search Grant Awardee:

ING SUN, PH.D. • YALE UNIVERSITY

Project: Regulation of Leukocyte: Matrix Interact

Many patients with scleroderma (systemic sclerosis, SSc disease (SSc-ILD)), and pulmonary involvement is the 1 disorder. Current therapies fail to target the specific curative. It is likely that elucidation of the mechanisms enable development of more effective interventions. P. tissue reveals the presence of inflammatory leukocyte appearing extracellular matrix (ECM). The importance is unknown, but we speculate that they may critically o component of all tissues that supply three-dimensional (3D) archit. Proliferation, and differentiation of many cell types. Therefore, the bioengineering-based methods that were originally construct can be adapted for the study of innate and adaptive i variety of modeling systems. We refined this strategy to study leuko decellularized human lung explants from patients with scleroder. Mononuclear cells (PBMCs) from subjects with and without SSc-ILD graft chymal phenotype consistent with fibrocytes and that SSc-ILD leuko. The findings are enacted via a mechanism involving the neuron guidance protein, which controls mechanotransduction and cell/matrix interactions via a pathway involving an adaptor protein with factor receptor bound protein-2 (GRB2). Preliminary data indicates this pathway regulates fibrocyte fication in our ex vivo modeling system and regulates experimentally induced lung fibrosis and fibrocyte ation in the bleomycin mouse model of pulmonary fibrosis. However, it is not clear whether these processes lated via Ntn-1 mediated mechanotransductive responses to the excessive stiffness of the SSc-ILD lung or tic interactions with diseased lung ECM proteins. In addition, the contribution of GRB2 has not been defined. nt will test the hypothesis that Ntn-1 regulates leukocyte:matrix interactions, fibrocyte accumulation and entally induced lung fibrosis via the combinational influence of ECM components and mechanotransduction. we will use PBMCs obtained from SSc-ILD and control subjects and a novel bioengineering-based culture of “tunable” hydrogels constructed from purified human lung ECM to decouple the contribution of ECM and tissue stiffness to Ntn-1’s modulation of leukocyte differentiation and fibrocyte development. The t of GRB2 will be evaluated as well. In Aim 2 we will use mice with cell-specific deletion of Ntn-1 gene to evalu. The therapeutic potential and mechanism of Ntn-1 and GRB2 in the regulation of fibrosis in two separate of SSc-ILD. It is hoped that these studies will result in improved understanding of SSc-ILD and perhaps facilitate ment of mechanism-based therapies for patients with this disease.

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 gear 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 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-cytosolic 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.

KATHRYN TOROK, M.D. • CHILDREN'S HOSPITAL OF PITTSBURGH AND UNIVERSITY OF PITTSBURGH

SCLERODERMA CENTER • THE KAO FAMILY FOUNDATION SCORE GRANT

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