The antifibrotic role of hepatocyte growth factor in dermal fibroblasts of systemic sclerosis

Sirs,

Hepatocyte growth factor (HGF) is a multifunctional mediator involving in cell proliferation and differentiation and has been previously shown to be a key antifibrotic cytokine that displays a potent therapeutic potential in preventing tissue fibrosis in systemic sclerosis (SSc, or scleroderma) (1, 2). High concentration of HGF inhibited collagen production in cultured SSc fibroblasts (3). HGF transfected into bleomycin-treated mice ameliorated dermal sclerosis (4). However, the information concerning the signalling pathways through which HGF acts against fibrogenesis are not fully understood. Here the antifibrotic role of HGF was further investigated in normal and SSc dermal fibroblasts cultured according to previous description (5).

Treatment of cultured SSc dermal fibroblasts with HGF (25ng/ml, R&D) after 24 hours considerably resulted in down-regulation of connective tissue growth factor (CTGF), collagen type I and fibronectin (FN) protein in cellular lysates, sustained until 48 hours, as determined by Western blot (Fig. 1A). We further examined the effect of HGF on TGF-β1-induced fibrosis. Treatment with 10ng/ml TGF-β1 (R&D) for 24 hours increased the production of CTGF, collagen type I and FN in SSc dermal fibroblasts (Fig. 1B). However, pretreatment with 25ng/ml HGF essentially abrogated TGF-β1-induced CTGF and collagen type I production, which is consistent with the anti-TGF-β1 action of HGF in sarcoidosis (6) and renal fibrosis (7).

Elevated phosphorylation of Akt contributes to profibrotic protein expression in SSc fibroblasts (8). In addition, Smad1 signaling was also activated in SSc and contributes to activation of SSc fibroblasts (9). Thus, we further define if HGF may alleviate ECM production through abolishing Akt and Smad1 signals. HGF at 25ng/ml increased ERK-1/2 phosphorylation level within 15 minutes in SSc dermal fibroblasts, and this level regressed to normal level and reduced in 24 hours. However, HGF reduced AKT and Smad1 phosphorylation at 15 minutes after incubation, and this decrease was sustained until 24 hours. The level of ERK-1/2, AKT and Smad1 in SSc dermal fibroblasts did not change in the presence of HGF (Fig. 2A). In normal human dermal fibroblasts, ERK, AKT and Smad1 were markedly activated upon 10ng/ml TGF-β1 stimulation at 15 min, such activation of ERK was not changed by HGF, while the TGF-β1-induced phosphorylation of AKT and Smad1 was decreased by HGF at 15 min (Fig. 2B).

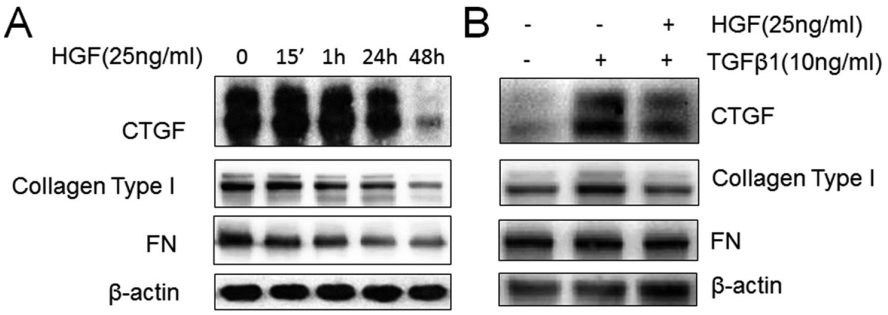

Fig. 1. A: Treatment of HGF (25ng/ml) repressed the expression of CTGF, collagen and FN in SSc dermal fibroblasts after 24 hours and sustained until 48 hours.
B: Pretreatment of HGF (25ng/ml) blocked matrix overproduction (including CTGF, collagen and FN) induced by TGF-β1 (10ng/ml) after 24 hours in normal human dermal fibroblasts. Immunoblots are a representative of 6 independent experiments.

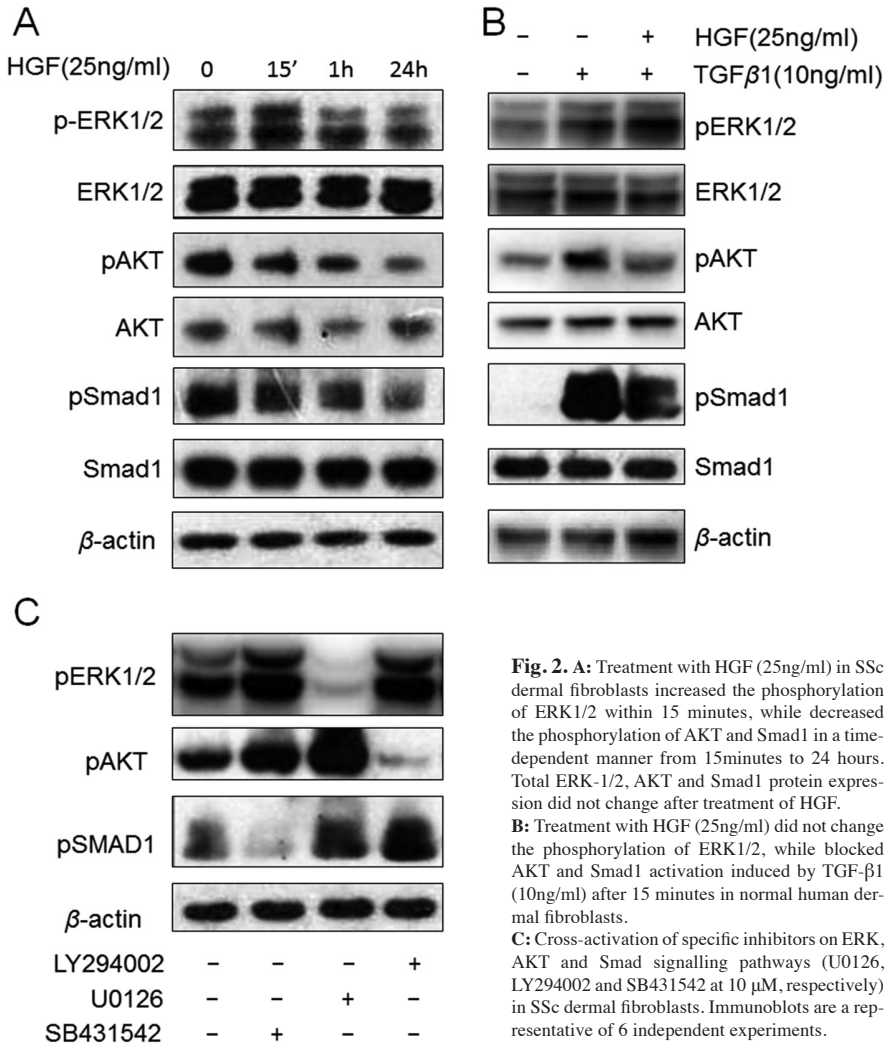

Fig. 2. A: Treatment with HGF (25ng/ml) in SSc dermal fibroblasts increased the phosphorylation of ERK1/2 within 15 minutes, while decreased the phosphorylation of AKT and Smad1 in a time-dependent manner from 15minutes to 24 hours. Total ERK-1/2, AKT and Smad1 protein expression did not change after treatment of HGF.
B: Treatment with HGF (25ng/ml) did not change the phosphorylation of ERK1/2, while blocked AKT and Smad1 activation induced by TGF-β1 (10ng/ml) after 15 minutes in normal human dermal fibroblasts.
C: Cross-activation of specific inhibitors on ERK, AKT and Smad signalling pathways (U0126, LY294002 and SB431542 at 10 μM, respectively) in SSc dermal fibroblasts. Immunoblots are a representative of 6 independent experiments.

To confirm the interplay between ERK, Akt and Smad1 pathways, specific inhibitors, U0126 (MEK-1/2 inhibitor), LY294002 (PI3K/Akt inhibitor) and SB431542 (ALK5 inhibitor) at 10 μM were included to incubate scleroderma dermal fibroblasts for 24h, respectively. U0126 inhibited the phosphorylation of ERK1/2, but increased the phosphorylation of AKT and Smad1 (Fig. 2c). LY294002 inhibited the phosphorylation of AKT, but increased the phosphorylation of Smad1 and ERK1/2 (Fig. 2C). SB431542 inhibited the phosphorylation of Smad1, but increased the phosphorylation of ERK1/2 and AKT (Fig. 2C). Therefore, it seems that ERK, Akt and Smad1 signaling are really having crosstalks in SSc dermal fibroblast. Taken together, our results re-

veal that HGF may reduce fibrosis in SSc through inhibiting AKT and Smad1 signals. ERK, Akt and Smad1 signals may compensate each other in the absence of other signals in SSc fibroblasts.

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