

FINAL REPORT

Novel approaches to scleroderma using animal models and biomarker studies

Overview

During the period of this research award (2010-2011), we have made excellent progress. The proposal focussed on scleroderma lung disease. **The goals of the research project generously supported by the KBSF Award were two-fold: to develop a novel mouse model of scleroderma lung disease, and to identify biomarkers for scleroderma that could be ultimately used in the clinical setting to evaluate the activity, severity or progression of fibrotic complications of the disease.**

Our **Aim 1** was to develop a new model of scleroderma lung disease. Animal models of disease are vital research tools for both understanding the cause and mechanism of damage, as well as for testing potential new treatments (preclinical testing). Currently there are no good models for scleroderma. Moreover, a commonly used mouse model for lung injury, which is the instillation of the anti-cancer drug bleomycin into the airways, is not a very accurate model to mimic lung disease occurring in patients with scleroderma. The approach we are taking is to induce chronic lung fibrosis in normal C57BL mice by injecting bleomycin directly into the skin (subcutaneous), rather than by intratracheal bleomycin installation. The commonly used intratracheal approach results in an acute ARDS-like pathology with mild and reversible lung fibrosis. We believe that this model is not particularly suitable to study potential scleroderma treatments. The lung pathology seen with the intratracheal bleomycin model is quite different from the pathology seen in patients with scleroderma lung disease. Therefore, this approach has little – if any – utility for studying scleroderma-associated lung disease, and for evaluating the efficacy of potential new treatments.

We believe that the results of our pilot studies supported by the KBS Award bring us a step closer to having a bona fide mouse model for scleroderma-associated lung fibrosis. As shown in the histology figure, subcutaneous injection of bleomycin (right panel) causes slowly progressive lung fibrosis which is centered around the blood vessels. This pattern of changes is congruent with scleroderma lung disease. In contrast, when mice are given intratracheal bleomycin (ie. installation directly into the airways), they develop less severe, transient and bronchiolocentric lung fibrosis that only imperfectly recapitulates scleroderma lung disease (left panel).

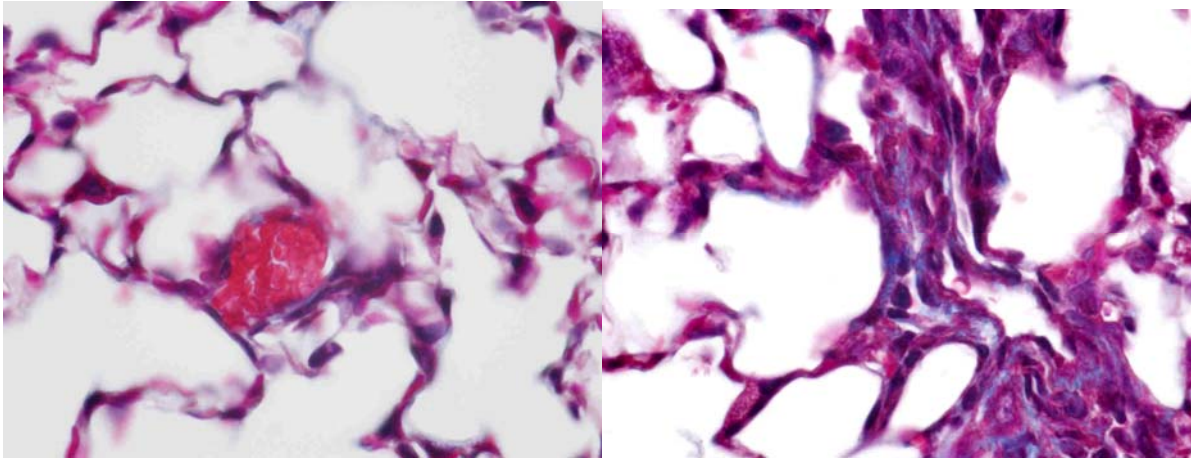


Figure 1. Bleomycin-induced lung fibrosis in C57BL mice. Left, intratracheal installation of bleomycin, right, subcutaneous injection of bleomycin for 14 days, and mice sacrificed on day 21. The lungs were removed, fixed and stained with trichrome (blue identified excessive collagen). Note in right panel the severe fibrosis and perivascular fibrosis and collagen accumulation resembling the lesion seen in patients with scleroderma-associated pulmonary fibrosis. This is not seen in the lungs from mice that received intratracheal bleomycin (left panel). Representative images.

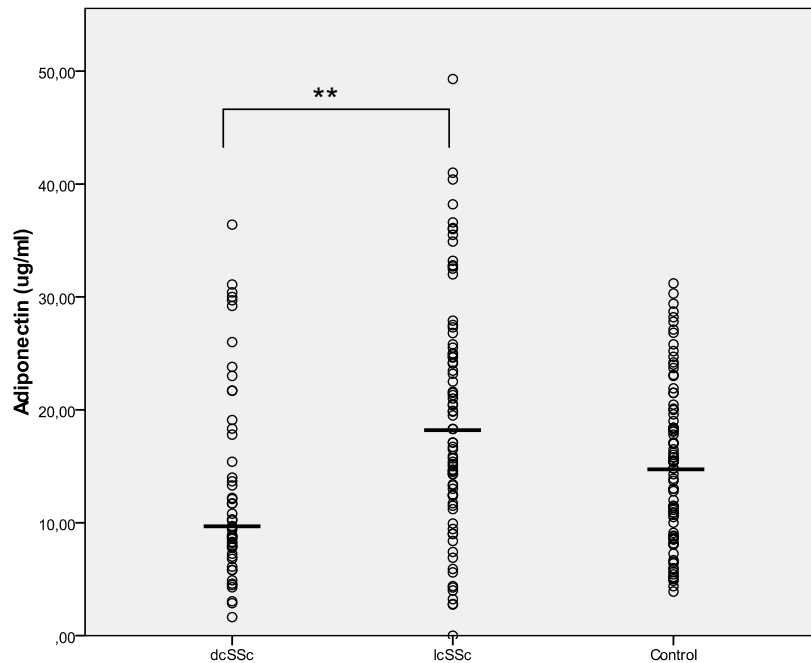
The goal of Aim 2 is the discovery and validation of novel biomarkers. Biomarkers are serum components that can be a) readily measured and b) used in both clinical research and clinical practice. The purpose of a biomarker is to be used as a clinical tool to assess disease extent, severity and activity, monitor for progression or regression with treatment, and for use in therapeutic trials. We are using ELISA and Multiplex assays for measuring a series of proteins and bioactive lipids in the serum, and correlating levels with clinical parameters.

In initial studies we examined the levels of a protein called adiponectin in the serum. We focused on adiponectin because it is produced under the influence of PPAR-gamma, and we had previously shown that PPAR-gamma is defective in patients with scleroderma. We therefore reasoned that low PPAR-gamma, as measured by the levels of adiponectin, might be a reflection of activity or severity of scleroderma. We studied a multi-ethnic cohort of 129 clinically well-characterized scleroderma (SSc) patients using the multiplex assay. 50 diffuse cutaneous (dcSSc) and 79 limited cutaneous SSc (lcSSc) patients were compared with 86 healthy Caucasian controls. The patients are drawn from the Northwestern scleroderma program biorepository. These samples are obtained with informed consent approved by the IRB, and are annotated and stored prior to testing. The table below indicates the demographic features of the study subjects and the control.

	SSc (129 sera)	Blood donors (86 sera)
Age	52 (21-82) years	43 (19-64) years
Gender	20M; 109F	56M; 30F

Ethnicity	91 Caucasian, 17 African- Americans; 7 Asian; 9 Hispanic; 2 other	all Caucasian
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As shown below, patients with diffuse cutaneous SSc have significantly reduced levels of serum adiponectin. Moreover, adiponectin levels in SSc correlate with extent of skin involvement (Rodnan skin score), suggesting potential utility of this assay as a clinical biomarker of fibrosis. This however will need to be independently validated in larger patient cohorts.



These data will be further analyzed using appropriate statistical tests, including Kruskal-Wallis, Spearman’s rank correlation, and Bonferroni correction for multiple comparisons. We will also examine how age, gender, race, BMI and disease duration might independently influence adiponectin levels. Importantly, we will seek to validate these findings in other independent patient cohorts. This step is important in order to assess the generalizability of our findings. In subsequent studies that we are initiating, we will examine variations (polymorphisms) in the gene for PPAR-gamma in a cohort of scleroderma patients and correlate the presence of specific genetic variants with serum levels of adiponectin. We hypothesize that patients with low activity of PPAR-gamma (hypofunctional) have reduced production of adiponectin, and therefore will have low serum levels of this protein. These findings might a) indicate a

potential genetic basis for reduced PPAR-gamma activity; and b) lead us in the direction of novel therapies targeting the PPAR-gamma pathway to control or prevent fibrosis in scleroderma.

The results summarized in this report are currently being finalized for submission to peer-reviewed publications.