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Altered proteoglycan gene expression and the tumor stroma

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Abstract. Tumor stroma is a specialized form of tissue that is associated with epithelial neoplasms. Recent evidence indicates that significant changes in proteoglycan content occur in the tumor stroma and that these alterations could support tumor progression and invasion as well as tumor growth. Our main hypothesis is that the generation of tumor stroma is under direct control of the neoplastic cells and that, via a feedback loop, altered proteoglycan gene expression would influence the behavior of tumor cells. In this review, we will focus primarily on the work from our laboratory related to the altered expression of chondroitin sulfate proteoglycan and its role in tumor development and progression. The connective tissue stroma of human colon cancer is enriched in chondroitin sulfate and the stromal cell elements, primarily colon fibroblasts and smooth muscle cells, are responsible for this biosynthetic increase. These changes can be reproduced in vitro by using either tumor metabolites or co-cultures of human colon carcinoma cells and colon mesenchymal cells. The levels of decorin, a leucine-rich proteoglycan involved in the regulation of matrix assembly and cell proliferation, are markedly elevated in the stroma of colon carcinoma. These changes correlate with a marked increase in decorin mRNA levels and a concurrent hypomethylation of decorin gene, a DNA alteration associated with enhanced gene expression. Elucidation of decorin gene structure has revealed an unexpected degree of complexity in the 5′ untranslated region of the gene with two leader exons that are alternatively spliced to the second coding exon. Furthermore, a transforming growth factor beta (TGF-β)-negative element is present in the promoter region of decorin gene. This regulatory domain is likely to be implicated in the silencing of decorin gene by TGF-β and may contribute to the regulation of this matrix gene in the tumor stroma.

Key words. Proteoglycan; chondroitin sulfate; decorin; gene expression; tumor stroma; DNA methylation.

Introduction

Tumor stroma has been considered for over a century as a tissue without 'character or nature', a mere nonspecific response to the injury produced by the advancing malignant cells. During the past decade, however, the connective tissue stroma has emerged as a lively, structured and vital compartment, which is indispensable to the long-term survival and final outcome of cancer. Whether one considers the tumor stroma as a supportive or as a reactive entity, this stroma ultimately plays two fundamental roles: the supply of nutrients and the clearance of toxic metabolites. As an appropriate 'military defense' the tumor stroma is endowed with an armamentarium of specialized constituents. These include immunological tools, both cellular and soluble, morphoregulatory and neuroendocrine modulators, growth factors and matrix macromolecules. The balanced control of all these facets will dictate the outcome of malignant tumors. The goal of this brief review is to summarize the altered expression of chondroitin sulfate proteoglycan decorin in the stroma of human colon cancer, a system that has been extensively investigated in our laboratory. These data will be reviewed in the context of the current knowledge of decorin gene structure and expression in other systems and its roles in matrix assembly and cell proliferation. A model of decorin gene activation and extracellular matrix production in the tumor stroma is proposed.

Evolving concept of tumor stroma: from amorphous to morphous substance

Cancer comprises two major compartments: the transformed epithelial cells and the tumor stroma[20]. The recent advances in the biochemistry and cellular biology of extracellular matrix have led to the realization that the whole tumor-matrix complex needs to be studied in order to fully understand the process of cancer growth. During neoplastic development, a process that may take months or years to reach clinical manifestations, there is a continuous remodelling of infrastructures under the control of the neoplastic cells. This constant interplay between the clonal tumor cells and the stromal elements ultimately leads to a growth advantage of a clonal cell population and the invasion of surrounding tissues and blood vessels. The extracellular matrix of tumors comprises a highly complex and 'historically' important neoformation with an imprint of all the past cellular interactions. In contrast to unscheduled remodelling, which accompanies wound healing and repair, the formation of the tumor stroma appears to be 'scheduled' and tumor-specific. There are at least three lines of reasoning that establish criteria of specificity for the

tumor stroma. First, the stromal reaction to tumors is present in selected cancers, primarily in tumors arising from glandular-forming epithelial cells, such as breast, pancreas, colon, and prostate. Second, an aberrant stromal formation is a phenotypic trait that is maintained or sometimes accentuated at the site of distant metastases. A clear example of this is the well-established osteoblastic lesion of prostate cancer metastatic to vertebral bones. Third, there is evidence that tumor-associated stromal changes in proteoglycan gene expression are not found in the inflammatory remodelling of tissues, but correlate with the presence of a neoplastic cell population[2]. Proteoglycans are intrinsic constituents of this extracellular matrix and occupy strategic positions such as the cell surface and basement membranes[29,41]. By virtue of their polyanionic nature, their expanded configuration in solution, and, most of all, their ability to interact with a variety of growth factors and cytokines, proteoglycans can affect all the major physiological processes. The central hypothesis of our research is that the formation of this tumor stroma is under the direct control of cancer cells and that altered proteoglycan gene expression would in turn affect the progression of human cancer.

Abnormal expression of decorin: a leucine-rich proteoglycan involved in the control of cell proliferation and matrix assembly

Decorin, a ubiquitous small proteoglycan also known as DS-PG II or PG-40, belongs to an expanding family of gene products that share the ability of binding other proteins[41]. Thus, its eponym, originally proposed for its periodic 'decoration' of collagen fibers, probably reflects a more generalized function as specific biological ligand. The proteoglycan has a relatively simple structure with an amino terminal region containing a single glycosaminoglycan chain (either chondroitin or dermatan sulfate) and two asparagine-linked oligosaccharides, while the remainder of the protein core is composed of a series of leucine-rich tandem repeats[32,42]. The biological roles of decorin include specific and high affinity interactions with collagen type I and II[52], fibronectin[45], collagen type VI[6], and growth factors[53,54]. Consequently, decorin is directly involved in the regulation of fundamental biological functions including matrix assembly, cell attachment/detachment, migration and cell proliferation[42]. Most of these properties appear mediated by the protein core, probably by domains located in the leucine-rich regions. The binding to collagen type I is quite specific since decorin has been localized by immunoelectron microscopy at the d-band of collagen type I[41] with a periodicity of approximately 70 nm (fig. 1). This localization is similar to that observed using cationic dyes such as cuprolinic blue[46,48]. The formation of a collagen network is profoundly affected by the amount of decorin present. For example, collagen molecules incubated under physiological conditions aggregate into fibrils and the kinetics of fibrillogenesis can be followed under experimental conditions in vitro[52]. Exogenously added decorin induces a reproducible inhibition of fibrillogenesis for both the fibrillar collagens of vascular (type I) and avascular (type II) connective tissues[52]. More recently, decorin has been shown to interact specifically with fibronectin[45], thereby preventing the adhesion of cells to substratum, and to collagen type VI[6]. Since collagen type VI microfibrils also bind collagen type I and the cell surface[8], decorin may be involved in the bridging of polymers to the cell surface. The importance of these effects of decorin on the structure and assembly of extracellular matrix can be exemplified by a physiological process in which there is extensive remodelling of connective tissue, namely the dilatation of the uterine cervix during pregnancy[31]. In this process, the softening of the cervix correlates with a progressive increase in decorin/collagen ratio, while in the post-partum period this ratio normalizes again. One explanation, in light of the observations summarized above, is that decorin decreases the stiffness of the cervix by inhibiting the formation of a well-organized collagen network, thereby facilitating cervical dilatation[31].

Decorin is also directly involved in the control of cell proliferation and this function appears to be mediated by its ability to bind and neutralize TGF-β. Chinese hamster ovary cells transfected with decorin cDNA, following selection for gene-amplified transfectants, synthesize and release large amounts of decorin and acquire a behavior of more 'normal' contact-inhibited cells[53]. Because the untransfected wild cells produce tumors in nude athymic mice, this phenotypic reversal following decorin-transfection suggests that this proteoglycan is directly involved in the control of cellular proliferation. Subsequently, however, the action of decorin has been shown to be indirect. Decorin binds to and sequesters TGF-β[54], which is an autocrine growth-stimulatory factor for the Chinese hamster ovary cells[16]. It is important to note that since TGF-β also has negative growth effects on a number of cells[35], its removal could contribute to the overall modulation of growth in a tumor. The growth-factor binding properties, together with the other properties summarized above, make decorin a suitable candidate for mediating some of the intercellular communications occurring between the growing neoplastic cells and the stromal elements. We have previously shown[22–25] that the connective tissue stroma of human colon cancer contains elevated concentrations of chondroitin sulfate. Abnormal expression of proteoglycans with properties similar to those described in the colon have been reported in a wide variety of tumors including those from prostate (Iozzo, unpublished study), breast, stomach and

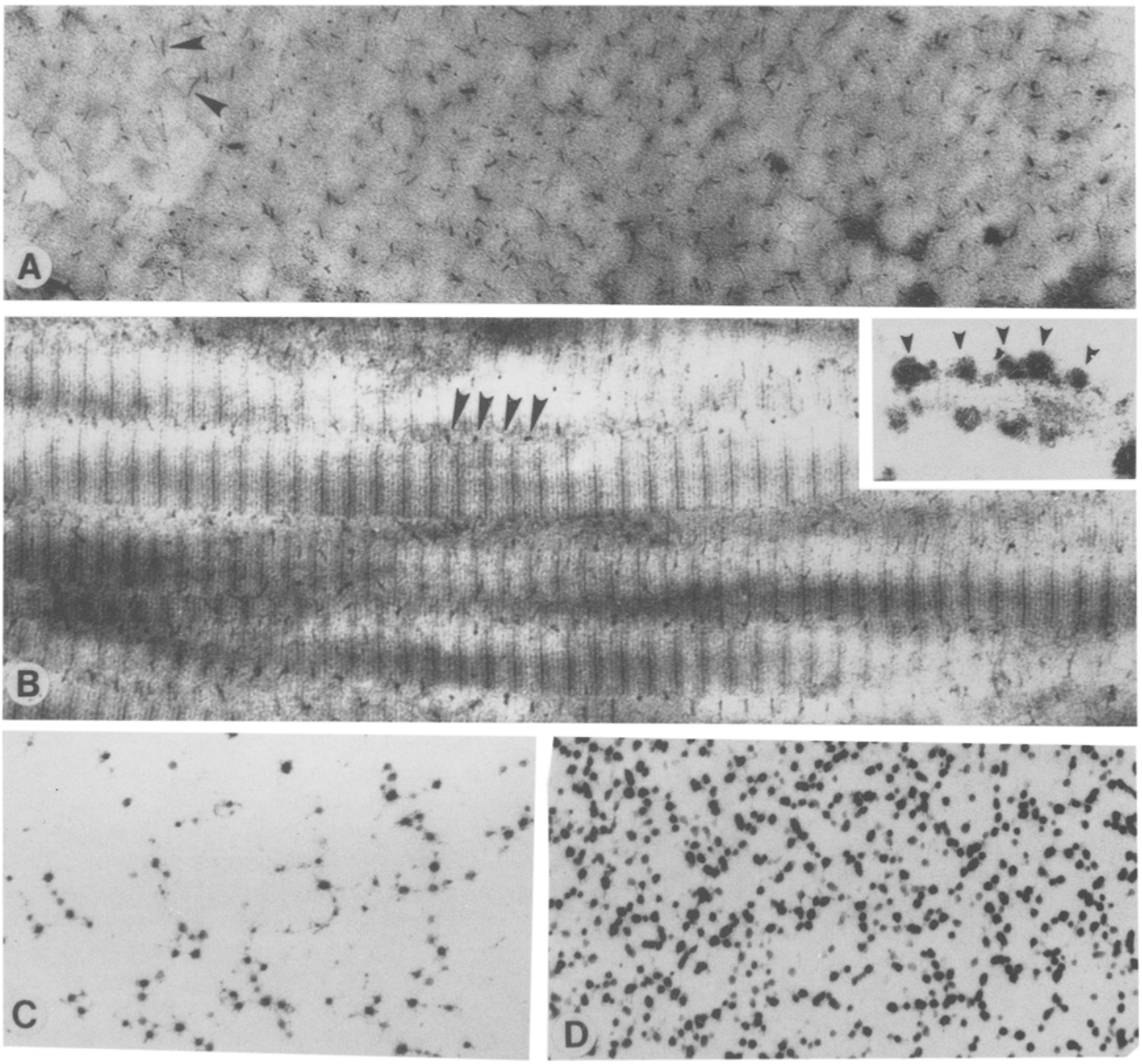

Figure 1. Ultrastructural localization of decorin in connective tissues using cationic dyes and specific anti-decorin antibodies. Panel *A* is a cross section of bovine pericardium stained with cuprolinic blue, a cationic dye that retains proteoglycan in tissues. Notice the presence of decorin proteoglycans appearing as needle-like structures (arrow heads) surrounding cross-sectioned collagen fibers. In panel *B*, one can better appreciate the periodicity of the proteoglycan decorin which binds to the d band of collagen type I. The inset in *B* is an immunoelectron microscopy demonstration of antigen-antibody reaction exhibiting a periodic deposition along the collagen molecular. Decorin, however, can also be free in the extracellular matrix as visualized by ruthenium red staining of colon (*C*). In the matrix of colon cancer, the decorin granules are much more increased in numerical density and decreased in size (note that panel *D* is printed at a higher magnification). These structural changes may reflect smaller chondroitin sulfate chains and thus less cationic dye bound to the protein core. For additional details, see the text. (*A*, ×66000; *B*, ×81000; *C*, ×60000; *D*, ×92000).

liver[50,55]. Autoradiographic and biosynthetic studies have shown that this up-regulation of chondroitin sulfate proteoglycans occurs in the stromal elements and that some of these changes can be reproduced in vitro by co-culturing colon cancer cells and mesenchymal cells of the colon, namely fibroblasts or smooth muscle cells[19,24]. These initial observations have led to the hypothesis that neoplastic cells may modulate their extracellular milieu by inducing stromal elements to synthesize a proteoglycan-rich extracellular matrix[19,20]. Analogous modulation of proteoglycan metabolism has been reported in other cellular systems[30,34]. These in vitro observations are supported by an experimental animal study performed with a transplantable V2 rabbit carcinoma[23]. Following an intraperitoneal injection of carcinoma cells there is a dramatic and progressive increase in thickness of the rabbit mesentery, which under normal conditions is a transparent fibrovascular membrane lined on either side by a monolayer of mesothelial cells. The most striking feature of this

system is that the increase in overall volume is not due to proliferating tumor cells, but rather due to an overall net increase in extracellular matrix and fibroblasts. This matrix induction is tumor specific since injection of normal liver cells induces no changes. Collagen type I, chondroitin sulfate proteoglycan and hyaluronan levels are all markedly elevated to produce a well-hydrated and hypertrophic mesentery[23]. Interestingly, close ultrastructural analysis of the proteoglycan network reveals a marked increase in the size and numerical density of collagen-associated proteoglycans, consistent with increased biosynthesis and deposition of decorin. In contrast, in human colon cancer, quantitative studies revealed that the proteoglycans that accumulate in the colon carcinoma stroma are relatively smaller but are much more numerous than their normal counterparts[22]. More recently, using a number of monoclonal antibodies directed against the chondroitin sulfate epitopes or the amino terminus peptide of decorin, our laboratory has shown that the amount of chondroitin sulfate proteoglycan and decorin epitopes are markedly elevated in the stroma of colon cancer[1]. The co-localization of undersulfated chondroitin sulfate and decorin epitopes suggest that in colon cancer stroma decorin protein core is substituted with this glycosaminoglycan, a more primitive species found in abundance in developing organs and the stroma of various epithelial tumors[20,21]. Analysis of steady state levels of decorin mRNA revealed several fold increase in the tumor as compared to the normal colon. However, in agreement with the immunohistochemical data, isolated colon carcinoma cells did not express decorin, while colon fibroblasts did express the two typical transcripts of ≈ 1.9 and 1.6 kb[1]. We further showed[2] that the steady state mRNA levels of versican[56], the other major chondroitin sulfate proteoglycan of human colon, are not significantly altered in the colon cancer. Collectively, these observations support the concept of an altered proteoglycan gene expression in the stroma of human tumors and suggest that these changes are intimately associated with the transformed phenotype.

Mechanisms of decorin gene activation: hypomethylation of decorin in the tumor stroma

The overexpression of decorin gene could be due to a number of factors, including gene rearrangement, amplification or hypomethylation (deactivation) of the gene[7]. These possibilities were tested experimentally by using Southern blotting and restriction endonucleases which were either sensitive or insensitive to methylation[1]. When a number of DNA samples from normal and neoplastic colon were analyzed by Southern blotting, both the pattern and relative intensity of decorin-specific bands in colon cancer tissue was identical to those of normal colon. This indicates that there is no significant rearrangement or amplification of decorin gene in the colon cancer. Therefore we investigated the methylation of CpG sites in human decorin gene.

Previous studies have shown that methylation of CpG islands influences gene expression by altering chromatin structure, by preventing binding of specific factors to regulatory regions of DNA, or by facilitating binding of certain proteins to DNA[7]. Gene activation is often associated with a modification of key regulatory regions in the degree of cytosine methylation, a covalent modification of the mammalian genome that occurs almost exclusively at the dinucleotide CpG[7]. When the degree of methylation of decorin gene was investigated, decorin gene was found to be hypomethylated in the colon cancer[1]. We have also attempted to pinpoint the cellular source of hypomethylation by analyzing the state of decorin gene methylation in isolated colon cancer cells and colon fibroblasts. As predicted, the colon carcinoma cells, which do not express decorin in the living organism, also maintain a fully-methylated and thus silenced gene, while colon fibroblasts, which express decorin, manifest a completely undermethylated gene[1]. Utilizing a quantitative polymerase chain reaction method coupled with digestion of DNA template by methylation-sensitive restriction endonucleases, we[3] showed that a specific site in the 3′ end of the gene is hypomethylated in colon cancer and adenomatous polyp, a premalignant condition. Interestingly, no such changes were observed in DNA from ulcerative colitis tissue[3]. Taken together, these findings indicate that hypomethylation of decorin gene may be a key regulatory factor in controlling the overt expression of this gene in colon cancer. The finding that DNA isolated from adenomatous polyps also contains hypomethylated decorin gene further suggests that these changes precede frank malignant transformation. Lack of a change in decorin methylation in ulcerative colitis, an inflammatory bowel disease characterized by severe ulceration and marked tissue remodelling, indicates that these tumor-associated alterations in decorin gene methylation are specific and suggest that inflammation alone is not necessary to induce these changes in 5-methylcytosine. It is noteworthy that changes in versican gene methylation are also observed in colon cancer stroma, although the steady state levels of versican mRNA are not significantly altered[2]. It should be emphasized, however, that the evidence is only indirect and the precise cellular source can only be implied given the complexity of the human system and the multitude of cellular elements. Nevertheless, these findings are quite provocative since one plausible conclusion is that demethylating events occur not only in the activated tumor genes but also in the genome of surrounding stromal cells. Hypomethylation of decorin gene in the tumor stroma 'conditioned' by the tumor cells may play a role in the abnormal expression of this gene in colon cancer and perhaps in other human tumors.

Significance of altered proteoglycan gene expression and implications for tumor progression

The finding of enhanced expression of decorin in the stroma of human cancer raises several important points. First, because decorin binds collagen fibrils and inhibits fibrillogenesis, a neoplastic-induced overexpression of decorin gene would result in a 'disorganized' extracellular matrix, a well established characteristic of cancer. An obvious consequence of depressed matrix organization is that tumor cells have an added degree of freedom and their mobility would not be limited by well-formed matrix scaffoldings. A further regulatory mechanism is offered by another proteoglycan of human colon, perlecan, a large heparan sulfate proteoglycan whose full cDNA sequence has been recently completed in our laboratory[14,36]. This molecule is present in the basement membrane and cell surface of colon cancer cells and is localized diffusely throughout the extracellular matrix, around colon fibroblasts and smooth muscle cells, and within the blood vessel wall[36]. Perlecan proteoglycan is a chimeric molecule with a number of potentially important domains including domains analogous to low density lipoprotein receptor, laminin, epidermal growth factor, and neural cell adhesion molecule. These structural modules could play significant roles in cell-matrix interactions during tumor progression. For instance, the protein core of perlecan is highly hydrophobic and capable of binding a number of extracellular matrix and cell surface molecules as well as basic fibroblast growth factors[51]. Moreover, Chinese hamster ovary cells defective in heparan sulfate biosynthesis fail to induce tumors in nude mice[17]. An increase in decorin and perlecan contents in the newly-formed tumor stroma could generate not only a well-hydrated tissue but also a growth-enriched micro-environment. Thus, both decorin and perlecan, via distinct binding domains, capture active cytokines and store them within the newly-organized extracellular matrix. This results in the efficient removal of the growth factors from the immediate cells and in their protection from proteolytic attack[41]. This is probably one of the most important biological characteristics of proteoglycans and contributes to the generation of an extracellular matrix that serves as tumor cell-growth stimulator. An additional feature of this biological system is that these two proteoglycans are subjected to a close regulation by the same cytokine-growth factors that they bind[15,35]. Therefore, decorin and perlecan may provide a feed-back control loop that modulates growth factor activities and can directly influence the proliferation of mesenchymal cells around tumors and the production of extracellular matrix. It is tempting to speculate that tumor cells capable of up-regulating these matrix products may possess a growth advantage over clonal cells that lack this ability. A plausible in vivo scenario is that the neoplastic epithelial cells express high levels of decorin and perlecan which bind to and protect locally-released growth factors. These factors would cooperatively maintain the transformed phenotype, stimulate the growth of tumor cells in an autocrine fashion and regulate the proliferation of surrounding host cells by potentiating paracrine growth mechanisms.

Unraveling the organization of human decorin gene: heterogeneity in the 5′ untranslated region and alternative splicing of leader exons

In order to make a molecular understanding of decorin gene regulation possible, we have recently cloned the entire human decorin gene and characterized the intron/exon junctions including several kilobases of 5′ and 3′ flanking regions[12]. The results from this study have provided a number of unexpected surprises both in terms of gene organization and alternative splicing. First, the gene is over 40 kb in size, surprisingly large given the relatively small size of the coding sequence. Second, we found two leader exons in the 5′ untranslated region, which we call exons Ia and Ib, respectively. These two exons are alternatively spliced to the second coding exon. The existence of these two exons in transcripts from normal cells has been confirmed by polymerase chain reaction (PCR) amplification of cDNAs containing either of the two exons spliced to exon II (fig. 2). Using reverse transcriptase-PCR, we have recently established the widespread expression of both exons in a variety of human cells and tissues[12]. The significance of these two leader exons is further strengthened by the finding of highly homologous (75–90%) sequences in the avian[33] and bovine[13] decorin cDNAs. The conservation of these exons across species, which diverged about 300 million years ago, suggest that these structures are evolutionarily important. It should be noted, however, that, since the alternate exons are in the 5′ untranslated region the ultimate protein sequence is not affected. What is then the functional implication of alternative splicing of leader exons? In most cases described so far, genes with heterogeneous 5′ regions express these exons in either a developmental- or tissue-specific manner[9]. Amongst the proposed roles, alternatively-spliced non-coding exons could affect the translational capacity of a transcript by forming unusual secondary structures[9]. Furthermore, utilization of a tissue-specific promoter would regulate the specific expression of certain genes useful or indispensable to the growth of that tissue[9]. There are at least three examples of extracellular matrix genes that exhibit heterogeneity in their 5′ untranslated regions, including the genes for $\alpha 1(IX)$[38], $\alpha 2(I)$[5], and $\alpha 2(VI)$[43] collagens. Interesting structures were also found in the 3′ untranslated region of decorin gene with several potential polyadenylation sites which would explain the two

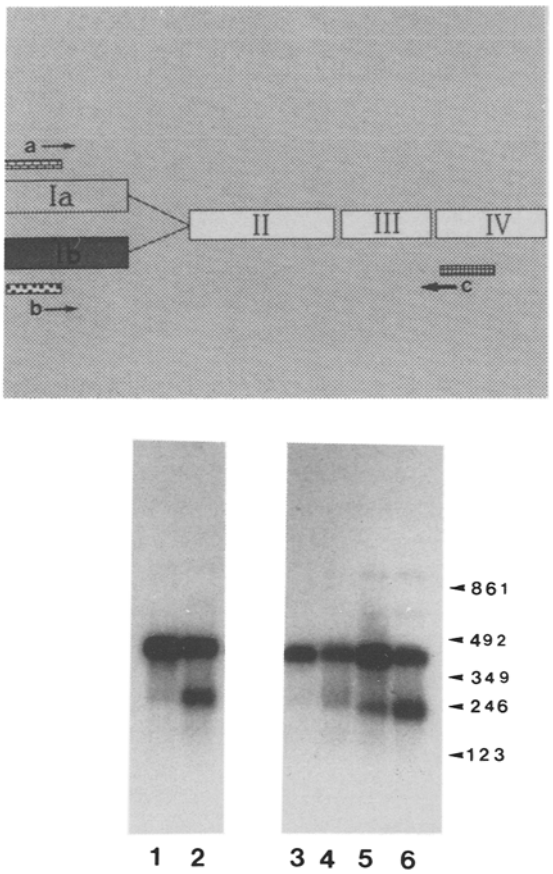

Figure 2. Heterogeneity in the 5′ untranslated region of decorin. The graphic at the top depicts the two leader exons of human decorin gene, exons Ia and Ib, respectively[12]. The direction of the arrows indicate the orientation of the three primers (labeled a, b and c) used to amplify by polymerase chain reaction various cDNA libraries. The amplified products of ≈450 bp are visualized in the Southern blotting (lower panel). Lanes 1, 3 and 5 represent amplified product pertaining to exon Ia, while lanes 2, 4 and 6 represent amplified fragments pertaining to exons Ib. Notice that a specific ≈450 bp fragment is present in cDNA library generated with mRNA from human fibroblasts (lanes 1 and 2), human heart (lanes 3 and 4), and human placenta (lanes 5 and 6). The Southern blot was hybridized under stringent conditions with a 411 bp fragment of 5′ decorin which was labeled with [^{32}P]dCTP to high specific activity (≈ 10^8 cpm/μg DNA) by random priming. The autoradiogram was exposed for 30 min. The lower molecular weight band was not constantly observed.

major transcripts of decorin of ≈1.6 and 1.9 kb[32]. It is intriguing that the 3′ untranslated region of human decorin contains AT-rich sequences that have been involved in the rapid degradation of mRNAs and are under the control of interleukins[47]. Consistent with this view is the observation that the steady state levels of decorin mRNA are modulated by interleukin-1[18].

A simplified model of matrix gene activation: balance of positive and negative signals

A comprehensive, yet simple, explanation that could account for the formation of tumor stroma is difficult to be formulated, given our limited understanding of the principal controlling mechanisms and the multitude of transcription factors probably involved in such a process. However, overwhelming evidence for a direct role of cytokines in the control of matrix biosynthesis and mesenchymal cell growth[35] indicates that cellular mediators, such a TGF-β, may play pivotal roles in the stroma formation during neoplastic growth. Below, we propose a 'reductionistic' model of matrix activation that may lead to desmoplasia. An emerging picture of TGF-β effects on proteoglycan metabolism is that this potent cytokine down-regulates decorin mRNA levels[26] while it up-regulates the levels of biglycan[10,26,40], versican[26], and perlecan[15] mRNAs. A plausible explanation for TGF-β-mediated inhibition of decorin gene expression is provided by our finding[12] of a TGF-β negative cis-acting element in the promoter region of human decorin gene (fig. 3). This sequence has been demonstrated to be a negative element in the promoter regions of transin/stromelysin[28], elastase[49], and collagenase, three genes which are also down-regulated by TGF-β. These three enzymes are involved in the degradation of all the major extracellular matrix proteins, including proteoglycans. The TGF-β-negative element functions as a transcriptional silencer and binds to the c-Fos protooncogene product[28], among other transcription factors. Thus, it is possible that decorin transcriptional control would involve TGF-β and c-Fos proteins. Interestingly, TGF-β exerts profound anabolic effects on several macromolecules including collagen type I, fibronectin and elastin[11,27,37,39] and is also a growth-inducer for the majority of mesenchymal cells. It is noteworthy that TGF-β accumulates in the activated fibroblasts and macrophages in the area of pulmonary fibrosis and this cytokine may represent the major humoral stimulus for the aberrant expression of connective tissue genes[11].

A simple model with TGF-β as a central player is presented in figure 3. Accordingly, synergism in matrix activation could be achieved by the concurrent down-regulation of enzymes which degrade matrix macromolecules and the up-regulation of genes encoding such matrix macromolecules. Why, among the extracellular matrix constituents, is decorin singled out and transcriptionally silenced? One plausible explanation is that decorin would have some detrimental effect in this marvelous biological loop. Indeed, decorin avidly binds to TGF-β[54], thereby making this cytokine unavailable to TGF-β-dependant cells. Furthermore, lack of secreted decorin would remove the negative modulatory activity of this proteoglycan on collagen fibrillogenesis and thus more collagen deposition would ensue. It is quite plausible, thus, that any significant derangement in decorin gene expression, could have profound effects on the overall outcome of the stroma as a whole. If decorin gene escapes the negative control of TGF-β (for example, via positive transcription factors modulated by

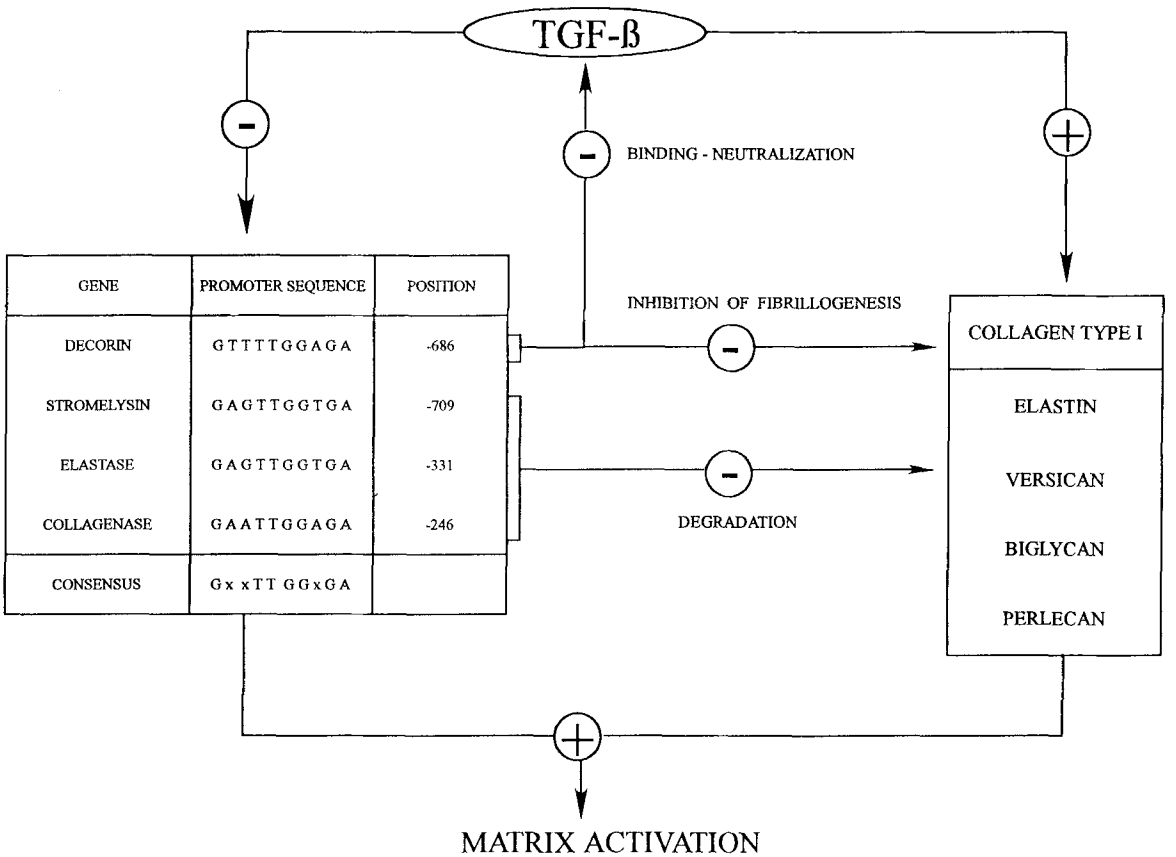

Figure 3. A model of matrix activation based on the multifunctional activities of transforming growth factor β (TGF-β). This cytokine inhibits transcription of decorin and of three major connective tissue lytic enzymes (stromelysin, elastase and collagenase) via a cis-acting sequence in the promoter region of the gene. The consensus sequence[28] and the corresponding cis-acting sequences in each promoter region are provided on the left box. The protooncogene FOS may play a direct role in silencing these genes since it is a part of the transcription factors which specifically bind this negative element. This TGF-β-mediated transcriptional block prevents degradation of elastin, collagen and various proteoglycans, while preventing the decorin-mediated inactivation of TGF-β itself. On the other hand, TGF-β up-regulates the expression of collagen type I, fibronectin and proteoglycans carrying either dermatan/chondroitin (biglycan, versican) or heparan sulfate (perlecan) chains. The concurrent positive and negative actions of TGF-β would potentiate extracellular matrix production and the formation of tumor stroma. For additional details, see the text.

interleukins[18]), the over-produced protein core would bind more TGF-β and would signal the cells to manufacture and release more of this cytokine. This, in turn, would amplify the production of extracellular matrix and the formation of tumor stroma. Eventually, this loop would result in a growth factor-enriched matrix. It may be that the major difference between the self-contained process of wound healing and the uncontrolled growth of tumor stroma may reside in an imbalance of this biological loop. This simple model obviously does not address the entire picture and some other factors would likely participate in such a process. However, it seems quite clear that during the initial stages of tumor development, when the changes in matrix can be both temporally and spatially defined, the roles played by cytokine-matrix interactions could set the stage for an uninterrupted and self-perpetuating process of matrix activation. A derepression of decorin gene transcription together with an input of TGF-β at the invasive front of tumors would be sufficient to alter tissue homeostasis and trigger the series of events described above. It is on this loop that we need to act both pharmacologically and immunologically to prevent the establishment of the invasive phenotype.

Conclusions and perspectives

In this brief synopsis we have tried to discuss the role of proteoglycans in the formation of tumor stroma. The elucidation of the molecular organization of human decorin gene has given important clues on the possible mechanisms involved in its regulation. The immediate future will undoubtedly reveal unsuspected functions for these proteoglycans. It is not inconceivable that decorin could act as an oncogene under appropriate condition of deranged expression. Abnormally high and prolonged production of the proteoglycan could lead to an abnormal proliferative response that is no longer regulated. The development of transgenic animals in which decorin, for example, is simply over-expressed

could clarify whether or not this gene is implicated in tumors. Do transgenic animals carrying high copy numbers of the gene become more prone to tumor formation? And if so, what kind of tumors? If decorin is such an important gene, what happens when the gene is knocked out by homologous recombination? Is any region of the gene necessary for its proper binding activities? Is lack of proper decorin gene expression lethal? Some of these fundamental questions are being addressed experimentally in our laboratory and the expectations from such research is what makes the research in proteoglycan worthwhile and rewarding.

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