Concise report

Differential gene expression of MMP-1, TIMP-1 and HGF in clinically involved and uninvolved skin in South Africans with SSc

Jacqueline Frost1,2, Michèle Ramsay2, Ridwan Mia3, Laeka Moosa4, Eustasius Musenge5 and Mohammed Tikly1

Abstract

Objective. To investigate the differential expression of MMP-1, tissue inhibitor of metalloproteinase-1 (TIMP-1) and hepatocyte growth factor (HGF) in clinically involved (affected) and uninvolved (unaffected) skin in patients with SSc.

Methods. Punch biopsies from affected forearm and unaffected upper back skin of 16 black South Africans with dcSSc and skin samples of 15 ethnically matched healthy controls were studied. Quantitative mRNA expression of MMP-1, TIMP-1 and HGF was performed by relative reverse transcription quantitative PCR.

Results. Compared with controls, TIMP-1 expression was significantly upregulated in patients, a 796- and 397-fold difference for affected and unaffected skin (P < 0.00001 for both), respectively. Conversely, MMP-1 expression was significantly decreased in patients, a 10- and 12.5-fold difference for affected and unaffected skin (P = 0.0004 for both), respectively. HGF expression was up-regulated in both affected and unaffected skin, a 14- and 18-fold difference (P = 0.004 and P = 0.002), respectively. Within the patient group, HGF expression in affected skin of patients correlated significantly with the European scleroderma disease activity score (r = 0.60, P = 0.013).

Conclusion. Perturbations in gene expression of TIMP-1, MMP-1 and HGF were evident in both affected and unaffected skin of the dcSSc patients. Targeting TIMP-1, which showed the greatest dysregulation, needs to be explored as a way of reducing collagen deposition and fibrosis in dcSSc.

Key words: scleroderma, fibrosis, tissue inhibitor of metalloproteinase-1, matrix metalloproteinase-1, hepatocyte growth factor, African.

Introduction

Fibrosis is a hallmark feature of SSc. Fibroblasts show both phenotypic changes similar to that of myofibroblasts and functional changes resulting in pathological accumulation of collagen in the extracellular matrix (ECM).

The fibroblast, which has a pivotal role in ECM homeostasis, produces two main families of enzymes that maintain collagen homeostasis: (i) the MMPs mainly degrade collagen, and (ii) tissue inhibitors of metalloproteinases (TIMPs) protect ECM integrity by locally inhibiting MMPs [1]. In SSc, excess TIMP-1 relative to the MMPs under the influence of TGF-β is thought to promote a pro-fibrotic state. Cultured myofibroblasts isolated from SSc skin samples show increased synthesis of TIMP-1 and collagen compared with fibroblasts from healthy subjects [2].

Hepatocyte growth factor (HGF) is a potent epithelial cell mitogen and has anti-fibrotic properties [3]. In vitro studies show that HGF inhibits TGF-β-induced myofibroblast transformation of rat lung epithelial cells [4],...
decreases collagen production by skin fibroblasts [5] and increases production of MMP-1 in both SSc and control skin fibroblasts [6].

SSc patients of African extraction have more aggressive and extensive skin involvement and frequently have pulmonary fibrosis [7, 8]. In the present study we investigated the differential mRNA expression of MMP-1, TIMP-1 and HGF in clinically affected and unaffected skin of black South Africans with dcSSc. The study was approved by the Ethics Committee of the Faculty of Health Sciences, University of the Witwatersrand, and all participants consented in accordance with the Declaration of Helsinki.

Patients and control subjects

Sixteen black South Africans (14 females and 2 males) fulfilling the classification criteria for dcSSc [9] and disease duration <6 years were studied. Two punch biopsy samples of 4 mm each were taken from clinically involved skin on the extensor surface of the left forearm, 4 cm proximal to the ulnar styloid (affected skin) [10] and clinically uninvolved skin on the upper back (unaffected skin). The European SSc disease activity score (ESDAS) [11] and modified Rodnan skin score (mRSS) [12] were documented at the time. Control samples were taken from 15 otherwise healthy and age-matched patients undergoing reconstructive plastic surgery, in all from the forearm except one, where it was taken from the groin.

RNA isolation and relative quantitative PCR

Each sample was placed in a cryotube and immediately immersed in liquid nitrogen to prevent RNA degradation. RNA was extracted from the skin samples using the commercially available RNeasy Micro Plus kit (Qiagen, Hilden, Germany). Approximately 1–2 mg of the tissue sample was used for RNA extraction, yielding on average 1 mg of total RNA. The RNA was used directly for RT-PCR using the ImProm-II Reverse Transcription Kit (Promega, Fitchburg, WI, USA) to generate cDNA.

Relative quantitative PCR (qPCR) was done in triplicate using GAPDH as the endogenous control. Primers were designed for each gene so that they spanned two adjacent exons.

<table>
<thead>
<tr>
<th>MMP-1:</th>
<th>5'-GAGCAAAACATGCAGGTACAGGA-3' (forward)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5'-TTGCCCAGATCCTCCTGAGCA-3' (reverse)</td>
</tr>
<tr>
<td>TIMP-1:</td>
<td>5'-CCGGCCTCAGTCAGGCAACG-3' (forward)</td>
</tr>
<tr>
<td></td>
<td>5'-CTATCTCTAATCACCTGTCA-3' (reverse)</td>
</tr>
<tr>
<td>HGF:</td>
<td>5'-GAAACCTGCGACACGGCACTCTG-3' (forward)</td>
</tr>
<tr>
<td></td>
<td>5'-CCATGATGATGATGATGATTTC-3' (reverse)</td>
</tr>
</tbody>
</table>

The Roche LightCycler 2.0 was used for the gene expression analysis, making use of the standard protocol for qPCR. Relative quantification is calculated using the ΔCq method [13]. For each sample the triplicate Cq values were averaged, and the s.d. together with the coefficient of variance was calculated (data not shown).

Statistical methods

The Mann–Whitney U-test was applied to compare gene expression between groups and Spearman’s correlation test to assess correlations between gene expression ratios and continuous clinical variables. A Bonferroni-corrected P = 0.017 (0.05/3) was considered significant.

Results

Patients were relatively young with early active aggressive disease [mean (s.d.) age, disease duration, ESDAS and mRSS of 35.6 (10.6) years, 24.7 (17.2) months, 3.4 (1.7) and 24.7 (17.2), respectively]. A quarter had pulmonary fibrosis and all were ANA positive, of whom a quarter were anti-topo-1 positive.

TIMP-1 expression was markedly increased in patients, with a 795.7- and 387-fold difference for affected and unaffected skin, respectively, compared with the control group (Table 1). There was a trend towards increased expression in affected skin compared with unaffected skin within the patient group. Conversely, MMP-1 expression was significantly decreased in patients, but the difference was less marked, a 10- and 12.5-fold difference for affected and unaffected skin, respectively, compared with the control group. HGF expression was up-regulated in both affected and unaffected skin, a 14.3- and 17.9-fold difference, respectively, compared with the control group. No significant correlations were observed in gene expression between TIMP-1, MMP-1 and HGF in either affected or non-affected skin (data not shown). HGF expression in unaffected skin correlated with the ESDAS (r = 0.60, P = 0.013). No other significant associations between clinical features, in particular the mRSS, and gene expression were observed.

Discussion

In this cohort of early, clinically active dcSSc, we found dysregulation of all three mediators of fibrosis that we studied. TIMP-1, which in normal skin is minimally expressed [14], was markedly up-regulated in both affected skin and unaffected SSc skin. TIMP-1 levels were previously found to be increased in supernatants of cultured SSc fibroblasts in early disease (<2.5 years), which decline in late disease to levels not significantly different from controls [15]. A microarray gene expression study of ECM proteins in SSc skin suggests that TIMP-1 is of fibroblast origin, with a 5-fold increase in expression in cultured fibroblasts relative to whole biopsy specimens [16].

Conversely, MMP-1 was down-regulated in both affected and unaffected SSc skin, but the dysregulation was not nearly as marked as in the case of TIMP-1. Previous studies have produced conflicting results, with an early study of SSc-cultured fibroblasts showing unchanged collagenase activity compared with control fibroblasts [17] and a study of serum levels of TIMPs and MMP-1 in SSc showing only TIMP-1 levels to be increased [18]. Other studies support our findings showing that MMP-1 activity in cultured fibroblasts is decreased.
IQR: interquartile range. *P: affected skin vs controls; **P: unaffected skin vs controls; ***P: affected vs unaffected skin.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Affected skin</th>
<th>P*</th>
<th>Unaffected skin</th>
<th>P**</th>
<th>P***</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1, median (IQR)</td>
<td>0.04 (0.009–0.07)</td>
<td>0.004 (0.003–0.01)</td>
<td>0.0004</td>
<td>0.005 (0.003–0.007)</td>
<td>0.0004</td>
<td>0.87</td>
</tr>
<tr>
<td>TIMP-1, median (IQR)</td>
<td>0.0004 (0.0003–0.02)</td>
<td>0.319 (0.13–1.2)</td>
<td>&lt;0.0001</td>
<td>0.155 (0.06–0.5)</td>
<td>&lt;0.00001</td>
<td>0.05</td>
</tr>
<tr>
<td>HGF, median (IQR)</td>
<td>0.0014 (0.0003–0.008)</td>
<td>0.02 (0.006–0.03)</td>
<td>0.004</td>
<td>0.025 (0.008–0.04)</td>
<td>0.002</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Suboptimal HGF production might explain why extensive skin disease and lung fibrosis are more common in SSC patients of African extraction [7]. HGF levels in both bronchoalveolar lavage fluid and plasma are lower in African Americans than in Caucasian Americans with SSC [23]. Moreover, recombinant HGF abolished CTGF expression and collagen synthesis in lung fibroblasts from Caucasian but not African American patients. Functional polymorphisms of the highly polymorphic HGF and c-met receptor genes might account for these ethnic differences [24, 25].

An intriguing finding is the extent of dysregulation of gene expression in unaffected skin of dcSSc patients. Abnormal endothelial activation and procollagen production has been shown in clinically unaffected skin [26]. FII1, a transcription factor that inhibits collagen gene transcription, is found to be down-regulated in cultured fibroblasts in unaffected skin, but not to the same extent as in affected skin [27]. DNA microarray studies show almost indistinguishable patterns of gene expression between affected and unaffected skin [10]. The clinical phenotypic differences between the affected and unaffected skin might be due to additional factors like hypoxia, which can up-regulate several ECM proteins, including proα2(Ⅰ) collagen, fibronectin-1 and TGF-β [28].

The strength of the present study was that gene expression studies were done on whole skin biopsy samples rather than cultured fibroblasts. Due to financial and technical constraints we were unable to perform immunohistochemistry studies to determine cell-specific dysregulation of these mediators of fibrosis and also to measure other markers such as CTGF, collagen and α-smooth muscle actin (α-SMA). The small sample size did not allow any meaningful conclusions to be drawn on the relationship of clinical features and gene expression.

In summary, perturbations in gene expression of TIMP-1, MMP-1 and HGF were evident in both affected and unaffected skin in black South Africans with dcSSc. Targeting TIMP-1, which showed the greatest dysregulation, needs to be explored as a way of reducing collagen deposition and fibrosis in dcSSc.

**Rheumatology key messages**

- TIMP-1, MMP-1 and HGF gene expression is dysregulated in affected and unaffected scleroderma skin.
- Therapies that down-regulate TIMP-1 synthesis may potentially reduce fibrosis in scleroderma.

**Acknowledgements**

**Funding:** The Connective Tissue Disease Research Fund from the University of the Witwatersrand Medical School, Johannesburg, South Africa.

**Disclosure statement:** The authors have declared no conflicts of interest.

**References**

3. Esposito C, Parrilla B, Cornacchia F et al. The antifibrogenic effect of hepatocyte growth factor (HGF) on renal...
15 Zurita-Salinas CS, Krotzsch E, Diaz de Leon L et al. Collagen turnover is diminished by different clones of skin fibroblasts from early- but not late-stage systemic sclerosis. Rheumatol Int 2004;24:283–90.