Differential Expression of Transmembrane Proteoglycans in Vascular Smooth Muscle Cells*

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Rat aortic vascular smooth muscle (VSM) cells synthesize the transmembrane proteoglycan syndecan (Cizmeci-Smith, G., Asundi, V., Stahl, R. C., Teichman, L. J., Chernousov, M., Cowan, K., and Carey, D. J. (1992) J. Biol. Chem. 267, 15729–15736). The present work demonstrates that VSM cells synthesize the related transmembrane proteoglycan fibroglycan and that increased expression of these two proteoglycans is stimulated under different conditions. Fibroglycan synthesis by cultured rat aortic VSM cells was demonstrated by Northern blot analysis with a rat fibroglycan cDNA probe and immunoblot analysis with anti-rat fibroglycan antibodies. Effects of growth factors and vasoactive substances on syndecan and fibroglycan expression were examined by Northern blot analysis. Syndecan mRNA levels increased in response to stimulation of VSM cells with serum, platelet-derived growth factor, or angiotensin II. VSM cells stimulated with platelet-derived growth factor contained more syndecan core protein and processed syndecan than control cells. Fibroglycan mRNA levels either decreased or remained unchanged in response to these agents. Fibroglycan mRNA levels increased following transforming growth factor-β stimulation, while syndecan mRNA levels decreased. Other agents, including basic fibroblast growth factor, endothelin, and carbacyclin did not alter the expression of either proteoglycan. Syndecan and fibroglycan mRNA levels also varied as a function of cell density. These data demonstrate that syndecan and fibroglycan expression are regulated differently in VSM cells and lend support to the hypothesis that these proteoglycans carry out distinct physiological functions.

Biomedical and molecular biological studies have identified a gene family of four sequence-related transmembrane proteoglycans called syndecan (Saunders et al., 1989), fibroglycan (Marynen et al., 1989), N-syndecan (Carey et al., 1992), and ryudocan (Kojima et al., 1992). The core proteins of these proteoglycans consist of an amino-terminal ectodomain, a single hydrophobic transmembrane domain, and a short COOH-terminal cytoplasmic domain. The amino acid sequence homology of the membrane and cytoplasmic domains of these proteins is very high (approximately 50% amino acid identity), including 4 cytoplasmic tyrosine residues that are 100% conserved. The ectodomains, in contrast, display little amino acid sequence homology. All of these proteoglycans possess covalently attached heparan sulfate chains. These proteoglycans have been referred to as the syndecan family of transmembrane proteoglycans (Bernfield et al., 1992).

The biological functions of the syndecan family proteoglycans are not known. In general, they appear to function as cell surface binding sites for biological regulatory molecules, such as extracellular matrix adhesive proteins and polypeptide growth factors. Syndecan, the most thoroughly studied member of this gene family, has been shown to bind a variety of extracellular matrix molecules in vitro, including fibronectin (Saunders and Bernfield, 1988), collagen type I (Koda et al., 1985), thrombospondin (Sun et al., 1989), and tenasin (Salmivirta et al., 1991) and has been proposed to function as a receptor for interstitial matrix. In addition, syndecan has been shown to bind basic fibroblast growth factor (bFGF)1 (Kiefer et al., 1990; Bernfield and Hooper, 1991). The structural variability of the syndecan family ectodomains suggests that different members of the gene family carry out distinct functions, governed by differences in their binding interactions, although this has yet to be demonstrated.

Consistent with the idea of distinct functions for these proteoglycans are data which indicate there is cell- and development specific regulation of their expression. Syndecan, which is synthesized by epithelial cells (Hayashi et al., 1987), has also been shown to be synthesized by other cell types, including mesenchymal cells of developing teeth (Salmivirta et al., 1991) and vascular smooth muscle (VSM) cells (Cizmeci-Smith et al., 1992) but not cells in the central nervous system. Syndecan synthesis by epithelial cells of the skin is increased during wound healing (Elensius et al., 1991). N-Syndecan, in contrast, is expressed at high levels in the developing central and peripheral nervous systems, at lower levels in adult brain, and is not expressed in liver (Carey et al., 1992). The chicken homologue of N-syndecan (syndecan-3) is expressed at high levels in developing limb buds at the time of mesenchymal cell condensation prior to cartilage matrix deposition but thereafter decreases (Gould et al., 1992).

The mechanisms that regulate expression of these proteins have not been thoroughly studied. Transforming growth factor-β (TGF-β) has been shown to alter the posttranslational modification of syndecan in a cultured epithelial cell line, but not synthesis of the protein (Rapraeger, 1989). Recently, it has been shown that bFGF and TGF-β together, but not individually, cause increased levels of syndecan mRNA and

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1 The abbreviations used are: bFGF, basic fibroblast growth factor; VSM, vascular smooth muscle; TGF-β, transforming growth factor-β; PCR, polymerase chain reaction; bp, base pair(s); DMB, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum, PDGF, platelet-derived growth factor; TFMS, trifluoromethanesulfonic acid.
proteoglycan in 3T3 cells but not in an epithelial cell line (Elenius et al., 1992).

We are interested in the function of cell surface proteoglycans in VSM cells. Both the normal development and abnormal proliferation and migration of VSM cells that occur in response to vascular injury are regulated by interactions with growth factors and extracellular matrix (Ross, 1986; Hamati et al., 1988; Carey, 1991). Because of their potential roles in cellular interactions with these regulatory molecules we have initiated an investigation of the expression of syndecan family proteoglycans in VSM cells. In the present study we provide evidence for the differential regulation of expression of syndecan and fibroglycan in VSM cells.

MATERIALS AND METHODS

Cloning of Rat Fibroglycan cDNA—A neonatal rat aorta 

C DNA library (Clonetech Laboratories, Palo Alto, CA) was used to amplify rat fibroglycan core protein cDNA by polymerase chain reaction (PCR) amplification using nested primers based on the published human fibroblast fibroglycan sequence (Marynen et al., 1989). For the first PCR amplification, the sense primer was 5'-CCGAAATTCTCGGAAAGGAGGAGGAGGACCCGGGGGG-3', and the antisense primer was 5'-CCGAAATTCTCGGATATAAATTCCCTT-3'. The product of this reaction was used as the template for a second PCR amplification using the sense primer 5'-CCGAAATTCTTGGACCCGTGCTCTCCTCGAGATCCG-3' and the antisense primer 5'-GGATGCTGCTCTGTGTCTTCTTTCCTCGGTA-3'. The resulting 880-bp product was gel purified and subcloned into the plasmid pGEM-3zf (Promega Biotec, Madison, WI) to construct plasmid C2. The subcloned fragment was sequenced by the dideoxy chain termination method (Sanger, 1977).

Expression and Purification of Rat Fibroglycan Fusion Protein—A 410-bp fragment encoding part of the ectodomain of fibroglycan core protein was amplified by PCR using plasmid C2 as template and primers 5'-CCGAAATTCTCGAATTCTGAGGAGGAGGAGGACCCGGGGGG-3' and antisense primer 5'-CCGAAATTCTTGGACCCGTGCTCTCCTCGAGATCCG-3'. The amplified fragment was gel purified and subcloned into the bacterial expression vector pEX2 (Clonetech). The orientation of the insert and the maintenance of the reading frame were verified by DNA sequence analysis. The resulting β-galactosidase-fibroglycan fusion protein was expressed in Escherichia coli strain NS426 (American Type Culture Collection, Rockville, MD) and purified as described previously for syndecan fusion protein (Cizmeci-Smith et al., 1992). Antibodies were raised in rabbits by immunizing with purified fusion protein emulsified with RAS adjuvant (Ribi, Hamilton, MT). The fusion protein antibodies were purified by affinity chromatography or a fusion protein-Sepharose column as described previously (Carey et al., 1992).

Cell Culture and Treatment of VSM Cells—VSM cells were obtained by enzymatic dissociation of medial strips dissected from aortas of male Sprague-Dawley rats and cultured as described previously (Hamati et al., 1988). Cells were used up to the third passage. To examine effects of serum and growth factors on proteoglycan expression the cells were plated and grown to approximately 80% confluence in DME supplemented with 10% fetal calf serum (FCS) and then made quiescent by switching them to DME containing 0.4% FCS. After 2 days the cells were switched to DME, 10% FCS or DME, 0.4% FCS plus purified growth factors as indicated under "Results." Media and serum were purchased from Life Technologies Inc.). Human bFGF and human PDGF were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). Huzan recombinant TGF-β1 was purchased from Life Technologies Inc. Endothelin was purchased from Peninsula Laboratories (Belmont, CA). Sat-1 antigen II was purchased from BACHEM FeinChemicalien AG (Switzerland). Carbacyn (carbaprostacyclin) was purchased from Cayman (Ann Arbor, MI).

Immunoblotting Experiments—Immunostaining of proteins electrophoretically separated on SDS-polyacrylamide gels and transferred to Immobilon membranes (Millipore) was carried out as described previously (Cizmeci-Smith et al., 1992). Antibody affinity purified polyclonal anti-syndecan and anti-N-syndecan have been characterized and described (Carey et al., 1992; Cizmeci-Smith et al., 1992). Trifluoromethanesulfonic acid (TFMPS) deglycosylation of proteoglycans was carried out as described (Carey et al., 1992).

Results—Cultured VSM cells were stained using the indirect immunofluorescence method as described previously (Carey and Suh, 1990). Anti-vinculin monoclonal antibodies were purchased from Sigma.

RNA Isolation and Northern Blot Analysis—Total RNA was isolated by SDS lysis as described previously (Huang and High, 1990). Equal amounts of total RNA/lane were fractionated on 1% agarose, 0.6 M guanidinium thiocyanate gels and transferred onto nylon membranes (Schleicher and Schuell), and immobilized by UV cross-linking. The filters were prehybridized for 2 h at 42 °C in 50% formamide, 5 × SSPE, 5 × Denhardt's reagent, 0.1% SDS, and 200 μg/ml denatured and sonicated salmon sperm DNA. The membranes were hybridized with 32P-labeled rat cDNA probes specific for syndecan or fibroglycan mRNAs. The syndecan probe (0.6 kb) corresponded to the ectodomain of rat syndecan (Cizmeci-Smith et al., 1992). The fibroglycan probe was a 650-bp cDNA corresponding to the ectodomain of fibroglycan cDNA derived from plasmid C2 described above. The probes were labeled by random prime labeling (Prime-a-Gene, Promega). Hybridization was carried out at 42 °C for 16-20 h. The final washes of the filters were with 1 × SSPE, 0.1% SDS at 65 °C. Autoradiography was performed using Kodak X-Omat AR film with intensifying screens at -70 °C. For all experiments the same filter was used for hybridization with both probes. After autoradiographic exposure and/or scanning following the first hybridization, the membranes were stripped of hybridized probe by soaking them in 50% formamide, 6 × SSPE at 65 °C for 30 min. Removal of the bound probe was confirmed by autoradiographic exposure before the membrane was used for the subsequent hybridization.

For quantitative comparisons of mRNAs levels equal amounts of total RNA were loaded per lane. The hybridization signals were quantitated by measuring band radioactivity directly using a radioanalytic scanner (Ambis), or by densitometry of x-ray films using a laser scanning densitometer (Molecular Dynamics). Identical results were obtained with the two methods. The signals were corrected for RNA loading by normalizing to the quantity of 18 S rRNA on the blots as determined by laser densitometric scanning of a photograph negative of the ethidium bromide-stained filter.

Purification of Syndecan Debris—Transmembrane proteoglycan debris were partially purified from homogenates of the decapitated heads of 2-day-old rats. Briefly, the tissue was homogenized in PBS (0.05 M sodium phosphate, 0.15 M NaCl, pH 7.4) containing 0.5 mM phenylmethylsulfonyl fluoride and 2 mM EDTA. After centrifugation at 20,000 × g for 1 h, the particulate fraction was extracted with 1% Nonidet P-40 in PBS. The detergent extract was loaded onto a column of Q-Sepharose fast flow (Pharmacia LKB Biotechnology Inc.) equilibrated with PBS containing 0.1% Nonidet P-40 and eluted with a linear gradient of 0-1 M NaCl in PBS, 0.1% Nonidet P-40. The syndecan- and N-syndecan-containing fractions (determined by immunoblotting) were pooled, dialyzed versus PBS, and loaded onto a 1-ml bed volume immunoaffinity column prepared by coupling anti-N-syndecan antibodies (Carey et al., 1992) to CNBr-activated Sepharose (Pharmacia). Material that was in the flow-through fractions was loaded onto a 1-ml immunoaffinity column prepared using anti-syndecan antibodies (Cizmeci-Smith et al., 1992). Bound proteins were eluted from the columns with 0.1 M triethylenediamine, pH 11.5. Material that was in the flow-through fraction of the syndecan immunoaffinity column was found to be enriched in fibroglycan but depleted of N-syndecan and syndecan (see "Results").

RESULTS

Fibroglycan Is Expressed by Rat Aortic VSM Cells—We have shown previously that rat VSM cells synthesize syndecan (Cizmeci-Smith et al., 1992). We cloned cDNA coding for rat fibroglycan core protein by PCR amplification using a neonatal rat aortic cDNA library as template. The sequence determined for this cDNA was identical to the rat fibroglycan cDNA sequence reported recently (Pierce et al., 1992). Hybridization of this cDNA under high stringency conditions to rat aortic VSM cell total RNA fractionated on an agarose/formaldehyde gel revealed three prominent RNA bands of 3.4, 2.4, and 1.2 kb (see below). These sizes were consistent with the previously reported sizes of rat fibroglycan mRNAs (Pierce et al., 1992). The multiple mRNA species apparently arise as the result of multiple polyadenylation and cleavage
sites in the mRNA. Thus, fibroglycan mRNA was detected in the rat aortic VSM cells.

A fragment of the rat fibroglycan cDNA corresponding to the ectodomain coding sequence was subcloned into the bacterial expression vector pEX2 to produce a recombinant β-galactosidase-fibroglycan fusion protein. The purified fusion protein was used to produce polyclonal antibodies in rabbits.

The specificity of the anti-fibroglycan antibodies was demonstrated by the results shown in Fig. 1. Enriched preparations of syndecan and N-syndecan were prepared from neonatal rat tissue by immunoaffinity chromatography as described under “Materials and Methods.” These, plus the syndecan- and N-syndecan-depleted proteoglycan fraction, were each subjected to immunoblot analysis with antibodies against syndecan, N-syndecan, or recombinant fibroglycan.

As shown in Fig. 1A, the anti-fibroglycan antibodies stained a high molecular weight smear in the syndecan- and N-syndecan-depleted material, but did not stain any material in the purified syndecan or N-syndecan preparations. These proteoglycans were stained by their respective antibodies (Fig. 1, B and C). To provide additional evidence for specificity of the antibodies, a tissue extract containing a mixture of proteoglycans was deglycosylated by treatment with TFMS and subjected to immunoblot analysis with anti-fibroglycan (Fig. 1D, lane 1), anti-syndecan (Fig. 1D, lane 2), or anti-N-syndecan (Fig. 1D, lane 3). Each of the antibodies stained specifically a core protein of the appropriate apparent molecular mass: 45 kDa for fibroglycan, 55 kDa for syndecan, and 120 kDa for N-syndecan.

When conditioned medium from VSM cells was subjected to immunoblot analysis and stained with anti-fibroglycan antibodies, a high molecular weight smear was observed (Fig. 2), consistent with the presence of processed fibroglycan. Anti-fibroglycan staining of TFMS-deglycosylated proteins revealed bands of apparent molecular mass = 45 and 90 kDa. This is identical to what has been reported for the core protein size of human fibroglycan (Marynen et al., 1989). These results, therefore, support the RNA hybridization data, and demonstrate that rat VSM cells synthesize and process fibroglycan.

The antibodies were also used to stain cultured rat aortic VSM cells by indirect immunofluorescence. As shown in Fig. 1, the rat aortic VSM cells were made quiescent by incubating them in medium containing 0.4% serum were exposed to these agents, and total RNA be shown to regulate syndecan or fibroglycan mRNA levels.

3, the cells were stained in a punctate pattern throughout the cytoplasm. VSM cells stained with anti-vinculin antibodies revealed areas of staining near the lateral edges of the cells, representing focal contacts. Comparison of the two patterns indicated that fibroglycan was not present in focal contacts.

Syndecan But Not Fibroglycan mRNA Levels Are Induced by Serum Stimulation of VSM Cells—Since VSM cells synthesize both syndecan and fibroglycan, and syndecan family proteoglycans have been implicated as growth factor “coreceptors,” we were interested in determining whether the levels of expression of these proteoglycans were regulated by agents that stimulate VSM cell proliferation. Subconfluent rat aortic VSM cells were made quiescent by incubating them in medium containing 0.4% FCS and then stimulated to proliferate by switching them to medium containing 10% FCS. Cells that were kept in low serum medium served as controls. Total RNA was isolated and subjected to Northern blot analysis with rat syndecan and rat fibroglycan cDNA probes. As shown in Fig. 4, exposure of the cells to medium containing 10% FCS caused a marked and rapid increase in the level of syndecan mRNA. A significant increase was detectable as early as 2 h after the initiation of the serum stimulation. In contrast, serum stimulation did not increase the level of fibroglycan mRNA. In most experiments, fibroglycan mRNA levels increased during incubation in low serum medium, and this increase was prevented by serum stimulation. Thus, syndecan and fibroglycan mRNA levels were regulated differently by serum stimulation.

Effects of Purified Growth Factors—VSM cells are responsive to a variety of polypeptide growth factors and other agents. We wanted to determine whether any of these could be shown to regulate syndecan or fibroglycan mRNA levels. For these experiments, VSM cells made quiescent in medium with 0.4% serum were exposed to these agents, and total RNA was isolated and used for Northern blot analysis.
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FIG. 3. Immunofluorescent staining of fibroglycan and vinculin in VSM cells. VSM cell cultures were processed for immunofluorescent staining with anti-fibroglycan (A), anti-vinculin (B), or non-immune (C) antibodies as described under "Materials and Methods." The bar = 50 μm.

FIG. 4. Serum induction of syndecan and fibroglycan mRNA. Quiescent VSM cells were switched to medium containing 10% FCS; control cells were given fresh medium containing 0.4% FCS. After 2 or 4 h total RNA was isolated, fractionated on an agarose/formaldehyde gel, and subjected to Northern blot analysis. Left, autoradiograms following hybridization with syndecan cDNA (top) and fibroglycan cDNA (center), and ethidium bromide stained membrane showing 18 and 28 S rRNAs (bottom). Samples were: 2 h control (lane 1), 2 h FCS stimulated (lane 2), 4 h control (lane 3), and 4 h FCS stimulated (lane 4). Right, quantitation of hybridization signals for syndecan (top) and fibroglycan (bottom) mRNAs. In each case the total signal for all hybridized bands was summed and normalized to the quantity of 18 S rRNA in the sample. The same filter was used for hybridization with both cDNAs. This experiment was carried out eight times with essentially identical results.

PDGF and bFGF are mitogens for VSM cells. As shown in Fig. 5, exposure of quiescent VSM cells to purified PDGF-AB resulted in an approximately 10-fold increase in the level of syndecan mRNA. In most experiments syndecan mRNA induction by PDGF-AB exceeded that produced by 10% serum (Fig. 6). Similar to serum stimulation, PDGF-AB caused a slight decrease in the levels of fibroglycan mRNA. An isoform of PDGF-AB, PDGF-BB, had a qualitatively similar effect on proteoglycan mRNA levels but was less potent than PDGF-AB (Fig. 6).

In contrast to these results, bFGF stimulation of the cells had no significant effect on the levels of either syndecan or fibroglycan mRNAs. The cells were responsive to bFGF stimulation under these conditions, however, since the growth factor caused an increase in [3H]thymidine incorporation into cellular DNA (data not shown). Since some growth factors have synergistic effects on cellular activity, we examined the consequences of stimulation with both PDGF-AB and bFGF. As shown in Fig. 5, this treatment produced a decrease in the levels of both mRNAs. Thus, bFGF was able to block the PDGF-AB induced increase in syndecan mRNA level.

We wanted to determine whether the increased syndecan mRNA levels in PDGF-treated cells resulted in an increase in the steady state level of the proteoglycan. Control cells and PDGF-treated cells were harvested, and the media and cell extracts were analyzed by immunoblotting with anti-syndecan antibodies. As shown in Fig. 7, the medium of the PDGF-treated cells contained significantly more processed syndecan, visible as a high molecular weight smear, than control cells. We have shown previously that in detergent extracts of VSM cells syndecan is present largely in the form of unprocessed core protein, which migrates on SDS gels at an apparent molecular mass of 55 kDa (Cizmeci-Smith et al., 1992). As shown in Fig. 7, detergent extracts of the PDGF-treated cells contained more syndecan core protein (arrow) than control cells.

We also examined the effects of other agents that regulate
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Fig. 5. Induction of syndecan and fibroglycan mRNA by purified growth factors. Quiescent VSM cells were switched to medium containing 0.4% FCS (control) or 0.4% FCS plus PDGF-AB (20 ng/ml), bFGF (20 ng/ml), or both growth factors. After 6 h total RNA was isolated and used for Northern blot analysis as described in Fig. 4. Left, autoradiograms following hybridizations with syndecan cDNA (top) and fibroglycan cDNA (bottom). Samples were control (lane 1), PDGF-AB stimulated (lane 2), bFGF stimulated (lane 3), and both growth factors (lane 4). Right, quantitation of hybridization signals for syndecan (top) and fibroglycan (bottom) mRNAs. This experiment was carried out at least five times with essentially identical results.

Fig. 6. Induction of syndecan mRNA by PDGF. Quiescent VSM cells were switched to medium containing 10% FCS or 0.4% FCS plus the indicated concentrations of PDGF-BB or PDGF-AB. After 6 h total RNA was isolated and used for Northern blot analysis of syndecan mRNA. The hybridization signals were quantitated and normalized to the amount of 18S rRNA in the samples. The values are expressed relative to the normalized signal obtained from cells treated with 10% FCS. This experiment was carried out three times with essentially identical results.

VSM cell activity for their effects on syndecan and fibroglycan mRNA levels. As shown in Fig. 8, stimulation of the cells with 10 nM angiotensin II, a peptide that induces contraction and causes hypertrophic growth of VSM cells (Geisterfer et al., 1988; Schelling et al., 1991) resulted in a selective increase in syndecan mRNA levels. Fibroglycan mRNA levels were not affected. Carbacyclin, a prostacyclin analogue that stimulates the production of cAMP in VSM cells and inhibits VSM cell proliferation (Thyberg et al., 1990), had no significant effect on the proteoglycan mRNA levels (Fig. 8). Similarly, treatment of cells with 100 nM endothelin, which induces smooth muscle contraction (Kai et al., 1989), had no significant effect on mRNA levels (data not shown).

In contrast to these results, treatment of VSM cells with transforming growth factor-β (TGF-β), an agent which is
inhibitory for VSM cell proliferation under most conditions, caused a selective increase in the level of fibroglycan mRNA, and a slight decrease in syndecan mRNA (Fig. 9).

Growth-dependent Alterations in mRNA Levels—VSM cells differ from most cultured cells in that they do not exhibit contact-dependent inhibition of proliferation. The cells continue to proliferate at significant rates subsequent to reaching confluent density, producing multiple layers of cells (e.g., Hamati et al., 1989). As the cell density increases, the VSM cells exhibit increased levels of terminal differentiation, as evidenced by the appearance of α-smooth muscle actin. We have shown previously that syndecan mRNA levels decrease as the cell density increases from subconfluent to post-confluent (multi-layered) (Cizmeci-Smith et al., 1992). To examine whether fibroglycan mRNA levels were also altered as a function of cell density, total RNA was isolated from sub-confluent, confluent, and post-confluent VSM cell cultures and subjected to Northern blot analysis with a radiolabeled rat fibroglycan cDNA probe. For comparison, the same blot was also hybridized to a radiolabeled rat syndecan probe. As shown in Fig. 10, in contrast to what was observed with syndecan mRNA, fibroglycan mRNA levels increased approximately 4-fold in postconfluent as compared to subconfluent cells. Because the syndecan mRNA levels decreased, the syndecan mRNA/fibroglycan mRNA ratio decreased approximately 10-fold between subconfluent and post-confluent cells.

Alterations in mRNA Processing—Both syndecan and fibroglycan mRNAs possess long 3'-untranslated segments with multiple potential polyadenylation and cleavage sites. In general, changes observed in response to growth factors affected the different sized mRNAs equally (Figs. 4, 5, and 8). We have observed that under certain conditions the relative amounts of these alternatively processed mRNAs are altered. Fig. 11 shows results of Northern blot analysis with rat syndecan and fibroglycan cDNA probes and RNA isolated from VSM cells stimulated with TGF-β in either DME, 0.4% FCS or serum free medium. The major difference between these media is the high concentration of insulin (10 μg/ml) in the serum-free medium. Whereas cells cultured in low serum DME contained roughly equal amounts of the three major fibroglycan mRNA bands (lane 2), cells cultured in serum free medium contained a preponderance of the largest mRNA species (lane 1). In cells cultured in serum free medium the ratio of the 3.4-1.2-kb fibroglycan mRNAs was approximately 4:1 in cells cultured in DME, 0.4% FCS this ratio was reduced to 0.4:1. Essentially identical results were obtained with PDGF-treated or quiescent cells (data not shown). A similar, but less pronounced, effect was also seen for syndecan mRNAs (Fig. 11, lanes 3 and 4). In spite of these differences between serum-free medium and DME-FCS cultured cells, the quali...
tive responses of the cells to serum or growth factor stimu-
lization were the same (data not shown), suggesting that only a
posttranscriptional processing step was affected.

DISCUSSION

In this paper we have presented evidence for the synthesis by
rat aortic VSM cells of the transmembrane heparan sulfate
proteoglycan fibroglycan. This conclusion was based on the
presence of fibroglycan mRNA in cultured VSM cells as well
as anti-fibroglycan immunoreactive protein of the correct
apparent molecular weight in the cells. Previously, we had
shown that VSM cells also synthesize syndecan (Cizmeci-
Smith et al., 1992), another member of this transmembrane
proteoglycan gene family.

We also have investigated factors that regulate the levels
of syndecan and fibroglycan mRNAs in VSM cells. These
studies focused on regulation by serum and growth factors.
The most interesting finding to come from these experiments
was that syndecan and fibroglycan expression appeared to be
regulated independently in VSM cells. In general, syndecan
mRNA levels were elevated by conditions that fostered rapid
cell proliferation, e.g. in FCS- or PDGF-stimulated cells, but
were decreased under conditions in which cell growth was
inhibited, e.g. in response to TGF-β or increasing cell density.
An exception to this pattern was our finding that bFGF, a
VSM cell mitogen, failed to increase syndecan mRNA levels.
This lack of responsiveness to bFGF is interesting in light of
recent findings that have implicated syndecan and other cell
surface heparan sulfate proteoglycans in bFGF signaling
mechanisms (Rapraeger et al., 1991; Yayon et al., 1991).
Fibroglycan mRNA levels, in contrast to what was observed
with syndecan mRNA, were not elevated by FCS or PDGF
stimulation of the cells, but increased in response to TGF-β
and with increasing cell density. Fibroglycan mRNA expres-
sion, also, was increased under conditions in which cell
proliferation was reduced, and paralleled the expression of
markers of VSM cell terminal differentiation, such as α-
smooth muscle actin. Table I summarizes these effects on
syndecan and fibroglycan mRNA levels.

In this study we also investigated vasoactive agents other
than growth factors for their effects on syndecan and fibro-
glycan mRNA levels in cultured VSM cells. Angiotensin II,
in addition to its vasoconstrictor effects has been shown to
act as a hypertrophic agent for VSM cells (Geisterfer et al.,
1988; Schelling et al., 1991). Angiotensin II-treated VSM cells
express 10–20-fold higher levels of PDGF-A chain mRNA
than control cells and increased rates of protein synthesis and
secretion (Naftilan et al., 1989). Angiotensin II induced hy-
pertrophy can be suppressed by antisense oligonucleotides
complementary to PDGF mRNA (Itoh et al., 1990). This
suggests that the increase in syndecan mRNA levels we ob-
served in response to angiotensin II may be due to the induc-
tion of PDGF by the peptide. It has also been shown that
expression of the extracellular matrix glycoprotein tenasin
is induced in VSM cells by angiotensin II (Sharifi et al., 1992).
Syndecan isolated from mesenchymal cells binds tenasin
(Salmivirta et al., 1991). Thus, angiotensin II causes an
increase in the levels of this matrix protein and a putative cell
surface receptor. In contrast to the effect of angiotensin II,
we did not find any effect of endothelin on VSM cell proteo-
glycan expression. Angiotensin II and endothelin have similar
vasoconstrictor effects on VSM cell contractility and mainte-
nance of vascular tone (Yanagisawa et al., 1988). The pro-
taglandin analogue carbacyclin, a potent vasodilator, also had
no effect on proteoglycan expression. This is consistent with
the idea that the effect of angiotensin II is related to its ability
to induce PDGF expression and not to its vasomotor activity.
The distinct patterns of expression in VSM cells of the
mRNAs encoding these proteoglycans is consistent with the
proteins having different functions. Functions that have been
ascribed to the cell surface proteoglycans usually invoke bind-
ing activity for a variety of extracellular ligands, including
extracellular matrix adhesive proteins (Saunders and Bern-
field, 1988; Salmivirta et al., 1991), polypeptide growth factors
(Cory et al., 1989; Kierfer et al., 1990), enzymes such as
lipoprotein lipase, and antithrombin (Kojima et al., 1992).
These binding functions are dependent on the glycosami-
no lign moieties of the proteoglycans. It is not clear to what
extent the differences in the structures of the ectodomains of
the syndecan family proteoglycans that lead to differences in the
number and placement of the glycosaminoglycan chains result
in functional alterations in binding characteristics of the
proteoglycans. Furthermore, it is possible there are struc-
tural differences in glycosaminoglycan side chains attached
to different syndecan family proteoglycans. This is clearly the
case for syndecan-1, which contains chondroitin sulfate
chains in addition to heparan sulfate. Additional structural
variation within a glycosaminoglycan type (e.g. heparan sul-
fate) can also occur, and may be functionally important. For
example, it has been shown that high affinity binding of bFGF
to heparan sulfate is dependent on blocks of specifically
modified sugars (Turnbull et al., 1992). Anti-proliferative
activity for VSM cells of heparin is also dependent on a
specific pattern of sulfation (Schmidt et al., 1992). At the
present time, factors that dictate the fine structure of glyco-
saminoglycan chains are poorly understood.

Our data indicate that the mechanisms responsible for
regulating the synthesis of these proteoglycans are complex.
Changes in mRNA levels appear to provide one mechanism
for regulating proteoglycan synthesis. These regulatory me-
chanisms may be cell type-dependent. For example, it has been
shown that in macrophages syndecan expression is regulated
by a cAMP-dependent pathway (Yeaman and Rapraeger,
1992). We found, however, that agents that elevate cAMP
levels in VSM cells, such as carbacyclin and forskolin, failed
to elevate syndecan mRNA levels. Moreover, endothelin,
which was shown to reduce cAMP accumulation by inhibiting
adenyl cyclase (Hilal-Dandan et al., 1992), also had no effect
on proteoglycan expression. This suggests that in VSM cells
regulation of syndecan expression occurs by cAMP-independent
mechanisms.

There is indirect evidence suggesting that additional post-
transcriptional regulatory mechanisms also exist. We found
that under some conditions the relative amounts of the dif-
ferently processed forms of the mRNAs were altered. The
effect of these changes on proteoglycan synthesis are not

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2 G. Cizmeci-Smith, R. C. Stahl, L. J. Showalter, and D. J. Carey, unpublished observations.
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known, but they could affect mRNA translation or mRNA stability. Additional experiments will be required to address these issues.

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