cells, SSTR2 expression was not detected despite being expressed in vascular structures.

The most interesting difference in the pulmonary expression pattern between integrin αvβ3 and SSTR2 was the presence of integrin αvβ3, but absence of SSTR2 on myofibroblasts. Integrin αvβ3 was constitutively expressed in normal human lung fibroblasts (NHLF) and showed a time-dependent increase after TGF-β-induced fibroblast activation. In contrast, mRNA and protein levels of SSTR2 were undetectable in NHLF neither at basal conditions nor after stimulation with TGF-β.

The last part of the article was about integrin αvβ3 and SSTR2 as potential imaging targets in ILD on mice model. Nuclear imaging was performed on SPECT/CT scans by using the 177Lu-DOTA-RGD as target for integrin αvβ3 and 177Lu-DOTA-NOC as target for SSTR2 on days 3, 7 and 14 after the BLM instillation. Authors confirmed that 177Lu-DOTA-RGD-SPECT/CT visualized inflammatory stages of lung fibrosis which was the most expressed on day 7 and 177Lu-DOTA-NOC-SPECT/CT visualized established lung fibrosis which was the most expressed on day 14.

A molecular-based rather than a clinical/histological-driven classification as the basis for sub-stratification of patients with ILD might open novel perspectives. This study suggests that specific visualisation of molecular processes in ILD by targeted nuclear imaging is possibly feasible and can direct clinician towards personally specified treatment of ILD.

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Lysyl oxidase enzymes mediate TGF-β1-induced fibrotic phenotypes in human skin-like tissues

Huang M, Liu Z, Baugh L, DeFuria J, Maione A, Smith A, Kashpur O, Black Iii LD, Georgakoudi I, Whitfield ML, Garlick J.. Lab Invest. 2018 Dec 19

Cutaneous fibrosis is a common complication seen in mixed connective tissue diseases. It often occurs as a result of TGF-β-induced deposition of excessive amounts of collagen in the skin. TGF-β1 is the isoform most commonly associated with skin fibrosis, as elevated levels of activated TGF-β1 are found in the systemic circulation of patients with fibrotic disease and TGF-β1 levels in skin lesions are correlated with the degree of skin fibrosis. Lysyl oxidases (LOXs), a family of extracellular matrix (ECM)-modifying enzymes responsible for collagen cross-linking, are known to be increased in dermal fibroblasts from patients with fibrotic diseases, denoting a possible role of LOXs in fibrosis.

The authors developed two bioengineered, in vitro skin-like models: human skin equivalents (hSEs), and self-assembled stromal tissues (SASs) that contain either normal or systemic sclerosis (SSc; scleroderma) patient-derived fibroblasts. These tissues provide an organ-level structure that could be combined with non-invasive, label-free, multiphoton microscopy (SHG/TPEF) to reveal alterations in the organization and cross-linking levels of collagen fibres during the development of cutaneous fibrosis, which demonstrated increased stromal rigidity and activation of dermal fibroblasts in response to TGF-β1.

Specifically, inhibition of specific LOXs isoforms, LOX and LOXL4, in foreskin fibroblasts (HFFs) resulted in antagonistic effects on TGF-β1- induced fibrogenic hallmarks in both hSEs and SASs. In addition, a translational relevance of these models was seen as similar antifibrogenic phenotypes were achieved upon knocking down LOXL4 in tissues containing SSc patient-derived- dermal fibroblasts (SScDFs).
These findings point to a pivotal role of LOXs in TGF-β1-induced cutaneous fibrosis through impaired ECM homeostasis in skin-like tissues, and show the value of these tissue platforms in accelerating the discovery of anti-fibrosis therapeutics.