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Biochemical and Biophysical Research Communications 322 (2004) 22–28
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Differential regulation of biglycan and decorin synthesis by connective tissue growth factor in cultured vascular endothelial cells

Toshiyuki Kaji [a,*], Chika Yamamoto [a], Mami Oh-i [a], Takashi Nishida [b], Masaharu Takigawa [b]

[a] *Department of Environmental Health, Faculty of Pharmaceutical Sciences, Hokuriku University, Ho-3 Kanagawa-machi, Kanazawa 920-1181, Japan*
[b] *Department of Biochemistry and Molecular Dentistry, Okayama University Graduate School of Medicine and Dentistry, 2-5-1 Shikata-cho, Okayama 700-8525, Japan*

Received 2 July 2004
Available online 30 July 2004

Abstract

It is possible that connective tissue growth factor (CTGF) serves as either an independent regulator or a downstream effector of transforming growth factor-β (TGF-β) on the proteoglycan synthesis in vascular endothelial cells. Since TGF-β regulates endothelial proteoglycan synthesis in a cell density-dependent manner, dense and sparse cultures of bovine aortic endothelial cells were metabolically labeled with [^{35}S]sulfate or ^{35}S-labeled amino acids in the presence of CTGF, and the labeled proteoglycans were characterized by biochemical techniques. The results indicate that CTGF suppresses the synthesis of biglycan but newly induced that of decorin in the cells when the cell density is low; in addition, no change was observed in the hydrodynamic size and the glycosaminoglycan chain length of these two small chondroitin/dermatan sulfate proteoglycans. The regulation of endothelial proteoglycan synthesis by CTGF is completely different from that by TGF-β, suggesting that CTGF is not a downstream effector of TGF-β but an independent regulator in vascular endothelial cells with respect to the proteoglycan synthesis.

Keywords: Atherosclerosis; Biglycan; Connective tissue growth factor; Decorin; Endothelial cell; Dermatan sulfate; Glycosaminoglycan; Proteoglycan; Transforming growth factor-β; Vascular

Proteoglycans (PGs) are a heterogeneous group of macromolecules that consist of glycosaminoglycan (GAG) side chains and a core protein as a common feature [1]. Vessel PGs have been implicated in vascular properties such as viscoelasticity, permeability, lipid metabolism, hemostasis, thrombosis, and extracellular matrix assembly [2–4]. Vascular endothelial cells synthesize heparan sulfate PGs (HSPGs), including the major endothelial cell extracellular matrix PG, perlecan [5], members of the syndecan family of transmembrane PGs [6,7], and the cell surface-associated PG, glypican [6]. Endothelial cells also constitutively synthesize a small leucine-rich chondroitin/dermatan sulfate PG (CS/DSPG), biglycan [8]. In addition, the cells express another small leucine-rich CS/DSPG, decorin, in certain conditions, for example during formation of neovessels both in vitro [9] and in vivo [10].

It has been shown that growth factors and cytokines can regulate the PG synthesis in vascular endothelial cells. Basic fibroblast growth factor promotes the synthesis of biglycan in bovine aortic endothelial cells during migration [11]. Although endothelial PG synthesis is in general promoted by transforming growth factor-β (TGF-β) but suppressed by either interleukin-1β or tumor necrosis factor-α [12,13], we found that regulation by TGF-β is cell density-dependent [14]; TGF-β promotes the synthesis of both perlecan and biglycan

* Corresponding author. Fax: +81 76 229 6208.
E-mail address: t-kaji@hokuriku-u.ac.jp (T. Kaji).

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doi:10.1016/j.bbrc.2004.07.078

when the cell density is high whereas only biglycan synthesis is stimulated when the cell density is low. Furthermore, GAG chains are elongated only in biglycan at a high cell density.

Connective tissue growth factor (CTGF) is a cysteine-rich, heparin-binding peptide with molecular mass of 38 kDa, first identified in the conditioned medium of cultured human umbilical vein endothelial cells [15]. CTGF is expressed in vascular endothelial cells and promotes their own proliferation and migration in an autocrine fashion [16]. In human skin fibroblasts, CTGF mRNA is induced specifically by TGF-β but not by other growth factors including epidermal growth factor, platelet-derived growth factor, and both basic and acidic fibroblast growth factors [17], suggesting that a part of action of TGF-β may be mediated via an indirect mechanism involving CTGF [18,19]. Since TGF-β is a strong inducer of CTGF gene expression in vascular endothelial cells [20], it is likely that CTGF may serve as a downstream effector of TGF-β in vascular endothelial cells as well as fibroblasts.

However, the proliferation of vascular endothelial cells is inhibited by TGF-β [21] but stimulated by CTGF [22], suggesting that CTGF can serve as an independent regulator of endothelial cell functions. It is unclear whether CTGF serves as a downstream effector of TGF-β or an independent regulator on endothelial PG synthesis. In the present study, the regulation of PG synthesis by CTGF was investigated using a culture system of bovine aortic endothelial cells. Since the regulation of endothelial PG synthesis by TGF-β is cell density-dependent [14], dense and sparse cultures of the cells were treated with recombinant human CTGF, and synthesized PGs were characterized by biochemical techniques. The results indicate that the regulation of endothelial PG synthesis by CTGF is completely different from that by TGF-β, in other words, CTGF is not a downstream effector of TGF-β but an independent regulator with respect to endothelial PG synthesis.

Materials and methods

Materials. Vascular endothelial cells obtained from bovine aorta and Western blotting blocking reagent were from Dainihon Pharmaceutical (Osaka, Japan). RPMI 1640 medium and ASF 301 medium were from Nissui Pharmaceutical (Tokyo, Japan) and Ajinomoto (Tokyo, Japan), respectively. Fetal bovine serum was from Equitech-Bio (Kerrville, TX, USA); tissue culture dishes and plates were from Iwaki (Tokyo, Japan). [^{35}S]Na_2SO_4 (carrier free) and Tran ^{35}S-labeled metabolic labeling reagent which consists of ~70% L-[^{35}S]methionine, ~15% L-[^{35}S]cysteine, and other ^{35}S-labeled compounds were from ICN Biomedicals (Irvine, CA, USA). Sepharose CL-4B, Sepharose CL-6B, and PD-10 columns (disposable Sephadex G-25M), ECL Western blotting detection reagents, horseradish peroxidase-linked protein A, nitrocellulose membranes (Hybond ECL), Hyperfilm ECL, and Quick prep micro mRNA purification kit were from Amersham–Pharmacia Biotech (Little Chalfont, UK); DEAE–Sephacel, benzamidine, Tris base, dextran blue, phenylmethanesulfonyl fluoride, papain (1.7 U/mg solid), and heparitinase (EC 4.2.2.8, derived from *Flavobacterium heparinium*) were from Sigma–Aldrich Chemical (St. Louis, MO, USA). Urea, phenol red, and sodium dodecyl sulfate were from Wako Purechemical Industries (Osaka, Japan). Chondroitin ABC lyase (EC 4.2.2.5, derived from *Proteus vulgaris*) was from Seikagaku (Tokyo, Japan). Recombinant human CTGF was purified from the conditioned medium of CTGF overexpressing HeLa cells as described previously [23]. Rabbit antisera against biglycan core protein (LF-96) and decorin core protein (LF-94) were kindly provided by Dr. Larry Fisher (NIDR, National Institute of Health, Bethesda, MD, USA). Oligonucleotide primers for reverse transcription-polymerase chain reaction (RT-PCR) were from Invitrogen (Carlsbad, CA, USA). Cetylpyridinium chloride (CPC) and other reagents were from Nacalai Tesque (Kyoto, Japan).

Cell culture and dissociative PG extraction. Vascular endothelial cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum in 100-mm dishes in a humid atmosphere of 5% CO_2 in air until confluent. They were transferred into 24-well culture plates or 100-mm dishes at 1×10^4 cells/cm^2 and cultured for 24 h (“sparse cultures”) or until confluent (“dense cultures”). The medium was replaced by fresh serum-free ASF 301 medium after washing of the cells with the medium, and the cells were incubated at 37 °C for 24 h in 0.25 ml of fresh serum-free ASF 301 medium with CTGF (1, 10, 100 or 1000 ng/ml) in the presence of [^{35}S]sulfate (370 kBq/ml). After incubation, the conditioned medium was harvested and solid urea was added to a concentration of 8 M. The cell layer was washed with ice-cold Ca^{2+} and Mg^{2+}-free phosphate-buffered saline (CMF-PBS). After extraction with 8 M urea solution (pH 7.5) containing 0.1 M of 6-aminohexanoic acid, 5 mM benzamidine, 10 mM *N*-ethylmaleimide, 2 mM phenylmethanesulfonyl fluoride, 0.1 M NaCl, 50 mM Tris base, and 2% Triton X-100 at 4 °C for 15 min, the layer was harvested by scraping with a rubber policeman. The medium and cell extracts were chromatographed on PD-10 columns equilibrated 8 M urea buffer (pH 7.5) containing 2 mM EDTA, 0.1 M NaCl, 0.5% Triton X-100, and 50 mM Tris base to obtain high molecular mass (>3 kDa) macromolecules.

Incorporation of [^{35}S]sulfate into GAGs. The incorporation of [^{35}S]sulfate into GAGs was measured by CPC precipitation [24]. Briefly, aliquots of the medium and cell extracts were spotted on filter paper and washed five times for 1 h in 1% CPC with 0.05 M NaCl. The radioactivity of precipitate on the dried filter paper was determined by liquid scintillation counting.

Characterization of PGs. To separate PGs on the basis of differences in charge density, the macromolecules were applied to DEAE–Sephacel (5 ml of resin) in 8 M urea buffer (pH 7.5) containing 2 mM EDTA, 0.1 M NaCl, 0.5% Triton X-100, and 50 mM Tris base. Unbound radioactivity was removed from the column by washing with 30 ml of the buffer. Bound radioactivity was eluted from the column with a linear gradient of 0.1–0.7 M NaCl in the urea buffer (total volume of 50 ml). PG-containing two peaks were eluted at ~0.4 and ~0.5 M NaCl, and the second peak in the conditioned medium of the sparse culture was pooled because it contained decreased radioactivity in sample from the CTGF-treated culture (see Results). Pooled peak material was concentrated by application of the diluted samples to 0.3 ml DEAE–Sephacel columns and eluting bound radioactivity with sequential washes of 8 M urea buffer containing 3 M NaCl. Concentrated PGs were then chromatographed on a Sepharose CL-4B column (0.9 × 80 cm) in 8 M urea buffer containing 0.25 M NaCl to separate PGs on the basis of their hydrodynamic size. Separately, a portion of the concentrated PGs were precipitated with 3.5 volumes of 1.3% potassium acetate in 95% ethanol and 80 μg/ml carrier chondroitin sulfate for 2 h at −20 °C; the precipitation was repeated three times. The precipitated PGs was digested with 10 μg papain in 0.1 M acetate buffer (pH 7.0) containing 5 mM EDTA and 5 mM cysteine at 65 °C for 4 h or with 1.7 U/ml chondroitin ABC lyase in 50 mM Tris–HCl buffer (pH 8.0) containing 0.1 mg/ml bovine serum albumin and 3 mM sodium acetate at 37 °C for 4 h or with 1 U/ml heparitinase in 10 mM Tris–

HCl buffer (pH 7.0) containing 0.5 mM calcium acetate at 37 °C for 4 h. The digested samples were chromatographed on a Sepharose CL-6B column (0.9 × 80 cm) in 0.2 M Tris–HCl buffer (pH 7.0) with 0.2 M NaCl. The void volume and the total volume were estimated by the elution position of dextran blue and phenol red, respectively. Estimates of GAG chain size were made by comparison of Sepharose CL-6B elution K_{av} determined experimentally with a previously published curve of log M_r versus K_{av} on Sepharose CL-6B for chondroitin sulfate chains of various known M_r [25].

Analysis of PG core protein expression. Sparse cultures of vascular endothelial cells were incubated at 37 °C for 24 h in 6 ml of fresh ASF 301 medium with CTGF (100 ng/ml) in the presence of Tran ^{35}S-label reagent (1 MBq/ml). After incubation, radiolabeled PGs accumulated in the conditioned medium were extracted under a dissociative condition in the presence of 8 M urea. The extracts were concentrated on 0.3 ml DEAE–Sephacel columns and precipitated with 1.3% potassium acetate in 95% ethanol. Some precipitates were digested with chondroitin ABC lyase. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to the procedure of Laemmli [26] on acrylamide 4–12% gradient slab gels with a 3% stacking gel. The radiolabeled PG cores were visualized by autoradiography of dried gels exposed to Kodak XAR-2 film at −70 °C. For Western blot analysis, the SDS–PAGE gels were equilibrated in 25 mM Tris transfer buffer (pH 9.5) with 20% methanol and transferred to nitrocellulose membranes for 90 min with a semidry transfer apparatus (Atoo, AE-6677). The membrane was blocked and exposed to a primary antibody against biglycan (LF-96, diluted 1:1000) or decorin (LF-94, diluted 1:1000) overnight at 4 °C. After incubation of the blot with horseradish peroxidase-linked protein A, bands that bound to the primary antibody were visualized by an enzyme-linked chemoluminescence procedure. In another experiment, poly(A)$^+$ RNA was isolated from sparse endothelial cells before and after treatment with CTGF (100 ng/ml) for 4, 8, or 24 h, and biglycan and decorin core mRNAs were analyzed by quantitative RT-PCR as previously described [27,28]. PCR was conducted under quantitative conditions, which were determined by plotting signal intensities as a function of the template amount and cycle number. Oligonucleotide primers were designed on the basis of human cDNAs. After RT-PCR, an aliquot of the reaction mixture was electrophoresed on a 2% agarose gel containing 0.1 μg/ml ethidium bromide. The sequences of the upstream and downstream primers were as follows: 5′-AGGCCCTCGTCC TGGTGAACA-3′ and 5′-GAATGCGGTTGTCGTGGATGC-3′ for biglycan core cDNA; 5′-CAAAATAACCGAAATCAAAGA-3′ and 5′-CGTAAGGGAAGGAGGAAGACC-3′ for decorin core cDNA. The size of the PCR products for biglycan and decorin was 165 and 400 bp, respectively.

Results

CTGF decreases the accumulation of GAGs into the conditioned medium of sparse endothelial cells

Fig. 1 shows the incorporation of [^{35}S]sulfate into GAGs accumulated in the cell layer and the conditioned medium of vascular endothelial cells after exposure to CTGF. In dense cultures, CTGF failed to change the [^{35}S]sulfate incorporation; however, the growth factor at 10 ng/ml and more significantly decreased the [^{35}S]sulfate incorporation into GAGs accumulated in the conditioned medium of sparse cultures after a 24-h incubation. It is suggested that CTGF regulates the synthesis of PGs secreted into the medium when endothelial cell density is low.

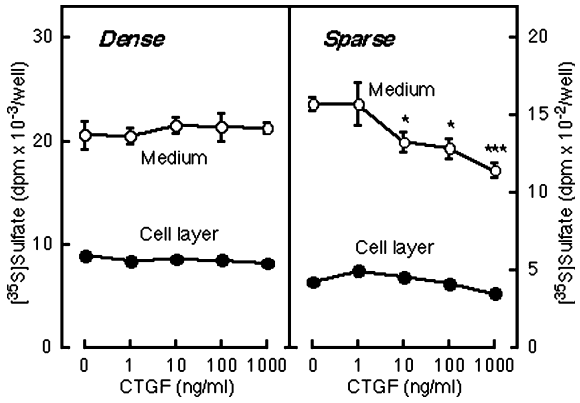

Fig. 1. The incorporation of [^{35}S]sulfate into GAGs accumulated in the cell layer and the conditioned medium of dense and sparse vascular endothelial cells after exposure to CTGF. Dense and sparse cultures of bovine aortic endothelial cells were incubated at 37 °C for 24 h with recombinant human CTGF (1, 10, 100 or 1000 ng/ml) in the presence of [^{35}S]sulfate. Values are means ± SE of four samples. Significantly different from the corresponding control, $*p < 0.05$; $***p < 0.001$ (Student's t test).

Characterization of PGs whose synthesis is regulated by CTGF

The cell layer and medium extracts of [^{35}S]sulfate-labeled sparse cultures were submitted to a DEAE–Sephacel column to separate PGs based on charge density differences. In the cell layer, either with or without CTGF treatment, incorporated [^{35}S]sulfate radioactivity eluted from the column by the NaCl gradient in two peaks, at approximately 0.45 and 0.55 M (Fig. 2). There was no difference between the DEAE–Sephacel profile of the control and that of CTGF treatment. In the conditioned medium, [^{35}S]sulfate-labeled PGs also eluted by NaCl at approximately 0.45 and 0.45 M. The radioactivity of the first peak was not changed, whereas that of the second peak was decreased by CTGF.

The DEAE–Sephacel second peak in the conditioned medium of sparse endothelial cells was pooled (Fig. 2, bars), and the equal radioactivity was separated on the basis of their hydrodynamic size by Sepharose CL-4B molecular sieve chromatography. As shown in Fig. 3, the peak was separated into two subclasses; a high M_r subclass (HMW) that eluted near the void volume and a predominant low M_r subclass (LMW) that eluted at a K_{av} of 0.2–0.8. However, the hydrodynamic size of the low M_r subclass was unaffected by CTGF, suggesting that CTGF specifically regulates the synthesis of small PGs without change in their hydrodynamic size.

The GAG composition of the pooled DEAE–Sephacel second peak was analyzed by Sepharose CL-6B chromatography to identify the type of PGs bound to the peak. As shown in Fig. 4, the PGs eluted near the void volume before and after heparitinase digestion (Figs. 4A–D); however, the peak shifted to K_{av} of 0.83 after chondroitin ABC lyase digestion in either the control or the CTGF

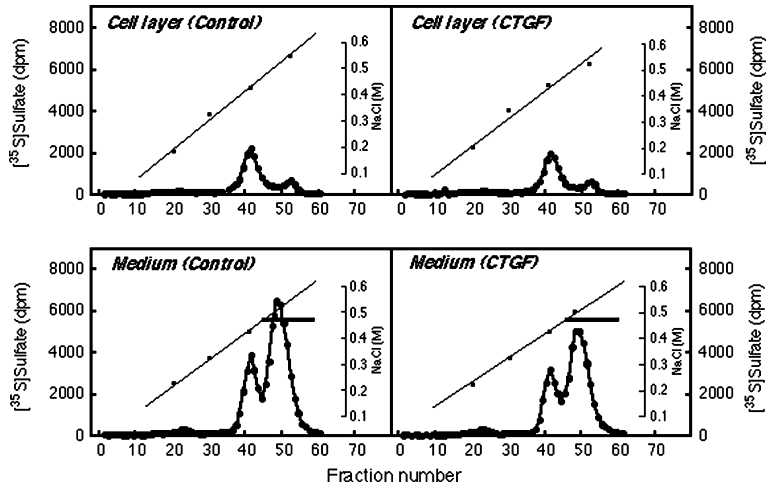

Fig. 2. DEAE–Sephacel ion exchange chromatography of [^{35}S]sulfate-labeled PGs extracted from the cell layer and the conditioned medium of sparse vascular endothelial cells after exposure to CTGF with a linear gradient of 0.1–0.7 M NaCl in 8 M urea buffer. Sparse cultures of bovine aortic endothelial cells were incubated at 37 °C for 24 h with recombinant human CTGF (100 ng/ml) in the presence of [^{35}S]sulfate. Horizontal bars indicate the fractions that were pooled and chromatographed on Sepharose CL-4B and CL-6B columns before and after digestion with heparitinase, chondroitin ABC lyase or papain (see Figs. 3 and 4).

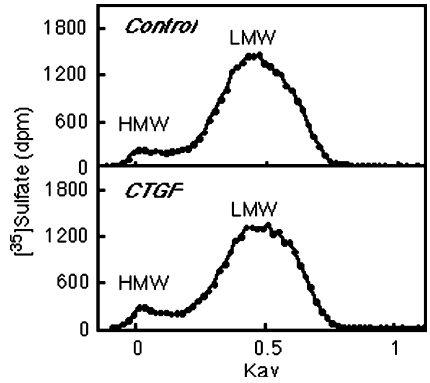

Fig. 3. Sepharose CL-4B molecular sieve chromatography of CS/DSPGs obtained from the conditioned medium of sparse vascular endothelial cells. The CS/DSPGs separated by DEAE–Sephacel ion exchange chromatography were chromatographed on a Sepharose CL-4B column in 8 M urea buffer. HMW, the high M_r subclass; LMW, the low M_r subclass.

treatment (Figs. 4E and F), suggesting that PGs bound to the DEAE–Sephacel second peak are not HSPGs but CS/DSPGs. When GAG chains were isolated by digestion of core proteins with papain, the radioactivity eluted at K_{av} of 0.35 in either the control or the CTGF treatment (Figs. 4G and H), indicating that the GAG chain size is M_r 44,000 and CTGF does not influence the chain elongation. This agrees with no change in the hydrodynamic size of small PGs obtained from the conditioned medium of the CTGF-treated sparse cells (Fig. 3).

CTGF suppresses the synthesis of biglycan core protein but newly induces that of decorin core protein

To examine whether the synthesis of specific CS/DSPG core proteins is suppressed or induced by CTGF, proteins were metabolically labeled with ^{35}S-labeled amino acids and analyzed by SDS–PAGE either with or without prior digestion with chondroitin ABC lyase (Fig. 5). Digestion with the enzyme generated CS/DSPG core protein bands with a molecular mass of approximately 50 kDa, and the core proteins were decreased by CTGF. Western blot analysis was performed because vascular endothelial cells can express both biglycan and decorin [9], small CS/DSPGs that have core proteins with a molecular mass of approximately 50 kDa. The 50-kDa CS/DSPG core proteins from the conditioned medium of the control cells reacted only with anti-biglycan antibody whereas those from the conditioned medium of CTGF-treated cells did with not only anti-biglycan antibody but also anti-decorin antibody, suggesting that the small CS/DS core proteins are composed of only biglycan and a mixture of biglycan and decorin in the control and the CTGF-treated cells, respectively. When the levels of core protein mRNAs were determined by quantitative RT-PCR, decorin mRNA was increased by CTGF after 8 h and longer, confirming the induction of decorin synthesis, although biglycan mRNA was unaffected by the growth factor (Fig. 6). It is suggested that CTGF suppresses the synthesis of biglycan but newly induces that of decorin, resulting in a decrease in the whole synthesis of small CS/DSPGs in sparse vascular endothelial cells.

Discussion

It has been hypothesized that CTGF functions as a downstream effector of TGF-β action on connective tissue cells, where it stimulates cell proliferation and extra-

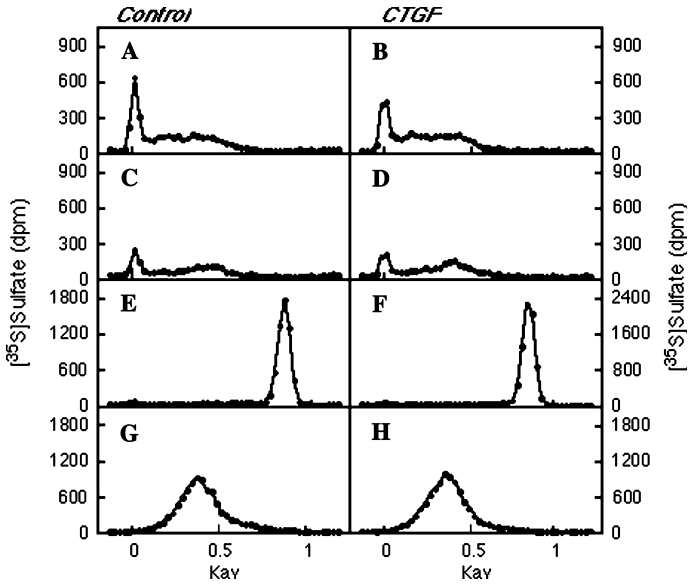

Fig. 4. Sepharose CL-6B chromatography of CS/DSPGs obtained from the conditioned medium of sparse vascular endothelial cells. The CS/DSPGs separated by DEAE–Sephacel ion exchange chromatography were chromatographed on a Sepharose CL-6B column in 0.2 M Tris–HCl buffer (pH 7.0) with 0.2 M NaCl before and after digestion with heparitinase, chondroitin ABC lyase, or papain. (A) Control, before enzyme digestion; (B) CTGF treatment before enzyme digestion; (C) control after heparitinase digestion; (D) CTGF treatment after heparitinase digestion; (E) control after chondroitin ABC lyase digestion; (F) CTGF treatment after chondroitin ABC lyase digestion; (G) control after papain digestion; and (H) CTGF treatment after papain digestion.

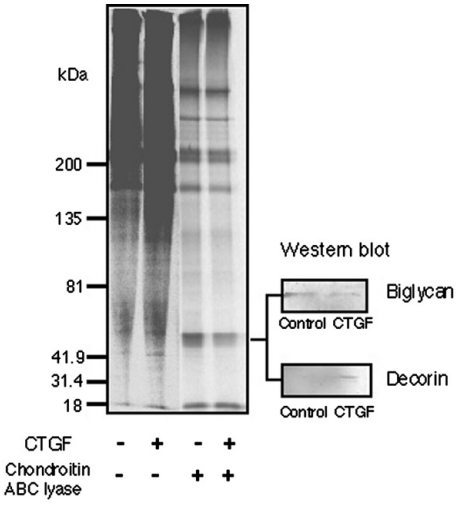

Fig. 5. Accumulation of biglycan and decorin core proteins in the conditioned medium of sparse vascular endothelial cells after exposure to CTGF. Sparse cultures of bovine aortic endothelial cells were incubated at 37 °C for 24 h with recombinant human CTGF (100 ng/ml) in the presence of ^{35}S-labeled amino acids. Samples were run on a 4–12% gradient slab gel before and after digestion with chondroitin ABC lyase. Separately, chondroitin ABC lyase-generated core proteins were probed with an antibody specific for either biglycan or decorin.

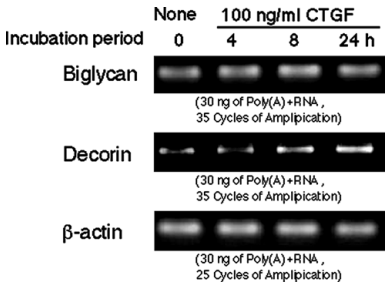

Fig. 6. Quantitative RT-PCR analysis of biglycan and decorin core mRNAs in sparse vascular endothelial cells after exposure to CTGF. Sparse cultures of bovine aortic endothelial cells were incubated at 37 °C for 4, 8 or 24 h with recombinant human CTGF (100 ng/ml).

cellular matrix synthesis [29]. In the present study, it was revealed that CTGF suppresses the synthesis of biglycan but newly induces that of decorin without change in length of their GAG chains in vascular endothelial cells when the cell density is low. The regulation of endothelial PG synthesis by CTGF is completely different from that by TGF-β [14]. For example, TGF-β promotes the synthesis of biglycan in both dense and sparse endothelial cells, while CTGF suppresses it in the sparse cells. CTGF induces the synthesis of decorin but TGF-β does not have such an effect. Thus, it is postulated that the regulation of endothelial PG synthesis by TGF-β is not mediated by CTGF; in other words, CTGF is not a downstream effector of TGF-β but an independent regulator with respect to endothelial PG synthesis at a low cell density.

CTGF is implicated in the progression of numerous fibrotic diseases including atherosclerosis that is initiated by functional damage of endothelial cells. The growth factor is undetectable in normal blood vessels but dramatically expressed in atherosclerotic plaques [30]. The expression is predominantly observed in vascu-

lar smooth muscle cells at the shoulder of fibrous cap and at margins along a lipid core or a necrotic core of the hyperplastic intima. CTGF-positive endothelial cells were also found at the luminal site of the plaques and in vasa vasorum inside the plaque lesions, suggesting that the cells would be exposed to CTGF during the repair process of damaged endothelium or angiogenesis. When vascular endothelial cells are injured, TGF-β is largely released from aggregated platelets at the damaged site [31] and appears to inhibit the migration and proliferation of the cells [21] near the damaged site. Then TGF-β would begin to induce the synthesis of CTGF [20] as well as biglycan [14] in proliferating endothelial cells in regenerating endothelium. Our results suggest that stimulated synthesis of biglycan after exposure to TGF-β may be reduced by CTGF in the proliferating cells. At that time, decorin core proteins derived from CTGF-exposed proliferating endothelial cells may bind and inactivate TGF-β [32]. As a result, inhibition of the proliferation [21] and fibrinolytic activity [33] of endothelial cells by TGF-β would be diminished by newly synthesized decorin. Therefore, induction of CTGF synthesis by TGF-β followed by induction of decorin synthesis by CTGF may serve as a negative feedback loop to limit the activity of TGF-β in proliferating cells in regenerating endothelium. However, CTGF may no longer modulate the activity of TGF-β after regrowth of the endothelial cell monolayer since CTGF does not appear to regulate endothelial PG synthesis when the cell density is high.

Decorin is a dermatan sulfate-containing PG present in the extracellular matrix of blood vessels [34]. Although decorin and biglycan have similar size core glycoproteins, the small CS/DSPGs are composed of distinct core proteins linked to one (decorin) or two (biglycan) chondroitin/dermatan sulfate chains [35]. The synthesis of biglycan and decorin is differentially regulated in vascular endothelial cells. The biglycan synthesis is stimulated by basic fibroblast growth factor [11] and TGF-β [14], whereas decorin is not synthesized constitutively but expressed with type I collagen during angiogenesis in vitro [9]. Although the function of biglycan is not necessarily certain, decorin has been shown to be involved in the fibrillogenesis of type I collagen [35–38]. In addition, decorin is bound to fibronectin and influences cell adhesion and migration [39,40]. When decorin is overexpressed in bovine aortic endothelial cells, their proliferation was unaffected but their migration is inhibited via a promotion of fibronectin fibrillogenesis [41]. From these results, an assumption can be made that CTGF-induced migration and angiogenesis [22] may be reduced by induction of decorin synthesis in proliferating and/or migrating endothelial cells. Consequently, CTGF may modulate its activity on vascular endothelial cell behavior by induction of decorin synthesis.

In summary, our results demonstrate that CTGF suppresses the synthesis of biglycan but newly induces that of decorin in vascular endothelial cells when the cell density is low. The results indicate that CTGF is not a downstream effector of TGF-β but an independent regulator with respect to PG synthesis in vascular endothelial cells. CTGF expressed in the proliferating endothelial cells after exposure to TGF-β may reduce the stimulation of biglycan synthesis by TGF-β. On the other hand, it is suggested that CTGF-induced decorin synthesis influences the action of not only TGF-β but also CTGF itself on vascular endothelial cell functions including proliferation, migration, and angiogenesis; the small leucine-rich CS/DSPG may be involved in the sequential regulation of endothelial cell behavior conducted primarily by TGF-β and subsequently by CTGF.

Acknowledgments

This work was supported in part by Grant-in-Aid for Scientific Research (to T.K.) from Japan Society for the Promotion of Science and by the Specific Research fund of Hokuriku University (to T.K.).

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