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What does global gene expression profiling tell us about the pathogenesis of systemic sclerosis?

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Abstract

Purpose of review—The purpose of this study is to review recent hypothesis-driven studies that utilize global gene expression data for elucidating the molecular basis of systemic sclerosis (SSc) and its various clinical manifestations.

Recent findings—The longitudinal skin gene expression studies indicate that the previously identified molecular subsets are stable over time and might identify inherent subgroups of SSc patients. Skin transcript follow-up studies indicate that the Wnt/ β -catenin pathway plays an important role in promotion of fibrogenesis in fibroblasts and preadipocytes. Furthermore, the transcript profile of sclerodermatous graft-versus-host disease (sclGVHD) mice resembles the skin transcriptomes of a subgroup of SSc patients with IL13/IL4-inducible skin signature wherein the profibrotic chemokine CCL2 plays a key role. The comparison of skin biopsies from SSc patients to skin lesions of patients with cutaneous lupus and dermatomyositis has provided valuable information about the interferon (IFN) signature in these autoimmune diseases. Furthermore, plasma IFN-inducible chemokines correlate with the *IFN* gene expression score in SSc patients, enabling researchers to examine this molecular signature in large SSc cohorts with serum or plasma collection.

Summary—Global gene expression profiling in skin and peripheral blood can contribute to a better understanding of SSc pathogenesis and identify novel biomarkers and therapeutic targets.

Keywords

gene expression profiling; pathogenesis; systemic sclerosis

INTRODUCTION

Global gene expression profiling with microarrays or high-throughput sequencing allows the simultaneous assessment of thousands of transcripts in a given tissue. This is an attractive prospect in complex diseases such as systemic sclerosis (SSc) because it allows examination of the interaction of multiple gene products along biological pathways. Genome-wide transcript profiling is now increasingly used to fingerprint pathological processes, subset diseases at the molecular level and predict disease outcome. In SSc, global gene expression profiling has been performed in affected end-organs such as skin [1–3] and lung [4] or in peripheral blood cells (PBCs) [5–8].

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Conflicts of interest

The authors have no disclosures.

Skin is a prominently affected end-organ in SSc that is easily accessible. SSc skin biopsy samples have a distinct gene expression profile compared with controls; an inflammatory activation pattern as well as a fibrotic signature have been observed [1,3]. A larger study [2] with 24 SSc patients was able to subset the diffuse SSc (dSSc) patients into three distinct groups and the limited SSc patients into two groups on the basis of the skin gene expression profiles. A subgroup of patients with limited and diffuse disease types showed an inflammatory pattern. Another subgroup of SSc patients with diffuse skin involvement showed a fibroproliferative gene expression profile containing tissue growth factor-beta (TGF- β) responsive genes [9]. Interestingly, a subgroup of limited and dSSc patients showed a normal-like gene expression signature.

PBC gene expression profiling in SSc patients reveals a distinct gene expression profile that includes interferon (IFN)-inducible genes and genes involved in cellular adhesion to the endothelium [6]. The presence of IFN signature in SSc blood has been confirmed in mononuclear, monocytes and CD4⁺ T-cells [7,10]. In a larger follow-up study [5], PBC gene expression profile of SSc ($n = 60$) was examined in comparison to controls and systemic lupus erythematosus (SLE). The SSc, SLE and healthy control study groups each had a distinct gene expression profile. However, IFN-inducible genes were overexpressed both in SSc and SLE patients. Approximately half of the SSc patients had an *IFN* gene expression signature while the remainder of patients had an inverse T-cell signature where in T-cell related pathways were downregulated.

Peripheral blood mononuclear cell (PBMC) transcript profiling of SSc patients with pulmonary arterial hypertension (PAH) has shown dysregulation of genes involved in angiogenesis such as vascular endothelial growth factor (VEGF) [11] and alternative macrophage/monocyte activation [8].

Lung tissue is not easily accessible and transcript profiling of SSc pulmonary tissue has only been studied in patients with end-stage disease ($n = 33$) undergoing lung transplantation [4]. Lung tissue from SSc interstitial lung disease (ILD) showed dysregulation of genes involved in fibrosis, insulin-like growth factor signalling and caveolin-mediated endocytosis, whereas PAH lung showed differential expression of genes in antigen presentation, chemokine activity and interleukin-17 signalling pathways.

Figure 1 shows selected pathways shown to be prominently dysregulated in global gene expression studies of peripheral blood, skin and lung tissues of patients with SSc.

REVIEW OF RECENTLY PUBLISHED FOLLOW-UP STUDIES

Herein, we review publications in 2012 that utilize global gene expression data to conduct hypothesis-driven follow-up studies for elucidating the molecular basis of SSc and its various clinical manifestations.

Wnt/ β catenin pathways: skin

Wei *et al.* [12] examined the role of Wnt/ β -catenin pathways in fibrogenesis. Global skin gene expression data revealed increased expression of Wnt receptor *FZD2* and the Wnt target *LEF1* and decreased expression of Wnt antagonists *DKK2* and *WIF1* in skin biopsies from the subset of SSc patients in the fibroproliferative group [2] compared with the other distinct subsets. However, there was no statistically significant difference between levels of the Wnt ligands (Wnt-1 to Wnt-11) among the five intrinsic subsets (including the normal-like subset). Further experiments focused on the well characterized canonical Wnt ligand Wnt-3a. Wnt-3a increased levels of activated β -catenin in fibroblasts, as well as fibroblast migration and proliferation. Wnt-3a stimulated *TGFB1* mRNA expression in a time and

dose-dependent manner, which was prevented by siRNA-mediated knockdown of β -catenin. On the contrary, TGF- β and Smad 2/3 inhibition abrogated the profibrotic effect of Wnt-3a. These results indicate that Wnt-3a exerts its profibrotic effects via Smad-dependent autocrine TGF- β signalling.

Explanted subcutaneous preadipocytes were used to examine the role of Wnt-3a in adipogenic differentiation. Recent studies have indicated that multipotent preadipocytes can differentiate into fibroblast-like cells and be a potential source of myofibroblasts [13]. Wnt-3a abrogated differentiation of preadipocytes into adipocytes. This finding was confirmed by the observation that Wnt-3a suppressed levels of stimulators of adipogenesis such as *PPARG1* and *PPARG2*. Furthermore, Wnt-3a induced differentiation of preadipocytes into myofibroblasts. In summary, these experiments indicated that Wnt/ β -catenin has profibrotic and antiadipogenic effects in preadipocytes.

The net effect of these combined stimulatory and inhibitory effects of Wnt/ β -catenin is a promotion of fibrogenesis in fibroblasts and preadipocytes. These effects parallel the clinical findings of SSc patients in whom an increased fibrosis in dermal layer is accompanied by loss of subcutaneous fat tissue.

In a related study [14], serum adiponectin levels were examined in SSc patients. Serum adiponectin levels tightly mirror PPAR-g activity and can be used as a surrogate marker for PPAR-g expression [15]. PPAR-g is not only a potent stimulator of adipogenesis but it can also block fibrogenic TGF- β responses *in vitro* and *in vivo* [16,17]. Reduced expression and function of PPAR-g in patients with SSc may contribute to progression of fibrosis [18,19]. Examining the role of adiponectin as a biomarker revealed that serum adiponectin levels were not higher in patients than controls after adjustment for age, sex and ethnicity. Although an adjustment for BMI was not performed, which can be an important confounder in this analysis. Patients with dSSc had lower adiponectin levels. Furthermore, adiponectin levels were lower in patients with early disease. Serum adiponectin level also inversely correlated with modified Rodnan Skin Score (mRSS) ($r = -0.26$), whereas it did not show a significant correlation with forced vital capacity (FVC) or diffusing capacity for carbon monoxide (DLco). These results indicate that PPAR-g might play a more prominent role in skin fibrosis than ILD in SSc patients. The significant associations within SSc patients were independent of demographic characteristics and BMI in the multivariable models. Longitudinal examination of adiponectin levels in 27 patients revealed that changes in serum adiponectin levels over time correlated inversely with changes in mRSS ($r = -0.52$).

Interleukin-13 pathway: skin

Greenblatt *et al.* [20] investigated global gene expression patterns of sclerodermatous graft-versus-host disease (scIGVHD) mice and compared them with transcript profiles seen in SSc skin. The main goal of this comparison was to investigate whether the observed phenotypic similarities between scIGVHD and SSc are due to intrinsic similarities in disease pathogenesis or a confluence of tissue damage patterns via separate molecular mechanisms. First, the skin transcripts of scIGVHD mice were compared with control mice. The upregulated genes belonged to pathways involved in immune responses, antigen presentation and protein degradation. The cross-comparison to skin gene expression patterns [2] revealed that the scIGVHD expression signature approximated the inflammatory subset of SSc ($r = 0.14$), although there was no correlation to the other SSc profile subsets. A total of 69 genes were common between the murine signature and the inflammatory subset of SSc. Chemoattractants for activated T cells, basophils, neutrophils and monocytes (*CCL2*, *CCL5*, *CXCL10*, *CCL4*), as well IFN-inducible gene were highly represented in the overlap. Furthermore, an IL-13 gene signature was derived by stimulating human dermal fibroblasts with IL-13. This IL-13 gene signature was enriched in the inflammatory subset of SSc but

not in the diffuse-proliferation subset, and only weakly in the limited and normal-like groups. The activation of the IL-13 pathway in sclGVHD was investigated using a previously published IL-13 induced transcript profile in a murine model of pulmonary fibrosis [21]. The sclGVHD gene signature was enriched with IL-13 induced transcript with an average correlation coefficient of 0.12. Pursuing the hypothesis that genes most central to disease pathogenesis would be among the overlap of genes upregulated within the core sclGVHD: human inflammatory signature, IL-13 treated human dermal fibroblasts, as well as the lungs of IL-13 transgenic animals [21]. The chemokine gene *CCL2* emerged as the only gene present in all three of these data sets. SclGVHD mice treated with blocking antibodies to *CCL2* and its orthologue *CCL12* were almost completely protected from pathological disease manifestations. Furthermore, the *CCL2* transcript levels were determined in an independent sample of SSc patients. The *CCL2* levels highly correlated with mRSS and IL13-RA1 levels in this group.

In another study, *CCL2* serum level was implicated as a biomarker for SSc-related ILD, correlating with pulmonary vital capacity at the cross-sectional and longitudinal levels in 31 SSc patients. An adjustment for potential confounders was not performed because the sample size was small [22]. These studies support the notion that *CCL2* might be a therapeutic target in SSc.

Longitudinal examination of gene expression profiles: skin

Pendergrass *et al.* [23] studied skin global transcript profiles of 13 dSSc patients enrolled in an open-label study of rituximab in addition to nine dSSc patients. Longitudinal samples were available in 14 patients. The majority of samples were obtained 6 months after enrolment. Analysis of before and after rituximab treatment samples revealed no significant change in the gene expression profile, which was consistent with lack of clinical response in this trial [24]. The gene expression profile of these samples recapitulated the previously described transcript subsets: fibroproliferative, inflammatory and normal-like [2]. Genes that were consistently expressed at a single time point for each patient but had the most diverse expression between time points were identified (intrinsic-by-time point). The analysis of these genes revealed that the skin gene expression of each patient varied little across time. Specifically, patients in the inflammatory subset did progress to the fibroproliferative subset and vice versa. Furthermore, the disease duration of patients in the identified transcript subsets did not differ significantly. The inflammatory group had the widest range of mRSS scores, whereas the normal-like group consistently showed lower mRSS scores. Autoantibodies (anticentromere, topoisomerase I and anti-RNA polymerase III) did not show a significant association with the intrinsic subsets. The findings of this study suggest that the skin gene expression subsets are stable and inherent features of dSSc. Studies with larger sample sizes and longer follow-up time are needed to further investigate this important finding.

Interferon signature: skin

Wong *et al.* [25] compared the gene expression profile of the dermatomyositis skin lesions with other diseases including SSc. Histologically, dermatomyositis skin lesions are characterized by apoptosis/necrosis of keratinocytes, perivascular inflammation, increased dermal mucin deposition and endothelial cell deposition [26]. Active skin lesions from dermatomyositis patients were compared with controls and a dermatomyositis signature was identified. The most prominent profile in this signature was an IFN signature. In fact, 21 of the top 25 most upregulated genes were IFN-inducible genes [27–29]. Notably, the IFN signature was not present in the dermatomyositis patients with inactive skin disease, suggesting that they are induced preferentially in active skin lesions. Notably, downregulated transcripts included genes involved in ribosomal synthesis or lipid

metabolism. Many of the genes involved in lipid metabolism have been previously reported to be downregulated in other inflammatory skin disorders such as psoriasis and atopic dermatitis [30,31]. Subsequently, the dermatomyositis profile was compared with the skin gene expression profile of patients with inflammatory subtype of SSc skin, cutaneous lupus, psoriasis, atopic dermatitis, contact dermatitis, acne vulgaris and herpes simplex virus-2. Only the cutaneous lupus samples were investigated concomitantly with dermatomyositis samples. The remainder of the global gene expression profiles was obtained from publically available databases. Due to the use of different array platforms in this analysis, only 490 genes of the original 946 genes of the dermatomyositis signature were available in all of the experimental data sets. Reflecting the histological similarities between dermatomyositis and cutaneous lupus (autoimmune interface dermatitis), the transcript profiles of active dermatomyositis and SLE skin lesions were remarkably similar (Spearman rho = 0.87). Interestingly, the dermatomyositis samples also showed similarities to samples from herpes simplex-2 (Spearman rho = 0.64). The SSc gene expression profile was less similar; this might reflect histological differences between SSc and dermatomyositis skin lesion and/or hint towards different underlying pathophysiological processes.

The IFN upregulation was quantified using a consensus list of 117 IFN-stimulated genes derived from publically available data wherein various cell types (keratinocytes, lung epithelial cells, macrophages, endothelial cells and fibroblasts) were stimulated *in vitro* with either type I or type II IFNs. The advantage of this approach over performing stimulation studies with one cell line (e.g. fibroblasts) is that the resulting gene list might mimic more closely in-vivo conditions in which several cell types are responding to a stimulant. Nevertheless, it is difficult to compare in-vitro conditions in cell lines with skin tissue with multiple responding cell types and unknown concentrations of various IFN proteins. The comparison of IFN scores across the various diseases revealed that skin samples from patients with dermatomyositis, cutaneous lupus and herpes simplex virus-2 had the highest levels, followed by psoriasis. Skin lesions in the inflammatory subset of SSc patients showed a weaker IFN signature. These findings parallel previously published results in SSc PBCs, although the IFN signature in blood seems to be more prevalent (up to 50% of patients) than in SSc skin.

Interferon signature: blood

Liu *et al.* [32] investigated the correlation of plasma IFN-inducible chemokines with the *IFN* gene expression signature in SSc patients. Plasma levels of IP-10 (CXCL10) and I-TAC (CXCL11) correlated with the PBC *IFN* gene expression score [5]. A composite score of IP-10 and I-TAC showed a stronger correlation with the *IFN* gene expression signature than with the individual chemokines (Spearman's rho = 0.62). Interestingly, the plasma CCL2 levels did not correlate significantly with the *IFN* gene expression score in SSc patients, whereas this chemokine shows a tight correlation with the *IFN* gene expression score in SLE [33,34]. This finding might indicate that CCL2 is regulated by other cytokines such as IL13/IL4 in SSc.

Subsequently, the correlation of the IFN chemokine score with clinical features was examined in a large early SSc cohort ($n = 266$). This composite score did not correlate with disease duration but was associated with the presence of anti-RNP and the absence of anti-RNA polymerase III antibodies. The negative association of IFN chemokine score with anti-RNA polymerase III antibodies might have clinical implications because this antibody is highly associated with diffuse skin involvement, but significant ILD occurs infrequently in this subgroup.

The chemokine score correlated with the concomitantly obtained markers of muscle, skin and lung disease severity independently of potential confounders including treatment with immunosuppressive agents. The easier accessibility of plasma samples compared with the peripheral blood RNA samples will enable other investigators to examine this molecular signature in other SSc cohorts with stored serum or plasma samples.

Human leukocyte antigen: blood

Odani *et al.* [35] compared the global gene expression profiles of pooled RNA samples from the PBMCs of SSc patients with ILD with those without ILD. ILD was defined as presence of isolated ground-glass opacities, honeycombing, ground-glass attenuation and/or traction bronchiectasis on high-resolution chest computed tomography (CT). Four sets of RNA pools were prepared: four patients with ILD on conventional therapy; four patients without ILD on conventional therapy; four patients with ILD who received haematopoietic stem cell transplant (HSCT); and two patients without ILD who received HSCT. To normalize the treatment background of the patients, the first comparison performed was in the conventional therapy group, and the second was in the HSCT group. Only two genes were unregulated in patients with ILD versus in those without ILD in the HSCT group, whereas 17 genes were upregulated in patients with ILD compared with those without ILD in the conventional treatment group. Only *HLA-DRB5* was commonly upregulated in both comparisons. In this study, the pooling of samples has resulted in decreased power to detect a higher number of differentially expressed genes. However, the global gene expression results were confirmed in a larger sample of SSc patients by quantitative PCR (37 patients with ILD and 18 patients without ILD) showing overexpression of *HLA-DRB5* in the ILD group. The *HLA-DRB5* transcript levels were related neither to disease duration nor to treatment regimen. Subsequently, *HLA-DRB5* typing was performed using genomic DNA in a discovery cohort ($n = 70$) and confirmation cohort ($n = 79$). The genetic studies indicated that the frequency of *HLA-DRB5* gene carriers was higher in SSc patients with ILD than in those without ILD or in healthy controls. More specifically, *DRB5*01:05* was more frequently present in SSc patients with ILD than in patients without ILD or unaffected controls. These associations were independent of antibody status. Findings were consistent in the discovery and confirmation cohorts. This study exemplifies how the unbiased approach of global gene expression profiling in a limited number of microarray experiments ($n = 4$) can guide researchers to generate hypothesis that can be tested in follow-up studies.

CONCLUSION

The wealth of data generated by genome-wide gene expression profiling has contributed importantly to our understanding of pathogenesis and to molecular stratification of patients. Follow-up studies are needed to better understand the molecular basis underlying the observed transcript signatures. These expression profiles most probably result from intertwined biological processes in which several biological pathways interact with each other. Although development of molecular signatures by stimulating one cell type with a certain molecule can contribute to our understanding of main pathways involved, this should not restrict us in considering other contributing biological processes. This is especially important because the currently available signatures such as IFN, IL-13 or TGF- β have significant overlap and cannot fully capture the complexity of in-vivo milieu.

Despite the significant progress in recent years, the identified gene expression profiles can be further refined by examining larger number of patient and control samples to truly reflect the heterogeneity of SSc. Furthermore, longitudinal sample collections with careful clinical characterization are needed to develop molecular biomarkers that can predict disease course or response to treatment.

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KEY POINTS

- Wnt/ β Catenin and IL-13 pathways are dysregulated in subsets of systemic sclerosis patients and are potential therapeutic targets.
- Skin gene expression profiles of SSc might be stable over time and represent inherent subsets of disease.
- Interferon-inducible chemokines correlate with the *IFN* gene expression signature and disease severity in SSc.

FIGURE 1.

Selected pathways shown to be prominently dysregulated in global gene expression studies of peripheral blood, skin and lung tissues of patients with systemic sclerosis. IFN, interferon; IGFBP, insulin-like growth factor binding protein; IL-13, interleukin-13; IL-17, interleukin-17; IL-4, interleukin-4; TGF-β, tissue growth factor-beta; TIMP-1, tissue inhibitors of metalloproteases 1.