Collagen type XVI expression is modulated by basic fibroblast growth factor and transforming growth factor-β

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Abstract We investigated the effects of bFGF and TGF-β on the expression of type XVI collagen, a member of the fibril associated collagen family, in human dermal fibroblasts and arterial smooth muscle cells. We found that bFGF decreased the α1(XVI) collagen mRNA to 18–24% of the controls, while TGF-β increased the mRNA to 150–360%. Immunoprecipitation of metabolically labeled cells revealed corresponding, but less pronounced, changes at the protein levels. The results suggested that type XVI collagen expression is regulated by bFGF and TGF-β in a manner similar to their regulation of the major type I fibrillar collagen produced by these cells.

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Key words: Transforming growth factor-β; Basic fibroblast growth factor; Collagen XVI; Arterial smooth muscle cell; Dermal fibroblast

1. Introduction

Type XVI collagen is a minor collagen initially identified by cDNA cloning of the α1(XVI) collagen chain from human fibroblast and placenta cDNA libraries [1,2]. The deduced primary structure of the α1(XVI) collagen chain possesses structural features common to the family of fibril-associated collagens with interrupted triple-helices (FACIT) [3,4]. Members of this family, collagens type IX, XII, and XIV, localize on the surface of either type I or type II collagen fibrils [5]. Subsequent immunoprecipitation experiments show that the α1(XVI) collagen polypeptide of 220 kDa is synthesized and secreted as a homotrimer by dermal fibroblasts and arterial smooth muscle cells [6]. However, it is not known if type XVI collagen, as its structure suggests, colocalizes with the major collagen fibrils consisting of type I and III collagens deposited by these two cell types.

Cytokines and growth factors play important roles in regulating collagen synthesis during normal and pathological conditions [7]. Transforming growth factor-β (TGF-β) is a particularly important regulator of collagen synthesis [8]. It upregulates collagen gene expression during wound repair, and causes the abnormal accumulation that characterizes tissue fibrosis. Basic fibroblast growth factor (bFGF), a heparin-binding growth factor, stimulates vascular smooth muscle cell proliferation and migration following vascular injury. Deposition of collagen by arterial smooth muscle cells is considered to be a major contribution to the formation of arteriosclerotic plaques [9]. The autocrine TGF-β activity is thought to be responsible for elevated collagen synthesis by the proliferating smooth muscle cells [10]. However, bFGF exerts the opposite effect on collagen synthesis by downregulating type I collagen synthesis in arterial smooth muscle cells [11] and in dermal fibroblasts [12].

Little is known about the regulation of type XVI collagen gene expression. To investigate whether it is regulated similarly to the major fibrillar collagens, we examined the effects of bFGF and TGF-β on the expression of type XVI collagen mRNA and protein.

2. Materials and methods

2.1. Growth factors and cell cultures

Simian recombinant TGF-β2 was a generous gift from Dr. David R. Olsen, Celtrix Laboratories, Santa Clara, CA, USA. bFGF was purchased from Intergen (Purchase, NY, USA).

Human dermal fibroblasts, established from breast tissue of normal individuals of the same age-group after reduction surgery, were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco-BRL) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin sulfate (Gibco-BRL). Arterial smooth muscle cells, established from adult iliac arteries from brain-dead, heart-beating individuals [11], were grown in Medium 199 (Gibco-BRL), supplemented with 10% FBS, 2 mmol/l glutamine, 200 U/ml penicillin, and 200 μg/ml streptomycin in flasks coated with 0.1% gelatin. All cells were grown at 37°C under 5% CO_2.

Subconfluent fibroblasts and smooth muscle cells were treated with 10 ng/ml TGF-β2 in their respective media supplemented with 0.5% FBS, or with 50 ng/ml bFGF in media containing 3% FBS.

2.2. RNA extraction and Northern blot analysis

Total RNA was extracted using the RNeasy kit from Quiagen (CA, USA). Twenty μg of total RNA was loaded onto 1% formaldehyde-agarose gels, electrophoretically separated, and transferred onto nylon membranes (Amersham) by standard methods [13]. The membranes were hybridized with a [^{32}P]dCTP labeled 4.0-kb α1(XVI) collagen cDNA probe, F352 [1], as described [13]. RNA was stained with ethidium bromide, photographed, and quantified by densitometric analysis (Bio-Rad, CA, USA) for evaluation of RNA loading differences. Quantitative evaluation of α1(XVI) collagen expression was obtained by exposing filters to phospho-storage screens and by subsequent scanning with the phospho-imaging system Si 445 (Molecular Dynamics, CA, USA).

2.3. Metabolic labeling and immunoprecipitation

Cells grown in 35-mm dishes were washed twice with phosphate buffered saline (PBS), and then metabolically labeled for 4 h with

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Abbreviations: TGF-β, transforming growth factor-β; bFGF, basic fibroblast growth factor; ECM, extracellular matrix

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40 μCi/dish of [^{35}S]Cys (>1000 Ci/mmol; Amersham) in 0.8 ml of deficient DMEM (without FBS, sodium pyruvate, L-methionine and L-cysteine) containing 50 μg/ml ascorbic acid, with or without 50 ng/ml bFGF or 10 ng/ml TGF-β2. Following the labeling period, the culture medium was collected, and 200 μl 5× immunomix buffer supplemented with protease inhibitors [6] was added and the mix incubated on ice for 30 min. Cell layers were washed twice with PBS, and then extracted with 1 ml of 1× immunomix with protease inhibitors and 0.1% SDS on ice for 30 min. After removing the soluble cytoplasmic extract (cell), the remaining extracellular matrix (ECM) was solubilized by incubating on ice for 30 min in 0.5 ml PBS containing 6 M urea, then was frozen, thawed, and centrifuged to remove the insoluble material. The supernatant was adjusted to 1 ml with 1× immunomix and protease inhibitors. For immunoprecipitation, 500-μl aliquots were precleared with 10 μl protein A agarose, and incubated for 2 h at 4°C with 10 μl rabbit antiserum, specific for the NC11 domain of the α1(XVI) collagen chain [14]. The immunoprecipitated material was washed as previously described [6], except for an additional wash with 0.75 ml buffer containing 10 mM Tris-HCl, pH 8.5, 0.6 M NaCl, 0.1% SDS, 0.05% NP-40 after the second washing step. The immunoprecipitated material was separated on 4–15% SDS-polyacrylamide gradient gels (Ready gel, Bio-Rad) and run at 40 mA for 45 min. Gels were fixed in a solution of 45% methanol/15% acetic acid, immersed in autoradiography enhancer solution (DuPont-NEN), dried and subjected to fluorography at −70°C.

3. Results

3.1. Basic FGF and TGF-β modulate steady state levels of the α1(XVI) collagen mRNA in arterial smooth muscle cells and in dermal fibroblasts

Human arterial smooth muscle cells were treated for increasing time periods with 50 ng/ml bFGF, a dose that previously was shown to exert the maximal effect on type I collagen gene expression [11]. Total RNA was prepared from the control and treated cultures at 24, 48, 72, and 96 h, and was subjected to Northern blot analysis for the α1(XVI) collagen mRNA. As shown in Fig. 1, the steady state levels of the 5.5-kb α1(XVI) collagen mRNA were down-regulated by bFGF in a time dependent manner. A detectable decrease was observed after 24 h of the bFGF treatment and the mRNA levels were reduced significantly after 48–96 h. Treatment of human dermal fibroblasts with bFGF produced a similar result. The degree of inhibition in both cell types was quantified by densitometric analysis of 2–4 separate Northern blots. Maximal inhibition to 18–24% of the control levels were observed after 72 h of bFGF treatment (Fig. 2A).

To determine the effect of TGF-β on α1(XVI) collagen mRNA expression, arterial smooth muscle cells and dermal fibroblasts were treated with 10 ng/ml TGF-β2 for 6, 12, 24, and 48 h. TGF-β caused an increase in the steady state mRNA levels in both cell types. The increase was detectable as early as 6 h after treatment, and maximal induction was observed 24 h after treatment (Fig. 2B). The induction was more pronounced in smooth muscle cells (360%) than in dermal fibroblasts (150%).

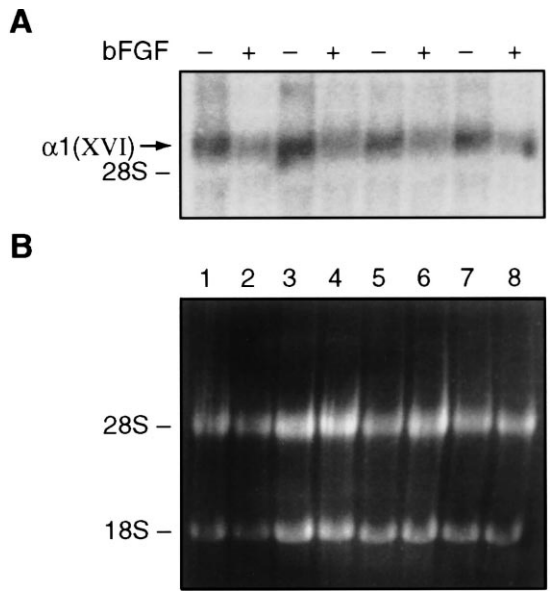

Fig. 1. A: A representative Northern blot analysis of total RNA from human arterial smooth muscle cells treated with 50 ng/ml bFGF. Twenty μg per lane total RNA was hybridized with a 4.0-kb α1(XVI) collagen cDNA probe, F352, resulting in specific binding to the 5.5-kb type XVI collagen mRNA. RNA isolated from cells treated without (lanes 1, 3, 5, 7) and with 50 ng/ml bFGF (lanes 2, 4, 6, 8) for either 24 h (lanes 1, 2), 48 h (lanes 3, 4), 72 h (lanes 5, 6) or 96 h (lanes 7, 8). B: Ethidium bromide staining of the 28S and 18S ribosomal RNA as controls for RNA loading. Filters were exposed to phospho-storage screens overnight and analyzed by scanning with the phospho-imaging system SI 445.

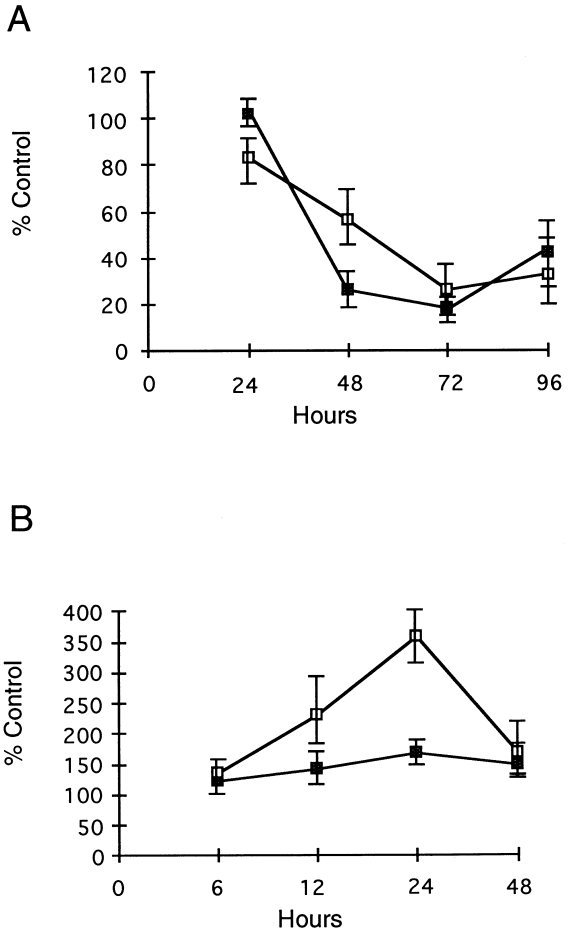

Fig. 2. Relative changes of α1(XVI) mRNA expression in response to bFGF (panel A) and TGF-β (panel B) at different time points. Northern filters were exposed overnight to phospho-storage screens and analyzed by phospho-imaging. The levels of α1(XVI) collagen mRNA were normalized to the 28S rRNA stained with ethidium bromide. Data represent the mean ± standard deviation of 3 or 4 separate Northern blots, and are expressed as percent of controls, arbitrarily set at 100%. Solid symbols denote dermal fibroblasts, and open symbols denote arterial smooth muscle cells.

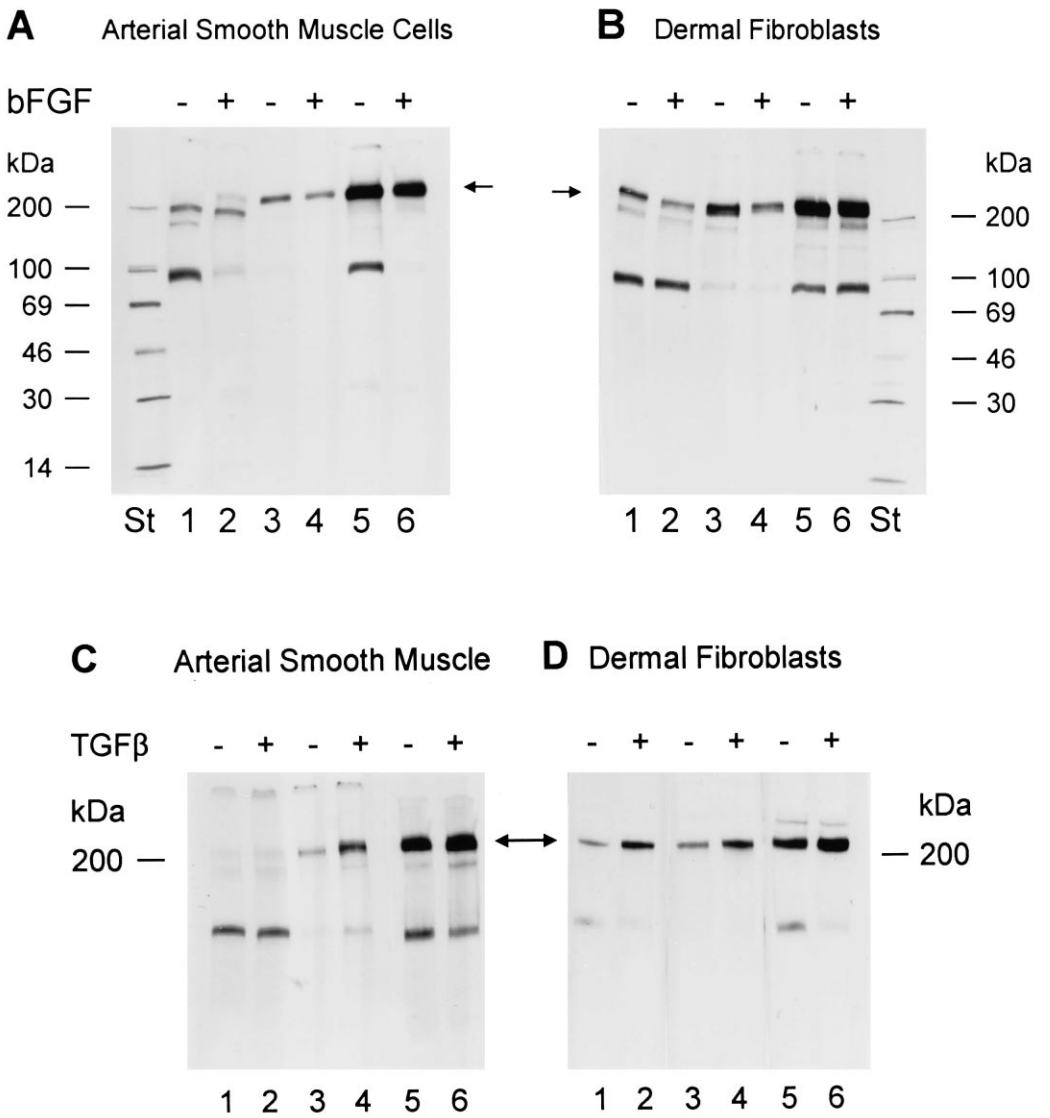

Fig. 3. SDS-PAGE analysis of cell, ECM and medium extracts immunoprecipitated with the α1(XVI) collagen specific NC 11 antiserum. A: Human arterial smooth muscle cells treated with 50 ng/ml bFGF for 96 h (lanes 2, 4, 6) and untreated control samples (lanes 1, 3, 5). B: Human dermal fibroblasts treated with 50 ng/ml bFGF for 72 h (lanes, 2, 4, 6) and untreated control samples (lanes 1, 3, 5). C: Human arterial smooth muscle cells treated with 10 ng/ml TGF-β for 24 h (lanes 2, 4, 6) and untreated control samples (lanes 1, 3, 5). D: Human dermal fibroblasts treated with 10 ng/ml TGF-β for 24 h (lanes 2, 4, 6) and untreated control samples (lanes 1, 3, 5). In all panels, cell extracts are analyzed in lanes 1, 2; ECM extracts are shown in lanes 3, 4 and medium extracts are separated in lanes 5, 6. Arrows denote the 220-kDa α1(XVI) collagen specific band. Gels were subjected to autoradiography for 12 h (panel A), 18.5 h (panel B), 9.5 h (panel C) and 6.5 h (panel D) at −70°C.

3.2. *Basic FGF and TGF-β modulate collagen type XVI protein biosynthesis*

Cells were metabolically labeled during the final 4 h of the treatment to investigate if these two growth factors cause the corresponding changes in the α1(XVI) collagen protein production. The medium, cell, and ECM extracts were immunoprecipitated with an antibody specific for the N-terminal domain NC11 [14] and analyzed by gel electrophoresis. There was essentially no change in the amount of total protein synthesis in all three fractions when cells with and without treatment were compared (data not shown). Incubation of fibroblasts with bFGF resulted in an approximately 20% decrease in the 220-kDa band, corresponding to the migration of the α1(XVI) collagen chain in the cell and in ECM, but a negligible decrease in the medium (Fig. 3B, Fig. 4A). When smooth muscle cells were treated with bFGF, the ECM and medium fractions displayed a 20% decrease in the 220-kDa band, but this band in the cell extract was very faint (Fig. 3A, Fig. 4A). Instead, a more prominent 180-kDa band appeared. This band previously was noted in smooth muscle cells and could be a partially degraded α1(XVI) collagen chain [6]. The antibody also precipitated an 85-kDa polypeptide, which was thought to be unrelated to the α1(XVI) collagen chain [6].

Administration of TGF-β to both smooth muscle cells and to fibroblasts for 24 h led to a marked increase in the 220-kDa band in cell and in ECM extracts, while only a moderate increase was observed in the media (Fig. 3C,D, Fig. 4B).

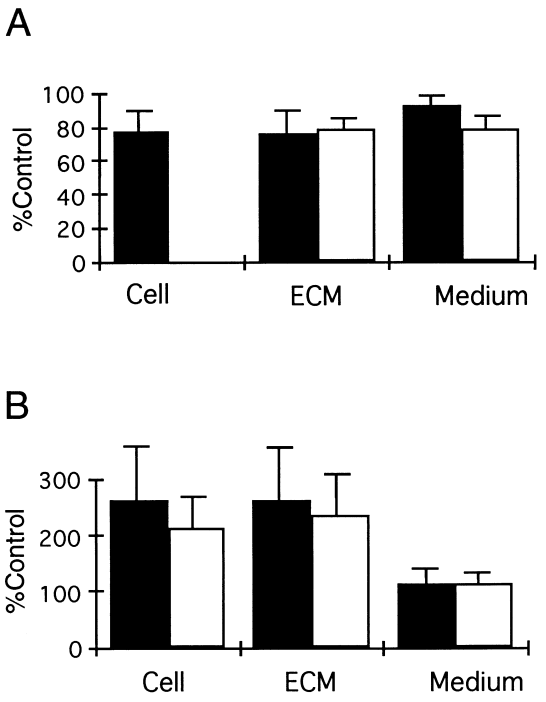

Fig. 4. Relative changes of the α1(XVI) collagen chain in the cell, in ECM, and in medium extracts in response to bFGF (panel A) and to TGF-β (panel B). Autoradiographs were scanned with a densitometer and the 220-kDa bands were quantified by a densitometer. Solid bars denote dermal fibroblasts, and open bars denote smooth muscle cells. In panel A, the 220-kDa bands in the cell extracts of smooth muscle cells were faint, and thus not quantified. Data represent the mean ± standard deviation of 3–5 separate experiments, and are expressed as percent of controls, arbitrarily set at 100%.

4. Discussion

We demonstrate in this study that TGF-β causes an increase in the steady state level of α1(XVI) collagen mRNA in dermal fibroblasts and in smooth muscle cells, while bFGF elicits the opposite effect in both cells. The modulation of mRNA by these two growth factors followed different time courses. bFGF exerts substantial effects after 48 h of treatment, while TGF-β exhibits a more rapid response in that an incubation time of 12–24 h results in maximal stimulation. Similar time courses have been reported for these two growth factors on type I collagen gene expression [11]. Thus, there appears to be a coordinate regulation between type XVI collagen, a member of the FACIT collagen family, and the major type I fibrillar collagen synthesized by fibroblasts and smooth muscle cells. It has been shown recently that bFGF upregulates collagenase gene expression within 24 h of treatment [11], and the effect is exerted at the transcriptional level through an AP-1 consensus sequence in its promoter [15]. This suggests that the bFGF effects on types XVI and I collagen expression may be secondary to initial events triggered by the growth factor, which are mediated through high affinity transmembrane FGF receptors with tyrosine kinase activity and lower affinity binding of bFGF to cell surface proteoglycans [16].

TGF-β is a potent stimulator of various collagen types, including types I, III, V, XI fibrillar collagens, and types IV, VI, VII non-fibrillar collagens [17–21]. TGF-β acts primarily at the transcriptional level and transcription factors NF-1, Sp1, AP-1 and TBE have been implicated in mediating its action [22–25]. Our results with type XVI collagen add another example to the repertoire of collagens upregulated by TGF-β. Whether type XVI collagen is regulated at the transcriptional level remains to be determined.

Upregulation of the α1(XVI) collagen mRNA by TGF-β is reflected at the protein levels in the cell and in ECM extract, but not in the medium. The lack of corresponding response in the medium may be explained by the short labeling time of the experiments. Downregulation of the α1(XVI) collagen expression by bFGF is less pronounced at the protein than at the mRNA level. This suggests that post-transcriptional mechanisms such as translational control or protein stability may play a role in regulating type XVI collagen protein expression.

TGF-β and bFGF elicit diverse effects on extracellular matrix gene expression in a variety of cell types in both normal and pathological conditions. We recently detected substantial synthesis of collagen type XVI in other cell types such as chondrocytes and keratinocytes (Grässel et al., in preparation). We also localized, by indirect immunofluorescence, type XVI collagen expression in the dermal-epidermal junction of skin and blood vessels of heart tissue. Collectively, the data suggest that type XVI collagen participates with fibrillar collagens in the development and maintenance of various types of connective tissues. Further investigation of collagen XVI regulation is needed to clarify the role of this FACIT collagen member in pathogenesis of connective tissue diseases.

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References

[1] Pan, T.-C., Zhang, R.-Z., Mattei, M.-G., Timpl, R. and Chu, M.-L. (1992) Proc. Natl. Acad. Sci. USA 89, 6565–6569.
[2] Yamaguchi, N., Kimura, S., McBride, O.W., Hori, H., Yamada, Y., Kanamori, T., Yamakoshi, H. and Nagai, Y. (1992) J. Biochem. (Tokyo) 112, 856–863.
[3] Mayne, R. and Brewton, R.G. (1993) Curr. Biol. 5, 883–890.
[4] Brown, J.C. and Timpl, R. (1995) Int. Arch. Allergy Immunol. 107, 484–490.
[5] Nishiyama, T., McDonough, A.M., Bruns, R.R. and Burgeson, R.E. (1994) J. Biol. Chem. 269, 28193–28199.
[6] Grässel, S., Timpl, R., Tan, E.M.L. and Chu, M.-L. (1996) Eur. J. Biochem. 242, 576–584.
[7] Bornstein, P. and Sage, H. (1989) Prog. Nucleic Acid Res. Mol. Biol. 37, 67–106.
[8] Massague, J. (1990) Annu. Rev. Cell Biol. 6, 597–641.
[9] Ross, R. (1986) New Engl. J. Med. 314, 488–500.
[10] Rasmussen, L.M., Wolf, Y.G. and Ruoslahti, E. (1995) Am. J. Pathol. 147, 1041–1047.
[11] Kennedy, S.H., Qin, H., Lin, L. and Tan, E.M.L. (1995) Am. J. Pathol. 146, 764–771.
[12] Tan, E.M.L., Rouda, S., Greenbaum, S.S., Moore, J.H., Fox, J.W. and Sollberg, S. (1993) Am. J. Pathol. 142, 463–470.
[13] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Edn., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[14] Tillet, E., Mann, K., Nischt, R., Pan, T., Chu, M.-L. and Timpl, R. (1995) Eur. J. Biochem. 228, 160–168.
[15] Aho, S., Rouda, S., Kennedy, S.H., Qin, H. and Tan, E.M.L. (1997) Eur. J. Biochem. 247, 503–510.
[16] Mason, I.J. (1994) Cell 78, 547–552.
[17] Varga, J., Rosenbloom, J. and Jimenez, S.A. (1987) Biochem. J. 247, 597–604.
[18] Lawrence, R., Hartmann, D.J. and Sonenshein, G.E. (1994) J. Biol. Chem. 269, 9603–9609.
[19] Heckmann, M., Aumailley, M., Chu, M.-L., Timpl, R. and Krieg, T. (1992) FEBS Lett. 310, 79–82.

[20] Grande, J., Melder, D., Zinsmeister, A. and Killen, P. (1993) Lab. Invest. 69, 387–395.
[21] Rudnicka, L., Varga, J., Christiano, A.M., Iozzo, R.V., Jimenez, S.A. and Uitto, J. (1994) J. Clin. Invest. 93, 1709–1715.
[22] Rossi, P., Karsenty, G., Roberts, A.B., Roche, N.S., Sporn, M.B. and de Crombrugghe, B. (1988) Cell 52, 405–414.
[23] Inagaki, Y., Truter, S. and Ramirez, F. (1994) J. Biol. Chem. 269, 14828–14834.
[24] Chung, K.-Y., Agarwal, A., Uitto, J. and Mauviel, A. (1996) J. Biol. Chem. 271, 3272–3278.
[25] Ritzenthaler, J.D., Goldstein, R.H., Fine, A. and Smith, B.D. (1993) J. Biol. Chem. 268, 13625–13631.