Background: LncRNAs are emerging as important regulators of immune function with increasing evidence of autoimmune disease relevance. Here, we leveraged RNA-seq, extensive bioinformatic data, and CRISPR technology to identify and functionally characterize LINC01871 as a potential mediator of the dysregulated T cell inflammatory response pathways implicated in SS pathogenesis.

Conclusion: LncRNAs are emerging as important regulators of immune function with increasing evidence of autoimmune disease relevance. Here, we leveraged RNA-seq, extensive bioinformatic data, and CRISPR technology to identify and functionally characterize LINC01871 as a potential mediator of the dysregulated T cell inflammatory response pathways implicated in SS pathogenesis.

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DYSREGULATED EXPRESSION OF THE LONG NON-CODING RNA, LINC01871, EXPRESSED IN SJÖGREN’S SYNDROME PATHOGENESIS


Background: Sjögren’s syndrome (SS) is a chronic, heterogeneous autoimmune disease characterized by inflammatory destruction of the exocrine glands. Long non-coding RNAs (lncRNAs) have emerged as a functionally diverse class of non-protein coding RNA (ncRNA) with increasing implications in interferon signaling, immune cell regulation, and autoimmune disease pathology. The potential role of IncRNAs in SS pathogenesis is unknown.

Objectives: To identify and characterize candidate IncRNAs with potential relevance to SS pathology.

Methods: RNA-seq was used on whole blood from SS patients (n=30) and healthy controls (n=27) to identify differentially expressed (DE) IncRNAs (log2 fold change (FC) ≥ 2 or ≤ -2; p < 0.05). Bioinformatic and pathway analyses were used to predict IncRNA function. In vitro time course experiments in HSB2 T cell lymphoblasts stimulated with PMA/Ionomycin (PMA/I) or type I interferon (IFN) were used to assess biological relevance. LINC01871 function was further investigated by RNA-seq on a single cell clone of HSB2 with confirmed CRISPR-targeted LINC01871 deletion (LINC01871Δ).

Results: We identified a total of 1054 unique DE ncRNAs between Ro+, Ro- and/or a combined analysis relative to HC; of these, 45 (1 long intergenic ncRNA (lincRNA), 1 antisense, 43 pseudogenes) were overexpressed in all 3 SS subsets. To begin investigating the function of the previously undescribed lincRNA, LINC01871 (SSRo+: FC=2.85; padj=1.1x10^-4), we performed a correlation analysis of the SSRo+ and SSRo- datasets. LINC01871 was strongly correlated with several immune-related pathways involved in immune regulation (THEMIS, TBX1, IL10RA, IL6RB, among many others). Similarly, Ingenuity Pathway Analysis of the SS transcriptome compared to HC, as well as several gene ontology enrichment analyses of publicly available RNA expression correlation databases, identified shared immune-related pathways including cytokytic T cell, natural killer cell, and T cell regulation. To further study the role of LINC01871 in cytokytic T cells, we used qRT-PCT to resolve the effects of PMA/I and IFN stimulation on LINC01871 expression in the T lymphoblastoid HSB2 cells. LINC01871 expression was downregulated after PMA/I stimulation, but unchanged with type I IFN stimulation. To explore the regulatory function of LINC01871 in T cells, we targeted LINC01871 in HSB2 cells using CRISPR. To this end, we generated a single cell LINC01871Δ clone with no RNA expression by qPCR and confirmed homozygous deletion using DNA sequencing. RNA-seq analysis of LINC01871Δ confirmed to unmodified HSB2 cells identified 1166 DE transcripts. Pathway analyses clustered the DE transcripts into similar immune regulatory, cytokytic and T cell pathways identified in SS whole blood RNA-seq and publicly available RNA-seq databases. Further, several prominent T cell regulatory transcripts that exhibited correlated upregulation with LINC01871 in SS whole blood RNA-seq also demonstrated downstream regulation after LINC01871 deletion: CD109 (FC=9.7; p=5.3x10^-10), IC22 (FC=-8.1; p=7.6x10^-1), PDCD1 (FC=-6.2; p=1.1x10^-6), THEMIS (FC=3.8; padj=2.7x10^-15) and TBX21 (FC=2.1; p=3.3x10^-3).

Conclusion: LncRNAs are emerging as important regulators of immune function with increasing evidence of autoimmune disease relevance. Here, we leveraged RNA-seq, extensive bioinformatic data, and CRISPR technology to identify and functionally characterize LINC01871 as a potential mediator of the dysregulated T cell inflammatory response pathways implicated in SS pathogenesis.

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HIGH DIMENSIONAL ANALYSIS REVEAL A NETWORK OF CERTAIN TRANSCRIPTION FACTORS THAT LINK VASCULOPATHY AND ORGAN FIBROSIS IN SYSTEMIC SCLEROSIS


Background: Since vascular manifestations such as Raynaud’s phenomenon often precede the onset of other clinical manifestations of systemic sclerosis (SSc), the identification of pathways linking vasculopathy to organ fibrosis might thus provide important insights into early disease mechanisms and allow early targeted intervention for both fibrotic and vascular events. Developed down to high dimensional (HD) analyses to identify mediators that link vasculopathy to organ fibrosis.

Methods: HD techniques including RNA-seq, ChIP-seq, ATAC-seq and FISH-seq have been performed to identify mediators in vessels and fibrotic lesions of human skin samples of SSc patients and healthy volunteers. In addition, murine skin and lung tissue samples were analyzed by multi-channel immunofluorescence (IF) and confocal laser scanning microscopy. Microvascular endothelial cells, smooth muscle cells and fibroblasts have been further processed to identify functional attributes with regard to their proliferative, migratory and chemotactic capacity. In vivo models and ex vivo mouse fetal metatarsal assays were performed to study fibrotic and angiogenic processes.

Results: Bioinformatic HD analyses revealed the ETS transcription factor PU.1 as molecular checkpoint of a network of factors that drive matrix production and fibrotic imprinting in SSc. Within this network ATF3 was significantly upregulated in fibroblasts of skin biopsies of SSc patients and of various organs of fibrosis models. ATF3 deficiency ameliorated fibrosis in various mouse models. Notably, ATF3 was significantly upregulated in vascular cells of fibrotic tissues of SSc patients. Multi-channel IF and confocal laser scanning microscopy of skin and lung biopsies of SSc patients revealed an increased expression of ATF3 expression in fibroblasts and microvascular endothelial cells. ATF3 expression in smooth muscle cells led to an extensively enhanced proliferation and increased migratory capacity whereas endothelial cells showed a SSc-like phenotype with reduced proliferation and migration. After ATF3 overexpression, tube formation capacity was completely altered as assessed by cumulative tube length, tube numbers and capillary sprouting. To investigate vessel growth from a different perspective, we used the ex vivo fetal mouse metatarsal assay. ATF3 knockout mice showed a completely altered angiogenic response as assessed by tube length, number of branches and number junctions compared to wild type controls.

Conclusion: We identified PU.1 and ATF3 as key factors in disturbed vasculature and endogenous activated fibroblasts suggesting this axis as a potential therapeutic target intervening both fibrotic and vascular manifestations.

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