Systemic sclerosis
A bird's eye review of the recent literature

edited by C. Tani

Pathogenesis and new therapeutic targets

Authors: E. Hsu, H. Shi, R.M. Jordan, J. Lyons-Weiler, J.M. Pilewski, C.A. Feghali-Bostwick
Title: Lung tissues in patients with systemic sclerosis have gene expression patterns unique to pulmonary fibrosis and pulmonary hypertension
Arthritis Rheum 2011; 63; 783-94.
Summary: In this study the authors aimed at investigating the molecular profiling of lung tissues and primary fibroblasts from patients with systemic sclerosis (SSc) with varying degrees of pulmonary involvement (pulmonary fibrosis- PF- and pulmonary arterial hypertension- PAH- or both, n=33) compared to patients with idiopathic forms of lung disease (IPF n=10, or IPAH n=8) and normal lung samples.
This study absolutely represents the largest molecular profiling analysis of lung tissue from patients with SSc revealing, overall, unique gene expression profiles that partially matched those of corresponding idiopathic forms of lung disease; thus, these results provide additional important insights into the pathogenesis of SSc-related lung disease.
In detail, the authors found that in SSc-PF and SSc-PAH lungs, 73 and 83 genes, respectively, were differentially expressed compared to normal lungs and both SSc groups had concordant expression of 39 genes. Tissue from SSc-PF and IPF lungs shared 53 differentially expressed genes and SSc-PAH and IPAH lungs had concordant expression of 42 genes. Interestingly, the unsupervised clustering of all the lung samples showed clustering of samples by disease phenotype (PF or PAH) and similarities in gene expression in fibrotic lungs (SSc-PF and IPF) and PAH lungs (SSc-PAH and IPAH) were found. Genes that specifically showed increased expression in SSc-PF and IPF lungs included matrix metalloproteinase 7, insulin-like growth factor binding proteins, and osteopontin; on the other hand, the genes that were increased in SSc-PAH and IPAH lungs included chemokines and haemoglobin genes. Moreover, enriched functional groups analysis showed that fibrotic lungs were enriched for genes involved in fibrosis (genes for type I and type III collagen, IGFBPs, tissue inhibitors of metalloproteases 1, and interferon-γ receptor) and in IGF signalling (genes for IGFBP, secretory leukocyte peptidase inhibitor, and connective tissue growth factor) while PAH lungs shared functional groups enriched for genes for interferon, IL-4, IL-17, and antigen presentation signalling.
As far as lung primary fibroblast is concerned, in SSc-PF and SSc-PAH fibroblasts, 78 and 97 genes, respectively, were differentially expressed compared to normal fibroblasts and both groups shared 31 differentially expressed genes. Moreover, fibrotic lung fibroblasts and PAH lung fibroblasts shared 19 and 24 differentially expressed genes, respectively. In contrast to the lung tissue, genes involved in caveolar-mediated signalling were significant in SSc- PAH and IPAH fibroblasts but not in SSc-PF and IPF Fibroblasts.
This different gene fingerprinting results were confirmed by real-time polymerase chain reaction on the RNA level and immunohistochemistry on the protein level in both lung tissues and fibroblasts.

Authors: J. Wei1, D. Melichian, K. Komura, M. Hinchcliff, A.P. Lam, R. Lafyatis, C.J. Gottardi, O.A. MacDougald, J. Varga
Title: Canonical Wnt signalling induces skin fibrosis and subcutaneous lipoatrophy: a novel mouse model for scleroderma?
Arthritis Rheum 2011 Epub March 2
Summary: The Wnt family comprises 19 glycoproteins involved both in transcriptional regulation and in cell-cell adhesion; they exert their function via canonical and non-canonical intracellular pathways and ß-catenin plays a fundamental role in canonical signalling process. Recent evidence points to the potential importance of Wnts in fibrosis.
In this paper the authors reported interesting findings suggesting that canonical Wnt signalling might be an important novel antifibrotic target in SSc.
First of all, they found that Wnt10b expression in the skin from patients with SSc is significantly higher than in healthy controls both in epidermal cells as well as in numerous stromal cells within the dermis. Moreover, in the skin from mice with bleomycin-induced scleroderma Wnt10b expression and mRNA levels of Axin2, a canonical Wnt target gene, was significantly increased compared to the controls.
According to these observations, the authors utilised transgenic mice to investigate the effect of Wnt10b; in Wnt10b transgenic mice they observed a progressive loss of subcutaneous adipose tissue accompanied by dermal fibrosis, increased collagen deposition, fibroblast activation and myofibroblast accumulation. Interestingly, in transgenic mice, the collagen fibres had a woven architecture distinct from the nearly parallel pattern seen in wildtype mice.
Moreover, in this model, Wnt activity throughout the canonical Wnt signalling correlated with collagen gene expression.
In addition, cultures of skin fibroblasts explanted from Wnt10b transgenic mice showed a marked and persistent elevation in Wnt10b mRNA levels as well as in levels of the canonical Wnt target Axin2, Type I collagen, α-SMA and CTGF mRNA; on the other hand, there was a decrease in the expression of adipogenic markers FABP4, PPAR-γ1 and PPAR-γ2.

Finally, the observation that dermal fibrosis in transgenic mice was associated with a reciprocal reduction of subcutaneous adipose tissue, was confirmed by *in vitro* experiments that showed that ectopic Wnt10b inhibits adipogenesis.
These observations underline a possible reciprocal relationship between adipogenic and fibrogenic gene expression, and a crucial role for Wnt10b/ß-catenin signalling in regulating the balance between adipogenesis and fibrogenesis.

Authors: A.M. Bujor, Y. Asano*, P. Haines, R. Lafyatis, M. Trojanowska
Title: The c-abl tyrosine kinase controls PKCδ induced Fli1phosphorylation in human dermal fibroblasts
Arthritis Rheum 2011 Epub Feb 14
Summary: Recent studies have revealed that the serine/threonine kinase PKCδ is involved in collagen gene regulation in normal and SSc fibroblasts, presumably via a Fli1-dependent mechanism; Fli1 is a transcription factor highly expressed in endothelial and haematopoietic cells and at lower levels in fibroblasts, it has an important antifibrotic action by controlling the expression of various extracellular matrix genes.
In a previous work, the authors described that in response to TGFβ, Fli1 activity is repressed through a series of sequential posttranslational modifications, leading to Fli1 protein degradation.
In this study, the authors aimed to further investigate the upstream events that lead to Fli1 phosphorylation in response to TGFβ.
To this end, fibroblasts were isolated from SSc and matched control patients; Western blot, quantitative real time PCR and immunocytochemistry analysis were conducted.
The authors found that cultured SSc fibroblasts showed upregulation of P-Fli1; in addition, enhanced Fli1 phosphorylation correlated with an increase in Fli1 acetylation and collagen gene expression, suggesting that it could directly contribute to the pathogenesis of SSc fibrosis.
They also demonstrated that the activation of PKCδ in response to TGFβ and their nuclear traslocation is mediated through a c-abl-dependent mechanism; in fact, c-abl was required for the TGFβ induced phosphorylation of Fli1. Inhibition of c-abl signalling through the pharmacologic inhibitor Imatinib and specific siRNA against c-abl resulted in a significant increase (approximately 2-fold) in the total levels of Fli1, suggesting that c-abl is a negative regulator of Fli1 protein.
Thus, it could be argued that increasing or restoring the expression of Fli1 by blocking the TGFβ/c-abl/PKCδ/P-Fli1 pathway could represent an exciting potential therapeutic possibility against SSc fibrosis.

Authors: C. Beyer, J.H.W. Distler, Y. Allanore, M. Aringer, J. Avouac, L. Czirják, M. Cutolo, N. Damjanov, F. Del Galdo, K. Fligelstone, S. Guiducci, O. Kowal-Bielecka, J.M. van Laar, M. Martucci-Cerinic, U. Müller-Ladner, G. Riemekasten, I.H. Tarner, A. Tyndall, A. Tyrrell Kennedy, G. Valentini, S. Vettori, U.A. Walker, C. Denton, O. Distler; the EUSTAR Biobanking Group
Title: EUSTAR biobanking: recommendations for the collection, storage and distribution of biospecimens in scleroderma research
Ann Rheum Dis 2011 Epub Feb 1
Summary: Systemic sclerosis (Ssc) is a rare disease with multifaceted clinical presentations; in this context, multicentre and multinational large study cohorts have become of pivotal importance for clinical and biomolecular research. For this purpose, the European League Against Rheumatism Scleroderma Trials and Research Group (EUSTAR) has established an online database which, to date, has enrolled 8200 patients with SSc. Given that high-quality biospecimens are the basis for successful biomolecular studies, the EUSTAR biobanking group has developed recommendations to standardise the collection, storage and distribution of SSc biospecimens at EUSTAR centres.
In this paper, the authors proposed evidence-based approaches and concepts of good laboratory practice which cover the following topics: collection and processing, storage, biological material tracking, retrieval, packaging and shipping, legal and ethical issues, standardised protocols, quality control and quality assurance, training and safety.
The authors encourage the strict adherence to these recommendations in daily laboratory routine in order to participate in EUSTAR biobanking; in addition, these recommendations could represent a useful tool for other SSc consortia to plan exchange of biosamples between different SSc initiatives.
Finally, the authors underlined that such an initiative is absolutely the first in the rheumatologic field, thus it could represent a useful example for similar projects in other rheumatic diseases.

Treatment

Authors: The Idiopathic Pulmonary Fibrosis Clinical Research Network
Title: A controlled trial of sildenafil in advanced idiopathic pulmonary fibrosis
N Engl J Med 2010; 363: 620-8.
Summary: Idiopathic pulmonary fibrosis is a chronic, progressive lung disease of unknown origin for which no pharmacologic therapies have definitively been shown to improve survival or patients' quality of life.
In this study, the Sildenafil Trial of Exercise Performance in Idiopathic Pulmonary Fibrosis (STEP-IPF), the authors aimed to evaluate the effect sildenafil, a phosphodiesterase-5 inhibitor, on functional and respiratory performances, as well as quality of life in patients with advanced idiopathic pulmonary fibrosis.

A total of 303 patients with a diagnosis of idiopathic pulmonary fibrosis with a diffusing capacity for carbon monoxide of less than 35% of the predicted value were eligible for this 12-week double-blind, randomised, placebo-controlled trial of oral sildenafil; of these, 180 were enrolled (89 and 91 in the sildenafil and placebo group, respectively).

The primary outcome was an improvement of at least 20% in the 6-minute walk test at 12 weeks, as compared with baseline.

No significant differences were observed between groups in terms of improvement in the 6-minute walk, nor in mortality rate or in the rate of acute exacerbations of idiopathic pulmonary fibrosis. Moreover, there were no significant differences in the occurrence of adverse events.

However, the authors found clinically meaningful differences favouring sildenafil in some secondary outcomes, including the arterial oxygenation, carbon monoxide diffusion capacity, degree of dyspnea, and quality of life.

In conclusion, although the primary outcome has not been reached, sildenafil was associated with symptomatic improvement in such advanced patients, thus this data could represent an intriguing starting point to conduct further studies.