Fli1 deficiency promotes CXCL13 expression from macrophages, contributing to the development of systemic sclerosis

Takashi Taniguchi, Yoshihide Asano, Takashi Yamashita, Kouki Nakamura, Ryosuke Saigusa, Yohei Ichimura, Takehiro Takahashi, Tetsuo Toyama, Ayumi Yoshizaki, Shinichi Sato.
Dermatology, University of Tokyo Graduate School of Medicine, Tokyo, Japan.

INTRODUCTION

Systemic sclerosis (SSc) is a multisystem autoimmune and vascular disease resulting in extensive organ fibrosis. CXCL13 induces chemotaxis of mature B cells, follicular helper T cells, T helper (Th) 17 cells, and regulatory T cells through its receptor CXCR5, while inhibiting angiogenesis by interfering with basic fibroblast growth factor. To investigate the role of CXCL13 in SSc, we examined the influence of Fli1 deficiency, a potential predisposing factor of this disease, on CXCL13 expression and assessed clinical correlation of serum CXCL13 levels by multivariate regression analysis.

METHODS

Gene silencing of *FLI1* and qRT-PCR

Cells were transfected with 10 nM of *FLI1* siRNA or scrambled non-silencing RNA using HiPerfect transfection reagent for 72 hours. mRNA levels of the *FLI1* and *CXCL13* genes in human cells and those of the *Fli1* and *Cxcl13* genes in murine macrophages were examined by qRT-PCR and normalized to mRNA levels of the *GAPDH* or *Gapdh* genes.

The measurement of serum CXCL13 levels

Specific ELISA kits were used to measure serum CXCL13 levels in 56 SSc patients (52 women, 4 men; age, median [25 - 75 percentile], 59 years [51.5 - 69]; disease duration, 5 years [2 - 13]) after getting informed consent.

RESULTS

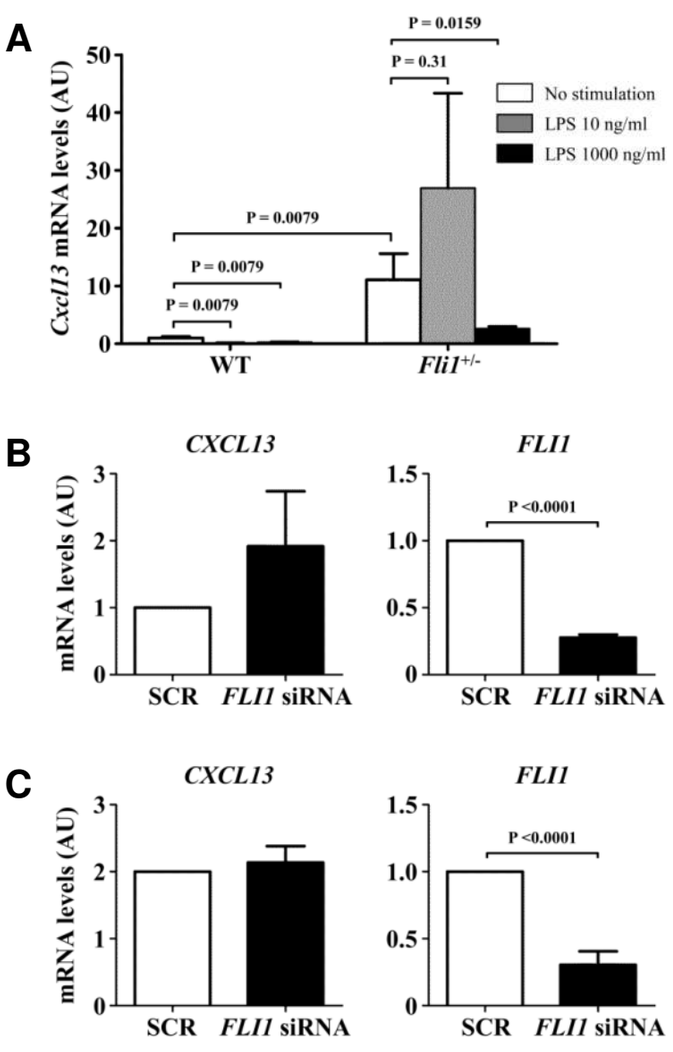

Figure 1. Fli1 deficiency regulates CXCL13 expression in macrophages.
(A) *Cxcl13* mRNA levels were determined by qRT-PCR in murine *Fli1*$^{+/-}$ and wild type (WT) peritoneal macrophages treated or untreated with lipopolysaccharide (n = 5). (B, C) *CXCL13* mRNA expression was evaluated by qRT-PCR in human dermal fibroblasts (n = 8, B) and human dermal microvascular endothelial cells (HDMECs; n = 4, C) treated with *FLI1* siRNA or scrambled non-silencing RNA (SCR). Results of controls or relative value compared with the controls are expressed as means ± SEM. Statistical analysis was carried out with Mann-Whitney U-test. Statistical significance was determined after Bonferroni correction in experiments with macrophages.

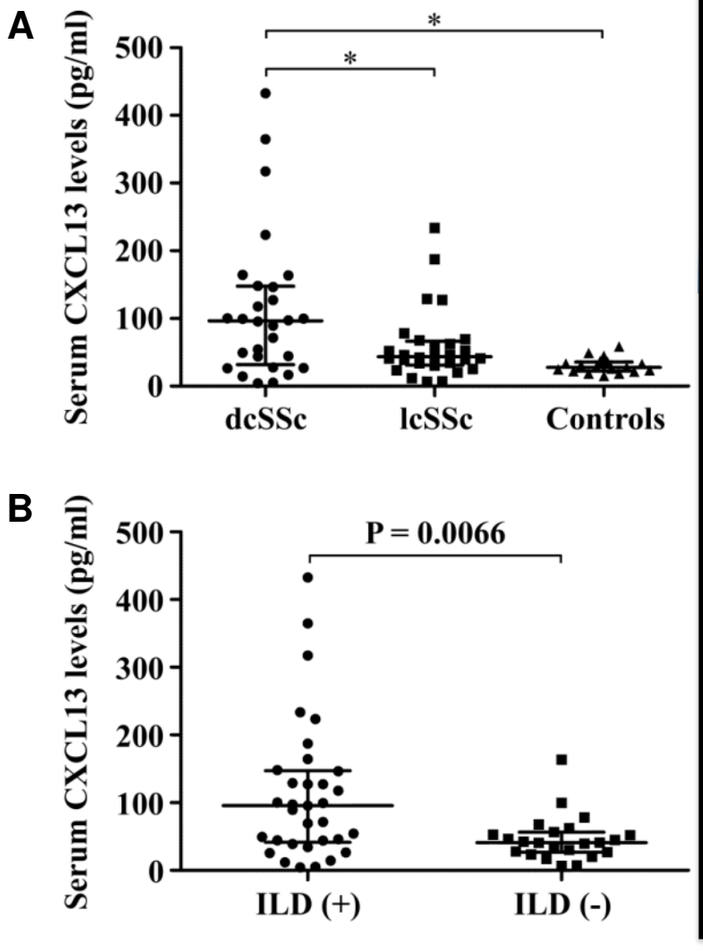

Figure 2. Serum CXCL13 levels are elevated in SSc patients, especially in those with diffuse cutaneous involvement and interstitial lung disease.
(A) Serum CXCL13 levels were measured in diffuse cutaneous SSc (dcSSc) patients, limited cutaneous SSc (lcSSc) patients, and healthy controls. Statistical analysis was carried out with one-way ANOVA followed by Turkey *post hoc* test (*P <0.05). (B) Serum CXCL13 levels were compared between SSc patients with interstitial lung disease (ILD) and those without. Statistical analysis was carried out with Mann-Whitney U-test.

Figure 3. Serum CXCL13 levels correlate with mRSS and pulmonary function test results (%VC and %DLco).
(A-C) Serum CXCL13 levels correlated positively with modified Rodnan total skin thickness score (mRSS, A) and negatively with the percentage of predicted vital capacity (%VC, B) and the percentage of predicted diffusion lung capacity for carbon monoxide (%DLco, C). Statistical analysis was carried out with Spearman's rank correlation test.

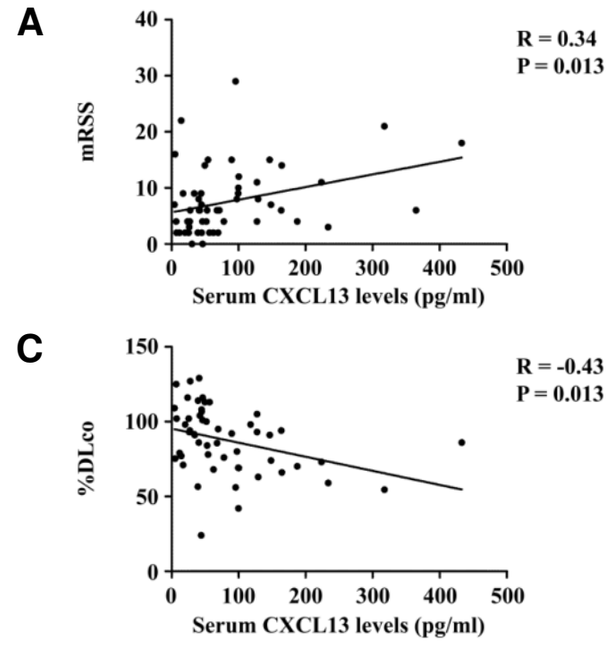

Table 1. Clinical correlation of serum CXCL13 levels in SSc

	Serum CXCL13 levels		
	Elevated (n = 27)	Not elevated (n = 29)	P values
Sex, no. male : no. female	2 : 25	2 : 27	1.0
Age, mean years	59 (54 - 70)	58 (49.5 - 69)	0.62
Disease duration, mean years	7.5 (3 - 20)	3 (1.5 - 10)	0.034
Skin sclerosis (dcSSc: lcSSc)	18 : 9	10 : 19	0.032
Clinical features, %			
Pitting scars	48 (13/27)	7 (2/29)	0.0007
Digital ulcers	63 (17/27)	7 (2/29)	<0.01
Raynaud's phenomenon	100 (27/27)	72 (21/29)	0.0046
Telangiectasia	67 (18/27)	33 (9/27)	0.029
Nailfold bleeding	63 (17/27)	69 (20/29)	0.78
Organ involvements, %			
Esophageal dysfunction	81 (22/27)	66 (19/29)	0.23
Interstitial lung disease	78 (21/27)	41 (12/29)	0.0073
Decreased %DLco	36 (9/25)	7 (2/28)	0.016
Decreased %VC	27 (7/26)	7 (2/28)	0.072
Elevated RVSP	26 (7/27)	7 (2/29)	0.073
Heart involvement	37 (10/27)	0 (0/29)	0.0002
Scleroderma renal crisis	11 (3/27)	0 (0/29)	0.11
Laboratory findings, %			
Elevated serum IgG	30 (8/27)	4 (1/27)	0.024
Elevated ESR	60 (15/25)	35 (9/26)	0.095
Increased CRP	26 (7/27)	24 (7/29)	1.0

Table 2. Factors predicting serum CXCL13 levels determined by multivariate regression analysis.

	Estimate	Standard error	P value
Intercept	28.4	18.2	P = 0.12
Digital ulcers	53.4	22.3	P = 0.020
Interstitial lung disease	67.2	21.5	P = 0.0029

CONCLUSION

CXCL13 expression is up-regulated by Fli1 deficiency in macrophages, potentially contributing to the development of tissue fibrosis, vasculopathy, and immune activation in SSc, especially ILD and digital ulcers.
This pathological process may be mediated by a multifaceted role of CXCL13, such as the regulation of macrophage/B cell interaction, Th17 cell infiltration, and angiogenesis.
The association of Fli1 deficiency with CXCL13 up-regulation in macrophages further supports the notion that Fli1 deficiency is a critical predisposing factor of SSc.

Conflict of interest

The authors declare no conflict of interest related to this study.