

2016 Scleroderma Foundation Research Grant Awardees

SANDEEP AGARWAL, M.D., PH.D. - BAYLOR COLLEGE OF MEDICINE



Project: Targeting Stat3 in Systemic Sclerosis and Dermal Fibrosis

Scleroderma (systemic sclerosis, SSc) is a devastating multisystem disorder clinically manifesting as progressive fibrosis of skin and visceral organs. There is a clear, unmet need to advance understanding of the pathogenesis in order to identify novel SSc therapeutic targets. STAT3 is one such candidate. STAT3 is a transcription factor that is activated by IL-6 family cytokines. Our preliminary data demonstrate that STAT3 is activated (phosphorylated) in human fibrotic lungs and affected skin from patients with SSc. Furthermore, inhibition of STAT3 phosphorylation with a novel small-molecule inhibitor (C188-9), administered in the fibrotic phases of the model, decreases lung and skin fibrosis induced by bleomycin. Finally, preliminary in vitro studies demonstrate that STAT3 regulates TGF-beta responses, but it is not completely understood how that occurs. We hypothesize that Stat3 phosphorylation regulates TGF-beta responses, is an important driver of the development of fibrosis, and is an excellent potential therapeutic target in SSc. The current proposal seeks to understand the extent to which STAT3 contributes to the development of dermal fibrosis and regulates TGF-beta responses. In Aim 1, we will utilize C188-9 to determine the extent to which STAT3 inhibition prevents and treats the development of dermal fibrosis in the subcutaneous bleomycin dermal fibrosis model and the Tsk/+ mouse model. In Aim 2, we will also use a cell lineage specific targeting of STAT3 to investigate which cellular populations (macrophages or fibroblasts) are regulated by STAT3 during the development of dermal fibrosis. Finally, in Aim 3, we will define the mechanisms that underlie the cross-talk between TGF-beta and in murine and human SSc dermal fibroblasts. These data, combined with the preliminary data, should truly bring the role of STAT3 activation in dermal fibrosis and SSc to a significant level of understanding and provide the foundation for the potential of STAT3 as a therapeutic target in SSc.

SWATI BHATTACHARYYA, PH.D. - NORTHWESTERN UNIVERSITY, FEINBURG SCHOOL OF MEDICINE



Project: Profibrotic Role of TLR4 in Scleroderma: A Novel Target for Therapy

Fibrosis, the hallmark of scleroderma, represents the transformation of normal wound-healing into a deregulated self-sustaining process, thus contributing to high morbidity and mortality of this disease. While multiple intracellular signaling pathways are implicated in scleroderma fibrotic responses, the nature of their persistent deregulation in pathological inflammation and fibrosis remain poorly understood. Elucidation of the mechanism underlying the switch from self-limited repair to intractable scar is essential for designing rational therapies for scleroderma. Our recent studies demonstrated that an immune signaling receptor toll-like receptor 4 (TLR4) and its endogenous ligand fibronectin-EDA and tenascin C—are markedly elevated in scleroderma skin. Stimulation of TLR4 in fibroblasts was associated with the induction of extracellular matrix remodeling and tissue repair programs as well as synergistic enhancement of TGF-beta-mediated fibrotic responses. Thus, in a fibrogenic milieu enriched with TGF-beta and endogenous TLR4 ligands, fibroblasts expressing elevated TLR4 cause uncontrolled collagen synthesis and myofibroblast differentiation, contributing to progression of fibrosis. Disrupting persistent TLR4 signaling with a novel small-molecule TLR4 inhibitor with unique mechanism of action (by blocking ligand interaction to its receptor) represents a potential strategy for breaking the vicious cycle of progressive fibrosis in scleroderma.

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In this proposal, I will evaluate the effect of a novel small-molecule TLR4 inhibitor for anti-fibrotic efficacy in vivo in complementary mouse models of scleroderma using prevention and regression approaches, in ex vivo skin equivalent model mimicking scleroderma skin. To investigate the fibroblast-specific function of TLR4, I will generate mice with conditional TLR4 deletion in mesenchymal cells and determine how TLR4 loss modulates the development of skin inflammation and fibrosis. Finally, I will derive a genome-wide TLR4 responsive gene signature by overexpressing constitutively active TLR4 in fibroblasts. This will be an important step for developing targeted anti-TLR4 therapy for scleroderma for identifying optimal responders likely to benefit in future clinical trials (beyond the scope of this proposal). As scleroderma has no approved viable therapy, and its multiple molecular subsets remain poorly understood, successful accomplishment of these goals comprising mechanistic and preclinical therapeutic approaches is expected to lead to rapid translation into clinical trials for scleroderma.

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GALINA BOGATKEVICH, M.D., PH.D. - MEDICAL UNIVERSITY OF S.C THE MARK FLAPAN AWARD



Function and Antifibrotic Mechanism of M10 in Scleroderma

Interstitial lung disease (ILD) is a major complication and the leading cause of mortality in scleroderma (systemic sclerosis, SSc). The molecular mechanisms underlying the pathogenesis of SSc-ILD are not well understood, and there is a great need for more effective treatment. Recently, we identified a C-terminal fragment of MET, designated as “M10,” as a peptide with strong antifibrotic properties. Our preliminary data demonstrate that M10 has robust antifibrotic effects in the bleomycin-induced mouse model of lung fibrosis and in human lung fibroblasts. Our overarching hypothesis is that M10 is a natural antifibrotic peptide generated from MET that negatively modulates TGF- β signaling pathways. The goals of this project are to identify signal transduction pathways and molecular mechanisms mediating antifibrotic effects of M10, and to determine the therapeutic potential of M10 in vivo in a preclinical model of scleroderma lung disease. To accomplish these goals, we propose two specific aims. Aim 1 is to define signaling pathways and downstream targets of M10 in SSc lung fibroblasts. We propose that M10 binds Smad-ubiquitination-related factor (Smurf)2 targeting type 1 TGF- β receptors (T β RI) for ubiquitin-mediated degradation. Additionally, M10 directly binds Smad2, interfering with its downstream pathways in scleroderma fibroblasts. We will determine M10-mediated negative regulation of TGF- β and Smad signaling in scleroderma fibroblasts and in TGF- β -stimulated normal fibroblasts by studying ubiquitination of T β RI, phosphorylation of Smad2/3, and transcription of TGF- β -induced genes. Aim 2 is to determine the efficacy of M10 in reducing inflammation and fibrosis in vivo in a bleomycin-induced murine model of scleroderma lung disease. We will perform pharmacokinetic and biodistribution studies and will examine therapeutic effects of M10 on bleomycin-induced scleroderma-like disease. The completion of these specific aims will provide important mechanistic information to clarify antifibrotic effects of M10 in SSc. Furthermore, it will provide fundamental, preclinical information about the feasibility and efficacy of M10 as a new therapeutic approach for the treatment of SSc and, particularly, SSc-ILD.

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**ROBERTA MARANGONI CONCALVES, M.D., PH.D. - NORTHWESTERN UNIVERSITY
FEINBERG SCHOOL OF MEDICINE**



Project: Adiponectin Drives Fibrosis in Systemic Sclerosis

The pathogenesis of fibrosis in systemic sclerosis (SSc) is poorly understood, and effective treatments are lacking. Recent findings point to a previously unappreciated, complex relationship between intradermal adipose tissue (IAT) and fibrosis in SSc. Adipocytes are not simply a storage reservoir of fat; they critically control metabolism as an endocrine organ by secreting adipokines. Adiponectin is the most abundant adipose-specific adipokine with anti-fibrotic activities. Loss of IAT is associated with dermal fibrosis in SSc and precedes the onset of dermal fibrosis in mouse models of scleroderma, suggesting that it is a primary event in pathogenesis. Our preliminary

results showed reduced levels of adiponectin in the serum and skin in SSc and an inverse correlation with extent of skin fibrosis. Adiponectin has potent anti-fibrotic effects, and loss of adiponectin in the mouse results in exacerbated fibrosis. I hypothesize that loss of IAT results in persistent reduced levels of adiponectin in SSc that contributes to fibroblast activation and progression of skin fibrosis. In this proposal I will elucidate the mechanisms by which decreased adiponectin is permissive to skin fibrosis in animal models of scleroderma and determine the effects of adiponectin agonists in skin fibrosis as a potential novel therapeutic strategy. These results will contribute to a better understanding of fibrosis and indicate if augmenting adiponectin may be a potential approach to therapy in SSc.

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VALERIE HORSLEY, PH.D. - YALE UNIVERSITY MARIE A. COYLE RESEARCH GRANT



Project: Functional Analysis of the Contribution of Adipocytes to Scleroderma's Skin Fibrosis

Systemic sclerosis, an autoimmune disease of unknown origin, is characterized by progressive fibrosis that leads to excessive extracellular matrix protein deposition and increased contractile fibroblasts within the stroma of many organs, including the skin. A major outstanding question in the field is how fibrosing cells arise within the stroma of different tissues. The identification of specific cells and molecules that control fibrosis is in its early stages and has the potential to ameliorate systemic sclerosis. Interestingly, dermal adipocytes are lost during skin fibrosis, yet how these cells function during fibrosis development is not well understood. The overall objective of this proposal is to define whether mature adipocytes interact with fibroblasts to influence

skin fibrosis. We will use a combination of in vivo mouse models of scleroderma combined with mouse and human cell cultures models to define if adipocytes can alter fibroblast function during fibrosis development. We will also use metabolic assays to characterize fibroblast metabolism during fibrosis to determine whether fibroblast metabolism can influence fibrosis development and to identify whether adipocytes can modulate fibroblast metabolism to alter fibrosis development. These experiments will allow us to better understand how fibroblasts are altered during skin fibrosis, a key presentation of scleroderma. We will also be able to use these experiments to define potential therapeutics for fibrotic phenotypes.

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HUANXING SUN, PH.D. - YALE UNIVERSITY



Project: Regulation of Leukocyte: Matrix Interactions by Netrin-1 in Scleroderma ILD

Many patients with scleroderma (systemic sclerosis, SSc) are affected by interstitial lung disease (SSc-ILD), and pulmonary involvement is the leading cause of death for patients with this disorder. Current therapies fail to target the specific factors driving disease and are not curative. It is likely that elucidation of the mechanisms regulating SSc-ILD pathogenesis will enable development of more effective interventions. Pathologic examination of SSc-ILD lung tissue reveals the presence of inflammatory leukocytes juxtaposed to normal and diseased-appearing extracellular matrix (ECM). The importance of these leukocyte:ECM interactions is unknown, but we speculate that they may critically regulate disease activity. The ECM is an essential component of all tissues that supply

three-dimensional (3D) architecture required for organ structure and the adhesion, proliferation and differentiation of many cell types. Therefore it is notable that fibrocytes—a leukocyte-derived population of cells with the inflammatory properties of monocytes and the tissue remodeling properties of fibroblasts—have been detected in the lungs and blood of patients with SSc. Recent work from our group and others shows that the bioengineering-based methods that were originally constructed for the development of biomimetic, organotypic lung tissue can be adapted for the study of innate and adaptive immune cell subsets and mesenchymal cells in a variety of modeling systems. We refined this strategy

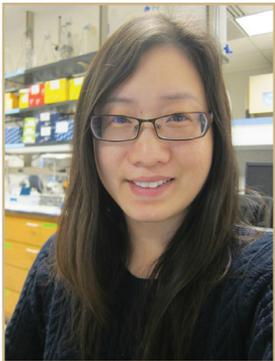
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HUANXING SUN, PH.D. (CONTINUED)

to study leukocyte:ECM interactions in SSc-ILD by employing decellularized human lung explants from patients with scleroderma. Our data indicate that peripheral blood mononuclear cells (PBMCs) from subjects with and without SSc-ILD grown in decellularized SSc-ILD lungs adopt a mesenchymal phenotype consistent with fibrocytes and that SSc-ILD leukocytes display enhanced responsiveness to the fibrotic lung scaffold. These findings are enacted via a mechanism involving the neuronal guidance protein Netrin-1, which controls mechanotransduction and cell:matrix interactions via a pathway involving an adaptor protein called growth factor receptor bound protein-2 (GRB2). Preliminary data indicates this pathway regulates fibrocyte accumulation in our ex vivo modeling system and regulates experimentally induced lung fibrosis and fibrocyte accumulation in the bleomycin mouse model of pulmonary fibrosis. However, it is not clear whether these processes are regulated via Netrin-1 mediated mechanotransductive responses to the excessive stiffness of the SSc-ILD lung or via specific interactions with diseased lung ECM proteins. In addition, the contribution of GRB2 has not been defined. This grant will test the hypothesis that Netrin-1 regulates leukocyte:matrix interactions, fibrocyte accumulation and experimentally induced lung fibrosis via the combinatorial influence of ECM components and mechanotransduction. In Aim 1 we will use PBMCs obtained from SSc-ILD and control subjects and a novel bioengineering-based culture platform of “tunable” hydrogels constructed from purified human lung ECM to decouple the contribution of ECM proteins and tissue stiffness to Netrin-1’s modulation of leukocyte differentiation and fibrocyte development. The contribution of GRB2 will be evaluated as well. In Aim 2 we will use mice with cell-specific deletion of Netrin-1 to define the therapeutic potential and mechanism of Netrin-1 and GRB2 in the regulation of fibrosis in two separate models of SSc-ILD. It is hoped that these studies will result in improved understanding of SSc-ILD and perhaps facilitate development of mechanism-based therapies for patients with this disease.

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PEI-SUEN (ELIZA) TSOU, PH.D. - UNIVERSITY OF MICHIGAN MARTA MARX FUND FOR THE ERADICATION OF SCLERODERMA



Project: Histone Deacetylases in Scleroderma: Investigation of Their Roles in Dysregulated Angiogenesis

Scleroderma (SSc) is a multifactorial disorder that is characterized by early inflammation, excessive extracellular matrix deposition, and vasculopathy including dysregulated angiogenesis. Vascular abnormalities represent a fundamental event in the pathogenesis of SSc in that the endothelial cell (EC) damage triggers a self-fueling process ending in pathological tissue fibrosis. Therefore, therapies aimed at preventing the loss of normal vasculature in SSc or increasing the amount of compensatory angiogenesis would have a significant impact on the lives of these patients. In this proposal, ECs isolated from SSc skin will be utilized to examine the role of epigenetic mechanisms, specifically histone deacetylases, on dysregulated angiogenesis in SSc.

Preliminary data showed that these cells have a reduced response to pro-angiogenic stimuli such as vascular endothelial growth factor (VEGF). In addition, the expression of histone deacetylases (HDACs) appears to be dysregulated in these cells. Interestingly, in addition to determining the acetylation status of histones, HDACs are also involved in controlling endothelial function. Specifically, HDAC5, which is overexpressed in SSc ECs, appears to be anti-angiogenic. Phosphorylated HDAC7, which is absent in SSc ECs, is involved in VEGF-mediated angiogenesis. Taken together, we propose the following hypotheses: (1) the overexpression of HDAC5 in SSc ECs gears these cells to an anti-angiogenesis state by repressing pro-angiogenic genes; (2) the machinery of VEGF-induced phosphorylation and nuclear export of HDAC7 is impaired in SSc ECs, leading to their inability to respond to VEGF. Methodologies used in this proposal

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PEI-SUEN (ELIZA) TSOU, PH.D. (CONTINUED)

include in vivo and in vitro angiogenesis assays, assay for transposase-accessible chromatin coupled with sequencing (ATAC-seq) and RNA-seq to determine the gene profiles governed by HDAC5, chromatin immunoprecipitation with sequencing (ChIP-seq) to determine HDAC7-repressed genes, and various biochemical assays to examine the nuclear-cytoplasmic shuttling mechanism of HDAC7 in SSc ECs. Few studies deal with the actual mechanism of how dysregulated angiogenesis in SSc may occur, largely due to the difficulty in isolating ECs from skin biopsies. We have mastered the skills in purifying ECs from both healthy subjects and SSc patients and utilized these cells in various studies. With this valuable tool and the novel concepts proposed in this application, we will be able to evaluate SSc angiogenesis in ways not previously explored. Moreover, identifying the mechanisms of reduced angiogenesis in SSc skin is critical to the development of therapies aimed at promoting blood vessel growth in the skin of these patients. The study design and techniques developed through this work will also be applicable to other diseases associated with angiogenesis issues.

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KATHRYN TOROK, M.D. - CHILDREN'S HOSPITAL OF PITTSBURGH AND UNIVERSITY OF PITTSBURGH SCLERODERMA CENTER SCLERODERMA FOUNDATION MULTI-CENTER RESEARCH GRANT (SCORE)



Project: Identifying Juvenile Scleroderma Immunophenotype Subsets

Scleroderma disorders in children encompass systemic sclerosis (SSc) and localized scleroderma (LS). Both forms are characterized by an initial inflammatory phase followed by a later fibrotic phase of collagenization, skin thickening and atrophy. Skin biopsies are typically indistinguishable between SSc and LS, suggesting shared pathophysiology, though clinical differences indicate that each disease also has unique pathways. Most translational studies in scleroderma focus on adult-onset disease. There are, however, important differences between adult and childhood disease, including the high frequency of extracutaneous involvement in juvenile LS, low frequency of anti-centromere antibody and different specific HLA associations in juvenile SSc, and the unique morbidity of abnormal growth and development for both juvenile LS and SSc. Our overarching

goal is to identify specific immunophenotypes of pediatric SSc and LS in order to elucidate pathogenic pathways and biologically define clinical subsets of disease to enable improved management and outcomes. The proposed collaboration between pediatric SCORE sites (University of Pittsburgh, Seattle Children's Research Institute and Hackensack University) and the Childhood Arthritis and Rheumatology Research Alliance (CARRA) will take advantage of the established strengths of each site and the power of the CARRA registry and biorepository to enable the feasibility of this large-scale project. We hypothesize that a combination of clinical features with antibody, cellular and cytokine profiles will both categorize and predict immunological/clinical subtypes of pediatric scleroderma. Defining subtypes will greatly augment the clinician's ability to screen for potential organ involvement, assess disease activity, and predict treatment responses, enabling better patient stratification for clinical trials. Novel data analyses utilizing a data-mining platform strategy will allow these immunological subtypes to be identified. The main aims of the proposal include the determination of immunophenotype, circulating cytokine signature, and antibody profiling of SSc and LS in context of detailed clinical features, standard inflammatory laboratories and longitudinal outcome measures.