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Effect of transforming growth factor-beta1 on expression of the connective tissue growth factor (*CCN2/CTGF*) gene in normal human gingival fibroblasts and periodontal ligament cells

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Background and Objective: Connective tissue growth factor (CCN2/CTGF) plays an important role in wound healing and regulation of the extracellular matrix in periodontal tissue. However, the functional relationship between altered transforming growth factor-beta1 levels and CCN2/CTGF has not been extensively investigated in human gingival fibroblasts and periodontal ligament cells. This study investigated the effects of transforming growth factor-beta1 on the expression of the *CCN2/CTGF* gene in human gingival fibroblasts and periodontal ligament cells *in vitro*.

Material and Methods: Cells were isolated from normal periodontal tissues and cultured in Dulbecco's modified Eagle's minimal essential medium/F12 containing 10% fetal bovine serum. Subconfluent cells were maintained under serum deprivation for 24 h then treated with Dulbecco's modified Eagle's minimal essential medium/F12 containing 0.5% fetal bovine serum (control) and 0.1, 1, 5 or 10 ng/mL of transforming growth factor-beta1 for 24, 48 or 72 h. The effects of transforming growth factor-beta1 on CCN2/CTGF mRNA expression were measured by reverse transcription–polymerase chain reaction. CCN2/CTGF protein was quantitatively analyzed using enzyme-liked immunosorbent assay. Subcellular distribution of CCN2/CTGF protein in both human gingival fibroblasts and periodontal ligament cells was observed using immunofluorescence microscopy.

Results: In both human gingival fibroblasts and periodontal ligament cells, the expression of CCN2/CTGF mRNA and CCN2/CTGF protein was significantly increased, in a dose- and time-dependent manner, in the presence of transforming

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growth factor-beta1. Moreover, immunofluorescence analysis indicated that immunoreactivity to CCN2/CTGF showed a granular pattern of protein localization.

Conclusion: The expression of CCN2/CTGF mRNA and protein was induced by transforming growth factor-beta1 in human gingival fibroblasts and periodontal ligament cells. These results suggest that CCN2/CTGF plays an important role in wound healing and in the regeneration of periodontal tissue.

After periodontal surgery, postoperative care to promote proper wound healing is critical. Wound healing of periodontal tissue involves tightly regulated processes that restore the epithelial barrier along with capillary and connective tissue structure and function. This wound healing is divided into four stages – inflammatory, proliferative, fibroblastic and maturation – and many questions remain regarding the growth factors that appear from the inflammatory stage to the fibroblastic stage.

Several growth factors, such as vascular endothelial growth factor (1), fibroblast growth factor (2), transforming growth factor-beta (3), platelet-derived growth factor (4) and connective tissue growth factor (CCN2/CTGF), flourish in the inflammatory and proliferative stages of wound healing. These growth factors are produced by various cells and tissues, including fibroblasts, endothelium, vascular smooth muscle and macrophages. Upon wounding, these growth factors interact and lead to angiogenesis (1,4), extracellular matrix production, and remodeling (2), including collagen synthesis (3). These events are orchestrated by the regulated release and activation of various growth factors and cytokines at the wound site.

Regeneration therapy is a new approach to enhance or reconstruct the healing potential of damaged regions using exogenous materials and cells. Growth factors are key for regeneration therapy (5). In fact, many types of periodontal therapy, including guided tissue regeneration (6,7), enamel matrix derivative (8) and platelet-rich plasma (9), use growth factors as part of standard therapeutic regimens.

Connective tissue growth factor (CCN2)/CTGF is a growth factor of 38–40 kDa that acts as a potent fibroblast mitogen and angiogenic factor. CCN2/CTGF belongs to the CTGF, Cyr61 and Nov (CCN) family of growth factors (10). CCN2/CTGF exhibits diverse cellular functions that are cell-type dependent; they include extracellular matrix production (11), angiogenesis (12,13), normal growth and development of certain tissues (14), tissue regeneration (14), and cell migration and adhesion (15). In periodontal tissues, one of the most important biological actions of CCN2/CTGF may be stimulation of the synthesis of extracellular matrix components.

Recently, the role of CCN2/CTGF in wound healing, systemic scleroderma (16), fibrosis (17,18) and scar formation (19,20) has been reported. However, in the dental field, other than reports on the role of CCN2/CTGF in gingival overgrowth (21,22), no studies have examined the wound-healing effects of CCN2/CTGF in human periodontal tissue.

In addition to CCN2/CTGF, transforming growth factor-beta is a representative growth factor during wound healing (23), and important links between CCN2/CTGF and transforming growth factor-beta have been reported, adding weight to the concept that CCN2/CTGF plays an important role in scarring. For example, transforming growth factor-beta is known to be a strong inducer of *CCN2/CTGF* gene expression (23,24).

In this study, we investigated the effect of transforming growth factor-beta on CCN2/CTGF in both human gingival fibroblasts and periodontal ligament cells. We selected transforming growth factor-beta for evaluation because it has been used in the regeneration of soft tissue (25) and bone (26) in the field of dentistry.

Material and methods

Cell culture

Induction of *CCN2/CTGF* gene expression has already been reported in human fibroblasts obtained from dental papillae (27). Therefore, we used normal gingiva over wisdom teeth, which is different from the typical cellular source, to examine whether *CCN2/CTGF* induction was a universal event among gingival fibroblasts.

Human gingival fibroblasts were obtained from human normal gingiva during extraction of impacted wisdom teeth (n = 4, 23–34 years of age). Periodontal ligament cells were obtained from normal human periodontal tissue attached to permanent lower premolar teeth that were extracted during orthodontic therapy (n = 3, 23–28 years of age). This study was approved by the Institutional Ethical Review Committee of The Nippon Dental University. Before treatment, each patient received an explanation of how periodontal tissue would be used, and informed consent was obtained.

The gingiva were cut into small pieces. The periodontal ligament was exfoliated from the apical third of the tooth. Fragments of both types of tissue were transferred to a 60 mm dish (Iwaki & Co., Asahi Techno Glass, Tokyo, Japan) containing a few drops of Dulbecco's modified Eagle's minimal essential medium/F-12 (Invitrogen Corp., Carlsbad, CA, USA) supple-

mented with 1 mM minimal essential medium/nonessential amino acids solution (Invitrogen Corp.), 50 units/mL of penicillin–streptomycin, 0.25 μg/mL of amphotericin B and 20% fetal bovine serum (Moregate, Bulimba, Australia) (Dulbecco's modified Eagle's minimal essential medium/F-12 plus 20% fetal bovine serum, hereafter designated as 20% fetal bovine serum/Dulbecco's modified Eagle's minimal essential medium/F-12) until dissociated cells adhered to the bottom of the dish. The cells were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2. The medium was replaced every 3 or 4 d until subconfluence was achieved. When the cells reached subconfluence, 0.25% trypsin–EDTA (Invitrogen Corp.) was used to remove the cells, which were then transferred to a 100-mm tissue-culture dish (Iwaki & Co.) for continued growth. The cells were maintained in 10% fetal bovine serum/Dulbecco's modified Eagle's minimal essential medium/F-12. Cells were used between the fourth and eighth passages for all experiments.

Reverse transcription–polymerase chain reaction analysis

Human gingival fibroblasts and periodontal ligament cells were plated at a density of 2×10^5 cells in 100-mm tissue-culture plates (Iwaki & Co.) containing 0.5% fetal bovine serum/Dulbecco's modified Eagle's minimal essential medium/F-12 for subconfluence. Cells were maintained under serum deprivation for 24 h, then treated with Dulbecco's modified Eagle's minimal essential medium/F-12 containing 0.5% fetal bovine serum (control), or 0.1, 1, 5 or 10 ng/mL of transforming growth factor-beta1, for 24, 48 and 72 h. Under each condition, cells were washed three times with PBS (−). Total cellular RNAs were isolated from human gingival fibroblasts and periodontal ligament cells using RNA-Bee™ (Tel-Test, Inc., Friendswood, TX, USA). The concentration and purity of RNA in each sample were determined by spectrophotometric absorption at 260/280 nm. The expression of CCN2/CTGF mRNA transcripts was semiquantitively analyzed by reverse transcription–polymerase chain reaction (RT-PCR) (annealing temperature 59°C), and standardized to the expression level of the glyceraldehyde-3-phosphate dehydrogenase housekeeping gene transcripts. RT-PCR was performed using Ready-To-Go PCR beads (Amersham Biosciences, Bucks., UK). PCR was performed in a reaction buffer of 50 μL containing a cDNA sample, corresponding primers and nuclease-free water, as well as the beads. The primers used are shown in Table 1. PCR products were then separated on a 2% agarose gel. The size of the PCR product was 300 bp, as anticipated. After visualization by staining with 15% ethidium bromide, the luminescence densities of each band were calculated using a densitograph lane analyzer (Atto, Tokyo, Japan).

Enzyme-linked immunosorbent assay

Cell culture supernatants of human gingival fibroblasts and periodontal ligament cells were sampled at the indicated time-points after the addition of transforming growth factor-beta1. At the same time, the cell lysates were prepared using a lysis buffer (Epitomics, Inc., Burlingame, CA, USA). The level of CCN2/CTGF in both cell culture supernatants was evaluated using a sandwich enzyme-linked immunosorbent assay (ELISA) system, which was kindly provided by Nichirei Corporation (Tokyo, Japan), as described previously (28,29). During incubation at 37°C for 2 h, CCN2/CTGF in the samples was captured on ELISA strips precoated with monoclonal anti-human CCN2/CTGF (clone 8-64 provided by Nichirei Corporation). Thereafter, horseradish peroxidase-conjugated antibody (clone 8-86) was added, and incubation was carried out for 1 h at 37°C. Signals were developed using tetramethylbenzidine and quantified as optical densities at a wavelength of 450 nm.

Table 1. Primers used in the reverse transcription–polymerase chain reaction analysis

Gene	Sequence (5′ → 3′)	Number of cycles	Product length (bp)
CTGF	Forward: AAGGTGTGGCTTTAGGAGCA Reverse: TTCACTTGCCAACCGCTGTC	35	300
GAPDH	Forward: ACCACAGACCATGCCATCAC Reverse: TCCACCACCCTGTTGCTGTA	30	452

CTGF, connective tissue growth factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Immunofluorescence

Human gingival fibroblasts and periodontal ligament cells were plated at 1×10^3 cells per well in eight-well culture chamber slides (BD Falcon, Franklin Lakes, NJ, USA) and incubated at 37°C in humidified air containing 5% CO_2 until subconfluence was reached. The cells were maintained under serum deprivation for 24 h, then treated with Dulbecco's modified Eagle's minimal essential medium/F12 containing 0.5% fetal bovine serum, with or without (control) 0.1, 1, 5 or 10 ng/mL of transforming growth factor-beta1 for 24, 48 and 72 h. Following incubation under each condition, cells were washed three times in 0.01 M phosphate-buffered saline(−) and fixed in 4% paraformaldehyde (Wako Pure Chemical Industries, Osaka, Japan) for 30 min. Thereafter, the cells were blocked with 10% normal goat serum for 30 min. The cells were incubated for 30 min with an anti-human CTGF immunoglobulin (SC25440; Santa Cruz Biotechnologies, Santa Cruz, CA, USA) diluted at 1:200, then incubated with Alexa Fluor 488 goat anti-rabbit IgG (H + L) (A11008, Invitrogen, Corp.) at a dilution of 1:200 for 2 h. After washing three times with 0.01 M phosphate-buffered saline(−), cells were incubated with (DAPI) (Cambrex Bio Science, Walkersville, MD, USA), mounted using Vectashield (Vector Laboratories, Inc., Burlingame, CA, USA) and then observed under epiflu-

orescent microscopy (Leica Microsystems, Wetzlar, Germany). Cells treated without primary antibody were used as negative controls.

Statistical analysis

All data are presented as mean ± standard deviation. Analysis of variance and Fisher's protected least significant difference were used to evaluate whether there were significant differences between the treatment groups. A p-value of < 0.05 was considered as significant. Significance was evaluated using STATVIEW (Hulinks, Inc., Tokyo, Japan) software for Macintosh.

Results

Expression of CCN2/CTGF mRNA by transforming growth factor-beta1 in human gingival fibroblasts and periodontal ligament cells

To examine the ability of transforming growth factor-beta1 to induce the

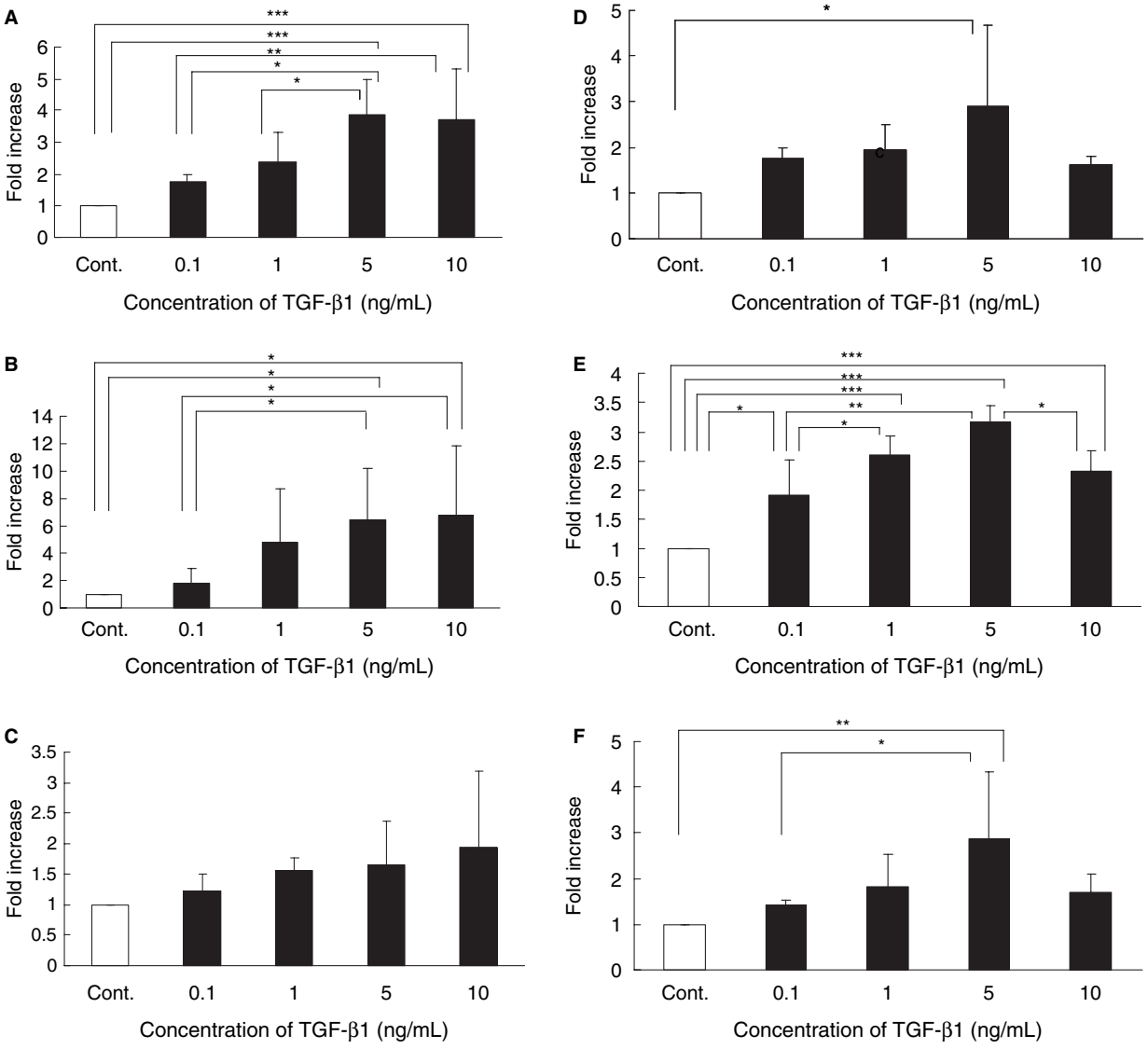

Fig. 1. Quantitative analysis of connective tissue growth factor (CCN2/CTGF) mRNA induced by transforming growth factor-beta1 in human gingival fibroblasts and periodontal ligament cells, as evaluated by reverse transcription–polymerase chain reaction. Cells were cultured to subconfluence in a 100 mm dish, starved of serum for 24 h, then treated for 24, 48 or 72 h with transforming growth factor-beta1. Data are presented as relative expression levels against those of the corresponding controls. In all cases, expression of CCN2/CTGF was significantly increased, in a dose-dependent manner, by stimulation with transforming growth factor-beta1 up to 5 ng/mL. (A) Human gingival fibroblasts, 24 h. (B) Human gingival fibroblasts, 48 h. (C) Human gingival fibroblasts, 72 h. (D) Periodontal ligament cells, 24 h. (E) Periodontal ligament cells, 48 h. (F) Periodontal ligament cells, 72 h. The results are the mean ± standard deviation obtained from human gingival fibroblasts ($n = 4$) and periodontal ligament cells ($n = 3$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; significant differences as indicated by brackets. TGF-β1, transforming growth factor-beta1.

production of CCN2/CTGF, expression of mRNA in human gingival fibroblasts and periodontal ligament cells was analyzed using RT-PCR. CCN2/CTGF mRNA was detectable even in control samples of both cell types (i.e. in the absence of transforming growth factor-beta1) (Fig. 1). The expression of CCN2/CTGF mRNA was significantly induced by transforming growth factor-beta1 in both cell types and increased over time (Fig. 1). In human gingival fibroblasts, after 24 h of incubation, 5 and 10 ng/mL of transforming growth factor-beta1 stimulated an increased expression of CCN2/CTGF mRNA, of approximately 3.84- and 3.71-fold, respectively, compared with controls ($p < 0.001$) (Fig. 1A). After 48 h, the increase reached 6.41- and 6.76-fold, respectively, at 5 and 10 ng/mL of transforming growth factor-beta1 ($p < 0.05$) (Fig. 1B). However, there were no significant differences after 72 h of stimulation (Fig. 1C). In periodontal ligament cells, after 24 h of incubation, 5 ng/mL of transforming growth factor-beta1 stimulated an increase in the CCN2/CTGF mRNA level by 2.9-fold compared with controls ($p < 0.05$) (Fig. 1D), and after 48 h of incubation, 0.1, 1, 5 ng/mL and 10 ng/mL of transforming growth factor-beta1 stimulated an increase in the CCN2/CTGF mRNA level by 1.90-, 2.60-, 3.16- and 2.31-fold, respectively, compared with controls ($p < 0.001$ for all comparisons) (Fig. 1E). After 72 h of stimulation, the CCN2/CTGF mRNA expression level showed a dose-dependent increase with increasing concentration of transforming growth factor-beta1 up to 5 ng/mL, and a 2.87-fold increase at 5 ng/mL was observed compared with controls ($p < 0.01$) (Fig. 1F).

In periodontal ligament cells, CCN2/CTGF mRNA expression was highest at 48 h with 5 ng/mL of transforming growth factor-beta1, and the mRNA expression of CCN2/CTGF was lower at the higher concentration (10 ng/mL) of transforming growth factor-beta1 used, and at 72 h ($p < 0.001$).

Time-dependently, the largest increase in CCN2/CTGF mRNA occurred at 48 h of stimulation, which produced an increase higher than that seen at 72 h in both cell types and at every concentration greater than 1 ng/mL of transforming growth factor-beta1. Overall, the expression of CCN2/CTGF mRNA in human gingival fibroblasts was higher than in periodontal ligament cells (Fig. 2).

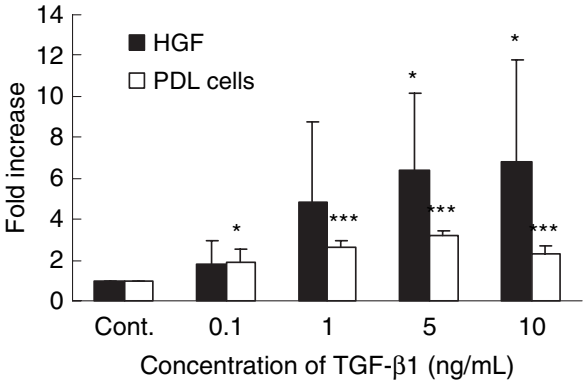

Fig. 2. Comparison of connective tissue growth factor (CCN2/CTGF) mRNA expression levels between human gingival fibroblasts ($n = 4$) and periodontal ligament cells ($n = 3$) after treatment with transforming growth factor-beta1 for 48 h. Results are mean values ± standard deviation. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; significant differences compared with the control. Cont., control; HGF, human gingival fibroblast; PDL, periodontal ligament; TGF-β1, transforming growth factor-beta1.

Production of CCN2/CTGF from human gingival fibroblasts and periodontal ligament cells after transforming growth factor-beta1 stimulation

To examine the production of CCN2/CTGF after stimulation, both cell types were incubated with transforming growth factor-beta1 for 24, 48 and 72 h, and the culture supernatants and cell lysates were analyzed using ELISA.

The levels of production of CCN2/CTGF protein increased over time, with peak production occurring at 48 h in both cell types. Thereafter, the level of CCN2/CTGF decreased, as shown by the data obtained at 72 h, and as observed in the mRNA analysis (data not shown). Thus, we decided to evaluate in greater detail the production of CCN2/CTGF protein with the sample obtained at 48 h of stimulation.

In the supernatants, the production of CCN2/CTGF protein was significantly increased by transforming growth factor-beta1, in a dose-dependent manner, in human gingival fibroblasts ($p < 0.01$) and periodontal ligament cells ($p < 0.001$) (Fig. 3A,B, respectively) at 48 h. In particular, the concentration of CCN2/CTGF protein induced by stimulation of human gingival fibroblasts with 1, 5 and 10 ng/mL of transforming growth factor-beta1 was approximately 70-, 84- and 92-fold higher, respectively, than seen in the controls ($p < 0.01$) (Fig. 3A; in periodontal ligament cells, the corresponding values were 30-, 31- and 31-fold ($p < 0.001$), respectively, compared with controls (Fig. 3B. Of note, transforming growth factor-beta1-enhanced production of CCN2/CTGF was remarkably more vigorous in human gingival fibroblasts than in periodontal ligament cells (Fig. 3C).

CCN2/CTGF is a matricellular protein and thus can accumulate in the cell fraction as well as being secreted. Therefore, to evaluate more precisely the effect of transforming growth factor-beta1 on the production of CCN2/CTGF, we also quantitatively evaluated CCN2/CTGF in the cell fractions. Because 5 ng/mL of transforming growth factor-beta1 yielded maximal effects on *CCN2/CTGF* gene expression and protein secretion, evaluation was performed at this dose. Consistent with the results obtained with the culture supernatants, after 48 h of incubation, cell-associated CCN2/CTGF significantly increased by 6-fold in human gingival fibroblasts ($p < 0.05$) and by 7.6-fold in periodontal ligament

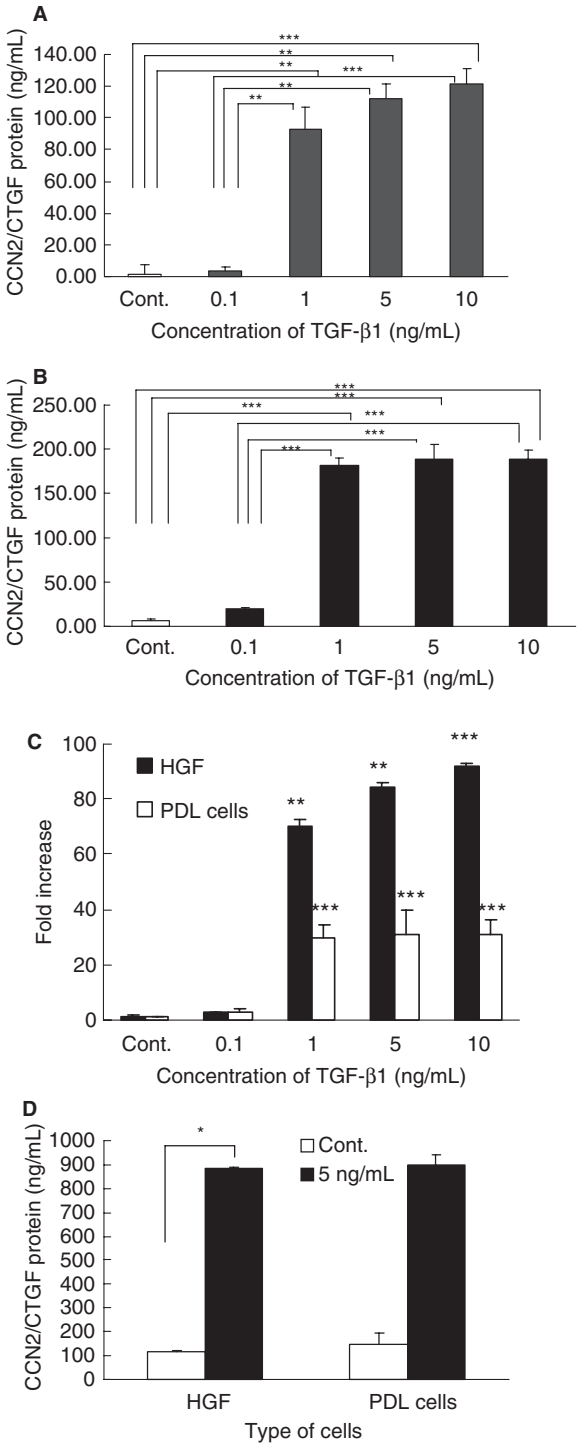

Fig. 3. Connective tissue growth factor (CCN2/CTGF) in the cell culture supernatant (ng/mL) of human gingival fibroblasts ($n = 4$) and periodontal ligament cells ($n = 3$) 48 h after stimulation with transforming growth factor-beta1 (0–10 ng/mL). Cell lysate was analyzed under the same conditions with no or with 5 ng/mL of transforming growth factor-beta1. The results are mean values ± standard deviation. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; significant differences are indicated by brackets. (A) Human gingival fibroblast supernatants. (B) Periodontal ligament cell supernatants. (C) Comparison of human gingival fibroblast and periodontal ligament cell supernatants. (D) Comparison of human gingival fibroblast and periodontal ligament cell lysates. Cont., control; HGF, human gingival fibroblast; PDL, periodontal ligament; TGF-β1, transforming growth factor-beta1.

cells following stimulation with 5 ng/mL of transforming growth factor-beta1 (Fig. 3D).

Intracellular accumulation of CCN2/CTGF protein by transforming growth factor-beta1 stimulation

To characterize the cell-associated CCN2/CTGF produced after stimulation with transforming growth factor-beta1, CCN2/CTGF protein in both cell cultures was observed using immunofluorescence. Immunofluorescence indicated that both cell types showed granular immunoreactivity for CCN2/CTGF protein. This immunoreactivity was dramatically increased by transforming growth factor-beta1 in a dose-dependent manner (Fig. 4). Interestingly, although CCN2/CTGF was distributed throughout the whole cell, we observed a dose-dependent accumulation of CCN2/CTGF, in particular in/around the nuclei in both cell types.

Discussion

Wound healing starts at the inflammatory stage, when cross-linked fibrins are formed. During this stage, the production of a number of inflammatory cytokines, such as tumor necrosis factor-alfa, is increased to confer various inflammatory responses. During the proliferative stage, fibroblasts migrate and proliferate, and the extracellular matrix, which contains collagen fiber as a major component, is created to form granulation tissue. In the fibroblastic stage, transforming growth factor-beta1 and other growth factors flourish and closely interact with each other, and gradually shift the healing from the fibroblastic stage to the maturation stage to complete the wound-healing process. Therefore, fibroblasts are key players in the wound-healing process, and transforming growth factor-beta1 is a key molecule needed for fibroblasts to produce collagen for tissue reconstruction. During growth, maturation and wound healing of connective tissues, transforming growth factor-beta1 promotes the migration of fibroblasts and macrophages, angiogenesis and

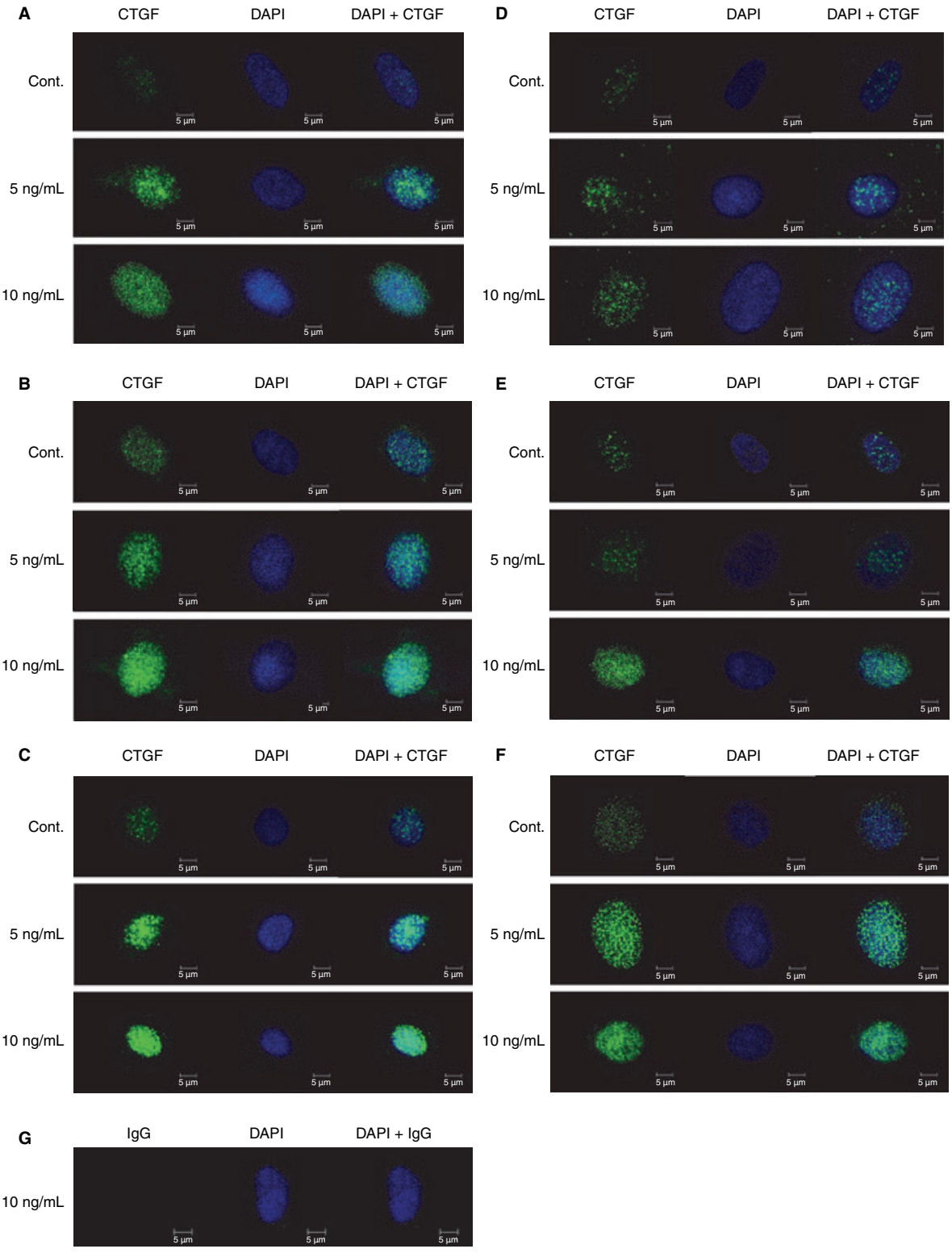

Fig. 4. Distribution of cell-associated connective tissue growth factor (CCN2/CTGF) protein in human gingival fibroblasts and periodontal ligament cells stimulated with transforming growth factor-beta1, as observed by immunofluorescence. The transforming growth factor-beta1 dose is indicated at the left of each panel. (A) Human gingival fibroblasts, 24 h. (B) Human gingival fibroblasts, 48 h. (C) Human gingival fibroblasts, 72 h. (D) Periodontal ligament cells, 24 h. (E) Periodontal ligament cells, 48 h. (F) Periodontal ligament cells, 72 h. (G) Human gingival fibroblasts, 24 h, incubated only with a secondary antibody against rabbit IgG (control). Cont., control; DAPI, 4,6-diamidino-2-phenylindole.

collagen metabolism. It should be noted that CCN2/CTGF is a critical collaborator of transforming growth factor-beta1 in collagen biogenesis. In fact, it has been suggested that CCN2/CTGF also has an important role in wound healing (30).

Periodontal tissue is open to the external environment and thus is always subject to minor injuries and mechanical stresses during oral activities. This study demonstrated the induction of CCN2/CTGF by transforming growth factor-beta1 in both human gingival fibroblasts and periodontal ligament cells in periodontal tissue. The induction of CCN2/CTGF was reported in 1999 by Hong *et al*., who used gingival fibroblasts obtained from healthy dental papillae (22). However, the effect of transforming growth factor-beta1 has not been evaluated in other gingival fibroblasts with different cellular backgrounds that are determined by microenvironments *in vivo*. Thus, in this study, normal gingival tissues covering wisdom teeth were used for evaluation. Transforming growth factor-beta1 induced significantly higher levels of CCN2/CTGF mRNA in both human gingival fibroblasts and periodontal ligament cells, which was dependent on the dose of transforming growth factor-beta1 as well as the time of exposure. The results obtained with human gingival fibroblasts are consistent with previous findings from dental papillae fibroblasts, indicating that induction of CCN2/CTGF is a general response against transforming growth factor-beta1 stimulation among gingival fibroblasts at different locations in the oral cavity. In addition, this is the first report to show the effect of transforming growth factor-beta on the *CCN2/CTGF* gene in human normal periodontal ligament cells. It is striking that even untreated control cells showed significant expression of CCN2/CTGF mRNA. This finding suggests that CCN2/CTGF is expressed in human gingival fibroblasts and periodontal ligament cells, albeit at low levels. We hypothesize that this expression is caused by the fact that gingival and periodontal ligament fibroblasts must always be prepared to regenerate gingiva and periodontal ligaments in response to frequent mechanical stress and minor damage that occurs in the oral environment.

After the addition of transforming growth factor-beta1, peak levels of RNA and protein were observed at 48 h in both cell types compared with controls. After 72 h, an apparent decrease in the effect of transforming growth factor-beta1 was seen. This is probably because the number of cells increased, almost to the level of confluence, at 72 h (data not shown). At confluence, *CCN2/CTGF* gene expression has been shown to be attenuated. Indeed, it has been reported, following analyses of the results obtained using *in situ* hybridization, that strong, positive signals for CCN2/CTGF were observed in sparse cultures of a mouse periodontal ligament cell line, but not in confluent cultures (31).

Immunofluorescence analysis indicated that both cell types showed granular immunoreactivity for cell-associated CCN2/CTGF protein. Immunoreactivity was increased by transforming growth factor-beta1 and occurred in a dose- and time-dependent manner. Interestingly, CCN2/CTGF strongly accumulated in/around the nuclei of the cells. According to previous reports, CCN2/CTGF can be taken up into the cells and transported to the nuclei of mesangial cells and chondrocytes (32,33). Nuclear localization of CCN2/CTGF has been confirmed also in growth plate chondrocytes *in vivo* (32). Although its nuclear function is not yet clear, CCN2/CTGF probably plays a significant role in the nuclei of various cells.

CCN2/CTGF regulates a variety of cellular functions in many cell types, including periodontal tissue cells. CCN2/CTGF is known to be involved in various biological processes, such as production of extracellular matrix (10), cytodifferentiation (34), normal growth and development of certain tissues (15), wound healing, tissue regeneration (15), angiogenesis (12,13), cell adhesion (10), cell proliferation (34) and chemotaxis (10). This multifunctional characteristic may be of great benefit as a therapeutic tool for the regeneration of damaged periodontal tissue. Recent advances in tissue engineering, known as regenerative medicine, are remarkable. In particular, the use of growth factors, including CCN2/CTGF in *ex vivo* regenerative therapeutics, is currently being established.

In summary, we investigated the interaction between transforming growth factor-beta1 and CCN2/CTGF systems in human gingival fibroblasts and periodontal ligament cells. The results indicated that regulation of the gene expression and production of CCN2/CTGF by transforming growth factor-beta1 occurred in both cell types. Collectively, these data strongly suggest that CCN2/CTGF is induced by transforming growth factor-beta1 and is probably a key regulator in human periodontal wound healing. Periodontal regeneration is dependent on a sequence of associated events, including cellular proliferation, migration and attachment to components of the extracellular matrix, as well as organic matrix synthesis and mineralization (35). A better understanding of the complex wound-healing systems not only helps our approach to promote wound healing after periodontal surgery, but also helps to expand the possibilities of periodontal regeneration techniques.

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