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Molecular Framework for Response to Imatinib Mesylate in Systemic Sclerosis

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Systemic sclerosis (SSc) is an autoimmune disease in which the tyrosine kinases platelet-derived growth factor receptor (PDGFR) and Abl are hypothesized to contribute to the fibrosis and vasculopathy of the skin and internal organs. Herein we describe 2 patients with early diffuse cutaneous SSc (dcSSc) who experienced reductions in cutaneous sclerosis in response to therapy with the tyrosine kinase inhibitor imatinib mesylate. Immunohistochemical analyses of skin biopsy specimens demonstrated reductions of phosphorylated PDGFRβ and Abl with imatinib therapy. By gene expression profiling, an imatinib-responsive signature specific to dcSSc was identified ($P < 10^{-8}$). The response of these patients and the findings of the analyses suggest that PDGFRβ and Abl play critical, synergistic roles in the pathogenesis of SSc, and that imatinib targets a gene expression program that is frequently dysregulated in dcSSc.

Systemic sclerosis (SSc) is an autoimmune disease characterized by fibrosis of the skin and internal organs and widespread vasculopathy. Current therapies for SSc focus on treating specific symptoms, but disease-modifying agents targeting the underlying pathogenesis are lacking.

The pathogenesis of SSc involves activation of profibrotic pathways, with overexpression of the cytokines transforming growth factor β (TGFβ) and platelet-derived growth factor (PDGF). A recent study showed that SSc patients have autoantibodies against the PDGF receptor (PDGFR), which stimulate the production of reactive oxygen species and expression of type I collagen (1). PDGFRs are up-regulated in the skin and bronchoalveolar lavage fluid of patients with SSc, and when activated, lead to fibroblast and myofibroblast proliferation (2,3). PDGF participates in the smooth muscle cell recruitment and mitogenic signaling that underlie the vasculopathy associated with pulmonary arterial hypertension (PAH), a complication of SSc associated with high mortality (4). In addition, stimulation of the TGFβ profibrotic pathway involves activation of c-Abl (2). Thus, the PDGF and TGFβ pathways are thought to contribute to the fibrotic and vascular complications in SSc.

Imatinib mesylate is a small molecule that antagonizes specific tyrosine kinases that mediate fibrotic pathways, including c-Abl (a downstream mediator of TGFβ [2]) and PDGFRs (5). Imatinib has been shown to inhibit bleomycin-induced lung and dermal fibrosis in

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mouse models (6,7), and proliferation of synovial fibroblasts derived from patients with rheumatoid arthritis (8). Imatinib has also been reported to provide benefit in the treatment of refractory idiopathic PAH, through its effects on vascular remodeling (9). Herein, we describe 2 patients with early diffuse cutaneous SSc (dcSSc) who experienced clinical improvement in response to imatinib therapy and report findings providing evidence that both c-Abl and PDGFR are targets of imatinib in involved skin of patients with SSc. Finally, we show that an imatinib-responsive gene signature is present in most patients with dcSSc.

CASE REPORTS

Patient 1. Patient 1, a 24-year-old woman with a 3-year history of dcSSc, presented with increasing tightness of the skin and shortness of breath. She had a history of severe Raynaud's phenomenon and digital ulcerations (Figure 1A) despite bilateral sympathectomies and treatment with multiple vasodilators. She had arthritis necessitating long-term prednisone treatment at 10 mg/day. The patient had noted increasing dyspnea on exertion, and high-resolution computed tomography (HRCT) of the chest showed bibasilar ground-glass opacities (Figure 1C) consistent with interstitial lung disease (ILD). Pulmonary function tests (PFTs) showed a forced vital capacity (FVC) of 48% of predicted and a diffusing capacity for carbon monoxide (DLCO) of 62% of predicted. Transthoracic echocardiography revealed a small pericardial effusion, but normal right ventricular systolic pressure. The patient was unable to tolerate intravenous immunoglobulins and mycophenolate mofetil. She declined cyclophosphamide therapy and was referred to our center for a trial of imatinib.

Prior to initiation of imatinib therapy, the patient's modified Rodnan skin thickness score (MRSS) (10) was 36 (scale 0–51), and she had 9 digital ulcers. Her complete blood cell count, creatine kinase level, and results of a comprehensive metabolic panel and urinalysis were within normal limits. The C-reactive protein (CRP) level was 2.8 mg/dl (normal <0.5). Skin biopsy demonstrated thickened, closely packed collagen bundles, with an average dermal thickness of 2.81 mm (Figure 1E).

After 3 months of oral imatinib at 100 mg twice daily, the patient reported softening of her skin, increased joint mobility, and decreased shortness of breath. Physical examination revealed an MRSS of 21 and 4 digital ulcers (Figure 1B). The CRP had normalized to 0.2 mg/dl and the prednisone dosage had been tapered to 5 mg/day. HRCT showed resolution of the interstitial changes (Figure 1D), and repeat transthoracic echocardiography showed no evidence of a pericardial effusion. Repeat PFTs showed a slight improvement in her FVC to 52% of predicted, but a decline in the DLCO to 54% of predicted. Repeat skin biopsy revealed more widely spaced, thinner collagen bundles, with an average dermal thickness of 2.31 mm (Figure 1F).

Patient 2. Patient 2, a 62-year-old woman with newly diagnosed dcSSc, presented to our clinic with progressive cutaneous sclerosis. The patient had a 2-year history of Raynaud's phenomenon and noted increasing tightening of her skin over the previous 6 months. Initial therapies included benazepril for her Raynaud's phenomenon and methotrexate (12.5 mg/week) and moderate-dose prednisone for her skin disease. The patient did not tolerate corticosteroid therapy and was referred to our center for investigational treatment with imatinib.

At initial evaluation, the patient was found to have prominent capillary dilation and dropout on nailfold capillaroscopy, and dermatologic examination revealed an MRSS of 36. Her creatine kinase level, erythrocyte sedimentation rate, and results of a complete blood cell count, comprehensive metabolic panel, and urinalysis were within normal limits. She had no evidence of ILD on HRCT of the chest, and PFT findings were unremarkable. Baseline transthoracic echocardiography showed a normal ejection fraction and right ventricular systolic pressure of 35 mm Hg with a small pericardial effusion.

After 6 months of oral imatinib at 200 mg daily, the patient had noted improvement in her skin tightening. Her Raynaud's phenomenon worsened in severity during the winter season, but she did not develop any digital ulcers. Her MRSS had improved to 20. Her PFT and HRCT results remained stable, and transthoracic echocardiography showed right ventricular systolic pressure of 23 mm Hg and resolution of the pericardial effusion.

METHODS

Lesional skin biopsy samples from the upper extremities (upper arm or forearm) were obtained at baseline and during imatinib therapy (at 3 months in patient 1 and at 1 month in patient 2) for histologic, immunohistochemical, and microarray analyses. The protocol was approved by the Institutional Review Board at Stanford University School of Medicine, and both patients provided written informed consent.

For immunohistochemistry analysis, skin biopsy tissue was fixed in formalin and paraffin embedded. Sections were stained with antibodies specific for the phosphorylated (activated) states of the tyrosine kinases PDGFRβ and c-Abl.

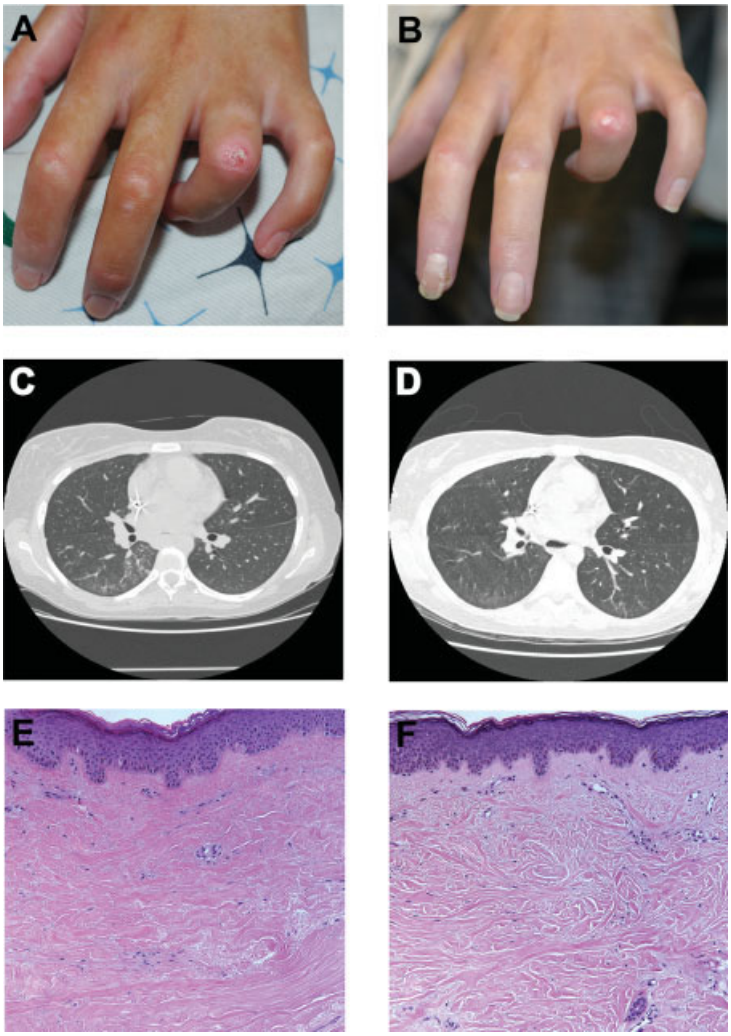

Figure 1. Effect of imatinib mesylate on digital ulcers, interstitial lung disease, and collagen architecture in a patient with systemic sclerosis (patient 1). **A**, Digital ulcer over the left fourth proximal interphalangeal joint prior to imatinib therapy. **B**, Healing of the digital ulcer after 3 months of imatinib therapy. **C**, High-resolution computed tomography (HRCT) of the chest prior to imatinib therapy, demonstrating patchy infiltrates associated with ground-glass opacities in the bilateral lower lobes. **D,** HRCT after 3 months of imatinib therapy, showing resolution of the ground-glass opacities. **E**, Hematoxylin and eosin (H&E)–stained skin biopsy specimen from the right arm, obtained prior to imatinib therapy, showing dense, eosinophilic, tightly packed collagen bundles in the papillary and reticular dermis, with an average dermal thickness of 2.81 mm (original magnification × 100). **F**, H&E-stained skin biopsy specimen from within 1 cm of the initial biopsy sample, obtained after 3 months of imatinib therapy, revealing normalization of collagen architecture, with loose spacing and thinning of collagen bundles and an average dermal thickness of 2.31 mm (original magnification × 100).

We next performed global transcriptional analysis of the skin, using oligonucleotide microarrays. Total RNA was extracted from snap-frozen skin biopsy specimens (from areas adjacent to those processed for paraffin embedding) before and after imatinib treatment, using an RNeasy fibrous tissue kit (Qiagen, Chatsworth, CA). RNA was amplified using an Amino Allyl MessageAmp II aRNA kit (Ambion, Austin, TX). Amplified skin RNA (labeled with Cy5) and amplified Human Universal Reference RNA (labeled with Cy3) (Stratagene, La Jolla, CA) were competitively hybridized to HEEBO (human exon evidence-based oligonucleotide) microarrays in duplicate as described (http://www.microarray.org/sfgf/heebo.do).

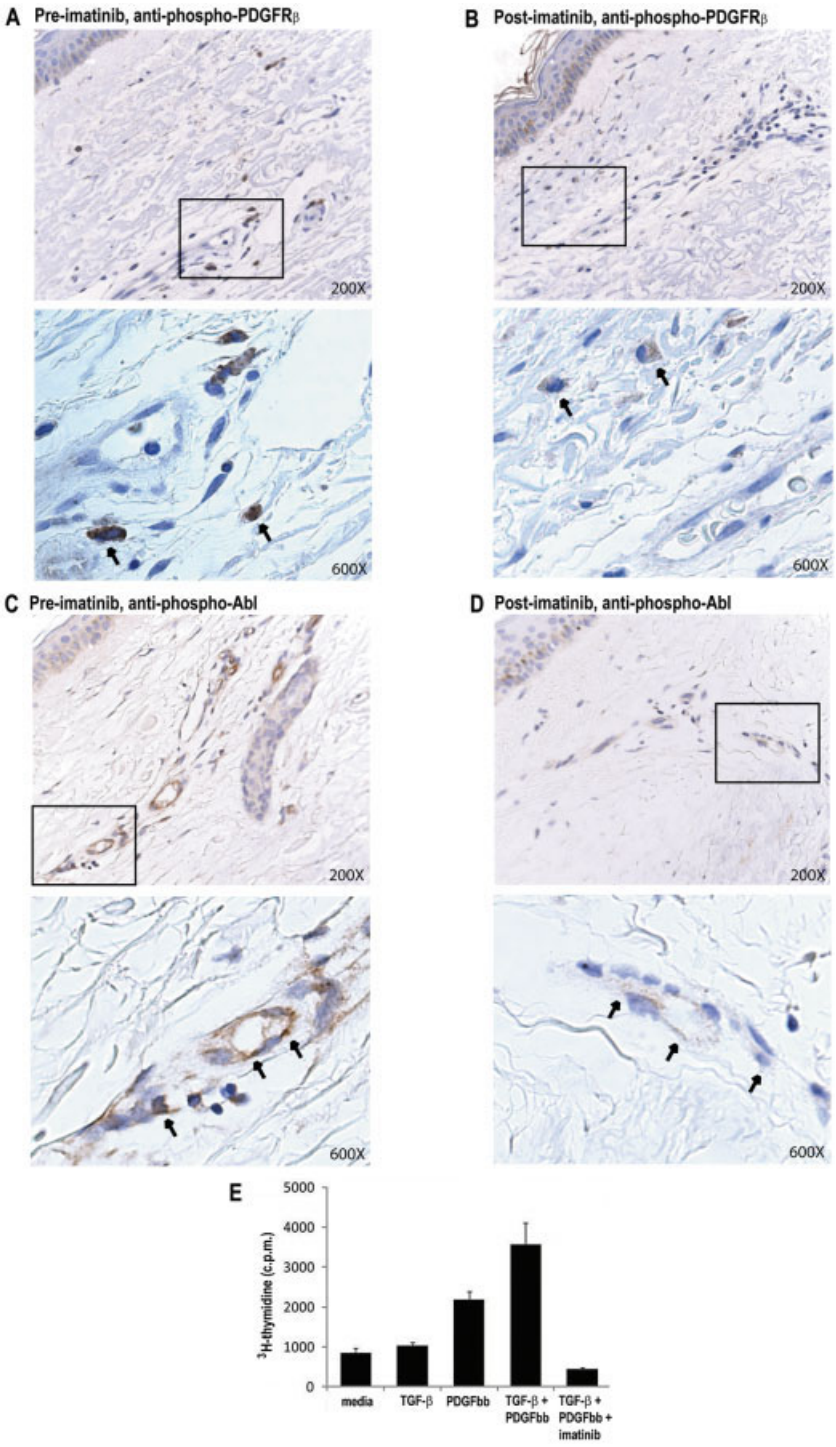

Figure 2. Imatinib-induced reduction of platelet-derived growth factor receptor β (PDGFRβ) and Abl activation in systemic sclerosis (SSc) skin and PDGFRβ and Abl function in SSc fibroblasts. **A–D,** Immunohistochemical staining of serial skin biopsy samples obtained pretreatment (**A** and **C**) and 1 month following the initiation of imatinib treatment (**B** and **D**). Specimens were stained with anti–phospho-PDGFRβ (**A** and **B**) or anti–phospho-Abl (**C** and **D**). Boxed areas in the upper panels are shown at higher magnification in the corresponding lower panels. Phospho-PDGFRβ was observed in interstitial fibroblasts as well as perivascular spindle-like cells and some cells resembling mast cells. Phospho-Abl was observed in endothelial cells in small vessels and in scattered dermal fibroblasts. Results are representative of those obtained in multiple sections from 2 patients. **E**, Stimulation of an SSc fibroblast line with transforming growth factor β (TGFβ) (0.5 ng/ml), PDGF (10 ng/ml), TGFβ plus PDGF, or TGFβ plus PDGF plus imatinib (1 μM). Proliferation was quantitated after 48 hours by measurement of ^{3}H-thymidine incorporation. Results are representative of experiments performed on 2 independent SSc fibroblast lines; similar results were obtained with normal fibroblast lines. Values are the mean and SD.

Genes were selected for analysis if they had a fluorescent hybridization signal ≥1.5-fold over local background in either the Cy5 or the Cy3 channel and if the data were technically adequate in ≥75% of experiments. Genes were analyzed by mean value centering within the data set for each patient. Imatinib-responsive genes were identified using Significance Analysis of Microarrays, with a false discovery rate (FDR) of <0.001. Samples were scored for their similarity to the transcriptional response of fibroblasts to serum, as previously described (11). The database of 75 SSc and control gene expression profiles is described elsewhere (12), and includes 75 microarray analyses on 61 skin biopsy specimens from 34 subjects, including samples from 18 patients with dcSSc, 7 with limited cutaneous SSc (lcSSc), 3 with morphea, and 6 healthy controls. Eight hundred seventeen of 1,050 imatinib-responsive genes were successfully mapped in the SSc database using EntrezGene ID, and their pattern of expression was analyzed by unsupervised hierarchical clustering. All data are publically available at Stanford Microarray Database (http://genome-www5.stanford.edu) and Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/; accession no. GSE11130). The imatinib-responsive gene signature is shown in Supplementary Table 1, which may be found in the online version of this article at http://www3.interscience.wiley.com/journal/76509746/home.

RESULTS

We performed immunohistochemical analysis of serial skin biopsy specimens obtained pretreatment and ≥1 month following initiation of imatinib therapy. An anti–phospho-PDGFRβ antibody strongly stained dermal cells with fibroblast-like morphology in the pretreatment samples (Figure 2A), and there was a significant decrease in staining 1 month after initiation of imatinib therapy (Figure 2B). Anti–phospho-Abl antibodies stained dermal vessels in the pretreatment samples (Figure 2C), and again there was a significant reduction in staining 1 month following initiation of therapy (Figure 2D).

To assess the ability of imatinib to inhibit PDGF- and TGFβ-induced fibroblast proliferation, titration curves for TGFβ and PDGF stimulation of SSc fibroblast proliferation were generated (data not shown). Concentrations of TGFβ (0.5 ng/ml) and PDGF (10 ng/ml) that submaximally stimulated SSc fibroblast proliferation were selected and used alone, in combination, or in combination with imatinib (1 μM) to stimulate SSc fibroblast lines (Figure 2E). As compared with the low-level proliferation induced by PDGF or TGFβ alone, costimulation with PDGF and TGFβ induced SSc fibroblast proliferation in a synergistic manner (the increase in proliferation of the costimulated fibroblasts was twice as high as the sum of the increases in proliferation observed with the individual stimuli). Imatinib completely abrogated SSc fibroblast proliferation induced by PDGF and TGFβ.

To gain further insights into the molecular mechanisms of action of imatinib, we established global gene expression profiles of lesional skin before and after imatinib treatment. Comparison of gene expression patterns in the 2 patients before and after treatment revealed a consistent set of 1,050 genes that were changed by imatinib in both patients (FDR <0.001). To test whether the gene targets of imatinib in SSc, as defined in these 2 patients, may be generalizable to other patients with SSc or other fibrotic diseases, we interrogated the pattern of activation of the imatinib-responsive signature in a database of 75 gene expression profiles of SSc and control samples (12). We found that samples both from patients with early dcSSc (≤3 years' duration) and from patients with late dcSSc (>3 years' duration) tended to express the imatinib-responsive signature, whereas most samples of normal skin or skin from patients with morphea or lcSSc/CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasias) did not ($P < 10^{-8}$ by chi-square analysis) (Figure 3A).

To determine which cell types may be contributing to the gene expression changes associated with imatinib therapy, we compared, using previously published data and methodology (13), the imatinib-responsive signature with the gene expression profiles of 11 individual cell types that are likely to be present in skin. These 11 comparison cell types include normal and SSc fibroblasts, myofibroblasts, T and B cells, epithelial cells, and endothelial cells. Results of this analysis (shown in Supplementary Figure 1, which may be found in the online version of this article at http://www3.interscience.wiley.com/journal/76509746/home) suggested that approximately half of the expression changes can be attributed to 1 of 3 single cell types, including fibroblasts, endothelial cells, and B cells, while the rest are likely expressed in multiple cell types.

In replicate array analyses, we assessed the transcriptional response of fibroblasts to serum (the "wound signature," in which PDGF plays an important role [11]) in the 2 patients with dcSSc. In both patients, the wound signature score was significantly lower after imatinib treatment compared with the pretreatment score ($P <$ 0.01 by Student's t-test) (Figure 3B).

DISCUSSION

We describe 2 patients with dcSSc who experienced clinical improvement with imatinib therapy. Similar to the patient reported by Sfikakis et al (14), both of our patients exhibited improvement in cutaneous sclerosis and resolution of inflammatory manifestations of their disease (i.e., ILD, arthritis, pericardial inflamma-

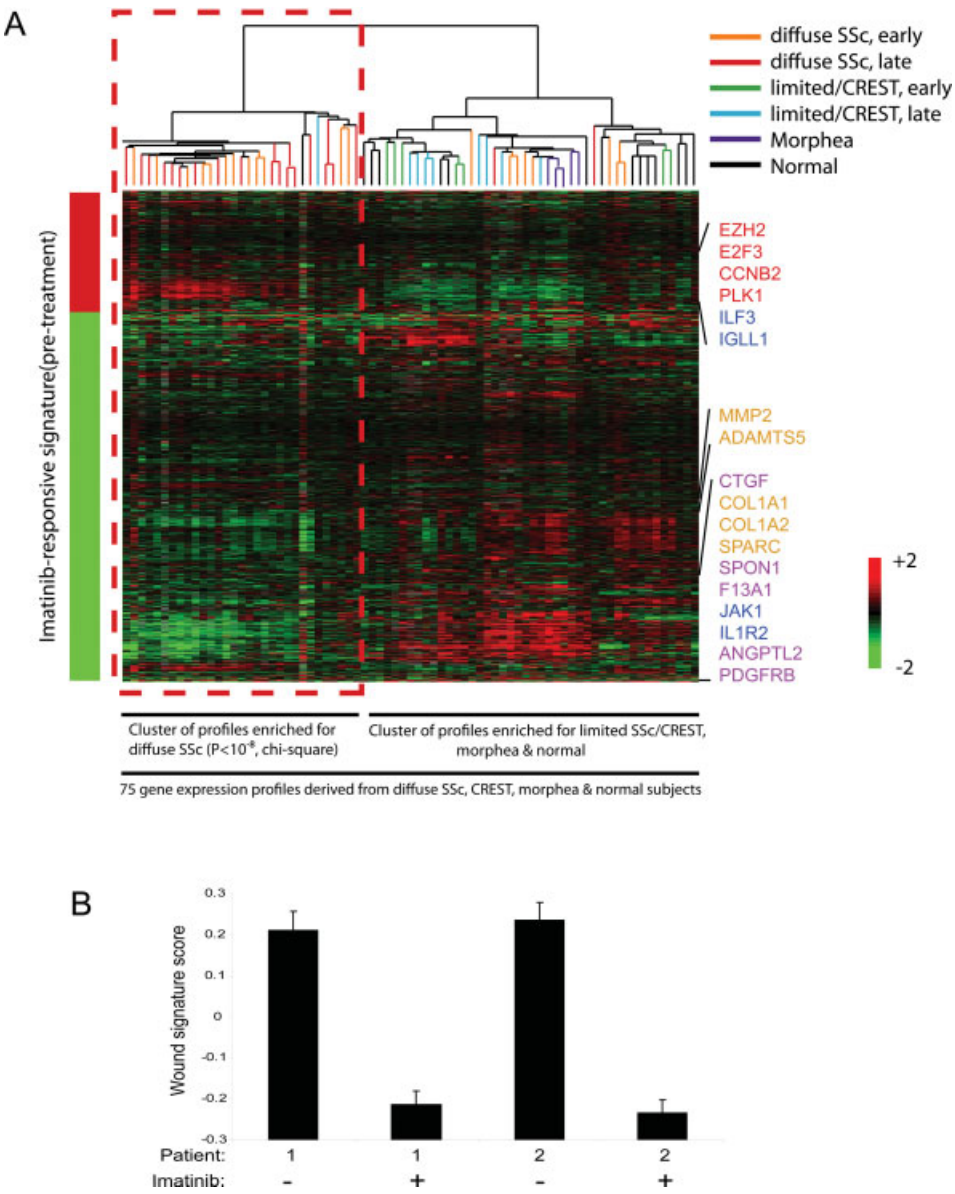

Figure 3. Presence of an imatinib-responsive signature in the majority of studied specimens from patients with diffuse cutaneous systemic sclerosis (dcSSc). **A**, The imatinib-responsive signature was determined by applying Significance Analysis of Microarrays (SAM) to identify genes with statistically significant differences in mRNA levels in posttreatment skin biopsy samples, as compared with pretreatment samples, derived from the 2 imatinib-treated SSc patients. SAM identified 1,050 genes that were changed by imatinib therapy in both patients (false discovery rate <0.001). This imatinib-responsive signature is represented by the bar to the left of the heatmap image. Red represents an increase, and green a decrease, in mRNA expression posttreatment; the genes comprising the imatinib-responsive signature are presented in Supplementary Table 1, which may be found in the online version of this article at http://www3.interscience.wiley.com/journal/76509746/home. The imatinib-responsive genes were then used to organize, via unsupervised hierarchical clustering, the 75 gene expression profiles derived from skin biopsy specimens from healthy controls and patients with dcSSc, limited cutaneous SSc (lcSSc)/CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasias), and morphea, contained in a database. The results of the hierarchical clustering are presented as a heatmap, with each column representing the mRNA profile of a sample, and rows representing the genes present in the imatinib-responsive signature. Unsupervised hierarchical clustering revealed 2 distinct clusters, with the imatinib-responsive gene expression pattern being similar to 1 of the clusters, and this cluster being highly enriched for dcSSc (29 of the 31 gene expression profiles contained in this cluster were from patients with dcSSc) ($P < 10^{-8}$ by chi-square analysis). This cluster of gene expression profiles derived from most of the dcSSc samples exhibited a pattern of gene activation and repression concordant with the imatinib-responsive signature, including alterations in the expression of genes involved in cell proliferation (red), immune signaling (blue), matrix remodeling (yellow), and growth factor signaling (pink) (indicated to the right of the heatmap). The other cluster contained most of the profiles derived from patients with lcSSc/CREST and morphea and from normal subjects, and the gene expression profiles from these subjects did not exhibit the imatinib-responsive signature (this cluster contains 44 gene expression profiles, including 14 from normal skin, 15 from patients with lcSSc/CREST, 5 from patients with morphea, and 10 from patients with dcSSc). **B,** Replicate array analysis was performed to determine the transcriptional response of fibroblasts to serum ("wound signature") in the 2 patients with dcSSc before and after imatinib treatment. The wound signature was significantly reduced after treatment in both patients ($P < 0.01$ by Student's *t*-test). Values are the mean and SD.

tion). Despite the concern that wound healing may be attenuated by PDGFR blockade (15), neither of our patients developed new digital ulcers, and patient 1 experienced healing of several ulcers while receiving imatinib therapy. Thus, although PDGFR blockade by imatinib may slow wound healing, it did not prevent healing in our patients. The patients tolerated imatinib well, with no evidence of bone marrow or liver toxicity. Both patients experienced gastrointestinal side effects, but these were mild and tolerable with the 200 mg/day dosage of imatinib. Immediately following her 6-month evaluation, patient 2 developed bronchitis necessitating oral antibiotic therapy, but there were no other infectious complications.

Immunohistochemistry analysis demonstrated high levels of phospho-PDGFRβ in dermal fibroblasts and phospho-Abl in vascular structures in pretreatment skin biopsy samples, and reductions in both phospho-PDGFRβ and phospho-Abl following initiation of imatinib therapy (Figures 2A–D). Imatinib binds to the ATP-binding pockets to inhibit phosphorylation of the tyrosine kinases PDGFRβ and Abl, and these results suggest that the clinical benefit observed is associated with imatinib-mediated inhibition of PDGFRβ and Abl activation.

We demonstrated that PDGF and TGFβ each stimulate proliferation of SSc fibroblasts, while costimulation with PDGF plus TGFβ has a synergistic effect on induction of proliferation. Addition of imatinib at 1 μM, a concentration achieved in human dosing, inhibited the proliferation induced by PDGF plus TGFβ (Figure 2E). These data provide further evidence that aberrant activation of PDGFRβ and Abl contributes to the pathogenesis of SSc, and that imatinib could provide benefit by inhibiting activation of these tyrosine kinases. Fibroblasts from patients with SSc have recently been shown to express increased levels of c-Kit (16), another tyrosine kinase that is potently inhibited by imatinib and that could play a significant role in the pathogenesis of SSc. The ability of imatinib to simultaneously inhibit multiple tyrosine kinase pathways involved in SSc pathogenesis likely contributes to its clinical benefit. Furthermore, the effects in SSc were observed with lower doses of imatinib than those typically used to treat cancers. This may be due to the involvement of wild-type kinases in the pathogenesis of SSc that are effectively inhibited with low doses of imatinib, while higher doses are needed to inhibit cancer cell growth mediated by mutated and aberrantly overexpressed kinases.

We characterized the global gene expression profiles in SSc skin before and after imatinib treatment (Figure 3). Because the posttreatment sample from patient 2 was obtained after only 1 month of treatment and before obvious clinical improvement had occurred, this gene expression signature may reflect the primary response of SSc to imatinib, rather than secondary changes associated with disease resolution. We identified an imatinib-responsive signature with genes involved in multiple functional pathways, including cell proliferation, matrix and vascular remodeling, immune signaling, and growth factor signaling. The imatinib-responsive gene expression program was specifically and frequently dysregulated in both early and late dcSSc. Importantly, consistent with the hypothesis that PDGF signaling may be activated in SSc, a gene signature of the transcriptional response of fibroblasts to serum (the wound signature) (11), a principal component of which is PDGF, was induced in both SSc samples and substantially reduced by imatinib treatment (Figure 3B).

The effects of imatinib on the fibrotic and vascular complications of SSc warrant further investigation, and randomized clinical trials are needed. While case reports can highlight new disease entities or treatment options, they are traditionally limited by the uncertainty of general applicability. Here we have used genomic profiling to bridge this gap. We have identified a specific gene signature of imatinib response in 2 SSc patients undergoing experimental therapy with imatinib. By comparison with a larger database of gene profiles from patients with fibrosing disorders, we found that the majority of patients with dcSSc, but not lcSSc or morphea, exhibit the same transcriptional signature. These data raise the possibility that patients with dcSSc who express this signature may benefit clinically from imatinib therapy.

AUTHOR CONTRIBUTIONS

Dr. Robinson had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Chung, Fiorentino, Chang, Robinson.

Acquisition of data. Chung, Fiorentino, BenBarak, Adler, Mariano, Paniagua, Milano, Connolly, Ratiner, Wiskocil, Whitfield, Robinson.

Analysis and interpretation of data. Chung, Fiorentino, Adler, Paniagua, Milano, Connolly, Ratiner, Whitfield, Chang, Robinson.

Manuscript preparation. Chung, Fiorentino, Whitfield, Chang, Robinson.

Statistical analysis. Adler, Paniagua, Chang, Robinson.

REFERENCES

1. Baroni SS, Santillo M, Bevilacqua F, Luchetti M, Spadoni T, Mancini M, et al. Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. N Engl J Med 2006;354:2667–76.
2. Ludwicka A, Ohba T, Trojanowska M, Yamakage A, Strange C, Smith EA, et al. Elevated levels of platelet derived growth factor and transforming growth factor-β 1 in bronchoalveolar lavage fluid from patients with scleroderma. J Rheumatol 1995;22:1876–83.

3. Yamakage A, Kikuchi K, Smith EA, LeRoy EC, Trojanowska M. Selective upregulation of platelet-derived growth factor α receptors by transforming growth factor β in scleroderma fibroblasts. J Exp Med 1992;175:1227–34.
4. Schermuly RT, Dony E, Ghofrani HA, Pullamsetti S, Savai R, Roth M, et al. Reversal of experimental pulmonary hypertension by PDGF inhibition. J Clin Invest 2005;115:2811–21.
5. Soria A, Cario-Andre M, Lepreux S, Rezvani HR, Pasquet JM, Pain C, et al. The effect of imatinib (Glivec) on scleroderma and normal dermal fibroblasts: a preclinical study. Dermatology 2008; 216:109–17.
6. Distler JH, Jungel A, Huber LC, Schulze-Horsel U, Zwerina J, Gay RE, et al. Imatinib mesylate reduces production of extracellular matrix and prevents development of experimental dermal fibrosis. Arthritis Rheum 2007;56:311–22.
7. Daniels CE, Wilkes MC, Edens M, Kottom TJ, Murphy SJ, Limper AH, et al. Imatinib mesylate inhibits the profibrogenic activity of TGF-β and prevents bleomycin-mediated lung fibrosis. J Clin Invest 2004;114:1308–16.
8. Paniagua RT, Sharpe O, Ho PP, Chan SM, Chang A, Higgins JP, et al. Selective tyrosine kinase inhibition by imatinib mesylate for the treatment of autoimmune arthritis. J Clin Invest 2006;116: 2633–42.
9. Souza R, Sitbon O, Parent F, Simonneau G, Humbert M. Long term imatinib treatment in pulmonary arterial hypertension. Thorax 2006;61:736.
10. Clements P, Lachenbruch P, Seibold J, White B, Weiner S, Martin R, et al. Inter and intraobserver variability of total skin thickness score (modified Rodnan TSS) in systemic sclerosis. J Rheumatol 1995;22:1281–5.
11. Chang HY, Nuyten DS, Sneddon JB, Hastie T, Tibshirani R, Sorlie T, et al. Robustness, scalability, and integration of a wound-response gene expression signature in predicting breast cancer survival. Proc Natl Acad Sci U S A 2005;102:3738–43.
12. Milano A, Pendergrass SA, Sargent JL, George LK, McCalmont TH, Connolly MK, et al. Molecular subsets in the gene expression signatures of scleroderma skin. PLoS ONE 2008;3:e2696.
13. Whitfield ML, Finlay DR, Murray JI, Troyanskaya OG, Chi JT, Pergamenschikov A, et al. Systemic and cell type-specific gene expression patterns in scleroderma skin. Proc Natl Acad Sci U S A 2003;100:12319–24.
14. Sfikakis PP, Gorgoulis VG, Katsiari CG, Evangelou K, Kostopoulos C, Black CM. Imatinib for the treatment of refractory, diffuse systemic sclerosis. Rheumatology (Oxford) 2008;47:735–7.
15. Rajkumar VS, Shiwen X, Bostrom M, Leoni P, Muddle J, Ivarsson M, et al. Platelet-derived growth factor-β receptor activation is essential for fibroblast and pericyte recruitment during cutaneous wound healing. Am J Pathol 2006;169:2254–65.
16. Del Papa N, Quirici N, Corti L, Graziani D, Fasoli E, Maglione W, et al. Clinical and molecular evidence for c-kit receptor as a therapeutic target in systemic sclerosis (SSc) [abstract]. Arthritis Rheum 2007;56 Suppl 9:S816.