Suppression of Syndecan-1 Expression in Endothelial Cells by Tumor Necrosis Factor-α*

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Varpu Kainulainen‡§, Lassi Nelimarkka‡, Hannu Järveläinen‡, Matti Laato**, Markku Jalkanen‡, and Klaus Elenius‡§‡‡
From the ‡Turku Center for Biotechnology and the Departments of §Medical Biochemistry, ¶Internal Medicine, and **Surgery, University of Turku, 20520 Turku, Finland

Syndecan-1 is a cell surface proteoglycan that binds extracellular matrix components and modulates the activity of heparin-binding growth factors. The expression of syndecan-1 is modified during development, carcinogenesis, and tissue regeneration. During cutaneous wound healing, syndecan-1 expression is transiently induced in newly formed capillaries of granulation tissue as well as in proliferating keratinocytes. To study the mechanisms underlying this regulation we investigated the effects of several growth factors/cytokines on syndecan-1 expression in two human cell lines: EA.hy 926 endothelial cells and HaCaT keratinocytes. None of these factors significantly altered syndecan-1 mRNA expression in cultured keratinocytes, but when given to endothelial cells, tumor necrosis factor-α (TNF-α) specifically and dose-dependently suppressed syndecan-1 expression at both mRNA and protein levels. TNF-α reduced the amount of syndecan-1 protein in EA.hy 926 cells in both the presence and absence of serum and, at the same time, induced the expression of intercellular adhesion molecule-1 (ICAM-1). The suppressive effect of TNF-α on endothelial syndecan-1 expression was reproducible in vivo experiments in which TNF-α-coated beads were administered directly to healing skin wounds of mice. Data supporting these findings were further obtained by injecting TNF-α into an experimental rat granulation tissue model. In this tissue TNF-α suppressed syndecan-1 mRNA expression by approximately 80%. These results indicate that TNF-α is capable of down-regulating syndecan-1 expression in endothelial cells both in vitro and in vivo and suggest that similar mechanisms may be responsible for the changes in syndecan-1 expression observed during various regenerative, developmental, and malignant processes.

Normal cutaneous wound healing requires collaboration between and within several cell types including fibroblasts, keratinocytes, endothelial cells, and macrophages. This interplay is based on paracrine, juxtacrine, and autocrine signaling mediated by growth factors and their receptors as well as on the mechanisms by which cells detect and regulate changes in adhesion. Many of the same mechanisms also participate in creating new tissues during embryogenesis. Furthermore, some of these molecular events are believed to be excessive or unrestricted during tumorigenesis.

One class of molecules that has been suggested to regulate both the growth factor responses and the adhesion of cells is the family of cell surface heparan sulfate proteoglycans named syndecans (1, 2). The members of this family (currently syndecans 1–4) consist of short conserved cytoplasmic and transmembrane domains and long dissimilar extracellular domains carrying variable numbers of glycosaminoglycan side chains. The predominant glycosaminoglycan type in all syndecans is heparan sulfate, but at least in the case of syndecan-1 a hybrid form bearing both heparan sulfate and chondroitin sulfate chains also exists (3).

The first and at the moment the best characterized syndecan is syndecan-1 (4). Syndecan-1 has been shown to bind via its heparan sulfate side chains to many extracellular matrix proteins, including fibronectin (5), fibrillar collagens (6), thrombospondin (7), and tenasin (8) as well as to antithrombin III (9) and fibroblast growth factor-2 (FGF-2; Refs. 10 and 11). Syndecan-like cell surface heparan sulfate proteoglycans have been reported to modulate the signal transduction activated by several heparin-binding growth factors. These include members of the FGF family (12–15), vascular endothelial cell growth factor (16, 17), heparin-binding epidermal growth factor-like growth factor (18, 19), and amphiregulin (20). Thus, syndecan-1 putatively has a function both in anchoring cells to surrounding extracellular matrix (possibly in concert with integrin-type matrix receptors) and as a growth factor co-receptor influencing activation of various receptor tyrosine kinases (1, 21). Furthermore, syndecan-like heparan sulfate proteoglycans expressed at the surface of endothelial cells have been suggested to have a role in regulating leukocyte endothelium interactions (22, 23).

In normal mature tissues syndecan-1 is expressed with few exceptions only in epithelia (24). However, during various regenerative and developmental states syndecan-1 expression is regulated both at transcriptional and posttranslational levels. For example, syndecan-1 expression is transiently induced in condensing mesenchymes during organogenesis (25, 26) and in proliferating keratinocytes and endothelial cells during wound healing (27). During malignant conversion of epithelial cells, on the other hand, syndecan-1 expression is usually suppressed.

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† Present address: Joint Program in Neonatology, Children’s Hospital, Harvard Medical School, Boston, MA 02115.
‡‡ To whom correspondence should be addressed: Dept. of Surgery, Children’s Hospital, Enders 10, 300 Longwood Ave., Boston, MA 02115.

1 The abbreviations used are: FGF, fibroblast growth factor; CPC, cetylpyridinium chloride; FBS, fetal bovine serum; GAPDH, glyceraldehyde 3-phosphate-dehydrogenase; ICAM-1, intercellular adhesion molecule-1; IFN-γ, interferon-γ; IL-1β, interleukin-1β; PBS, phosphate-buffered saline; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α.
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(28–30). This, together with transfection studies in which over-expression of syndecan-1 has been shown to result in down-regulation of growth factor responses (31, 32), has lead to a hypothesis that enhanced syndecan-1 expression is associated with “normal” nonmalignant proliferation and provides the cells a way to restrict excessive growth. Thus, transformed cells with suppressed syndecan-1 synthesis could have lost a molecule important for down-regulating proliferation.

To investigate the molecular mechanisms that regulate syndecan-1 expression we studied the effect of different growth factors/cytokines on syndecan-1 expression in two cell types that alter their syndecan-1 expression in response to wounding, i.e. keratinocytes and endothelial cells. Here we report that TNF-α, a proinflammatory cytokine produced in wound tissue by macrophages (33), specifically and dose-dependently reduces endothelial syndecan-1 expression both in vitro and in vivo. We speculate i) that cell type-specific regulation of syndecan-1 expression by locally available cytokines may prevail during various in vivo processes and ii) that some of the known effects of TNF-α on endothelial cells or angiogenesis could be influenced by its effects on syndecan-1 expression.

MATERIALS AND METHODS

Cell Culture and Growth Factor Treatment—EA.hy 926 human endothelial cells (34) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Life Technologies, Inc.), HAT (12 µg/ml hypoxanthine, 1 µg/ml aminopterin, 8 µg/ml thymidine; Sigma), penicillin (100 U/µl), and streptomycin (50 µg/ml). Fresh medium was changed every third day. HaCaT human keratinocytes (35) were cultured similarly but without HAT supplement. For RNA isolation, cells were plated at equal density on culture dishes and grown to 70–80% confluence. Twenty-four h before the addition of the growth factor(s), fresh medium with or without FBS was changed. For growth factor treatments, cells were washed with ice-cold PBS containing 0.5 mM EDTA. Cellsweredetachedfromculturedishesby scraping with a rubber policeman. The nuclei were washed with ice-cold PBS and incubated on ice for 10 min. The washed cells were resuspended in 1 ml of PBS, 10 µg/ml trypsin and incubated for 15 min on an ice bath, after which cells were centrifuged and the supernatants containing trypsin sensitive cell surface proteoglycans (44) were removed and stored at −20 °C. The pellets containing EA.hy 926 cells were further resuspended in 1 ml of PBS, 0.5 mM KCl, 1% Triton X-100, and after centrifugation, supernatants containing syndecan-1 were stored at −20 °C. One-fourth of the total volume of medium and of trypsin- and Triton-released samples was transferred to cetylpyridinium chloride (CPC)-impregnated filters to quantitate the amount of activity residing in sulfate-labeled proteoglycans (44). The filters were washed with distilled water, 10% trichloroacetic acid, and 95% ethanol. The radioactivity in the filters was estimated using a liquid scintillation counter.

Growth Factor-releasing Beads and Wound Healing Model—To expose healing wounds to exogenous growth factors we implanted coated agarose beads locally into the wounded skin of mice. These beads have previously been shown to slowly release growth factors when implanted to embryonic tissues (45, 46). Ten µl of Affi-Gel blue agarose beads (100–200 mesh, 75–150-µm diameter; Bio-Rad) were washed once with PBS and pelleted. Beads were then coated by an incubation with 100 ng/µl of TNF-α or FGF-2 in PBS in a total volume of 1 ml for 30 min at 37 °C. Beads incubated with PBS only were used as negative controls. After incubation the total volume was increased to 100 µl with PBS, and one-third of this final volume was injected to each of the three mice (see below) for each treatment. The wounds were prepared as described for the experiments above. After 2 days of healing, 5-mm full-thickness incisions were made vertically on the back skin of CO2 anesthetized 3-month-old male BALB/c mice. After operation, the mice were housed individually in cages. Two days after wounding, freshly coated agarose beads were injected into the granulation tissue area under the scab. Two days after injection, mice were sacrificed. Wound tissue was excised and bisected horizontally across the center of the wound. One-half of each wound tissue sample was fixed in 10% buffered formalin and embedded into paraffin. The other half was frozen immediately in liquid nitrogen and subsequently processed to frozen sections (30). This was found necessary because the CAM-1 antibody used (CRL 1878; ATCC) detected its antigen only in frozen sections, but the morphology of the tissue as well as the cell type-specific distribution of syndecan-1 antibody (281–2) was better restored in paraffin sections. Five-µm sections were immunostained using the avidin-biotin-peroxidase complex (ABC) technique (41), as described previously for paraffin sections (27) and cryosections (30). The following primary antibodies were used: a rat monoclonal antibody against mouse syndecan-1 ectodomain (281–2; Ref. 42), a rat monoclonal antibody against mouse CAM-1 (CRL 1878; ATCC) and a rat monoclonal antibody against mouse L-selectin (MEL-14; Ref. 43; negative control). The staining pattern of primary antibodies was visualized using Vectastain ABC Kit (Vector Laboratories).

Quantitation of 35SO4-labeled Proteoglycans from EA.hy 926 Cells—Cells cultured on 24-well plates were labeled for 24 h in 400 µl of sulfate-free medium supplemented with 100 µCi/ml 35SO4 in either the presence or absence of 25 ng/ml TNF-α. After removal of the medium, cells were washed with ice-cold PBS and incubated on ice for 10 min in PBS containing 0.5 µl EDTA. Cells were detached from culture dishes with a rubber policeman and washed twice by centrifugation and resuspension in medium to wash off growth factors with negative controls. Human TNF-α (Peprotech) was used at final concentrations of 5, 25, and 50 ng/ml. FGF-7 (Peprotech), FGF-2 (Peprotech and Boehringer Mannheim), interleukin-1β (IL-1β), interleukin-γ (IFN-γ) and transforming growth factor-β (TGF-β; all from Boehringer Mannheim) were used at concentrations of 50 ng/ml, 10 ng/ml, 5 IU/ml, 1,000 U/µl and 20 ng/ml, respectively, in all experiments. For cell number estimation, the media were removed, and the cells were washed once with phosphate-buffered saline (PBS) supplemented with 0.5 mM EDTA. Cells were dispersed and bisected horizontally across the center of the wound to prepare both paraffin and frozen sections from the same tissue for subsequent immunostainings (described above). To quantify the effects of bead treatments, morphologically distinct blood vessels over 50 µm in diameter showing positive or negative immunostaining were counted from the area of affected dermis (dermis below hypertrophic epidermis).

Granulation Tissue Model—To study the effect of exogenous TNF-α on syndecan-1 mRNA expression in granulation tissue, a standardized experimental wound model was used (47). Viscose cellulose sponge (Säteri OY) was cut into cylindrical pieces with a 3-mm diameter tunnel...
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**Fig. 1.** The effect of various cytokines on syndecan-1 mRNA levels in EA.hy 926 endothelial cells and HaCaT keratinocytes. Cells were grown in medium containing 10% FBS. Total RNAs were isolated 24 h after the beginning of cytokine treatments (concentrations are described under "Materials and Methods") and used for Northern blot analysis. Ten μg of total RNA was loaded on each lane. Two independent autoradiograms following hybridizations with human syndecan-1 (a Pst I fragment of a partial human syndecan-1 cDNA clone hsyn4) and GAPDH probes are shown.

**Fig. 2.** The effect of TNF-α on syndecan-1 mRNA expression in EA.hy 926 and HaCaT cells. A, cells were cultured for 24 h in medium containing 10% FBS and the indicated concentrations of TNF-α. Total RNA was isolated and subjected to Northern blot analysis using human syndecan-1- and GAPDH-specific probes. Ten μg of total RNA was loaded on each lane. Independent autoradiograms containing EA.hy 926 and HaCaT cell-derived material are shown. B, cells were grown in medium containing 10% FBS plus 25 ng/ml of TNF-α. Total RNA was isolated at the indicated time points and used for Northern blot analysis with human syndecan-1- and GAPDH-specific probes. The syndecan-1 signals were quantitated by densitometry and normalized according to the GAPDH signals. The control value obtained from cells not treated with TNF-α is expressed as 100%. The mean obtained by quantitating four independent filters is shown.

**RESULTS**

TNF-α Reduces Endothelial Syndecan-1 Expression in Vitro—We have previously shown that syndecan-1 expression is transiently induced in newly synthesized capillaries as well as in proliferating keratinocytes during wound healing in mice (27). The expression is both stimulated and subsequently down-regulated in a strictly coordinated manner. Concurrently with these phenomena, a large array of growth factors is released from several sources including recruited macrophages; damaged or activated endothelial cells, keratinocytes, and fibroblasts; and storage sites in extracellular matrix. To explore the molecular mechanisms leading to the regulation of syndecan-1 expression, several growth factors suggested to be involved during the inflammatory and proliferative phases of wound healing were tested for their capability to alter syndecan-1 expression in two human cell lines, EA.hy 926 endothelial cells and HaCaT keratinocytes.

When applied to endothelial cells, IFN-γ, FGF-2, IL-1β, TGF-β, and FGF-7 were not found to have any significant effect on syndecan-1 mRNA expression as analyzed by Northern blotting (Fig. 1). In contrast, TNF-α was shown to dose-dependently down-regulate syndecan-1 mRNA steady state level (Fig. 2A). The effect was already seen at a TNF-α concentration of 5 ng/ml and was further enhanced by increasing the amount to 25 and 50 ng/ml (Fig. 2A). None of the cytokines tested, including TNF-α, had an influence on syndecan-1 expression in Northern analysis of HaCaT keratinocytes (Figs. 1 and 2A), indicating that the regulation needs specific mechanisms characteristic of endothelial cells.

To study the time dependence of the effect of TNF-α on endothelial syndecan-1 mRNA expression, total RNAs were isolated from EA.hy 926 cells exposed to 25 ng/ml of TNF-α for various periods indicated in Fig. 2B. The signals from Northern blot filters hybridized with a human syndecan-1-specific cDNA probe were quantitated by autoradiography and densitometry. A suppression by approximately one-half was observed already at 6 h after the beginning of TNF-α treatment, and the effect was maximal (~70% inhibition) at 10–12 h (Fig. 2B). The suppression was reversible at later time points (48–72 h).

Comparable results were observed at the protein level by immunoprecipitation using the anti-P117 polyclonal antibody directed against the cytoplasmic domain of human syndecan-1 (39). Sulfate-labeled cell-associated material was isolated from EA.hy 926 cells 24 h after the addition of different amounts of TNF-α. To estimate the quantitative changes of cell-associated syndecan-1, the immunoprecipitated samples were analyzed on 0.75% agarose gels (Fig. 3A). While 5 ng/ml of TNF-α was enough to clearly diminish cell-associated syndecan-1 expression (by ~50%), 25 ng/ml caused the maximal reduction (~90%...
of ICAM-1 in endothelial cells has previously been reported to adhesion molecule, ICAM-1, in EA.hy 926 cells. The expression of ICAM-1 was studied at the protein level by preparing cell lysates from EA.hy 926 cells at various time points after TNF-α treatment (Fig. 3, A). The amount of cell-associated syndecan-1 was usually close to or marginally beyond the control level (Fig. 3, A). After 48 and 72 h the amount of cell surface syndecan-1 was usually close to or marginally beyond the control level (Fig. 3, B). The amount of cell-associated syndecan-1 remained unchanged 0.5, 2, or 6 h after TNF-α treatment (data not shown).

Suppression of Syndecan-1 Expression by TNF-α Is Selective—To confirm that the biological activity of TNF-α preparation was consistent with known TNF-α properties, we analyzed the effect of TNF-α on the expression of another cell surface adhesion molecule, ICAM-1, in EA.hy 926 cells. The expression of ICAM-1 in endothelial cells has previously been reported to be up-regulated in response to TNF-α (48), in contrast to the down-regulatory response in the case of syndecan-1 suggested in this paper. EA.hy 926 cells were cultured on coverslips and exposed for 24 h to TNF-α treatment. Cells were stained by indirect immunofluorescence using ICAM-1-specific monoclonal antibody against human ICAM-1. Untreated cells were faintly stained (Fig. 4D), while TNF-α-treated cells showed intense cell surface staining (Fig. 4, E and F). The morphology of the cells was also found to change to more elongated and spindle-like, along with the increasing TNF-α concentrations (Fig. 4, D, E, and F). Cells treated with 0, 0.5, or 25 ng/ml of TNF-α and stained with a mouse monoclonal antibody against human cytokeratin served as negative controls (Fig. 4, A, B, and C).

To evaluate the effect of TNF-α on the synthesis of other cell surface or medium-secreted proteoglycans of EA.hy 926 cells, total proteoglycan amounts were analyzed from the medium and from trypsin releasable and detergent-soluble fractions. This was accomplished by measuring the activity of 35SOS4-labeled material using a CPC filter method (see “Materials and Methods” and Ref. 44), which enables selective precipitation of proteoglycan molecules. The amount of total proteoglycan-derived radioactivity in conditioned culture medium was found to

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3 M. Jalkanen, unpublished observations.
be increased by TNF-α (Fig. 5A). The effect was not seen after 8 h of treatment, but at 24 h the induction was clearly reproducible (Fig. 5A). This result was not due to changes in cell number, because the influence of TNF-α on EA.hy 926 cell proliferation under these conditions was inhibitory (Fig. 5B).

On the contrary, the effect of TNF-α on the cell number makes the actual increase of medium proteoglycans (proteoglycans/cell) slightly more prominent than that presented in Fig. 5A.

The effect of TNF-α treatment on the amount of total cell-associated proteoglycans was evaluated from aliquots applied to CPC filters after a mild trypsin treatment or Triton-KCl extraction of cells. The total amount of proteoglycans in trypsin-(Fig. 5A) or Triton-KCl- (data not shown) extracted fractions was not found to be significantly changed in response to TNF-α treatment. These experiments suggest that the suppressive effect of TNF-α on syndecan-1 expression is selective and that the synthesis of other major medium-secreted or cell surface proteoglycans remains either unchanged or increases.

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In vitro experiments suggested to us that TNF-α produced in wounded tissue by macrophages could be responsible for down-regulating the transiently stimulated endothelial cell syndecan-1 expression observed in vivo (27). To test directly the effect of TNF-α on syndecan-1 expression within healing wound tissue we administered exogenous TNF-α into mouse granulation tissue using agarose beads as carriers (see "Materials and Methods"). Another angiogenic growth factor, FGF-2 (basic FGF), was studied for comparison. Beads were injected just beneath the scab 2 days after making a 5-mm-long full-thickness skin wound. Two days later (i.e. 4 days after incision) the wound tissue was excised, prepared to sections, and analyzed by immunohistochemistry.

Control wounds treated with uncoated beads and stained with 281–2 antibody against mouse syndecan-1 showed a strong signal in proliferating keratinocytes as well as a moderate signal in a subpopulation of small vessels within affected dermal tissue/granulation tissue (Fig. 6A), as described previously for this time point after wounding (27). No signal from endothelial cells from normal peripheral skin was detected (data not shown). Application of TNF-α or FGF-2-coated beads did not seem to have an effect on the strong syndecan-1 staining observed in the proliferating keratinocytes near the wound edge (Fig. 6B and data not shown). However, the amount of syndecan-1-positive vessels, as well as the intensity of the signal on the remaining positive endothelial cells was diminished in response to TNF-α treatment (Fig. 6B). To quantitate these results several paraffin sections were examined under a microscope, and all morphologically distinct blood vessels exceeding 50 μm in diameter were counted in the area of "affected dermal tissue" (defined here as tissue under hypertrophic epidermis). By this method the proportion of syndecan-1-positive vessels within this area was estimated to be reduced by approximately two-thirds after TNF-α treatment when compared with control samples (Fig. 6C). The relative number of syndecan-1-positive vessels was not significantly changed in re-
response to FGF-2 (Fig. 6C). Immunostaining with MEL-14 antibody against mouse L-selectin (negative control) showed no specific signal (data not shown). For comparison, halves of all the wound tissue samples were prepared to frozen sections and stained with an antibody against mouse ICAM-1. Quantitation of the absolute number of ICAM-1-positive vessels showed an approximately 7-fold increase against mouse ICAM-1. Quantitation of the absolute number of vessels (diameter >50 μm) was estimated to be increased rather than suppressed in response to TNF-α (data not shown).

To control these in vivo results in a more quantitative way and to get information of the level of syndecan-1 regulation, an approach to isolate RNA from TNF-α-exposed and -unexposed granulation tissue was established. For this purpose an experimental rat granulation tissue model (47) was chosen. In this model granulation tissue is formed in hollow sponge cylinders under the skin of a laboratory rat (see “Materials and Methods”). The tissue growing inside the sponge has been described to closely resemble “normal” granulation tissue filling the open space between wound edges (49). To study the effect of TNF-α on syndecan-1 mRNA expression, sterile sponges were implanted subcutaneously into rats, subjected to treatments by transcutaneous injections (inside the cylinder), and excised 7 days after implantation. Total RNA was isolated from the tissue grown into the sponges and analyzed for syndecan-1 mRNA content using a fragment of a mouse syndecan-1 cDNA clone (Fig. 7A). After seven daily injections of 50 or 200 ng of TNF-α the intensity of syndecan-1 mRNA signal was diminished by approximately 80% when compared with signal from PBS-treated tissue (Fig. 7B). Granulation tissue extracted from cylinders injected with similar quantities of another monokine, prostaglandin E₂, showed no reduction of syndecan-1 expression (data not shown).

In conclusion, we suggest that TNF-α down-regulates endothelial syndecan-1 expression both in vitro and in vivo.

**DISCUSSION**

Although it has been generally suggested that regulation of syndecan-1 expression makes an important functional contribution to both normal and malignant growth, little is currently known about the mechanisms behind this regulation. Some growth factors (11, 50) and a wound fluid-derived antibiotic peptide (51) have been reported to up-regulate syndecan-1 expression of mesenchymal cells in vitro. However, no data about factor(s) that could significantly restrict syndecan-1 expression of adherent cells or about the activity of these regulators in vivo have so far been reported. To investigate these mechanisms is potentially important, since suppression of syndecan-1 expression has been shown to be associated with malignant conversion both in vitro (29, 52) and in vivo (30).

Regulation of Syndecan Expression—In this study, we examined the effect of several growth factors on syndecan-1 expression of two cell types that have been shown to transiently regulate their syndecan-1 expression during cutaneous wound healing: endothelial cells and keratinocytes (27). The in vitro experiments were done using human EA.hy 926 endothelial and human HaCaT keratinocyte cell lines. As a result, TNF-α was found to selectively and dose-dependently reduce syndecan-1 expression in endothelial cells but not in keratinocytes. None of the other cytokines tested (FGF-2, FGF-7, TGF-β, IL-1β, and IFN-γ) had either remarkable inductive or suppressive effects on syndecan-1 expression of either cell type. Similarly, exogenous TNF-α suppressed syndecan-1 antigen expression in endothelial cells but not in keratinocytes in healing skin wounds of mice in vivo. TNF-α further decreased syndecan-1-specific mRNA expression in a rat granulation tissue model. Although the cell type(s) synthesizing syndecan-1 transcripts in this particular tissue was not directly demonstrated, these results suggest that suppression of syndecan-1 expression in response to TNF-α took place at the mRNA level both in vitro and in vivo.

These and earlier findings indicate that the mechanisms and factors responsible for the regulation of syndecan-1 expression vary between different cell types. Interestingly, also regulation of the expression of another member of the syndecan gene family, syndecan-2, requires different growth factors than the regulation of syndecan-1 (50, 51). This selective regulation of different cell surface proteoglycans by specific factors is supported by findings presented in this paper. Although syndecan-1 expression was clearly diminished, the total amount of trypsin-sensitive cell surface proteoglycans (probably including syndecan-2 and syndecan-4; Ref. 53) remained unchanged, and the total amount of medium secreted proteoglycans (including shed syndecan ectodomains and extracellular matrix proteoglycans) increased in response to TNF-α treatment of EA.hy 926 cells. Furthermore, total hexosamine and uronic acid content in the experimental rat granulation tissue model used in this study to demonstrate the reduction in syndecan-1 mRNA levels was not altered in response to TNF-α (54), suggesting stability in the overall proteoglycan synthesis. Thus, the differences in the distribution of the members of syndecan gene family both in adult organisms and during embryogenesis (for review see Ref. 1) may be a result of variability in responsiveness of individual syndecans (syndecan genes) to different effector molecules. Analysis of the genes of different syndecans may eventually give more information of the molecular mechanisms responsible for this variance. The genomic mouse syndecan-1 sequence (55, 56) has been shown to contain a binding site for at least one transcription factor known to mediate effects of TNF-α (NF-κB; Refs. 57 and 58), but no detailed data about the
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