Gene Signature in Systemic Sclerosis Modulated by a Human IgG1 Monoclonal Antibody that Neutralizes EphrinB2

Benjamin Wilks[1], Gabriela Dower[1], Juan-Pablo Zertuche[1], Gabriela Bunu[1], Allie Roach[1], Rezi Zawadski[1], Paul Yaworsky[1]

[1]Mediar Therapeutics, Boston, MA 02215, USA www.mediartx.com

Mediar Therapeutics

Background

Systemic sclerosis (SSc) is a progressive fibrosing disease of unknown etiology that affects multiple organ systems and has the highest mortality rate of any rheumatologic disease. Genetic and environmental factors are likely involved, which has led to work aiming to understand which genes mediate the disease process. EphrinB2 is a protein hypothesized to play a role in fibroblast activation and has been found to be increased in fibrosing diseases, such as SSc. A human IgG1 monoclonal antibody was developed to bind EphrinB2 with high specificity and affinity and was evaluated in preclinical in vitro and in vivo studies including bleomycin models of skin and lung fibrosis. We sought to identify genes and pathways relevant in SSc with evidence of preclinical modulation by anti-EphrinB2 antibody treatment.

Methods

Publicly available and proprietary human and mouse skin, lung, and heart gene expression datasets were analyzed, including RNA-seq and microarray data from preclinical fibrosis models and systemic sclerosis clinical trials. Differential expression analysis was performed using DESeq2 (for RNA-seq) and limma (for microarrays), followed by pathway and meta-analyses to identify consistent biological signals across datasets and to understand the transcriptional impact of a monoclonal antibody targeting EphrinB2.

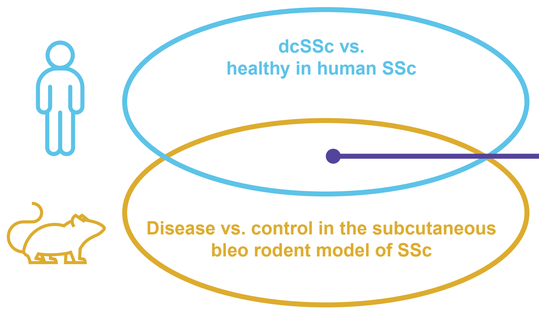
Results

Cross-species analysis revealed 1,618 upregulated and 1,341 downregulated genes significantly shared between human and mouse SSc datasets (FDR-adjusted $p < 0.05$). Shared upregulated pathways included TGF-β, JAK-STAT, NFκB, and EGFR signaling, along with processes related to immune activation, inflammation, epithelial-mesenchymal transition, and extracellular matrix remodeling. Comparative analysis with anti-EphrinB2 antibody data identified 442 genes upregulated in SSc but downregulated by anti-EphrinB2 treatment, and 605 genes downregulated in SSc but upregulated by anti-EphrinB2 treatment, suggesting potential reversal of disease-associated transcriptional programs. Among these, three of the most significantly dysregulated genes in the human SSc signature (CCN2, THBS1, and CD14) were also suppressed by anti-EphrinB2 treatment, highlighting potential therapeutic relevance.

Conclusions

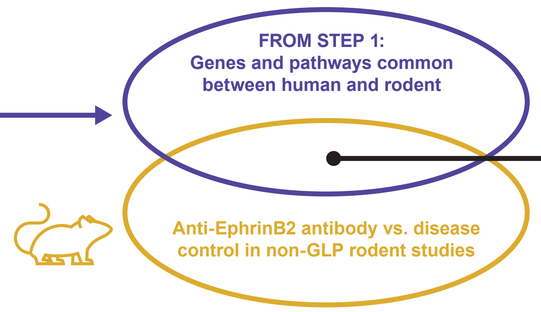
This meta-analysis identified key transcriptional similarities between human SSc and preclinical rodent models, highlighting conserved dysregulated pathways in disease, including TGFβ, JAK-STAT, NFκB, TNFα, and EGFR signaling. Anti-EphrinB2 administration reversed significant subsets of these dysregulated genes, with robust evidence supporting its impact on fibrosis- and inflammation-related pathways. These findings align with preclinical evidence and further support EphrinB2 neutralization as a promising strategy for SSc treatment. Notably, CCN2, THBS1, and CD14 emerged as top disease-relevant genes suppressed by anti-EphrinB2 treatment, reinforcing their potential use as biomarkers. A change in this gene signature could be evaluated in clinical studies of anti-EphrinB2 treatment in SSc patients to identify a potential early signal of efficacy.

STEP 1: Find overlap between human and rodent SSc data



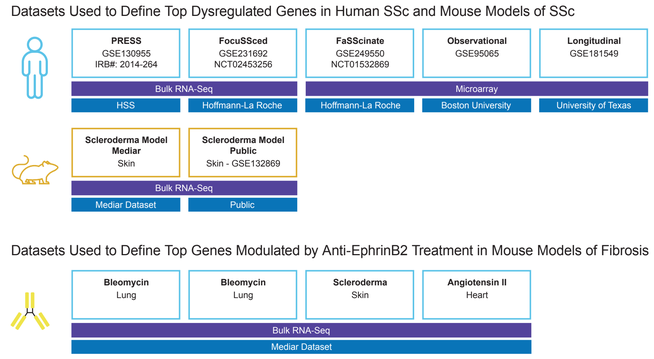
Genes and pathways that are common between human and rodent

STEP 2: Identify relevant signature to interrogate clinically

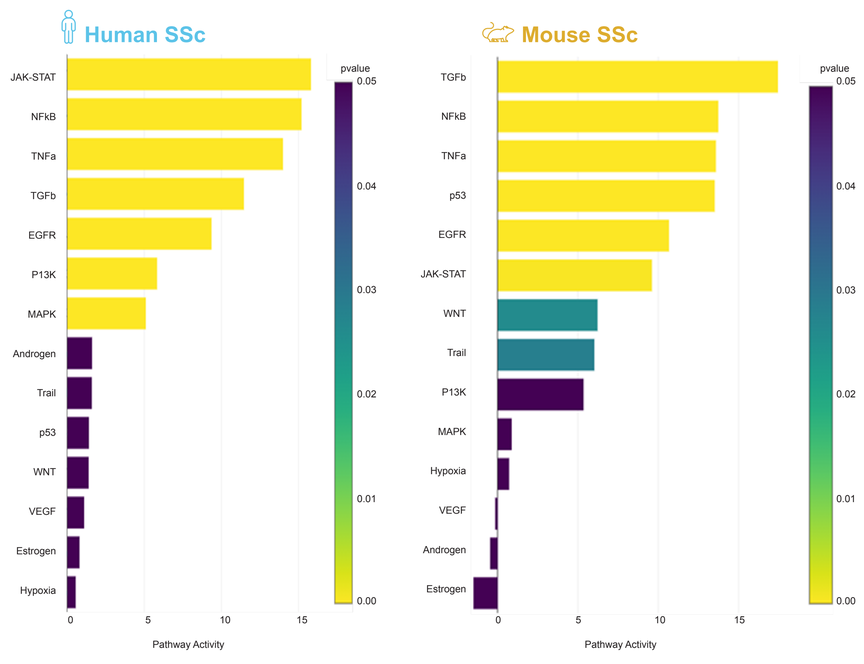


Human-relevant genes and pathways with preclinical evidence of being modulated by anti-EphrinB2 treatment

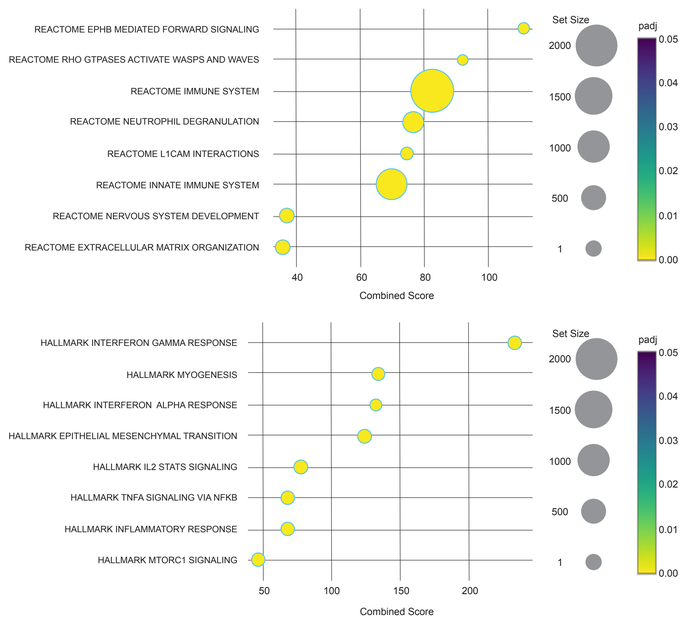
Strategy to Identify Human-relevant Genes and Pathways Modulated by Anti-EphrinB2 Treatment



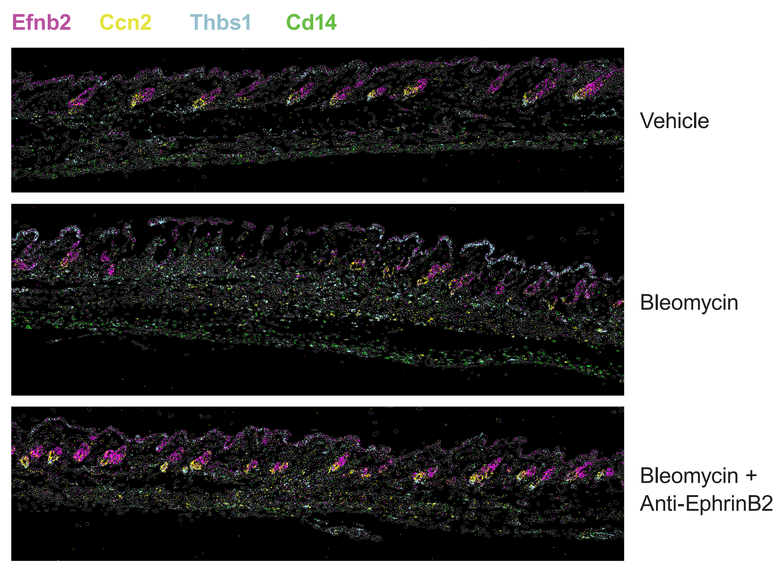
Pathway Analysis of Differentially Expressed Genes in Human SSc and Mouse Models of SSc



Over-enrichment Analysis of Genes Upregulated in Human SSc and Downregulated in Mouse SSc Following Anti-EphrinB2 Treatment



Vizgen spatial transcriptomics of skin bleomycin mouse model enables cell-type understanding of changes in gene expression in a spatially-resolved context



For additional *in vitro* and *in vivo* preclinical data on EphrinB2, see Wilks et. al. Mediar Therapeutics, May 2024: "Anti-EphrinB2 neutralizing antibody suppresses fibrosis in preclinical *in vitro* and *in vivo* models" www.mediartx.com/2024_EphrinCongress_AntiEphrinB2AntibodySuppressesFibrosis

EncompaSSc

MTX-474-S201 Phase 2 clinical trial