Fibrotic diseases such as scleroderma (systemic sclerosis, SSc) are characterized by an excessive production of extracellular matrix and profibrotic proteins such as connective tissue growth factor (CTGF). In normal dermal fibroblasts, CTGF is not expressed unless induced by proteins such as tumor growth factor-β (TGFβ). Conversely, in fibroblasts cultured from fibrotic lesions CTGF mRNA and protein are constitutively expressed, even in the absence of exogenously added TGFβ. Thus, studying the mechanism underlying CTGF overexpression in SSc fibroblasts is likely to yield valuable insights into the basis of the fibrotic phenotype of SSc and possibly other scarring disease. CTGF overexpression is mediated primarily by sequences in the CTGF promoter. In this report, we identify the minimal promoter element involved with the overexpression of CTGF in SSc fibroblasts. This element is distinct from the element necessary and sufficient for the induction of CTGF expression by TGFβ in normal fibroblasts. Within this region is a functional Sp1 binding site. Blocking Sp1 activity reduces the elevated, constitutive levels of CTGF promoter activity and protein expression observed in SSc fibroblasts. Relative to those prepared from normal dermal fibroblasts, nuclear extracts prepared from SSc fibroblasts possess increased Sp1 binding activity. Removal of phosphate groups from nuclear extracts enhanced Sp1 binding activity, suggesting that phosphorylation of Sp1 normally reduces Sp1 binding to DNA. Thus, the constitutive overexpression of CTGF in SSc fibroblasts seems to be independent of TGFβ signaling but dependent at least in part on Sp1.

Wound healing requires the de novo synthesis of connective tissue. If this process is not appropriately terminated, excessive matrix deposition occurs, resulting in pathological fibrosis (1, 2). Fibrotic diseases are among the largest groups of disease for which there is no known effective therapy, in part because the cause of these diseases remains elusive. Because TGFβ promotes fibroblast proliferation and matrix synthesis, attention has long been devoted to the potential role of this factor in initiation and maintenance of fibrosis (for reviews, see Refs. 3 and 4). For example, there is a clear correlation between TGFβ action and the initiation of fibrosis; in acute drug- or surgery-induced animal models, anti-TGFβ strategies, such as a neutralizing TGFβ antibody or overexpression of Smad7 (for reviews, see Refs. 3 and 4), are effective at attenuating the onset and severity of fibrogenesis.

However, the precise role that TGFβ plays in human fibrotic disease is not entirely clear. In the chronic fibrosing disorder scleroderma (SSc) TGFβ mRNA is localized to the leading edge of the fibrotic lesion (i.e. to the region of enhanced inflammatory response that is presumably involved with the expansion of the fibrotic response) but not to the lesional area itself (5). Although a general feature of fibroblasts taken from SSc lesions is that they show elevated levels of collagen relative to their normal counterparts, SSc fibroblasts do not show elevated TGFβ levels or binding activity or enhanced sensitivity to TGFβ treatment (6–8). Thus, TGFβ expression seems to be clearly associated with the initiation of the fibrotic phenotype of SSc; however, the role of this cytokine in the maintenance of the fibrotic phenotype in SSc, or of other fibrotic disease, for that matter, remains unclear.

In contrast to TGFβ, expression of the profibrotic protein CTGF correlates well with the severity of fibrotic phenotype of SSc (9, 10). Although not normally expressed in fibroblasts unless induced by TGFβ or other proteins, such as thrombin, CTGF is constitutively expressed in many fibrotic disorders, such as scleroderma, liver sclerosis, and pulmonary and renal fibrosis (for reviews, see Refs. 11 and 12). CTGF protein induces proliferation, collagen synthesis, and chemotaxis in mesenchymal cells (13–15). CTGF seems to be associated with matrix deposition. For example, a CTGF response element lies between nucleotides −376 and +58 of the type I collagen (Col1A2) promoter (16), which includes the TGFβ response element of this promoter (17). In addition, whereas subcutaneous injection of TGFβ into neonatal rats results only in a transient fibrotic response, coinjection of CTGF and TGFβ results in sustained fibrosis (18). This result could possibly be because CTGF can bind TGFβ and consequently may enhance the activity of TGFβ, at low concentrations, to bind its receptors (19). Collectively, these results suggest that TGFβ may initiate, but CTGF may sustain, the fibrotic response. Thus, examining the mechanism underlying the constitutive overexpression of CTGF in fibroblasts from fibrotic lesions should provide insights into the molecular mechanism underlying fibrosis.

CTGF expression seems to be controlled primarily at the level of transcription and involves both TGFβ-dependent and -independent mechanisms (20–24). For example, the TGFβ...
induction of the CTGF promoter requires consensus binding motifs for Smad and TEF transcription factors (22–24). Similarly, the elevated expression of CTGF protein observed in SSc fibroblasts is paralleled by elevated levels of CTGF promoter activity (20, 22). Thus, analyzing the relative contribution of TGFβ-dependent and -independent mechanisms to the elevated level of CTGF promoter activity in lesional SSc fibroblasts should yield valuable insights into the molecular basis of the maintenance of the SSc phenotype.

To gain insights into the molecular mechanism underlying the fibrotic phenotype of SSc, in this study, we identify regions of the CTGF promoter necessary for the overexpression of CTGF in lesional, dermal SSc fibroblasts. Our results provide new insights into the molecular mechanism underlying the maintenance of the fibrotic phenotype of the SSc fibroblast.

MATERIALS AND METHODS

Cell Culture, Reporter Assays, Transfections, and Western Analysis—Dermal fibroblasts from SSc lesions and healthy persons were taken from biopsies of age-, sex-, and anatomical site-matched volunteers, after informed consent was obtained. All patients fulfilled the criteria of the American College of Rheumatology for the diagnosis of diffuse SSc. Fibroblasts were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and used between passages 2 and 5 (18, 20). Transfections and reporter assays were carried out as described previously (20, 22), using 1 µg of a reporter construct and 0.25 µg of the control CMV-β-galactosidase plasmid (Clontech). For assays in which the role of trans-acting proteins were to be tested, 0.5 µg of reporter and 1 µg of empty vector or expression vector encoding the protein of interest were used. TGFβ2 was from R&D Systems or Cetrix. Data presented are means ± S.E. Statistical analysis was performed by the Student’s unpaired test. Values less than 0.05 were considered statistically significant. For Western blots, cells were cultured and 25 µl of media were electrophoresed through a 12% SDS/polyacrylamide gel (Novex) and blotted to nitrocellulose (Bio-Rad). CTGF protein was detected as described previously (20, 22). For studies involving mithramycin, 150 nm mithramycin (Sigma) was added 6 h before harvesting. Anti-actin antibody was from Sigma.

DNA Constructs—The CTGF promoter/SEAP reporter constructs were as described previously (20, 22). Point mutations in putative Sp1 sites were introduced (Stratagene) using primer (Sigma Genosys) for the 5′ Sp1 mutant 5′-CAGCTCATTTGCGCGGTGATACCCGGGAGGTGGTATAAAGGCCTC-3′ and for the 3′ Sp1 mutant 5′-GTATAACCCGGCGCGGTGATACCCGGGAGGTGGTATAAAGGCCTC-3′. The Sp1 expression vector was provided by Dimitris Kardassis (University of Crete, Greece).

Electrophoretic Mobility Shift Assays—An enzyme-linked immunosorbent assay detecting levels of Sp1 binding to a double-stranded oligomer containing a consensus Sp1 site was purchased (Clontech). A commercially available source of Sp1 was purchased (Promega). Fibroblast nuclear extracts were prepared and quantified as described previously (25). Gel shifts were performed with 2–5 µg of protein and a 0.5-ng probe (1–5 × 10⁶ cpm) as described previously (22). For competition experiments, 100-fold excess unlabeled competitor was added to the reaction mixture before incubation, when required. The SMAD oligomer was described previously (25); AP2 and Sp1 oligomers were contained either a consensus AP2 site or the SMAD element of AP2 sites. However, a 100-fold excess of oligomers containing either a consensus AP2 site or the SMAD element of the CTGF promoter (22) did not compete for factor binding.

RESULTS

The Region Lying between Nucleotides −86 and +17 of the CTGF Promoter Is Required for Its Overexpression in SSc Fibroblasts—To identify regions of the CTGF promoter important for its overexpression in SSc fibroblasts, we transfected a series of CTGF promoter deletion constructs (wt, −244 bp, and −86 bp) into normal and SSc dermal fibroblasts. All constructs contained an abundant CTGF promoter expression, suggesting that the elevated level of CTGF expression observed in SSc fibroblasts was controlled by the first 86 base pairs upstream of the transcription initiation start site (Fig. 1). This region of the CTGF promoter does not contain the TGFβ response that is necessary and sufficient for TGFβ to induce CTGF in fibroblasts (22–24).

To begin to identify elements of the CTGF promoter lying between −86 and +17 that might contribute to the elevated level of CTGF expression in SSc fibroblasts, we examined this region of the CTGF promoter for consensus sites of known transcription factors. We noted a TATA box flanked by two consensus binding sites for transcription factor Sp1, one 5′ to the TATA box (5′Sp1; Fig. 2A); the other 3′ to the TATA box (3′Sp1; Fig. 2A). To determine whether the CTGF promoter’s putative Sp1 elements were functional, we first assessed whether these sites could bind Sp1 protein. To do this, we synthesized and radiolabeled a double-stranded oligomer containing both putative Sp1 sites (the sequence shown in Fig. 2A) and performed gel-shift analyses using a commercially available source of Sp1 protein. We found that addition of Sp1 protein to the probe resulted in a shift that could be competed either by a 100-fold molar excess of either unlabeled probe or by an oligomer containing a consensus Sp1 site (Fig. 2B).

To test which of the putative Sp1 binding sites in the CTGF promoter could bind Sp1, we generated double-stranded oligomers that contained mutations in either the 3′Sp1 site or the 5′Sp1 site but were otherwise identical to the gel-shift probe and used them as competitors in our gel-shift assays. We found that a 100-fold molar excess of an oligomer containing a mutated 3′Sp1 site did not compete for factor binding; however, an oligomer containing a mutation in the 5′Sp1 site still competed for factor binding (Fig. 2B). Thus, Sp1 or Sp1-like binding activity binds to the consensus Sp1 site downstream from the TATA box in the CTGF promoter but not to the consensus site upstream of the TATA box.

To verify that the Sp1 binding site downstream of the TATA box was functional, we mutated the 5′Sp1 or the 3′Sp1 sites in the context of an otherwise wild-type, full-length CTGF pro-
Fig. 2. **Sp1 activates the CTGF promoter.** A, sequence of the CTGF promoter. Putative Sp1 sites are underlined (5′Sp1 and 3′Sp1) and are defined relative to the TATA box (gray). B, the sequence shown in A was synthesized as a double-stranded oligomer, radiolabeled, and used in a gel-shift assay with HeLa nuclear extracts that contained Sp1 binding activity (Promega). Competitor oligonucleotides were used as indicated at 100-fold molar excess. The 5′Sp1 and 3′Sp1 competitors are identical to the probe (WT), other than that they contain a mutation to a HindIII (AAGCTT) site in the appropriate putative binding site. Oligomers bearing consensus binding sites for known transcription factor binding sites (Ap2 and Sp1) were from Promega. Mutation of the 3′Sp1 site abolishes the ability of the competitor oligomer to compete for Sp1 binding to the labeled probe. C, effect of mutating putative Sp1 sites on basal and TGFβ-induced CTGF promoter activity. The wild-type CTGF promoter/SEAP reporter construct (wtCTGF, containing nucleotides −805 to +17 (20)) or otherwise identical constructs bearing mutations in either 5′Sp1 or 3′Sp1 site of the CTGF promoter (see “Materials and Methods”) were transfected into NIH 3T3 cells. After an 18-h incubation in serum-free media, TGFβ2 (25 ng/ml) was added for a further 24 h. Mutating the Sp1 site 3′ to the TATA box (m3Sp1) results in an equivalent reduction of basal promoter activity and TGFβ induction of CTGF; that is, mutation of the functional Sp1 site has no impact on the fold-induction of the promoter by TGFβ. All experiments were performed in 6-well plates. Cells were cotransfected with a CMV promoter-driven vector encoding β-galactosidase (0.25 μg) to control for differences in transfection efficiency. Values expressed are mean ± S.E. (n = 6). D, Sp1 transactivates the CTGF promoter. A wild-type CTGF promoter/SEAP reporter construct (wtCTGF (20)) or otherwise identical constructs bearing mutations in the Sp1 sites within the CTGF promoter at positions 5′ (m5′SP1) or 3′ (m3′SP1) of the TATA box (see Fig. 1) were transfected into NIH 3T3 cells. Cells were cotransfected with empty expression vector or vector encoding Sp1, as shown. All experiments were performed in 6-well plates. Cells were cotransfected with a CMV promoter-driven vector encoding β-galactosidase (0.25 μg) to control for differences in transfection efficiency. Values expressed are mean ± S.D. (n = 6).
normally suppresses Sp1 binding to DNA (Fig. 5). We found that removal of phosphates enhanced binding of Sp1 with calf intestinal alkaline phosphatase before use in EMSAs. Administration of 150 nm mithramycin also caused a reduction in CTGF promoter activity.

FIG. 3. Role of Sp1 in elevated CTGF promoter activity in dermal, lesional scleroderma fibroblasts. CTGF promoter/SEAP reporter constructs used contained either the wtCTGF (Fig. 3) or an otherwise identical construct containing a mutated SMAD site (ΔSMAD (22)) or a mutated 3′Sp1 site (m3′Sp1; Fig. 1). CTGF promoter/reporter constructs (0.5 μg/well) alone with expression vector (as indicated; 1 μg/well) were transfected into fibroblasts cultured from dermal scleroderma lesions. The construct containing a mutation in the 3′Sp1 site possesses 50% less activity than the full-length, wild-type construct. Addition of 150 nm mithramycin also caused a reduction in CTGF promoter activity.

Elevated Levels of Sp1 Binding Exist in Nuclear Extracts from SSc Fibroblasts—We then assessed whether the requirement for Sp1 for the elevated levels of CTGF observed in SSc fibroblasts was Sp1-dependent. Elevated Levels of Sp1 Binding Exist in Nuclear Extracts from SSc Fibroblasts—We then assessed whether the requirement for Sp1 for the elevated levels of CTGF observed in SSc fibroblasts was Sp1-dependent.

FIG. 4. Effect of mithramycin on CTGF expression in dermal fibroblasts. After culturing cells to 85% confluence, media were changed, and the Sp1 inhibitor mithramycin (150 nm) was added for 24 h to normal dermal fibroblasts, in the presence or absence of TGFβ1 (2.5 ng/ml), and to fibroblasts cultured from dermal lesions of patients with diffuse scleroderma. Cell layers were then lysed and equal amounts of protein (25 μg) were subjected to SDS/PAGE and Western blot analysis with an anti-CTGF antibody. Addition of mithramycin blocks the basal CTGF expression in scleroderma fibroblasts. Blots were also probed with an anti-actin antibody to establish that lanes were equally loaded.

results suggest that the elevated level of CTGF expression observed in SSc fibroblasts is dependent, at least in part, on an elevated level of Sp1 binding present in the nuclei of SSc fibroblasts and is relatively independent of sequences involved with the TGFβ induction of CTGF. These results are consistent with the notion that the fibrotic phenotype of the SSc fibroblasts is not caused solely by hyperactive or autocrine TGFβ signaling.

DISCUSSION

Previously, we identified the CTGF promoter’s TGFβ response element, which is both necessary and sufficient to confer TGFβ responsiveness to a heterologous promoter (22–24). However, this TGFβ response element is dispensable for the elevated, constitutive level of CTGF expression that is the hallmark of the fibrotic phenotype of the SSc fibroblast; removal of this element has no significant impact on the elevated level of CTGF promoter activity in SSc fibroblasts (22, current study). Here, we have found that Sp1 is involved in CTGF promoter activity. Targeting Sp1 markedly reduced CTGF expression in SSc fibroblasts. Furthermore, nuclear extracts from SSc fibroblasts possessed significantly elevated levels of Sp1 binding to DNA. These results suggest that targeting mechanisms involved with Sp1 or its activation may provide useful in developing antifibrotic therapies.

Our results are intriguing in light of recent observations concerning the role of Sp1 in matrix gene regulation. Functional Sp1 binding motifs have been found in the minimal promoters of several collagen genes. For example, mithramycin inhibits collagen α1 type I protein expression in normal fibroblasts (28), and Sp1 has been shown to activate expression of this gene’s promoter (29). Furthermore, analysis of the collagen α2 type I promoter has shown that TGFβ activates its expression through a complex process involving SMAD3 and -4 and Sp1 (30). In addition, Sp1 was shown to directly contribute to the expression of a wide variety of matrix genes (31). However, none of these studies examined the role of Sp1 and in the regulation of fibrogenic genes in a fibrotic setting. Intriguingly, Sp1 or its family members may contribute directly to the elevated level of expression of target genes in fibrosis. Intriguingly, increased phosphorylation of Sp1 has been shown to be a feature of scleroderma fibroblasts (32). However, the relevance of the phosphorylated residues to Sp1 binding or the elevation of matrix gene expression in SSc fibroblasts is not clear. In this study, we found that phosphorylation of Sp1 had little effect on the ability of Sp1 to bind to its consensus binding element; addition of alkaline phosphatase to nuclear extracts before performing a gel shift with an oligomer bearing a consensus
Sp1 element enhanced Sp1 binding to DNA. Our results are, in fact, consistent with data from other systems showing that Sp1 binding is repressed by phosphorylation and that removal of phosphate groups from Sp1 enhances binding to DNA (33–39). However, it is possible that phosphorylation of Sp1 or Sp1-like proteins could enhance the ability of Sp1 to form an active transcriptional complex with other nuclear factors. That said, our report is the first to directly assess the potential, functional contribution of Sp1 to the fibrotic phenotype of the SSc fibroblast.

The elevated level of Sp1 binding observed in our studies could arise from an increased amounts of Sp1 protein in SSc nuclear extracts compared with extracts prepared from healthy subjects. However, when we directly addressed this issue by using Western blot analysis of nuclear extracts with an anti-Sp1 antibody, we could show no appreciable differences in Sp1 protein levels between nuclear extracts prepared from healthy subjects and those prepared from persons afflicted with SSc (data not shown). Sp1 is a member of the Kruppel-like family of proteins that effectively bind to a consensus Sp1 binding site (40). Thus, it is possible that the elevated Sp1 binding activity observed in SSc nuclear extracts could be caused by other members of the Kruppel-like Sp1 family and that these members show elevated expression in lesional diffuse SSc fibroblasts relative to normal fibroblasts. In fact, we have shown that other Kruppel-like family members are capable of activating the CTGF promoter (data not shown). The precise identity of the proteins binding to the Sp1 binding site in the CTGF promoter, and whether expression of these proteins is elevated in SSc, is currently under investigation.

In conclusion, by investigating the constitutive CTGF expression from defined fibrotic settings, we have shown that the TGFβ response element in the CTGF promoter is dispensable for its constitutive up-regulation in SSc fibroblasts; rather, it seems to reflect an elevation of basal promoter activity as visualized by its dependence on Sp1. Sp1 generally acts with other transcription factors to potentiate transcription (for review, see Ref. 40), so it is likely that Sp1 acts with these factors to activate transcription of genes, such as CTGF, expressed in SSc fibroblasts. Identification of these additional factors is currently underway. That said, our results suggest that a simple model of autocrine TGFβ signaling seems to be insufficient to explain the fibrotic phenotype of the SSc fibroblast. For example, prolonged exposure of fibroblasts to TGFβ is insufficient to generate fibroblasts that constitutively overexpress collagen relative to their normal counterparts (8). Because CTGF has been reported to be an effective marker of fibrosis, and given the known profibrotic effects of this molecule and that small molecule inhibitors that suppress CTGF expression alleviate fibrotic effects in vivo and in vitro (41, 42), further identification of key transcription factors and signaling pathways involved in regulating CTGF promoter activity in SSc is highly likely to have a significant impact on the development of novel therapeutic agents that could combat this debilitating disorder.

REFERENCES

1. Gruschwitz, M., Muller, P. U., Sepp, N., Hofer, E., Fontana, A., and Wick, G. (1990) J. Invest. Dermatol. 94, 197–203
2. Peltonen, J., Kahari, L., Uitto, J., and Jimenez, S. A. (1990) J. Invest. Dermatol. 94, 365–371
3. Blobe, G. C., Schiemann, W. P., and Lodish, H. F. (2000) N. Engl. J. Med. 342, 1350–1358
4. Cotton, S. A., Herrick, A. L., Jayson, M. I., and Freemont, A. J. (1998) J. Pathol. 184, 4–6
5. Querfeld, C., Ecker, B., Huerkamp, C., Kreig, T., and Sollberg, S. J. (1999) J. Dermatol. Sci. 21, 13–22
Elevated CTGF Expression in Scleroderma Depends on Sp1

6. Kikuchi, K., Hartl, C. W., Smith, E. A., LeRoy, E. C., and Trojanowska, M. (1992) Biochem. Biophys. Res. Commun. 187, 45–50
7. Moreland, L. W., Goldsmith, K. T., Russell, W. J., Young, K. R., Jr., and Garver, R. I., Jr. (1992) Am. J. Med. 93, 628–636
8. McWhirter, A., Colosetti, P., Rubin, K., Miyazono, K., and Black, C. (1994) Lab. Invest. 71, 885–894
9. Igarashi, A., Nashiro, K., Kikuchi, K., Sato, S., Ihn, H., Grotendorst, G. R., and Takehara, K. (1995) J. Invest. Dermatol. 105, 280–284
10. Sato, S., Nagao, T., Hasegawa, M., Tamatani, T., Nakaniishi, T., Takigawa, M., and Takehara, K. (2000) J. Rheumatol. 27, 149–154
11. Moussad, E. E. A., and Brigstock, D. A. (2000) Mol. Genet. Metab. 71, 276–292
12. Leask, A., Holmes, A., and Abraham, D. J. (2002) Curr. Rheumatol. Rep. 4, 136–142
13. Bradham, D. M., Igarishi, A., Potter, R. L., and Grotendorst, G. R. (1991) J. Cell Biol. 114, 1285–1294
14. Brigstock, D. R., Steffen, C. L., Kim, G. Y., Vegunta, R. K., Diehl, J. R., and Harding, P. A. (1997) J. Biol. Chem. 272, 20275–20282
15. Duncan, M. R., Frazier, K. S., Abramson, S., Williams, S., Klapper, H., Huang, X., and Grotendorst, G. R. (1999) FASEB J. 13, 1774–1786
16. Shi-wen, X., Pennington, D., Holmes, A., Leask, A., Bradham, D., Beauchamp, J., Funeca, C., du Bou, R. M., Martin, G. R., Black, C. M., and Abraham, D. J. (2000) Exp. Cell Res. 259, 213–224
17. Inagaki, Y., Truter, S., and Ramirez, F. (1994) J. Biol. Chem. 269, 14828–14834
18. Mori, T., Kawate, M., Shinonaki, N., Hayashi, T., Kakinuma, A., Igarishi, M., Takigawa, T., Nakaniishi, T., and Takehara, K. (1999) J. Cell. Physiol. 181, 153–159
19. Abreu, J. G., Ketpura, N. I., Reversade, B., and De Robertis, E. M. (2002) Nat. Cell Biol. 4, 599–604
20. Abraham, D. J., Shiwen, X., Black, C. M., Sa, S., Xu, Y., and Leask, A. (2000) J. Biol. Chem. 275, 15220–15225
21. Grotendorst, G. R., Okochi, H., and Hayashi, N. (1996) Cell Growth Differ. 7, 469–480
22. Holmes, A., Abraham, D. J., Sa, S., Shiwen, X., Black, C. M., and Leask, A. (2001) J. Biol. Chem. 276, 10594–10601
23. Blom, I. E., Goldschmieding, R., and Leask, A. (2002) Matrix Biol. 21, 473–482
24. Leask, A., Holmes, A., Black, C. M., and Abraham, D. J. (2003) J. Biol. Chem. 278, 3008–3015
25. Leask, A., Byrne, C., and Fuchs, R. (1993) Proc. Natl. Acad. Sci. U. S. A. 88, 7948–7952
26. Blume, S. W., Snyder, R. C., Ray, R., Thomas, S., Koller, C. A., and Miller, D. M. (1991) J. Clin. Investig. 88, 1613–1621
27. Verrecchia, F., Chu, M., and Mauviel, A. (2001) J. Biol. Chem. 276, 17058–17062
28. Nehr, M. C., Brenner, D. A., Gruse, H. J., Dierbach, H., Mertelsmann, R., and Herrmann, F. (1993) J. Clin. Investig. 92, 2916–2921
29. Chen, S. J., Arlett, C. M., Jimenez, S. A., and Varga, J. (1998) Gene 215, 101–110
30. Zhang, W., Ou, J., Inagaki, Y., Greenwel, P., and Ramirez, F. (2000) J. Biol. Chem. 275, 39237–39245
31. Verrecchia, F., Rossert, J., and Mauviel, A. (2001) J. Investig. Dermatol. 116, 755–763
32. Ihn, H., and Tamaki, K. (2000) Arthritis Rheum. 43, 2240–2247
33. Leggett, R. W., Armstrong, S. A., Barry, D., and Mueller, C. R. (1995) J. Biol. Chem. 270, 25879–25884
34. Armstrong, S. A., Barry, D., Leggett, R. W., and Mueller, C. R. (1997) J. Biol. Chem. 272, 13489–13495
35. Borellini, F., Aquino, A., Josephs, S. F., and Glazer, R. I. (1999) Mol. Cell. Biol. 19, 5541–5547
36. Water, R. D., Nawrot, T., Potts, R. J., and Hart, B. A. (2003) Toxicology 184, 157–178
37. Lacroix, I., Lipeezy, C., Imbert, J., and Kahn-Perles, B. (2002) J. Biol. Chem. 277, 8998–9003
38. Mortensen, E. R., Marks, P. A., Shiotani, A., and Merchant, J. L. (1997) J. Biol. Chem. 272, 16540–16547
39. Daniel, S., Zhang, S., DePaulo-Roach, A. A., and Kim, K. H. (1996) J. Biol. Chem. 271, 14692–14697
40. Kaczynski, J., Cook, T., and Urritia, R. (2003) Genome Biol. 4, 206
41. Stratton, R., Shiwen, X., Martini, G., Holmes, A., Leask, A., Haberberger, T., Martin, G. R., Black, C. M., and Abraham, D. (2001) J. Clin. Investig. 108, 241–250
42. Stratton, R., Rajkumar V, Ponticos, M., Nichols, B., Shiwen, X., Black, C. M., Abraham, D. J., and Leask, A. (2002) FASEB J. 16, 1949–1951
Constitutive Connective Tissue Growth Factor Expression in Scleroderma Fibroblasts Is Dependent on Sp1
Alan Holmes, David J. Abraham, Youjun Chen, Christopher Denton, Xu Shi-wen, Carol M. Black and Andrew Leask

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