Case Study

Uncovering mechanistic insights for systemic sclerosis

Problem: The causative mechanism of systemic sclerosis is poorly understood

Systemic sclerosis is a rare and complex autoimmune disease classically characterized by the thickening and changing appearance of the skin. In more severe forms of the disease, other organs such as blood vessels, muscles, heart, digestive system, lungs, and kidneys can all also be affected. Despite its rarity, mortality associated with systemic sclerosis is higher when compared to other rheumatic diseases[1,2].

The primary pathology associated with systemic sclerosis symptoms is an overproduction of collagen, which is a component of connective tissue. The underlying cause of collagen overproduction—which can result in severe complications involving heart failure, kidney failure, high blood pressure, and cancer—remains elusive.

Currently available treatments include anti-inflammatory therapies such as corticosteroids, non-steroidal anti-inflammatory drugs, and other immunosuppressants, but these approaches are ineffective for many patients. To develop more targeted and effective treatments, it is critical to advance our understanding of the underlying mechanisms and pathogenesis of systemic sclerosis.

Solution: Identifying genes associated with systemic sclerosis via expression profiling

For approximately the last two decades, genome- and transcriptome-wide association studies have become an integral strategy for understanding the mechanisms underlying complex autoimmune diseases like systemic sclerosis. Several previous studies have identified differentially-expressed genes in systemic sclerosis associated with cardiovascular health and fibrotic pathways[3,4]. Other studies have implicated dysfunction of the immune system, extracellular matrix, DNA/RNA degradation, and apoptosis/autophagy pathways[5,6].

In a recent study by Xu *et al.* that employed powerful new statistical techniques, two different microarray datasets of skin biopsy samples from healthy and systemic sclerosis patients were studied, uncovering a total of 106 differentially-expressed genes that were common for systemic sclerosis samples between these two datasets[7].

After corroborating the two studies, Xu *et al.* used a STRING online database to predict protein-protein interactions specific to these differentially expressed genes. From this analysis, the authors found 10 hub genes associated with systemic sclerosis (*at right*).

The ability of each of these hub genes to independently predict systemic sclerosis disease was confirmed by respective receiver operator characteristic curve analysis, further demonstrating the power of expression profiling.

The ten hub genes included:

- Regulators of immunity (toll-like 4, interleukin-6, and suppressor of cytokine signaling 3)
- Regulators of fibrotic/growth pathways (cysteine rich angiogenic inducer 61 and bone morphogenetic protein 4)
- A regulator of extracellular matrix (tenasin C)
- Regulators of cell function (calumenin, secretogranin II, and serine protease 23)
- A regulator of apoptosis (clusterin)

Context: Using differential expression for mechanistic insight

Categorically breaking down the associated hub genes provides a specific framework for researchers and clinicians to begin to understand the various levels of dysregulation that occur in systemic sclerosis. As previous studies have implicated dysregulated immune pathways in systemic sclerosis pathology, interleukin-6 (IL-6) has emerged as a particularly interesting molecule that may be predictive of as well as a target of treatment for this disease.

IL-6 is a cytokine involved in a number of different immunological processes. While it acts as a pro-inflammatory cytokine in most tissues (*Figure 1*), it also produces anti-inflammatory effects when released from muscles as a myokine[8]. Its primary role is to help differentiate pro-inflammatory cytotoxic T cells and Th17 helper cells, decrease anti-inflammatory regulatory T cells, and to activate B cells to stimulate immune responses during the active phase immunological response[9,10]. The diverse immune-related functions of IL-6 suggest that its dysregulation could have important ramifications for autoimmune diseases.

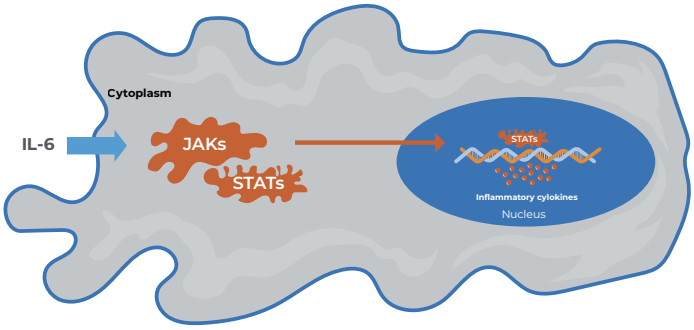

***Figure 1**. Simplified graphic of the IL-6 signaling pathway. Adapted from Chen* et al[11].

Indeed, in systemic sclerosis patients, increased IL-6 has been found in skin samples[12] and serum[13,14] versus healthy controls. IL-6 is correlated with alterations in B cell homeostasis[15], and pulmonary fibrosis[16] in systemic sclerosis patients. Lastly, it has recently been found that the IL-6/signal transducer and activator of transcription 3 (STAT3) axis is activated in various cell types of systemic sclerosis patients, with endothelial cells having particularly high expression[17].

In the bleomycin-induced mouse model of systemic sclerosis, deletion of the IL-6 gene reduced myofibroblast numbers and disease severity[18]. Genetic profiling of IL-6 and related pathways (*Figure 2*) is a powerful strategy to begin to understand the potential efficacy of anti-IL-6 treatments for systemic sclerosis.

Opportunities for applying the HTG EdgeSeq Immune Response Panel

Collectively, these findings highlight the potential benefit of gene expression analysis based on key immune regulatory networks. A focus on genetic profiling of IL-6 and its related signaling pathways could provide a framework to assess differential gene expression across potential therapies that target IL-6 pathways.

The HTG EdgeSeq Immune Response Panel includes immunity regulators highlighted by Xu *et al.* as well as several downstream signaling molecules in the IL-6 pathway such as the IL-6 receptor and genes in the JAK and STAT families. Gene expression profiling of the carefully curated 2,002 genes in the HTG EdgeSeq Immune Response Panel in patients with systemic sclerosis and related rheumatic diseases could identify additional regulatory pathways that help uncover mechanistic insights and highlight potential therapeutic strategies.

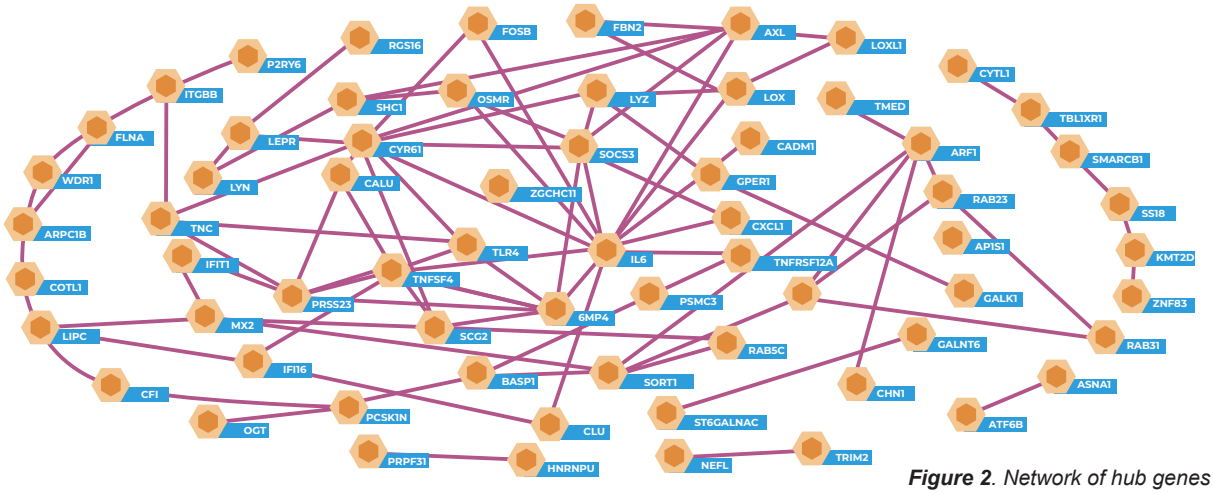

***Figure 2**. Network of hub genes identified by Xu* et al[7] *with IL6 at the center.*

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