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ORIGINAL ARTICLE

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Antifibrotic effects of hepatocyte growth factor on scleroderma fibroblasts and analysis of its mechanism

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Abstract We investigated the effect of hepatocyte growth factor (HGF) on collagen metabolism in cultured fibroblasts from scleroderma (SSc) patients and discussed the possible mechanism of its effect. Synthesis of matrix metalloproteinase-1 (MMP-1) and collagen and mRNA levels of various cytokines were examined by enzyme-linked immunosorbent assay and real-time polymerase chain reaction, respectively. Hepatocyte growth factor enhanced MMP-1 production and mRNA levels of MMP-1 and Ets-1 (a transcriptional factor of MMPs). In addition, HGF suppressed collagen synthesis and mRNA levels of procollagenα1(I) and connective tissue growth factor (CTGF) in SSc fibroblasts. Expression of transforming growth factor (TGF)-β1 was not inhibited significantly in SSc or control fibroblasts. Hepatocyte growth factor also increased interferon (IFN)-γ mRNA significantly in SSc and control fibroblasts. Addition of anti-HGF antibody neutralized these effects of HGF on MMP-1 and collagen synthesis. The results suggest that HGF can suppress collagen accumulation in SSc fibroblasts by increasing MMP-1 levels possibly via activation of Ets-1 and also by decreasing collagen synthesis, which may be partly related to inhibition of CTGF, and increasing IFN-γ levels rather than the effect on TGF-β1. The present study indicates that HGF may be a promising therapeutic agent for this intractable disease.

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Introduction

Scleroderma (systemic sclerosis, SSc) is a multisystem connective tissue disease characterized by inflammatory, fibrotic, and degenerative changes in the skin and other organs.[1,2] At present, the pathogenesis of SSc is unknown. Some phenotypes characteristic to cultured SSc fibroblasts have been demonstrated, such as increased production of collagen and glycosaminoglycans[1,2] and decreased level of matrix metalloproteinases (MMP) that can degrade overproduced extracellular matrix (ECM),[3,4] both of which can contribute to excessive accumulation of ECM. It has been suggested that several growth factors and cytokines released from inflammatory cells play an important role in the initiation and progression of fibrosis in SSc. Among these, transforming growth factor (TGF)-β has been focused as a key factor in the pathogenesis of SSc.[5] Transforming growth factor-β is known to stimulate collagen production and to injure endothelial cells, which may lead to skin and vascular lesion characteristically seen in SSc patients. Recently, connective tissue growth factor (CTGF), which is a downstream mediator of TGF-β1 and modulates fibroblast proliferation and ECM production,[6] also has been reported to be important to maintain fibrosis.[7,8]

Hepatocyte growth factor (HGF), originally identified as a potent mitogen for hepatocytes, is a multifunctional mediator that shows motogenic, morphogenetic, and antiapoptotic activity in a variety of cells.[9–11] Hepatocyte growth factor is also known to have anti-TGF-β action and has been shown to reverse fibrogenic processes in animal models.[12–14] In these reports, HGF inhibited ECM deposition and reduced amount of pre-existing ECM constituents including fibrillar collagen. In addition, renal tubulointerstitial fibroblasts treated with HGF in advance significantly reduced CTGF induction by TGF-β1.[14] Up to the present, there is only a

limited number of studies concerning the action of HGF in SSc cells. Kawaguchi et al. reported that HGF inhibited collagen production in SSc fibroblasts[15] and also showed an increased serum level of HGF in SSc patients.[16] Recently, transfection of human HGF gene into bleomycin-treated mice prevented dermal sclerosis,[17] and the antifibrotic effect of HGF on collagen and MMP-1 synthesis in cell culture medium of fibroblasts from SSc patients was reported.[18] However, the information concerning HGF effect on SSc fibroblasts is still limited, and the mechanism by which HGF acts against fibrogenesis, especially whether HGF inhibits TGF-β1 action directly or inhibits other fibrotic pathways, is not fully understood. In addition to suppression of collagen synthesis, degradation of pre-existing ECM is thought to be a necessary step in resolution of fibrosis. Although studies in animal models treated with HGF suggested that HGF stimulated ECM-degrading activity or made a shift toward the degradation in ECM metabolism,[12,17] underlying mechanisms remain to be clarified.

In the present study, we investigated the effect of HGF on ECM metabolism in SSc fibroblasts, especially its effect on the expression and synthesis of MMP-1, collagen, Ets-1, CTGF, and other cytokines considered to be related with fibrosis, and further discussed the possible mechanism of HGF action.

Materials and methods

Patients

Skin samples were obtained from six patients with SSc and five age- and sex-matched normal subjects. All SSc patients were diagnosed as definite SSc according to the American College of Rheumatology criteria for classification of scleroderma.[19] Three patients had limited cutaneous SSc and three had diffuse cutaneous SSc. All biopsy specimens were obtained from sclerotic skin at the dorsum of the patients' forearm and also from normal skin at the same area in normal controls. A small portion of SSc specimen, separated from biopsied skin sample for pathological examination, was used for subsequent culture. All subjects gave informed consent prior to sample acquisition. The study was approved by the Saga Medical School Ethics Committees. Characteristics of the patients and controls enrolled in the study are shown in Table 1.

Table 1. Characteristics of SSc patients and controls

	Age (years)	Sex	Disease type	Duration of illness (months)	Drugs
SSc patients					
1	61	M	Limited	4	NSAID
2	52	F	Diffuse	5	None
3	58	F	Diffuse	18	None
4	56	F	Limited	7	NSAID
5	75	F	Limited	60	None
6	42	F	Diffuse	15	None
Controls					
1	40	F			
2	48	F			
3	64	F			
4	45	M			
5	72	F			

NSAID, nonsteroidal anti-inflammatory drug; SSc, systemic sclerosis

Fibroblast culture

Skin explants were minced and placed in plastic dishes. After cells had adhered to the dish, culture medium consisting of Dulbecco's modified essential medium (Sigma–Aldrich, St. Louis, MO, USA) with 10% fetal calf serum (FCS) was added. Explant cultures were incubated at 37°C in 5% CO_2/95% air, followed by subcultures. The cells in the 3rd through 9th passages were used in the following experiments. The cells were plated at a density of 50000 cells/well in 24-well culture plates (Nunc, Roskilde, Denmark). When the culture reached confluency, the wells were washed and fresh medium with 5% FCS was added with or without recombinant HGF (R&D Systems, Minneapolis, MN, USA) at the indicated concentrations. At the end of the culture, supernatants were collected and stored at –80°C until a measurement of MMP-1 and collagen production. Cells in the wells were washed with phosphate-buffered saline (PBS) and stored –80°C until a measurement of cellular protein concentration. Cell protein was measured using BCA Protein Assay Kit (Pierce, Rockford, IL, USA).

Measurement of MMP-1 and collagen protein

The amount of MMP-1 accumulated in fibroblast culture medium was measured by a one-step sandwich enzyme immunoassay kit (Daiichi Fine Chemical, Toyama, Japan). Collagen concentration in the supernatant was measured by using a SIRCOL collagen assay kit (Biocolar, Belfast, UK).

Effect of neutralizing anti-HGF antibody

It was examined whether addition of anti-HGF antibody could neutralize HGF effect on MMP-1 and collagen production. Fibroblasts were plated in 24-well culture plates as described above, and after confluency the dish was treated with HGF (100 ng/ml) for 24 h in the presence or absence of 100 μg/ml of monoclonal anti-human HGF antibody (R&D Systems), which was mouse monoclonal (clone 24612) isotype IgG1 and was shown to specifically neutralize the biological activity of rHGF according to manufacturer's reference. Then, MMP-1 and collagen concentration in the supernatant was measured as described.

RNA isolation and real-time polymerase chain reaction (PCR)

For real-time PCR, fibroblasts were plated at a density of 10^6 cells/100-mm dish and after confluency the dish was treated

Table 2. Primers used in the study

Gene	Sequence	Fragment size (bp)
GAPDH	5′-ACGCATTTGGTCGTATTGGG-3′	
	5′-TGATTTTGGAGGGATCTCGC-3′	230
c-Met	5′-TCTTGGGACATCAGAGGGTC-3′	
	5′-TGACTGCAGGACTGAAATG-3′	222
MMP-1	5′-TCCAAGCCATATATGGACGTT-3′	
	5′-ACTTCATCTCTGTCGGCAAAT-3′	255
Procollagenα1(I)	5′-TGAATCTGGACGTGAGGGGG-3′	
	5′-CACGGTCACGAACCACATTGGCAT-3′	647
Ets-1	5′-GGGTAGCGACTTCTTGTTTG-3′	
	5′-GTTAATGGAGTCAACCCAGC-3′	274
CTGF	5′-GAGGAAAACATTAAGAAGGGCAAA-3′	
	5′-CGGCACAGGTCTTGATGA-3′	250
TGF-β1	5′-GCCCTGGACACCAACTATTGCT-3′	
	5′-AGGCTCCAAATGTAGGGGCAGG-3′	161
IL-6	5′-ATGAACTCCTTCTCCACAAGCGC-3′	
	5′-GAAGAGCCCTCAGGCTGGACTG-3′	628
IFN-γ	5′-TTCGGTAACTGACTTGAATGT-3′	
	5′-ACCTCGAAACAGCATCTGA-3′	121

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MMP-1, matrix metalloproteinase-1; CTGF, connective tissue growth factor; TGF-β1, transforming growth factor-beta 1; IL-6, interleukin-6; IFN-γ, interferon-gamma

with 100 ng/ml HGF for 0, 6, 24, or 48 h and total RNA was extracted using an Easy Prep RNA isolation kit (Takara, Shiga, Japan). To quantitatively estimate the mRNA expression of the relevant gene, PCR was performed on a Light-Cycler instrument system (Roche, Mannheim, Germany) using SYBR greenIdye in combination with the Light-Cycler DNA Master SYBR GreenIKit (Roche, Basel, Switzerland). Briefly, 1 μg of each total RNA was reverse transcribed with random primer. The resulting synthesized cDNA was used for real-time PCR amplification with primers specific for MMP-1, Ets-1, procollagen-α1(I), CTGF, TGF-β1, IL-6, and IFN-γ (Table 2). Polymerase chain reactions were performed in a total volume of 20 μl, containing 5 mM $MgCl_2$, 2 μM each primer, 2 μl of the cDNA. After 30 s denaturation at 95°C, PCRs were carried out with 55 cycles of 2 s denaturation at 95°C, 10 s annealing at 55°C, and 15 s extension at 72°C. Melting curves were obtained with a protocol consisting of a 0 s denaturation period at 95°C, a starting temperature of 65°C, an ending temperature of 95°C, and a rate of temperature increase of 0.2°C/s. Polymerase chain reaction and melting curves were detected online with the Light-Cycler instrument. The experiments were carried out in duplicate. The PCR product was quantified using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) plasmid as a standard, which was purified using QIA quick Gel Extraction Kit (Qiagen, Hilden, Germany), and the relative expression rate was calculated.

Statistical analysis

Statistical analysis was performed using SPSS version 10. Statistical comparisons were made using non-parametric Wilcoxon's signed rank test to measure the difference of the paired data, and the Mann–Whitney *U*-test to see the difference between SSc and control fibroblasts. A *P* value of less than 0.05 was considered to be significant for all tests.

Results

Effect of HGF on MMP-1 and collagen production

Treatment of cultured fibroblasts with HGF at various concentrations (0–1 μg/ml) resulted in a dose-dependent increase in MMP-1 synthesis (data not shown). Matrix metalloproteinase-1 protein secreted into the medium increased significantly both in SSc and control fibroblasts by treatment with 100 ng/ml of HGF at each treatment time (Fig. 1A). Without HGF treatment, SSc cells produced significantly less MMP-1 than control cells at 24 h culture. Although the MMP-1 increasing effect by HGF treatment seemed high in SSc cells as compared with control cells, the difference was not significant.

Production of collagen secreted into the medium by fibroblasts treated with HGF decreased both in SSc and control fibroblasts in a dose-dependent manner (data not shown). Without HGF treatment, collagen production was higher in SSc cells than in control cells at 48 h after incubation (Fig. 1B). The amount of collagen decreased significantly in SSc fibroblasts treated with HGF at all treatment times, while control fibroblasts showed significantly decreased collagen production only after 24 h of HGF treatment. Although the inhibitory ratio of collagen production by HGF treatment seemed high in SSc cells as compared with control cells, the difference was not significant.

Effect of HGF on various gene expressions

Real-time PCR analysis revealed that MMP-1 mRNA from SSc and control fibroblasts increased significantly after treatment with HGF (Fig. 2A). Peak expression of MMP-1 mRNA in control fibroblasts was seen after 24 h of HGF treatment, and then decreased after 48 h treatment, while in

SSc fibroblasts MMP-1 mRNA continued to increase during all treatment times. The expression of transcriptional factor of MMPs, Ets-1 increased significantly both in SSc and control fibroblasts after 6 h of HGF treatment, then gradually decreased (Fig. 2B). Procollagenα1(I) mRNA from SSc fibroblasts, which showed higher baseline level than from control cells, decreased significantly at 48 h of HGF treatment, while control fibroblasts showed no significant effect (Fig. 3A). Connective tissue growth factor mRNA in SSc fibroblasts decreased significantly after 48 h treatment with HGF, but not in control cells (Fig. 3B). Although TGF-β1 expression in SSc fibroblasts was extremely higher than controls, there was no effect of HGF treatment on TGF-β1 mRNA level in either cells (Fig. 3C). c-Met expression showed tendency toward somewhat higher level in SSc fibroblasts than controls, but the difference was not significant (Fig. 3D). In addition, there was no effect of HGF treatment on c-Met mRNA level in either cells. On the other hand, IFN-γmRNA, of which baseline level was significantly less in SSc than in control cells, increased significantly after 48 h treatment with HGF in both fibroblasts (Fig. 3E). Hepatocyte growth factor had no significant effect on IL-6 expression in SSc and control cells (data not shown).

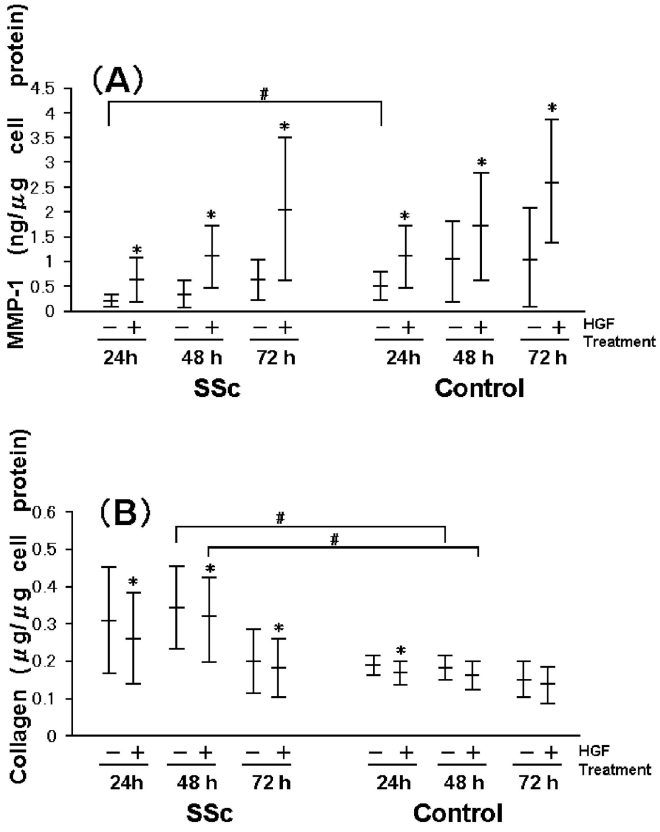

Fig. 1A,B. Effect of hepatocyte growth factor (*HGF*) on production of matrix metalloproteinase (*MMP*)-1 and collagen in systemic sclerosis (*SSc*) and control fibroblasts. MMP-1 (**A**) and collagen (**B**) protein secreted into the medium by fibroblasts treated with 100 ng/ml of HGF for 24, 48, and 72 h was determined by enzyme immunoassay and Sircol assay, respectively. ($n = 6$ for SSc group, $n = 5$ for control group values expressed as mean ± SD). *$P < 0.05$, estimated by Wilcoxon's signed rank test between HGF-treated and -untreated conditions at each treatment time in each cell group. #$P < 0.05$, estimated by Mann–Whitney *U*-test between SSc and control cells at each culture time

Neutralizing effect by the addition of anti-HGF antibody

Matrix metalloproteinase-1 and collagen produced by the fibroblasts incubated with or without anti-HGF antibody (100 μg/ml) in addition to HGF (100 ng/ml) for 24 h were measured (Fig. 4A,B). As described above, HGF significantly increased MMP-1 and decreased collagen production in SSc and control cells. Further addition of anti-HGF antibody restored these up- or downregulated productions approximately to untreated level in both cell groups. Addition of anti-HGF antibody alone caused even lower MMP-1 production and higher collagen production as compared with under untreated condition ($P < 0.05$).

Discussion

In the present study, SSc fibroblasts tended to produce a larger amount of collagen and less MMP-1 than control cells

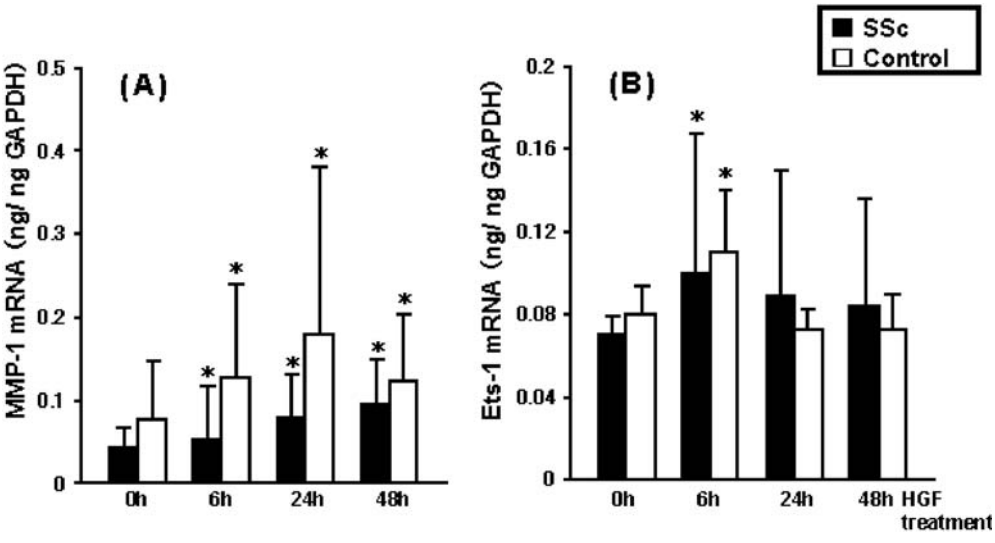

Fig. 2A,B. Effect of HGF on expression of MMP-1 and Ets-1 expression in SSc and control fibroblasts. mRNA level of MMP-1 (**A**) and Ets-1 (**B**) in cultured fibroblasts treated with 100 ng/ml of HGF for 0, 6, 24, and 48 h was analyzed by real-time polymerase chain reaction assay ($n = 6$ for SSc group, $n = 5$ for control group; values expressed as mean ± SD). *$P < 0.05$, vs 0 h in each cell group, estimated by Wilcoxon's signed rank test. *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase

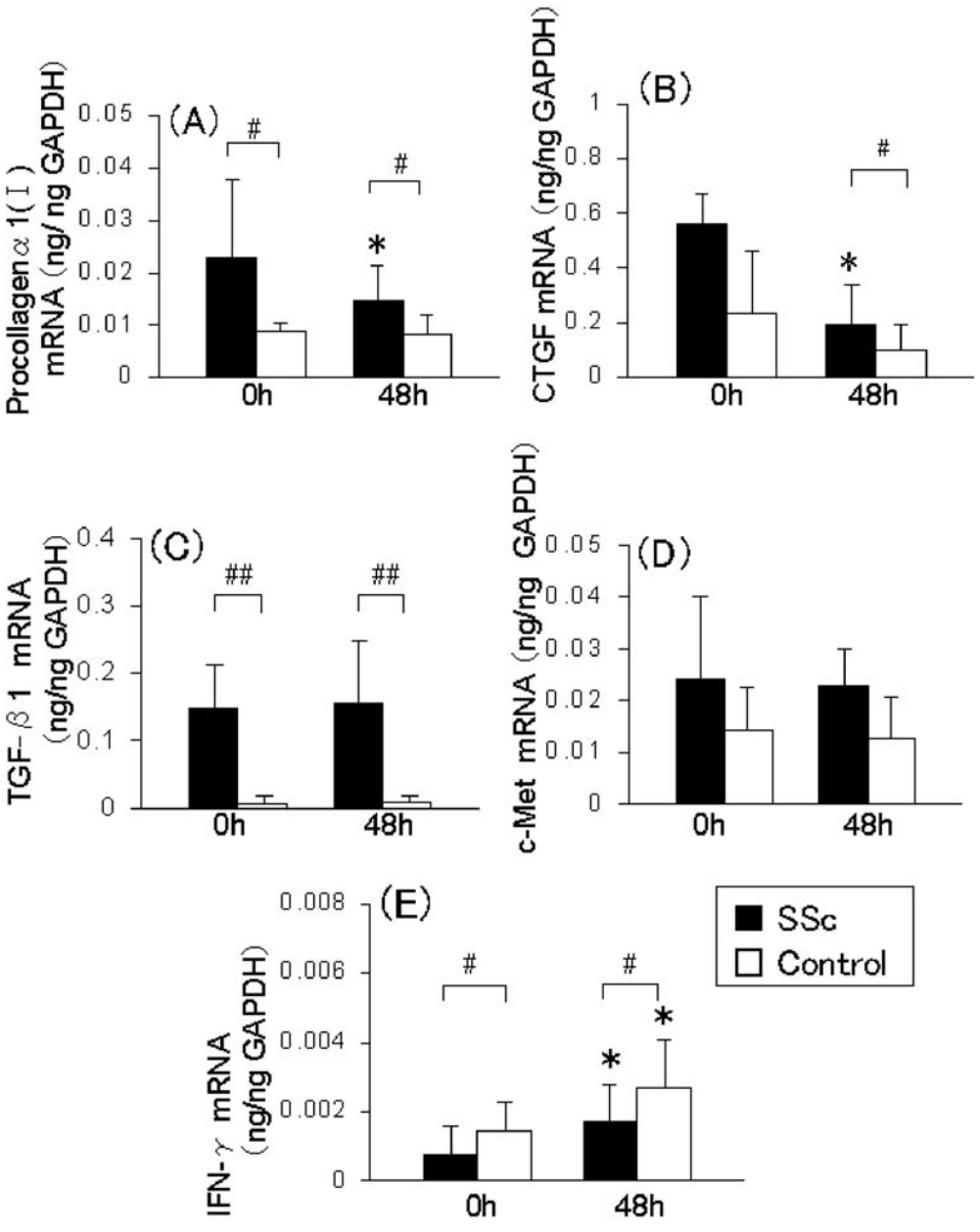

Fig. 3A–E. Effect of HGF on expression of procollagenα1(I), connective tissue growth factor (*CTGF*), transforming growth factor β1 (*TGF-β1*), c-Met, and interferon (*IFN*) expression in SSc and control fibroblasts. mRNA level of procollagenα1(I) (**A**), CTGF (**B**), TGF-β1 (**C**), c-Met (**D**), and IFN-γ (**E**) in cultured fibroblasts treated with 100 ng/ml of HGF for 48 h was analyzed by real-time polymerase chain reaction assay (n = 6 for SSc group, n = 5 for control group; values expressed as mean ±SD). $^{*}P < 0.05$, vs 0 h in each cell group, estimated by Wilcoxon's signed rank test. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, estimated by Mann–Whitney U-test between SSc and control cells

in the absence of HGF. Although the variation among the subjects was large and therefore the difference did not reach statistical significance at all of the treatment time in our study, these findings are considered to be a typical phenotype of SSc cells.[20,21]

Addition of HGF significantly increased production and mRNA expression of MMP-1 in SSc and control skin fibroblasts. These results were consistent with a previous report in which a hepatic cell line was used.[22] Although not significant, an increasing rate of MMP-1 production in HGF-treated SSc fibroblasts tended to be higher than in HGF-treated control cells. In control fibroblasts, after peak expression of MMP-1 mRNA at 24 h of HGF treatment, it decreased after 48 h. A similar result was reported in the experiment using hepatic stellate cells treated with HGF.[22] While in SSc fibroblasts, MMP-1 mRNA continued to increase during all treatment times. Thus, in our experiments HGF's enhancing effect on mRNA expression and protein synthesis of MMP-1 was demonstrated in SSc skin fibroblasts, showing a tendency toward greater action than in control cells.

Ets-1 has been shown to modulate transcription of several MMP genes including MMP-1.[23] Our study showed that Ets-1 expression significantly increased after 6 h of HGF treatment in SSc and control fibroblasts, then somewhat decreased at further treatment times. Ozaki et al. reported that peak expression of Ets-1 mRNA was seen after 12 h of HGF treatment, followed by an increase in MMP-1 mRNA, in hepatic stellate cells.[22] In addition, they demonstrated that HGF enhanced Ets-1 expression in a dose-dependent manner, and also that transfection of Ets-1 gene enhanced promoter activity of MMP genes and treatment with Ets-1

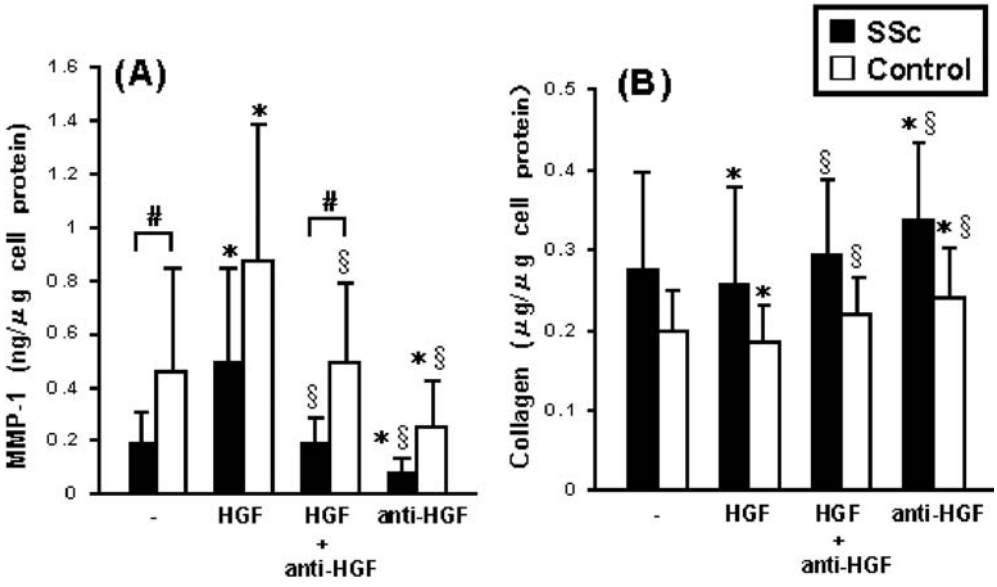

Fig. 4A,B. Inhibition of HGF effect on MMP-1 and collagen production by addition of anti-HGF antibody. MMP-1 (**A**) and collagen (**B**) protein produced by the fibroblasts incubated for 24 h with or without anti-HGF antibody (100 μg/ml) in addition to HGF (100 ng/ml) were measured ($n = 6$ for SSc group, $n = 5$ for control group; values expressed as mean ±SD). $^{*}P < 0.05$, vs untreated condition estimated by Wilcoxon's signed rank test. $^{\#}P < 0.05$, estimated by Mann–Whitney U-test between SSc and control cells. $^{\S}P < 0.05$, vs HGF (100 ng/ml)-treated condition estimated by Wilcoxon's signed rank test

antisense oligonucleotides downregulated HGF-induced MMP expression in hepatic stellate cells[22] and hepatocellular carcinoma cells,[24] indicating induction of several MMP genes by HGF via transcription factor Ets-1 in these cells. Also in our experiment, increased transcription of MMP-1 mRNA followed peak expression of Ets-1 mRNA, suggesting the induction of MMP-1 by HGF via Ets-1 in skin fibroblasts. However, in the present study we did not perform a confirmational study including Ets-1 transfection and translocation of Ets-1 protein into the nucleus; therefore, further experiments would be needed to clarify this mechanism in skin fibroblasts.

Collagen production was significantly decreased at 24, 48, and 72 h of HGF treatment in SSc fibroblasts, while in control fibroblasts a significant decrease was seen only at 24 h treatment. At the mRNA level, HGF suppressed procollagenα1(I) expression in SSc fibroblasts but not in control cells treated for 48 h. Therefore, our experiments revealed an inhibitory effect of HGF on procollagen expression and production in SSc skin fibroblasts, showing even greater action than in control cells.

In our study, there was a significant difference in TGF-β1 expression between SSc and control fibroblasts, and control cells showed very low expression. This finding was in contrast to that of a previous report showing constituted expression of TGF-β1 mRNA in normal fibroblasts.[25] This may be partly explained by the fact that we used cells from relatively old donors who were age-matched with the patients, because TGF-β1 expression was shown to decrease with aging in normal cells.[26,27]

To determine the possible mechanism of inhibitory action of HGF on collagen production, we examined the effect of HGF on the expression of several cytokines related to collagen metabolism. The results showed that HGF treatment for 48 h suppressed CTGF but not TGF-β1 expression in SSc cells. Recently, CTGF has been focused on to be a key factor for SSc to progress to fibrosis.[28] Connective tissue growth factor caused persistent procollagenα2(I) gene expression induced by TGF-β in animal model of skin fibrosis[29] and increased collagen synthesis in human dermal fibroblasts.[7] In addition, it was reported that pretreatment with HGF significantly reduced CTGF induction by TGF-β1, and consequently suppressed collagen production by renal tubulointerstitial fibroblasts.[14] In a mouse model of scleroderma treated with HGF, the ratio of TGF-β1 positive macrophage-like cells was found to be suppressed, but that of fibroblastic cells in established sclerotic skin was not.[17] Taken together, exogenous HGF attenuated the procollagenα1(I) gene expression in SSc fibroblasts, probably not through a reduction in TGF-β1 expression but via a direct blockade of CTGF induction, although this notion was derived indirectly from time-course analysis of HGF effect on cytokine expression, and further experiments would be needed to confirm this.

Recently, Van Beek et al. reported the cooperative participation of Ets-1 and Smad3 (intracellular signal transducer for TGF-β) in the induction of CTGF promoter by TGF-β1, indicating that Ets-1 upregulated CTGF transcription in NIH3T3 fibroblasts.[30] This seems to be inconsistent with our results showing that HGF enhanced Ets-1 and inhibited CTGF expression. Indeed, also in our experiments CTGF mRNA level increased transiently after 6 h of HGF treatment (data not shown), and thereafter decreased after 24 and 48 h treatment. Although further experiments are needed to elucidate the relationship between Ets-1 and CTGF especially regarding antifibrotic effects of HGF, one of the speculative mechanisms for HGF's inhibitory effect on CTGF expression is as follows. Hepatocyte growth factor was reported to block activated Smad 2/3 nuclear translocation in fibroblasts,[31] and therefore in the late course of HGF treatment in the present study, increased Ets-1 might not have a sufficient effect on CTGF without Smad3.

The present study also revealed that HGF enhanced IFN-γ expression in SSc and control fibroblasts. Interferon-γ is a potent inhibitor of collagen production[32] and it is suggested that inhibition of collagen synthesis induced by

HGF may be in part due to enhanced action of this antifibrotic cytokine. On the other hand, although IL-6 has been shown to stimulate dermal fibroblasts to produce increased amounts of collagen and glycosaminoglycan[33] and to be overexpressed in SSc fibroblasts,[34] our study showed that HGF had no significant effect on IL-6 expression in cultured skin fibroblasts, suggesting no significant role for IL-6 in antifibrotic effect of HGF.

A recent report by Jinnin et al.[18] also has shown the inhibitory effect on type I collagen and stimulatory effect on MMP-1 expression of HGF, with greater action observed in SSc fibroblasts than in normal cells. As to the difference in HGF effect between SSc and control cells, they suggested that increased TGF-β expression induced elevation of c-Met expression in SSc cells leading to a tendency toward a greater HGF effect seen in SSc fibroblasts. In the present study, although c-Met expression in SSc cells seemed somewhat increased as compared with normal cells, the difference was not significant either in the presence and absence of HGF. In addition, the present study revealed different findings from the report of Jinnin et al. that had shown little c-Met expression in control fibroblasts.[15] Similar to our results, a moderate amount of c-Met expression in fibroblasts from normal lung tissue was reported previously.[35] Since HGF had actually a considerable effect on both SSc and control fibroblasts as shown in the present study, we believe that normal fibroblasts also had constitutive expression of c-Met. In any event the tendency toward greater c-Met expression in SSc cells may be related to the result showing a relatively greater antifibrotic effect of HGF in SSc cells compared with normal cells.

Addition of anti-HGF antibody almost completely abolished the effect of HGF on MMP-1 and collagen synthesis seen in the experiment, indicating that this antifibrotic effect was truly due to HGF itself. Moreover, addition of anti-HGF antibody alone caused somewhat lower MMP-1 production and higher collagen production than in the untreated condition. It is probable that this may be due to the effect of anti-HGF antibody, which eliminated intrinsic action of HGF produced by fibroblasts themselves.

In the present study, there seemed to be no apparent difference in HGF effect between fibroblasts derived from limited SSc patients and those from diffuse SSc patients (data not shown). However, the number of analyzed subjects was too small, and further study with a sufficient sample size would be needed for more accurate analysis.

Hepatocyte growth factor has various biological activities, and there have been several contradictory results as to HGF function depending on target cells[11,36] and on administration dose in mouse model.[11–13,37] Further studies will be needed regarding practical use of HGF-related agents for human fibrotic diseases.

In conclusion, HGF enhanced MMP-1 production probably via a transcription factor, Ets-1, and inhibited collagen synthesis in cultured SSc fibroblasts, suggesting that HGF could suppress collagen accumulation by these two ways of antifibrotic action. In addition, HGF had no effect on TGF-β1 expression, but inhibited CTGF and enhanced IFN-γ expression in skin fibroblasts, suggesting that the latter two cytokines might be partly related to the inhibitory effect of HGF on collagen synthesis. Although some modifications are needed for a practical use, HGF could be a promising therapeutic agent for those patients having intractable fibrotic disease such as SSc.

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