Brief Report

Platelet-derived growth factor, basic fibroblast growth factor, and interferon γ increase type IV collagen production in human fetal mesangial cells via a transforming growth factor-β-dependent mechanism

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Abstract

Background. Glomerulosclerosis is characterized by glomerular accumulation of extracellular matrix following mesangial cell proliferation. The precise pathomechanism of glomerulosclerosis is still undetermined. Platelet-derived growth factor (PDGF) and basic fibroblast growth factor (b-FGF) are known to be mitogenic for mesangial cells, and interferon γ (IFN-γ) is known to have an inhibitory effect on mesangial cell proliferation. We attempted to clarify the role of these cytokines on mesangial matrix production using cultured human fetal mesangial cells (HMC).

Methods. HMC were incubated with these cytokines for 24–72 h and the levels of type IV collagen and TGF-β in the cell supernatants were measured by enzyme immunoassay.

Results. PDGF, b-FGF, and IFN-γ stimulated type IV collagen production by HMC in a dose- and time-dependent manner. The anti-TGF-β neutralizing antibody clearly inhibited their stimulatory effect on type IV collagen production. PDGF and b-FGF also stimulated TGF-β production by HMC in a dose-dependent manner, although IFN-γ did not.

Conclusion. PDGF, b-FGF, and IFN-γ stimulate type IV collagen production in cultured HMC via a TGF-β-dependent mechanism.

Keywords: b-FGF; IFN-γ; mesangial cell; PDGF; TGF-β; type IV collagen

Introduction

Glomerular accumulation of extracellular matrix (ECM) is the common pathological feature of glomerular injury, and an alteration in its synthesis or degradation or both may result in its accumulation in the glomerulus. Glomerular ECM has been shown to consist of type IV collagen, laminin, heparan sulphate proteoglycan, fibronectin, and other proteins. Glomerular deposition of type IV collagen has been reported in human glomerular diseases [1] and experimental animal models [2]. Platelet-derived growth factor (PDGF) has been shown to be a potent mitogen for mesangial cells in culture and is expressed in both experimental and human forms of glomerulonephritis in which mesangial cell proliferation occurs [3–6]. Basic fibroblast growth factor (b-FGF) is also thought to be mitogenic for mesangial cells in culture [7], while interferon γ (IFN-γ) is known to inhibit mesangial cell proliferation [8]. Mesangial cell proliferation may precede or be associated with ECM accumulation in mesangial proliferative glomerulonephritis. However, it is still undetermined how PDGF, b-FGF, and IFN-γ influence the glomerular accumulation of ECM in glomerulonephritis. Transforming growth factor β (TGF-β) is a multifunctional regulatory protein and is known to participate in the glomerular accumulation of ECM in glomerulonephritis. Therefore we studied the production of type IV collagen and TGF-β by cultured fetal mesangial cells (HMC) incubated with PDGF, b-FGF, and IFN-γ. We also examined the effect of anti-TGF-β antibody to clarify the role of TGF-β in type IV collagen production stimulated with these cytokines.

Subjects and methods

Reagents

Dulbecco’s modified Eagle’s minimum essential medium (DMEM) and Hanks’ balanced salt solution (HBSS) were purchased from Gibco Laboratories (Grand Island, NY, USA). Fetal calf serum (FCS) was purchased from Bioserum (Canterbury, Australia). Panaassay IV-C, an enzyme immunoassay kit for type IV collagen, was purchased from

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Daiichi Kagaku Yakuhin Inc. (Tokyo, Japan), and Quantikine: Human TGF-β-1 Immunoassay Kit was from R&D Systems, Inc. (Minneapolis, MN, USA). Bovine serum albumin (BSA) was from Sigma (St Louis, MO, USA). Recombinant human PDGF-BB, recombinant human b-FGF, and recombinant human IFN-γ were purchased from Pepro Tech, Inc. (Rocky Hill, NJ, USA). Anti-TGF-β neutralizing antibody was purchased from King Brewing Co. Ltd (Kakogawa, Japan).

**HMC culture**

HMC derived from human fetal kidneys (16 and 18 weeks gestation) were kindly provided by Dr M. R. Daha (University Hospital of Leiden, The Netherlands). The cells were cultured in DMEM with 20% FCS at 37°C in 5% CO2. The cells were identified as glomerular mesangial cells by standard criteria [9], i.e. they exhibited a typical stellate morphology when subconfluent, and typical hillocks were seen at confluence. Staining for actin, myosin, and type IV collagen was positive by immunofluorescence, and the cells were negative for cytokeratin and factor VIII. The cells were used between passages 6 and 9.

**Assay for type IV collagen**

The concentration of type IV collagen in culture supernatants was quantified by the Panaassay IV-C. HMC were cultured in 12-well plates (Falcon, Becton Dickinson, Franklin Lakes, NJ, USA) and grown to confluence. Subsequently, the cells were washed with HBSS and were cultured with DMEM containing 0.2% BSA for 24–72 h. Type IV collagen in the culture supernatants was then measured. The cells in each well were lysed in 1 N NaOH and the protein content was measured by the method of Lowry et al. [10] using BSA as a standard. The levels of type IV collagen in culture supernatants were expressed as ng per μg of lysed HMC protein. Type IV collagen was also measured in HMC lysates with 10 mmol/l Tris-HCl buffer (pH 7.2) containing 1% Triton × 100.

**Assay for TGF-β**

TGF-β was quantified by an enzyme-linked immunosorbent assay (ELISA) using a Quantikine: Human TGF-β-1 Immunoassay Kit. This assay is a sandwich ELISA using TGF-β soluble receptor type II and an enzyme-linked polyclonal antibody specific for TGF-β-1. This assay recognizes both natural human TGF-β-1 and recombinant human TGF-β-1. No significant cross-reactivity with other cytokines was observed.

Samples were obtained under the same conditions as those for type IV collagen assay. TGF-β levels were measured in the culture supernatants after 1 N HCl was added at 1:10 and then incubated for 10 min to activate latent form of TGF-β. Following neutralization with 1.2 N NaOH/0.5 M HEPES, the samples were subjected to the assay. TGF-β level was expressed as pg per μg of HMC protein, as well as type IV collagen.

**Effect of PDGF, b-FGF, and IFN-γ on the production of type IV collagen and TGF-β by HMC**

We measured type IV collagen and TGF-β in the culture supernatants of fully confluent HMC incubated with different concentrations of recombinant human PDGF-BB, recombinant human b-FGF, and recombinant human IFN-γ for 24–72 h. We also examined the effect of anti-TGF-β neutralizing antibody, which is a polyclonal rabbit antibody, on type IV collagen production stimulated with PDGF, b-FGF, and IFN-γ.

**Statistics**

All data are expressed as the means ± standard deviation (SD). Results were compared using one-way factorial ANOVA followed by multiple comparison tests, and two-way repeated-measures ANOVA. P < 0.05 was considered statistically significant.

**Results**

PDGF, b-FGF, and IFN-γ each significantly increased type IV collagen production by HMC in a dose- and time-dependent manner (Figure 1 and Figure 2). Type IV collagen in HMC lysates was not detected even when the HMC were stimulated with 10.0 ng/ml PDGF.

The neutralizing antibody to TGF-β inhibited the stimulatory effect of PDGF, b-FGF, and IFN-γ on type IV collagen production, while anti-TGF-β antibody itself did not influence type IV collagen production (Figure 3). PDGF and b-FGF significantly increased TGF-β production by HMC in a dose-dependent manner, although IFN-γ did not stimulate TGF-β production (Figure 4).

**Discussion**

In the present study, PDGF, b-FGF, and IFN-γ stimulated type IV collagen production in HMC in a dose- and time-dependent manner. We used fetal mesangial cells in this study. Fetal and adult mesangial cells are known to have similar characteristics, although it is not fully understood whether there are functional and phenotypic differences between them.

It is well known that PDGF stimulates proliferation of various kinds of cell, including mesangial cells in vitro. PDGF is thought to play an important role in mesangial cell proliferation in glomerulonephritis. Northern blot analysis revealed that PDGF and PDGF receptor mRNA expressions were enhanced in anti-Thy 1 nephritis [3], and the increase of PDGF mRNA was demonstrated by in situ hybridization [4]. The administration of antibody to PDGF significantly inhibited mesangial cell proliferation and matrix expansion in anti-Thy 1 nephritis [5]. Clinically the increased glomerular expression of PDGF was demonstrated in IgA nephropathy patients [11]. Basic FGF is also known to induce proliferation in various cultured cells, including fibroblasts, endothelial cells, and vascular smooth-muscle cells [12]. Basic FGF has been shown to stimulate mesangial cell proliferation, and cultured mesangial cells themselves produce b-FGF, which may
Fig. 1. Effect of PDGF, b-FGF, and IFN-γ on type IV collagen production by HMC. We measured type IV collagen in the supernatants of HMC incubated with PDGF-BB (1.0–10.0 ng/ml), b-FGF (1.0–100.0 ng/ml) and IFN-γ (1.0–10.0 ng/ml) for 72 h to determine their dose-dependent effects. PDGF, b-FGF, and IFN-γ stimulated type IV collagen production by HMC in a dose-dependent manner. Values are mean±SD for three wells and representative data from one of two experiments are shown. *P<0.0001 vs 0 and 1.0 U/ml PDGF; **P<0.0001 vs 0 and 1.0 U/ml PDGF, and P<0.002 vs 5.0 U/ml PDGF; ***P<0.0001 vs 0 and 1.0 U/ml b-FGF; ****P<0.0001 vs 0, 1.0 and 10.0 U/ml b-FGF; *****P<0.05 vs 0 U/ml IFN-γ; ******P<0.002 vs 0, 1.0 U/ml IFN-γ.

be involved in mesangial proliferative glomerulonephritis [7].

Increased b-FGF expression was observed segmentally in glomeruli and in areas of tubulointerstitial damage in IgA nephropathy and focal glomerular sclerosis [13]. However, it is uncertain whether these cytokines also stimulate type IV collagen production by mesangial cells. PDGF is suspected to stimulate matrix protein synthesis. Advanced glycosylation end-products have been shown to enhance type IV collagen mRNA expression in mouse mesangial cells and this augmentation was inhibited by anti-PDGF antibody [14]. In vivo observations that anti-PDGF antibody ameliorated the accumulation of type IV collagen as well as mesangial cell proliferation in anti-Thy 1 nephritis [5] are consistent with our present in vitro results showing that PDGF regulates type IV collagen production in HMC. Haensch et al. [15] reported that PDGF enhanced gene expression of type IV collagen and fibronectin in cultured human mesangial cells. Addition of neutralizing anti-PDGF antibody resulted in only 40% reduction of type IV collagen production stimulated by TGF-β, and TGF-β also stimulated gene expression of PDGF. Therefore, they suggest that TGF-β increases type IV collagen synthesis directly and indirectly via the synthesis of PDGF. Throckmorton et al. [16] showed that PDGF or TGF-β stimulates type III collagen production in rat mesangial cells. They described that while the neutralizing anti-TGF-β antibody prevented type III production stimulated by PDGF, the anti-PDGF antibody did not prevent type III collagen production stimulated by TGF-β. Therefore they suggest that PDGF may increase type III collagen production by stimulating the intermediate production of TGF-β. Paolo et al. [17] reported that high glucose concentration initially induces augmentation of PDGF BB mRNA and then an increase in TGF-β mRNA in human mesangial cells. They also demonstrated that the increase in TGF-β mRNA was markedly diminished by anti-PDGF antibody. They suggest that high glucose induces the overexpression of TGF-β through the activation of PDGF. Thus, previous reports on the reciprocal action between PDGF and TGF-β in ECM production are not consistent.

In our study, PDGF stimulated type IV collagen production and anti-TGF-β antibody clearly inhibited this effect of PDGF. Therefore it is suggested that PDGF stimulated type IV collagen production through a TGF-β-dependent mechanism.

Basic FGF is also a potent stimulator of mesangial matrix production. However, there are no reports on the stimulatory effect of b-FGF on type IV collagen production in vitro. Our study suggests that b-FGF
may stimulate type IV collagen production through a TGF-β-dependent mechanism as PDGF does.

IFN-γ is known to inhibit the proliferation of many types of cells. It was also reported that IFN-γ inhibits collagen production in fibroblast [18]. In our study IFN-γ stimulated type IV collagen production by HMC. This stimulating effect was blocked by anti-TGF-β antibody. It was reported that TGF-β mRNA expression was up-regulated in glomeruli of rats with IFN-γ [19]. Another report described that IFN-γ treatment enhanced type IV collagen mRNA in anti-Thy 1 nephritis, while it inhibited mesangial cell proliferation [20]. These in vivo observations are consistent with our in vitro results.

We also examined TGF-β production in HMC to confirm the TGF-β-dependent mechanism for type IV collagen production by PDGF-, b-FGF-, or IFN-γ-stimulated HMC. PDGF and b-FGF significantly increased TGF-β production in a time-dependent manner, although IFN-γ did not. Marra et al. [21] demonstrated that PDGF stimulated TGF-β production in mesangial cells, and IFN-γ has been also shown to stimulate TGF-β production in cultured murine macrophages [22]. In our study IFN-γ did not increase
TGF-β production. However, anti-TGF-β antibody inhibited IFN-γ-stimulated type IV collagen production. This suggests that IFN-γ stimulates type IV collagen production via a TGF-β-dependent mechanism as PDGF and b-FGF. There are two possible explanations. One is involvement of other isoforms of TGF-β in type IV collagen production by IFN-γ-stimulated HMC, as the anti-TGF-β antibody we used was not specific for TGF-β 1 and does not neutralize all isoforms of TGF-β. The other is that the amount of TGF-β may not be so important for type IV collagen production. PDGF and b-FGF increased TGF-β production by 25−50%, while a 5−10-fold increase in type IV collagen production was observed. Thus, only a small amount of increase of TGF-β, which cannot be detected by the assay used in the present study, may be enough to induce type IV collagen production. TGF-β is a multifunctional regulatory protein and is known to stimulate the synthesis of type IV collagen and fibronectin as well as proteoglycans, which are matrix components present in large amounts in normal glomeruli [23]. TGF-β has been shown to participate in the glomerular accumulation of extracellular matrix in glomerulonephritis [24]. We have reported that thrombin increased type IV collagen production by HMC and this effect of thrombin was inhibited by anti-TGF-β antibody as well as PDGF, b-FGF, and IFN-γ [25]. We have also demonstrated that thrombin increased TGF-β production in HMC [26]. The previous and present data indicate that PDGF, b-FGF, and IFN-γ increase type IV collagen production through a TGF-β-dependent mechanism and TGF-β is one of the most important factors in type IV collagen production in HMC.

References

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