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Three members of the connective tissue growth factor family CCN are differentially regulated by mechanical stress

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Abstract

Expression of connective tissue growth factor (CTGF), a member of the CCN gene family, is known to be significantly induced by mechanical stress. We have therefore investigated whether other members of the CCN gene family, including Cyr61 and Nov, might reveal a similar stress-dependent regulation. Fibroblasts growing under stressed conditions within a three-dimensional collagen gel showed at least a 15 times higher level of Cyr61 mRNA than cells growing under relaxed conditions. Upon relaxation, the decline of the Cyr61 mRNA to a lower level occurred within 2 h, and was thus quicker than the response of CTGF. The regulation was fully reversible when stress was reapplied. Thus, Cyr61 represents another typical example of a stress-responsive gene. The level of the Nov mRNA was low in the stressed state, but increased in the relaxed state. This CCN gene therefore shows an inverted regulation relative to that of Cyr61 and CTGF. Inhibition of protein kinases by means of staurosporine suppressed the stress-induced expression of Cyr61 and CTGF. Elevated levels of cAMP induced by forskolin mimicked the effects of relaxation on the regulation of Cyr61, CTGF and Nov. Thus, adenylate cyclase as well as one or several protein kinases might be involved in the mechanoregulation of these CCN genes.

Keywords: Mechanical stress; CCN; Cyr61; Connective tissue growth factor; CTGF; Nov; Collagen gel

1. Introduction

Mechanical forces play an important role in the homeostasis of our tissues during normal development. In addition, mechanical forces are involved in the generation of various pathological conditions, such as wound healing and fibrosis [1–3]. During the past 20 years, several in vitro models have been established that allow the application of defined mechanical stress to cultures of eukaryotic cells. These models made it possible to study the effects of mechanical stress in isolated systems in the absence of any biological side effects such as inflammation. The results of the studies demonstrated that mechanical stimulation of cells results in an altered pattern of gene expression. The cells must therefore be able to sense the mechanical stimuli and to respond to these changes by alterations in the metabolism of biomolecules and by adapting the surrounding extracellular matrix [1,4]. The studies also indicated that mechanical forces act in concert with the local matrix and with soluble cytokines to modulate the behavior of the cells.

The interplay between growth factors, growth factor signaling and mechanotransduction appears to be extremely complex. Transforming growth factor-β (TGF-β) and connective tissue growth factor (CTGF) have been identified as potent inducers of extracellular matrix synthesis [5,6]. Likewise, mechanical stress has been demonstrated to increase the synthesis of several components of the extracellular matrix [7–10]. On the other hand, mechanical stress induces the expression of TGF-β, CTGF and platelet-derived growth factor (PDGF), and these growth factors might contribute, via auto- and paracrine feedback loops, to mechanotransduction [10–15]. Moreover, the signaling of these growth factors is influenced by the mechanical state of the cells. Autophosphorylation of the PDGF receptor [16] and induction of CTGF synthesis by TGF-β strongly depend on the presence of mechanical stress [13]. A tight

Abbreviations: CCN, protein family named after Cyr61, CTGF and Nov; CTGF, connective tissue growth factor; Cyr61, cysteine-rich 61; DMEM, Dulbecco's modified Eagle's medium; EST, expressed sequence tag; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Nov, nephroblastoma overexpressed; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-β

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connection between growth factors and mechanical stress is finally demonstrated by the fact that the addition of PDGF and TGF-β increases the tractional forces of fibroblasts when seeded in collagen gels [17–19].

We have recently set out to analyze differences in gene expression between mechanically stressed and relaxed fibroblasts [13]. The gene array technology was used to screen many hundreds of gene products at the same time. Several stress-responsive genes were identified that showed a two- to six-fold difference in their relative expression, including the cytoskeletal proteins actin, tubulin and zyxin, and the extracellular matrix proteins SPARC and collagen I. CTGF was among those gene products that showed the most striking up-regulation by mechanical stress. Its regulation occurred at the transcriptional level and was fully reversible after stress relaxation.

CTGF belongs to a small family of structurally related polypeptides. At present, this family comprises six proteins, namely Cyr61, CTGF, Nov, Wisp-1, Wisp-2 and Wisp-3 [20–22]. The acronym CCN, which is derived from the first letters of three members (underlined), was coined for the novel family. The six members share an amino acid sequence identity of 30–50% and exhibit a conserved modular structure comprising an N-terminal domain related to IGF-binding proteins, a domain related to von Willebrand factor, a thrombospondin-like domain and a C-terminal cysteine-rich knot. The proteins are involved in many fundamental biological processes such as cell proliferation, attachment, migration, differentiation, wound healing and angiogenesis as well as in the development of several pathologic conditions including fibrosis and tumorigenesis [20–22].

Closing wounds and fibrotic tissues are known to be subjected to considerable tension. The goal of the present study was therefore to investigate whether other members of the CCN family might also show a stress-dependent regulation similar to CTGF.

2. Materials and methods

2.1. Cell culture in three-dimensional collagen gels

IMR-90 human lung fibroblasts (American Type Culture Collection, Manassas, VA) were used at passages 10–17. The cells were grown at 37 °C in the presence of 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 9% fetal bovine serum (FBS), 100 μg/ml streptomycin and 100 units/ml penicillin. For gel contraction experiments, 4×10^4 cells/cm^2 were cultivated within a three-dimensional collagen gel containing 0.1% rat tail collagen as previously described [13]. In brief, the collagen solution (collagen R, 2 mg/ml in 0.01% acetic acid, Serva, Heidelberg, Germany) was neutralized with 0.1 M NaOH and diluted to physiological pH and ionic strength with concentrated DMEM (10× DMEM w/o $NaHCO_3$, Biochrom, Berlin, Germany), $NaHCO_3$ and FBS (final concentration 9%). After having added the cells, the suspension was dispensed into 110 mm plastic dishes (5 ml/dish, Falcon 3003) and allowed to solidify at room temperature. Finally, the gels were overlaid with DMEM supplemented with 9% FBS. After incubation for 24 h, part of the gels were released from the plastic dishes with the help of a sterile spatula and allowed to contract as free-floating discs (relaxed condition), while another part remained attached to the culture dishes (stressed condition). In some cases, specific inhibitors were added 30 min before releasing the collagen gels ($t = -1/2$ h). Genistein, bpV(biby) and forskolin were purchased from Alexis Corporation (San Diego, CA). Actinomycin D and staurosporine were obtained from Sigma-Aldrich (St. Louis, MO). The Ser/Thr kinase inhibitor Y-27632 was bought from Tocris Cookson Inc., Ellisville, MO.

To study the reversibility of gene regulation, small polyethylene plugs were inserted into the collagen gels. Tensile stress was manipulated by moving these plugs relative to the center of the dishes as described by Trächslin et al. [8].

2.2. RNA isolation and Northern blots

Total RNA was purified from the three-dimensional fibroblast cultures by the guanidinium isothiocyanate method [23] as previously described [13]. The collagen gels were washed with phosphate buffered saline and dissolved in the guanidinium buffer. The resulting solution was extracted with phenol/chloroform, followed by chloroform, and the RNA was isolated using the RNeasy Midi Kit of Qiagen GmbH (Hilden, Germany). The RNA (10 μg/lane) was separated on a 1% agarose gel in the presence of 1 M formaldehyde and transferred to a Nylon membrane (GeneScreen, NEN, Boston, MA) by vacuum blotting. The blot was hybridized at 42 °C with various cDNA probes in a buffer containing 50% formamide. After 24 h, the blot was washed [24] and analyzed with a phosphorimager (Storm 840, Molecular Dynamics, Sunnyvale, CA).

The cDNA probes were obtained from the Resource Center of the German Human Genome Project (Berlin, Germany) and corresponded to the following expressed sequence tag (EST) clones: human Cyr61 (IMAGE ID 34-54581), human CTGF (1963467), human Nov (5273738), human WISP1 (2125289), human WISP2 (4691574), human WISP3 (4514456), mouse Cyr61 (4975880), mouse Nov (3494896) and mouse CTGF/Fisp12 (3589136). All cDNA probes were labeled with [α^{32}P] dCTP by the random primed oligolabeling method [25].

3. Results

3.1. Regulation of CCN genes by mechanical stress

Previous studies established a pronounced up-regulation of CTGF expression by tensile stress [13–15]. To investi-

gate the influence of mechanical stress on the expression of the other CCN proteins, the model system of human fibroblasts growing in a three-dimensional collagen gel was used. Under these conditions, the fibroblasts develop isometric tension as they try to contract the surrounding matrix (stressed condition) [26]. When the gels were released from the plastic support with the help of a spatula, the fibroblasts started to contract the collagen gel, which then floated as a disc in the culture media (relaxed condition). Total RNA was extracted from stressed and relaxed cultures at different time points and Northern blotting was performed with probes specific for the mRNAs of the six CCN proteins (Fig. 1).

Under stressed conditions, expression of the Cyr61 gene was fairly high and remained similar during the 24 h of the experiment (Fig. 1, S0 and S24). After stress release, the mRNA level of Cyr61 declined rapidly and was barely detectable after 2 h (Fig. 1, R1 to R24), demonstrating a pronounced, stress-dependent regulation of this gene product. In this respect, the adjustment of the Cyr61 mRNA to a lower level was even quicker than that previously reported for the CTGF mRNA [13]. Expression of CTGF was also high under stressed conditions. Upon stress release, the level of the CTGF mRNA increased slightly in the beginning (R1 and R2) before it decreased to a lower level after 6 h (R6 and R24). In contrast, expression of Nov was barely detectable in stressed fibroblasts (S0 and S24). After relaxation, the mRNA level of Nov increased and reached a clearly detectable level after 6 h that remained similar to the end of the experiment (R6 and R24). Expression of the other three CCN genes Wisp-1, Wisp-2 and Wisp-3 could not be detected on our Northern blots, either under stressed or under relaxed conditions (not shown). The mRNA level of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) remained constant during the entire experiment and served as a positive control (Fig. 1).

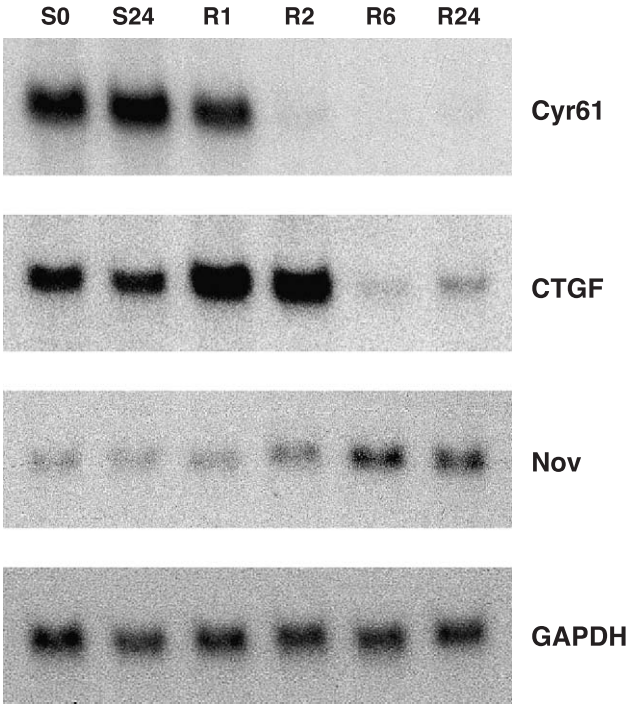

Fig. 1. Expression of CCN genes in stressed and relaxed collagen gels. The mRNA levels for Cyr61, CTGF and Nov were analyzed on Northern blots at the beginning (S0) and end (S24) of the experiment under stressed conditions as well as 1, 2, 6 and 24 h after stress relaxation (R1, R2, R6, R24). Expression of GAPDH was analyzed in parallel as a control. The mRNA levels for Wisp-1, Wisp-2 and Wisp-3 were too low to be detected by Northern blotting (not shown).

To test whether the regulation of gene expression by relaxation was reversible, tension was reapplied to relaxed fibroblast cultures. An experimental set-up was used [8], in which the collagen gels could be manipulated with the help of small polyethylene plugs that had been inserted into the gels (Fig. 2A). The cultures were kept in the stressed state for 24 h by applying tension to the polyethylene plugs. Then, relaxation was allowed to proceed for 6 h by moving the plugs towards the center of the dish. Finally, the gels were stressed again by applying tension to the plugs for the following 12 h (Fig. 2A, R6S12). Under these conditions, the level of the Cyr61 mRNA decreased during the transient relaxation period, but reached again the initial level after resumption of stress (Fig. 2B). The expression of CTGF showed a similar, reversible regulation. On the other hand, expression of Nov was up-regulated during the transient relaxation period as expected. After resumption of stress, however, the Nov mRNA level did not return to the initial level but further increased to a higher level (Fig. 2B). Thus, the induction of Nov expression by relaxation was not reversible.

3.2. Regulation at the transcriptional level

To distinguish between a possible effect of mechanical stress on RNA transcription or on mRNA stability, we determined the turnover rate of the mRNAs for Cyr61, CTGF and Nov (Fig. 3A). Actinomycin D, a potent inhibitor of RNA synthesis, was added to the cells in the three-dimensional collagen gels and after 30 min, part of the collagen gels were released from the plastic dishes while the rest remained attached. Northern blotting demonstrated that, within experimental error, there were no differences in the mRNA levels for Cyr61, CTGF and Nov between the stressed and the relaxed condition (S0 and R0, S6 and R6). Thus, mechanical stress modulates the expression of the three CCN genes at the level of transcription and not at the level of mRNA stability.

This experiment also allowed us to make a rough estimate of the half-lives for the three CCN mRNAs (Fig. 3A). The mRNA level for Cyr61 dropped to less than 50% within 30 min after addition of the inhibitor and was no longer detectable after 6 h. Although we do not know how long it will take for actinomycin D to enter the cells and block transcription, the half-life of the Cyr61 mRNA must be considerably shorter than 1 h. The mRNA level for

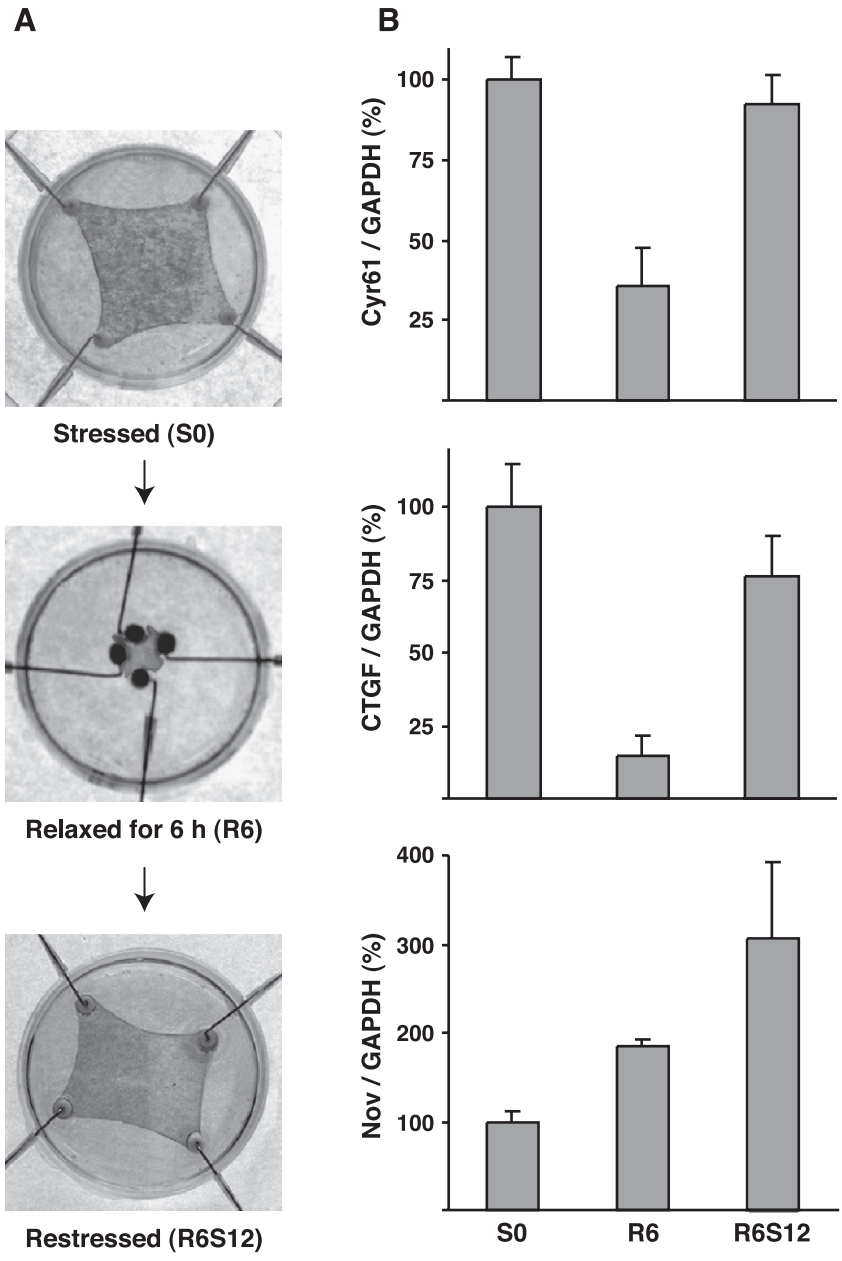

Fig. 2. Reversibility of CCN gene regulation by stress and relaxation. Fibroblasts within stressed collagen gels (S0) were relaxed for 6 h (R6) and stretched again for 12 h (R6S12) by the help of polyethylene plugs inserted into the gel (A). The mRNA levels for Cyr61, CTGF and Nov were analyzed on Northern blots (B). The intensities of the bands are depicted in relation to those of GAPDH. The results are the means with standard deviation from three independent experiments.

CTGF was also reduced after the initial 30 min, but it was still detectable after 6 h. Its half-life must therefore be in the range of a few hours. In sharp contrast, the mRNA level for Nov did not change during the entire experiment. Its half-life must therefore be considerably larger than 6 h, as was the half-life of the GAPDH mRNA that had been included as a control (Fig. 3A).

To exclude any side effect of the collagen matrix on Nov expression, we compared the mRNA levels of fibroblasts cultured on plastic dishes to those of fibroblasts grown under stressed and relaxed conditions within three-dimensional collagen gels (Fig. 3B). Consistent with the fact that fibroblasts on plastic dishes are mechanically stressed because they generate traction against a rigid substrate [8], Nov expression on plastic dishes was similar or even lower than that within stressed collagen gels.

3.3. Expression of CCN genes in different tissues

Since Nov showed a stress-dependent expression that markedly differed from those of CTGF and Cyr61, we investigated whether the three CCN genes might also

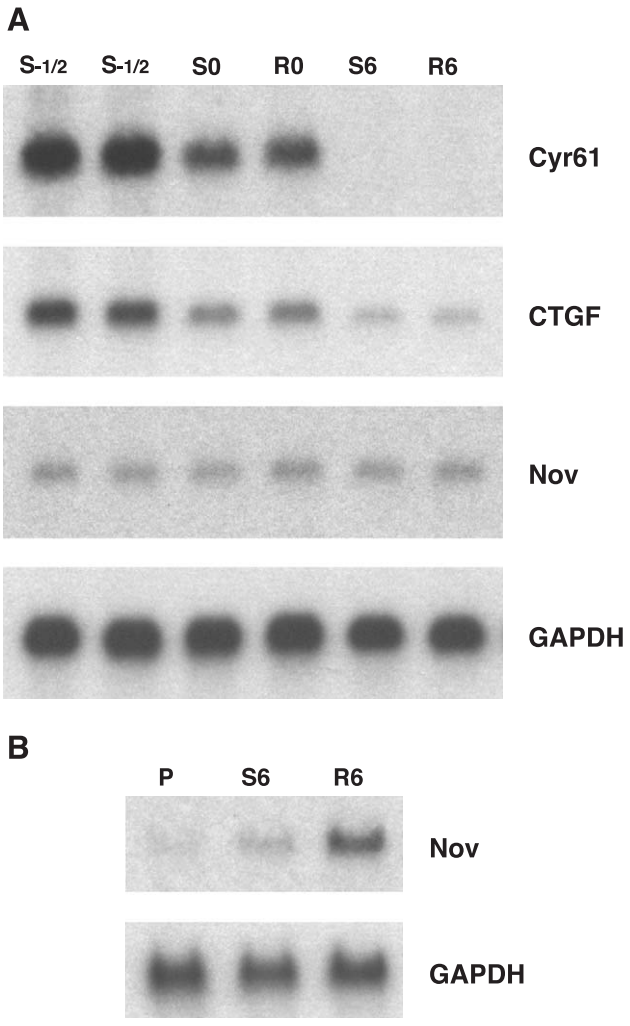

Fig. 3. Influence of mRNA stability (A) and the presence of the collagen matrix (B) on the expression of CCN genes. (A) Degradation of the mRNAs for Cyr61, CTGF and Nov was determined in stressed and relaxed fibroblasts. RNA synthesis was blocked 30 min before the onset of the experiment by the addition of actinomycin D (5 μg/ml). Part of the fibroblast cultures were released from the plastic dishes, while the other part remained attached. Total RNA was prepared prior to the addition of the inhibitor (S – 1/2), at the time of gel relaxation (S0, R0) and 6 h after relaxation (S6, R6). The RNA samples were analyzed on Northern blots with probes specific for Cyr61, CTGF, Nov and GAPDH as indicated. (B) Total RNA was prepared from fibroblasts cultivated on plastic dishes under stressed conditions (P), from fibroblasts grown in stressed collagen gels (S6) and from fibroblasts grown in relaxed collagen gels (R6). The RNA samples were analyzed on Northern blots with probes specific for Nov and GAPDH.

display distinct expression profiles in different tissues. RNA was extracted from 12 mouse tissues and hybridized on a Northern blot with probes specific for mouse Cyr61, CTGF and Nov (Fig. 4). The expression profiles of Cyr61 and CTGF were found to be nearly identical. Both genes were expressed at very high levels in the cartilage of the sternum and at high levels in the vertebrae, heart, aorta, tendon, lung and kidney. Very low expression was observed in spleen, brain, skeletal muscle and liver. The expression of Nov was considerably weaker, but it followed a similar pattern to Cyr61 and CTGF. Fairly high expression was observed only in aorta and heart.

3.4. Mechanotransduction

Since the three CCN genes are differentially regulated by mechanical stress, we investigated whether different signal transduction pathways might be involved in their expression under stressed and relaxed conditions. Specific inhibitors of various pathways were added to the fibroblasts in the three-dimensional collagen gels. Part of the gels were released from the plastic support and the expression of the CCN genes was analyzed, 6 h after relaxation, by Northern blotting (Fig. 5).

Genistein, a specific inhibitor of protein tyrosine kinases, did not appear to have any effect on either one of the three CCN genes (Fig. 5A). In contrast, staurosporine, a broad-range inhibitor of protein kinases, suppressed the expression of Cyr61 and CTGF in stressed fibroblasts to an extent that the mRNA levels became comparable to those of relaxed fibroblasts. On the other hand, the expression of Nov did not appear to be affected by staurosporine. Our results indicate that signaling via one or several protein kinases might be involved in the up-regulation of Cyr61 and CTGF by mechanical stress. Hence, we also investigated the effects of bpV(biby), a protein tyrosine phosphatase inhibitor. Addition of bpV(biby) completely suppressed the up-regulation of Cyr61 in stressed fibroblasts as observed above with staurosporine (Fig. 5A). In sharp contrast, bpV(biby) prevented the down-regulation of CTGF expression after stress relaxation, suggesting that Cyr61 and CTGF are regulated by different mechanisms. As noted above with genistein and staurosporine, the mRNA levels of Nov were barely affected by the addition of bpV(biby).

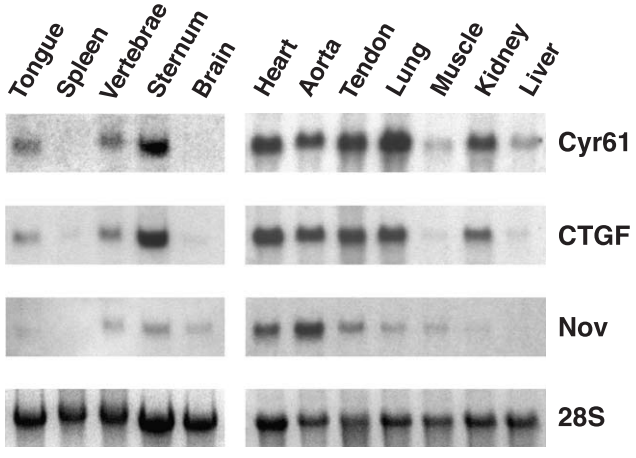

Fig. 4. Expression profile of the Cyr61, Nov and CTGF mRNA in 12 different mouse tissues. Total RNA was isolated from liver, kidney, skeletal muscle, lung, Achilles tendon, aorta, heart, brain, sternum, vertebrae, spleen and tongue and analyzed by Northern blotting. As a control for equal loading, the 28S subunit of ribosomal RNA stained with ethidium bromide was included (28S).

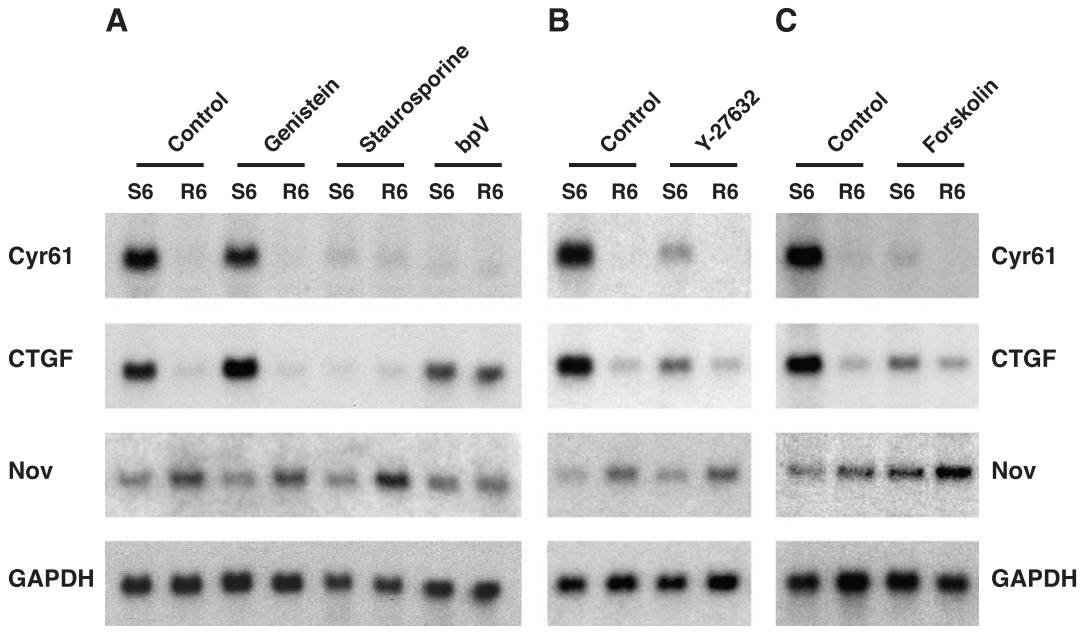

Fig. 5. Influence of several effectors on the expression of CCN genes in stressed and relaxed fibroblasts. Fibroblasts were cultivated within three-dimensional collagen gels. (A) Inhibitors of protein kinases and phosphatases were added (genistein 100 μM; staurosporine 10 nM; bpV(bipy) 100 μM) and 30 min after the addition, part of the gels were relaxed for 6 h (R6) while the other part remained stressed for the same time (S6). The mRNA levels for Cyr61, CTGF, Nov and GAPDH were analyzed by Northern blotting. (B) Fibroblasts in three-dimensional collagen gels were treated with a specific inhibitor of p160ROCK (Y-27632 10 μM) and analyzed as described above. (C) Fibroblasts in three-dimensional collagen gels were treated with forskolin (30 μM), an activator of adenylate cyclase, and analyzed as above.

To investigate the potential involvement of the cytoskeleton and the small G protein Rho, we tested the effects of Y-27632, a specific inhibitor of the RhoA dependent activation of the Ser/Thr kinase p160ROCK (Fig. 5B). When added to the cell cultures at 10 μM, this agent significantly reduced the expression of Cyr61 and CTGF in stressed fibroblasts, but it had no obvious effect on the expression of Nov. Thus, the Rho signaling pathway appears to be involved in the mechanoregulation of Cyr61 and CTGF, but not in that of Nov.

Expression of CTGF is known to be inhibited by cAMP [13]. We therefore analyzed the effects of the adenylate cyclase activator forskolin on the mechanoregulation of the three CCN genes under stressed and relaxed conditions (Fig. 5C). When added to the cultures at 30 μM, forskolin completely inhibited the up-regulation of Cyr61 expression in stressed fibroblasts. This effect was even stronger than the effect observed with CTGF. In contrast, forskolin evoked a slight increase in the mRNA level of Nov in the relaxed state. Thus, elevated levels of cAMP appear to have a similar effect to stress relaxation on the expression of Cyr61, CTGF and Nov.

4. Discussion

The CCN proteins Cyr61, CTGF and Nov represent three members of a small family of structurally related proteins that share up to 60% sequence similarity with one another. In previous studies, we as well as other researchers demonstrated that the expression of CTGF is dramatically induced in fibroblasts by tensile stress [13,14]. The goal of the present study was therefore to determine whether the susceptibility to mechanical stress might be a general feature of CCN genes.

Utilizing the model system of fibroblasts cultivated in a three-dimensional collagen gel under stressed and relaxed conditions, we found that expression of Cyr61 is significantly stimulated by tensile stress. In fact, the up-regulation of Cyr61 in the stressed state was even more pronounced than that of CTGF and the down-regulation upon relaxation occurred much quicker. Thus, Cyr61 belongs to those genes whose expression displays the most prominent effect upon mechanical stimulation. Our results are in line with the observations of Tamura et al. [27] who demonstrated that Cyr61 is significantly up-regulated when smooth muscle cells are subjected to cyclic stretching.

In contrast to the positive effect of mechanical stress on the mRNA levels of Cyr61 and CTGF, the level of the Nov mRNA was very low in the stressed state but increased after relaxation. This result suggests that the expression of Nov follows an inverted regulation when compared to that of CTGF and Cyr61. However, when the relaxed fibroblasts were stressed again, the Nov mRNA did not return to initial levels but increased even further. Thus, the stress-dependent expression of Nov is not reversible, while those of CTGF and Cyr61 are fully reversible. Part of this effect can be explained by the relatively slow turnover rate of the Nov

mRNA. The half-life of the mRNA for Nov was found to be much larger (>6 h) than that for CTGF (3–6 h) and Cyr61 (<1 h). It is therefore not possible to observe any down-regulation of Nov within the relatively short time of our experiments.

Considering the different regulation of Cyr61, CTGF and Nov, it is interesting to note that the biological functions of these CCN proteins are also counteracting. Overexpression of Nov in fibroblasts has been demonstrated to block progression through the cell cycle, probably at the transition of G2 to M phase [28]. On the other hand, CTGF accelerates DNA synthesis and progression through the cell cycle when EGF is present [6,29]. Likewise, Cyr61 acts in concert with other growth factors, including FGF-2, to augment DNA synthesis in fibroblasts [30].

In spite of their converse regulation, Cyr61, CTGF and Nov exhibit quite similar patterns of gene expression when different tissues are examined. This is consistent with previous findings that the three gene products occur in the same tissues [20]. However, in some tissues such as cartilage, they appear to be expressed in different zones and at different developmental stages as reviewed in detail by Perbal [20].

Since the expression of CTGF and Cyr61 is specifically stimulated by mechanical stress, one would expect to see a preferential expression of these genes in tissues that bear high tensile stress. While this appears to be the case for some tissues, such as aorta, heart and tendon, it does not apply to all the tissues subjected to high mechanical stress such as skeletal muscle where expression of all the three CCN genes is low. There is some correlation between elevated tension and increased CCN gene expression under pathological conditions. Clones for Cyr61 were found in a subtracted cDNA library prepared from cardiac muscle. This library had been created with the specific aim to identify genes that are affected by mechanical stress during load-induced cardiac hypertrophy [31]. Furthermore, Cyr61 as well as CTGF have been described as molecular markers for bladder wall remodeling after outlet obstruction [32]. Since increased mechanical stress will occur under these conditions, it is likely that mechanical stimulation is directly responsible for the altered gene expression.

Mechanical stress can modulate different signaling pathways. Integrins and stress-responsive ion channels have been discussed as the initial triggers of mechanotransduction. Despite these findings, the connection between the initial signaling events and the subsequent up-regulation of the stress-responsive genes has not yet been demonstrated in detail. It has also become increasingly clear that the mechanoregulation of different genes might involve completely different pathways [33]. In this study, we have tried to characterize the mechanoregulation of Cyr61 and Nov with the help of inhibitors that interfere with the mechanoregulation of CTGF as demonstrated in a previous study [13]. We found that at least one protein kinase must be involved in the up-regulation of Cyr61 and CTGF in stressed fibroblasts because staurosporine completely suppressed Cyr61 expression in the stressed state. The pathway affected might include the Rho-dependent kinase p160ROCK since the specific inhibitor Y-27632 partially blocked the stress-induced expression of Cyr61 and CTGF. A similar effect has also been noted by other researchers [27,34]. Interestingly, the decline of the Cyr61 mRNA level observed upon relaxation of the fibroblasts could not be prevented by the tyrosine phosphatase inhibitor bpV(biby). This is in sharp contrast to results obtained with CTGF, which revealed a nearly complete inhibition of the down-regulation after addition of bpV(biby) [13]. This observation leads to the conclusion that Cyr61 and CTGF are not regulated by the same mechanism.

Increased levels of cAMP induced by the addition of forskolin abolished the up-regulation of Cyr61 and CTGF in stressed fibroblasts and conversely, forced the up-regulation of Nov. Thus, an increase in the intracellular concentration of cAMP can mimic relaxation. This is in agreement with the observation that the contraction of fibroblast cultures in collagen gels leads to an increase in the intracellular level of cAMP [35].

Taken together our results demonstrate that mechanical stress regulates the two CCN genes Cyr61 and CTGF in an opposite way relative to Nov. This regulation appears to reflect the converse functions of the different CCN proteins since Nov is believed to counterbalance the stimulatory effects of Cyr61 [20]. Mechanical stress might therefore be involved in the altered expression of CCN genes under pathological conditions, such as fibrosis and wound healing.

Acknowledgements

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