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Decorin Transfection in Human Mesangial Cells Downregulates Genes Playing a Role in the Progression of Fibrosis

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The proteoglycan decorin inhibits TGF-β; therefore, it could antagonize progression of fibrotic diseases associated with activation of TGF-β_1. The effect of decorin transfection in human mesangial cells (HMCs) on the expression of genes related to kidney fibrosis was investigated. HMCs, isolated from glomeruli of healthy portions of human kidneys removed due to carcinoma, were histochemically typed. Decorin cDNA cloned in a eukaryotic expression vector was transfected into HMCs. Gene expression of fibrogenetic cytokines and fibrotic proteins TGF-β_1, PDGF-β, α_1 collagen type IV, α_1 collagen type I, fibronectin, and tenascin was analyzed, by reverse transcription polymerase chain reaction (RT-PCR), 24 hr after transfection. Immunoblotting analysis of protein extracts using anti-decorin IgG, revealed a positive signal of about 52 MDa, corresponding to the molecular weight of decorin, in cultures transfected with the decorin gene. Decorin mRNA increased about 12 times in cultures transfected with the construct pCR3.1-Deco. Cells with increased decorin synthesis showed a 61% decrease of TGF-β_1 mRNA, a 71% reduction of α1 collagen type IV mRNA, and a 29% reduction of fibronectin mRNA. This study is the first to investigate decorin transfection into *human* mesangial cells, and supports the use of the decorin gene to control the progression of glomerular and interstitial fibrosis in kidney diseases. J. Clin. Lab. Anal. 16:178–186, 2002. © 2002 Wiley-Liss, Inc.

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INTRODUCTION

The progression of glomerular and interstitial fibrosis, the hallmark of chronic renal failure, is normally treated with ACE inhibitors or antagonists of angiotensin II receptors. A new and different approach could be the use of decorin. The decorin gene is situated in chromosome 12q23 in a region of about 38 kb, which contains 8 exons and very large introns. Its deduced amino acid sequence shows high homology and an identical intro-exon junction with biglycan. The presence of two characteristc cysteine clusters flanking 11 leucine rich repeats suggests a closely related origin of decorin, biglycan, and fibromodulin (1,2). These proteoglycans are constituents of the extracellular matrix of connective tissues, which are responsible for maintaining hydration and interstitial pressure (3). Lack of decorin during ontogeny causes altered collagen fibril structure and skin fragility. Decorin is directly involved in the control of matrix organization, and binds to type I and type II collagen and fibronectin, affecting the rate of fibril formation (4–6).

In addition to its primary role as a modulator of extracellular matrix, decorin can inhibit cellular response to growth factors. In particular, decorin is a known inhibitor of TGF-β (7,9–11). This is a very relevant feature because activation of TGF-β_1 in response to injury or disease is known to be one of the major causes of extracellular matrix deposition, which leads to tissue fibrosis and loss of renal function (8).

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In vitro and in vivo studies have revealed that decorin core protein binds TGF-β_1, and neutralizes bioactivity of TGF-β_1, TGF-β_2, and TGF-β_3, the three isoforms expressed in mammals (9–11). Decorin expression in human and glioma cells not only abrogates TGF-β bioactivity by complex formation, but also inhibits TGF-β_1 and TGF-β_2 mRNA trascription and TGF-β protein synthesis (7). These observations together with findings that decorin is one of the matrix components induced by TGF-β overexpression, while in other tissues expression of decorin mRNA and protein is suppressed by TGF-β, support the hypothesis that decorin may act as an effector molecule in a feedback loop that regulates TGF-β. Therefore, decorin could be a natural regulator of TGF-β (12).

The fact that decorin can block activity of TGF-β, a cytokine known to induce fibrosis (13), supports decorin administration as a potential therapy in renal fibrotic diseases. The therapeutic benefit of inhibiting TGF-β_1 by injecting antibodies was demonstrated in several disease models, including kidney, lung, skin. In an experimental glomerulonephritic rat model, decorin administration through repeated injections of human decorin or transfer of decorin cDNA into rat skeletal muscle reduced glomerular levels of TGF-β_1 mRNA and TGF-β_1 protein, extracellular matrix accumulation, and proteinuria (9,14).

Further studies at the molecular level have produced additional information about a fine regulation of decorin gene expression. DNA binding motifs for AP1, AP5, NF-κB, TGF-β, and TGF-α have been described upstream from the translational start of decorin (1,15). Two transcripts of typically 1.6 and 1.9 Kb have been detected by northern blot in different tissues and cell types (1). The decorin gene has two promoters, P1 and P2, located upstream of two alternatively spliced leader exons, Ia and Ib (1,15). Both exons encode a portion of the 5′ untranslated region of the mRNA, and translation from either P1 or P2 results in the same protein (1). While both promoters have low basal expression levels in human mesangial cells (HMCs) (16), only the P2 promoter is functional in HeLa epithelial cells and MG-63 osteosarcoma cells (15). Mauviel et al. (17) reported a novel transcriptional activation of the decorin gene associated with induced quiescence of human dermal fibroblasts and HeLa cells. The same group also demonstrated the presence of TNF-α responsive elements and a dose-dependent transcription repression of decorin by TGF-α in fibroblasts. This effect was additionally increased by TGF-β.

Conflicting results have been reported concerning TGF-β's influence on decorin expression. TGF-β upreglates decorin expression in primary mesangial cells, lung fibroblasts, and epithelial cells (18), while it downregulates decorin expression in human skin fibroblasts and a human osteosarcoma cell line (19). Interestingly, a TGF-β inhibitor element is present in P2 and has been demonstrated to negatively regulate TGF-β expression (15). The transfection of a reporter gene in HMCs, driven either by P1 or P2, showed that P2 has little activity and P1 contains a high glucose and TGF-β_1 responsive element (20).

The tissue-specific expression of either Ia or Ib (1,15), and the presence of a TGF-β_1 responsive element in P1 and a TGF-β inhibitor element in P2 clearly indicate a cell-type specific control of decorin expression.

Since a possible way to introduce the decorin gene into the kidney is through engineering mesangial cells with the decorin gene, and reintroduction of decorin-expressing mesangial cells in the glomerulus through renal artery circulation (21,22), a first step toward application of decorin gene therapy for human glomerular fibrotic diseases is to study the effect of decorin engineering in primary HMC cultures.

In this study the decorin cDNA was introduced into HMCs by transfection. We then investigated the effect of increased decorin synthesis on the expression of growth factors that had been reported to induce glomerulosclerosis TGF-β_1 and PGDF-β, (13), and on the expression of the extracellular matrix components fibronectin, α1 collagen type I, α1 collagen type IV, and tenascin.

MATERIALS AND METHODS

Reagents

Collagenase 1A, d-valin, Hanks' salts, L-glutamin, MEM vitamins, transferrin, selenium, and insulin were purchased from Sigma (St. Louis, MO). RPMI-1640 without L-glutamin and $NaHCO_3$, fetal calf serum, and trypsin were from Sigma-Aldrich (Milan, Italy).

The mouse monoclonal IgG anti-human Thy-1 was from Chimax (Bradaschia, Milan, Italy). The mouse monoclonal IgG anti-human α-smooth muscle actin, and mouse monoclonal antibody IgG anti-human cytokeratin were from Dako (Giostrup, Denmark). The rabbit polyclonal IgG anti-human von Willenbrand factor was from Sigma; sheep polyclonal IgG raised against the C-terminal ammino acidic sequence of human decorin, CVYVRSAIQLGNYK, was from Anawa Biomedical Service and Products (Wangen, Zurich); and rabbit anti-sheep IgG conjugated with horseradish peroxidase (HRP) was from Dako (Giostrup, Denmark). The polyvinylidene difluoride membrane for western blotting was from Amersham (Buckingamshire, UK), and the pCR3.1 TA cloning eukaryotic expression vector was from Invitrogen (Groningen, The Netherlands).

Preparation and Characterization of HMC Cultures

Human glomeruli were isolated from macroscopically normal-appearing kidney cortical fragments obtained from nephrectomies removed for localized carcinoma, using a modification of a published methodology (23). Briefly, about 20 g of tissue were reduced in small fragments (approximately 1-mm^3 cubes), suspended in HBSS (Sigma), and passed through progressively smaller wire-mesh sieves. After treatment with 0.1% collagenase, the glomeruli were placed in 25-cm^2 tissue culture flasks and maintained at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air. The culture medium was RPMI-1640 supplemented with 20% fetal calf serum (FCS), 2 mM L-glutamin, 10 μg/mL insulin, 1 × MEM vitamins, 5.5 μg/mL transferrin, 5 ng/mL selenium, 100 μg/mL penicillin, 100 μg/mL streptomycin, and 2.5 μg/mL fungizone. After several days, when mesangial cell outgrowth was evident, nearly confluent cells were detached from culture flasks using 0.025% trypsin in EDTA and diluted at a ratio of 1:3. After the second passage of culture, the HMCs were stored in cryotubes containing 10% DMSO, 30% RPMI, and 60% serum, and kept in liquid nitrogen for further use.

Cells were used between the third and fifth passages of trypsinization. Each preparation of mesangial cells was characterized before use. Mesangial cells stained positively for α-smooth muscle actin and human Thy-1, and negatively for cytokeratin and von Willenbrandt factor, excluding epithelial and endothelial cell contamination.

Construction of the Decorin Expression Vector, pCR3.1-Deco

The decorin cDNA was amplified by polymerase chain reaction (PCR) from cDNA obtained by reverse transcription (RT) of RNA obtained from human fibroblasts, and cloned in the eukaryotic expression vector pCR3.1 under the control of the CMV promoter. The pCR3.1-Deco construct was transformed in *E. coli* DH5α, and plasmid DNA was purified by affinity chromatography on DNA purification columns (Quiagen Inc., Catworth, CA). The correct insert DNA sequence was additionally confirmed by automatic sequencing.

Transfection

About 1×10^5 HMCs were seeded in 3.5-cm-diameter wells of tissue culture plates, and cultured in 2 mL of culture medium, supplemented with 10% FCS. Then 100 μl of transforming solution containing 1 μg of plasmid DNA in calcium phosphate precipitate were added drop by drop to each cell culture. After 8 hr, cells were incubated for 2 min in 10% glycerol to enhance nuclear uptake of DNA, and washed three times with PBS. After 1 day of growth in tissue culture medium containing 500 μg/mL geneticin (to select for plasmid pUC3.1-Deco, which carries the cloramphenicol acetyltransferase gene under the control of the SV40 promoter), cells were harvested for further RNA and protein extraction.

Protein Extraction

Almost confluent HMCs were washed in PBS and lysed directly on tissue culture plates using gel SDS loading buffer, containing 100 mM TrisCl pH 6.8, 200 mM DTT, 4% SDS, and glycerol (24). Subsequently, samples were boiled for 10 min; DNA was sheared by repeated passages through a 23-gauge hypodermic needle and eliminated by centrifugation 10 min at 10,000 rpm. The protein concentration was determined using a protein colorimetric assay kit from BioRad Inc. (Segrate, Milan, Italy). Samples were stored at −80°C.

Immunoblotting

Protein samples (about 1-μg) were separated by electrophoresis on a 10% SDS/PAGE slab, using the procedure described by Laemmali (25). After electrotransfer to a polyvinylidene difluoride membrane, and blocking with 5% w/v nonfat powder milk, 0.1% Tween20, in 1 × TBS at pH 7.5, the membrane was incubated with primary antibody diluted 1:2,000 in 1 × TBS 5% w/v nonfat powder milk. After it was washed, the membrane was incubated in a 1:100 dilution in 1 × TBS of the secondary antibody. The primary antibody was sheep polyclonal IgG raised against the polipeptide CVYVRSAIQLGNYK, which is the C-terminal sequence of human decorin. The secondary antibody was rabbit anti-sheep IgG conjugated with horseradish peroxidase (HRP). Detection was by enzymatic activity of HRP.

RNA Extraction

Total RNA was isolated using guanidinium thiocyanate RNAzol-B reagent (Biotex, Houston, TX). HMC cells were lysed in 200 μl of RNAzolB solution by pipetting. A 0.1 volume of chloroform was added to the homogenate, shaken vigorously, kept in ice for 5 min, and centrifuged 20 min at 14,000 rpm at 4°C. The aqueous phase was transferred to a new eppendorf tube and precipitated using an equal volume of isopropanol, recovered by centrifugation, and washed with 70% ethanol. After it was dried, the RNA pellet was dissolved in diethyl pyrocarbonate-treated water. RNA

quality and concentration were analyzed by absorbance at 260 nm and 280 nm, and then the RNA was diluted to a concentration of 50 ng/μl.

RT-PCR Analysis

Equal amounts of total RNA (100 ng) from each sample were reverse-transcribed into cDNA, using 50 units MuLV reverse transcriptase (Perkin Elmer Inc., distributed by Applied Biosystem, Milan, Italy), 20 units RNAse inhibitor, 2.5 μM random exanucleotide primers, 1 mM dNTPs, and 5 mM $MgCl_2$, in a final volume of 20 μl. The reaction was carried out for 30 min at 42°C and was arrested by heating for 5 min at 99°C.

Equal amounts of the RT reaction (2 μl aliquots) were subjected to PCR amplification in a final volume of 50 μl containing 2 μl cDNA from RT reaction, 1× buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 μM primers, and 2 units of Taq. Amplification was started with 5 min of denaturation at 95°C followed by cycles of 45 sec at 94°C, 45 sec at 60°C, and 1 min at 72°C. The final extension was 7 min at 72°C in all instances. To quantify PCR products comparatively and to confirm the use of equal amounts of the initial RNA, the housekeeping gene G_3PDH was coamplified. Amplification products were analyzed by 7% polyacylamide gel electrophoresis, silver staining, and densitometric analysis of PCR product optical density (OD) using Gelpro software (© Media Cybernetics 1993–1997, MIlan, Italy). The mRNA levels of target genes were expressed as the ratio of OD target mRNA and OD G_3PDH mRNA.

Standard PCR reaction curves were constructed for each gene under investigation, taking out 5 μl of PCR product at 22, 24, 26, 28, 30, and 32 cycles of amplification. The amount of the RT reaction (2 μl) used for the amplification was selected as being nonsaturating for the PCR product of both G_3PDH and the gene under investigation before 30 cycles of amplification.

The sequences of primers were designed from the published sequences of the human genes and are listed in Table 1. Primers were designed to span one or more introns within the genes, in order to exclude genomic DNA contamination.

Statistical Analysis

Experiments were repeated in triplicate using three primary HMC cultures. All data are expressed as mean ± standard deviation (SD). Groups were compared using a paired Student's *t*-test and one-way analysis of variance (ANOVA). Differences were considered significant at P values <0.05.

RESULTS

Characterization of HMCs and Transfection

HMCs observed under phase-contrast microscopy presented their characteristic morphology. They appeared fusiform with indistinct cell borders and, when grown to confluence in medium containing FCS, they exhibited the characteristic "domes and valleys" appearance. Immunohistochemical characterization showed positive staining for α-smooth muscle actin and Thy-1, and negative staining for cytokeratin and von Willenbrandt factor, excluding epithelial and endothelial cell contamination (data not shown). After the transfection procedure cell survival was variable,

TABLE 1. Primers used for RT-PCR

Name	Sequences 5′-3′	Gene	Size
LEFT-DECO	AAGGTTCCCTGGTTGTGAAA	Decorin	
DECO-RSCR	CCACATTGC AGTTAGGTTTCC		1217 bp
LEFT-G_3PDH	TGAAGGTCGGAGTCAACGGATTTGGT	G_3PDH	
RIGHT-G_3PDH	CATGTGGGCCATGAGGTCCACCAC		986 bp
LEFT-TGFβ$_1$	GCCCTGGACAAAAACTATTGCT	TGFβ	
RIGHT-TGFβ$_1$	AGGCTCCAACTGTAGGGGCAGG		161 bp
LEFT-PDGFβ	GTTCCCTGACCATTGCTGA	PDGFβ	
RIGHT-PDGFβ	GTCACCGTGGCCTTCTTAAA		262 bp
LEFT-α$_1$IV	CTCTACGTGCAAGGCAATGA	α$_1$ collagen type IV	
RIGHT-α$_1$IV	TGGCGCACTTCTAAACTCCT		417 bp
LEFT-TN	CCGSSCGTACCAGGGACTTA	Tenascin	
RIGHT-TN	GTCTTGGTTCCGTCCACAGT		274 bp
LEFT-FN	GGACTTCCTATGTGGTCGGA	Fibronectin	
RIGHT-FN	GTTGGTAAACAGCTGCACGA		312 bp
LEFT-α$_1$I	AGAGAGAGGCTTCCCTGGTC	α$_1$ collagen type I	
RIGHT-α$_1$I	TCCAGAGGGACCTTGTTCAC		496 bp

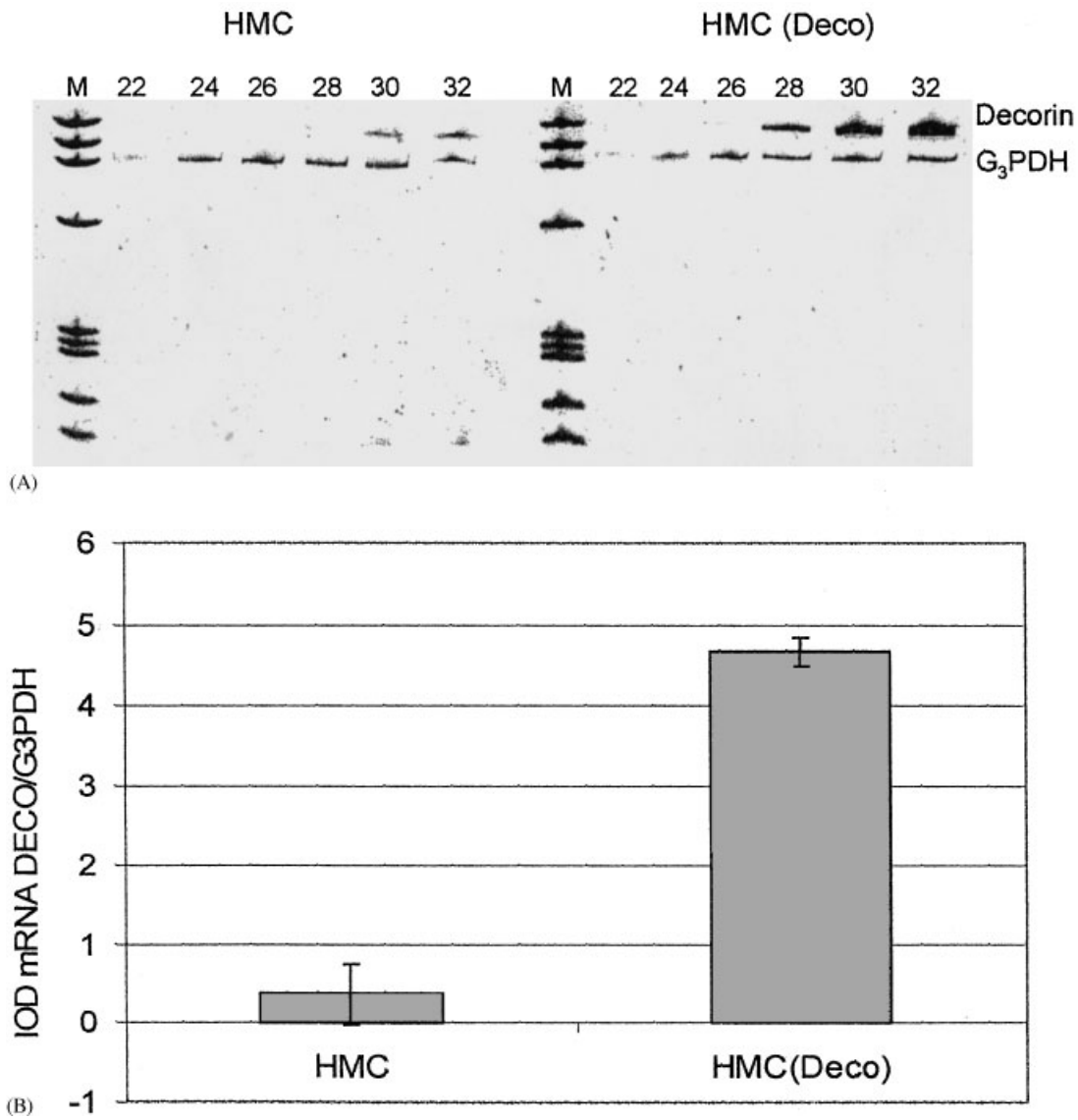

Fig. 1. Analysis of decorin mRNA in HCMs transfected with decorin cDNA. After RT of total RNA from HMC cultures transfected with pCR3.1-Deco, HMC(Deco) and control samples, HMC were amplified by quantitative PCR with primers for decorin. **A:** Expected 1,2 Kb decorin and 983 bp G_3PDH products after 22, 24, 26, 28, 30, and 32 cycles of amplification. **B:** Histogram of the decorin mRNA ratio, measured as decorin/G_3PDH OD obtained from densitometric scanning of silver-stained PCR products. Data are expressed as the mean $\pm$ SD of three experiments using three different primary HMC cultures.

from immediate death (especially when cells over the fifth passage of culture were used) to general survival (when cells before the fourth passage of culture were used).

Detection of Decorin Transcript in Transfected HMC Cultures

Decorin-specific primers (Table 1) were used to amplify decorin cDNA originating from RNA of HMC cultures transfected with pUC3.1-Deco and control cultures. Control HMC cultures showed no decorin amplification for two of the cell cultures tested, and a vanishing amplification band after the 32nd cycle of PCR for the third HMC cultures tested, indicating that decorin expression in mesangial cells is almost absent. This is in agreement with previously reported low basal expression of decorin in HMC (16). A DNA fragment of about 1.2 Kb, corresponding to expected amplification product size (Table 1), was amplified from cells transfected with the decorin gene (Fig. 1), confirming new decorin mRNA transcription from the

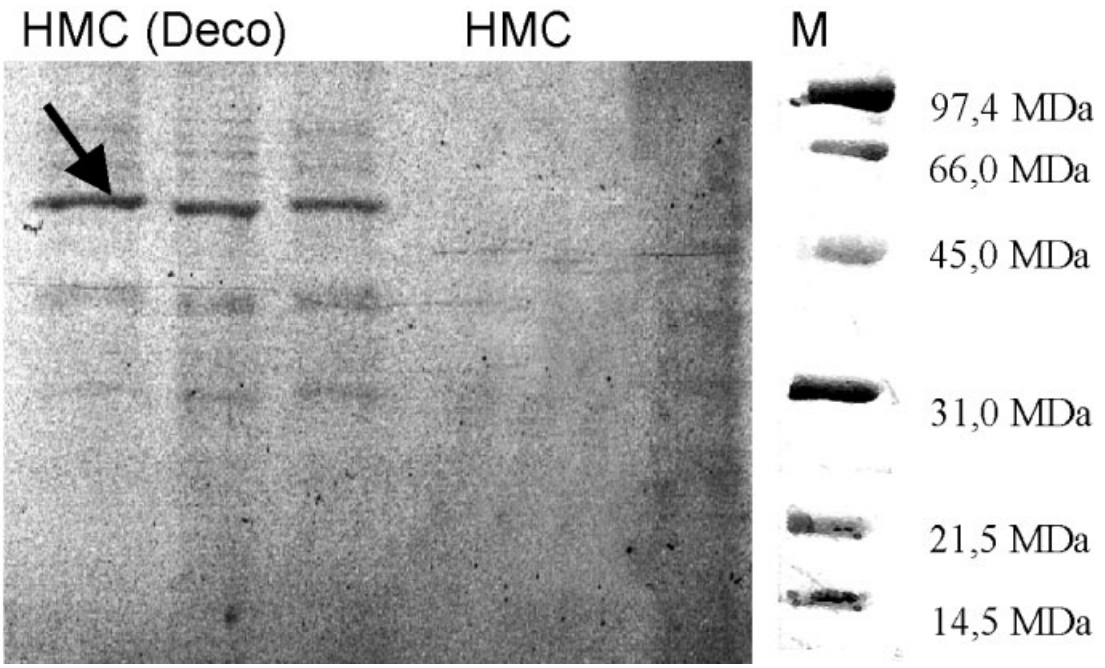

Fig. 2. Immunoblotting analysis of decorin. Total protein extracts from HMCs transfected with pCR3.1-Deco and HMC(Deco), and control cell culture HMCs were analyzed using antiserum raised against a C-terminal polypeptide of mature decorin core protein. On the right side of the figure is shown the migration of molecular weight marker proteins (phosphorylaseB, 97.4 MDa; bovine serum albumin, 66 MDa; ovalbumin, 45 MDa; carbonic anhydrase, 31 MDa; soybean trypsin inhibitor, 21 MDa; and lysozyme, 14.5 MDa).

pCR3.1-Deco construct. Decorin mRNA was increased about 12 times in cultures transfected with pUC3.1-Deco ($Deco_{26}/G_3PDH_{24}$ OD ratio 0.370 ± 0.381 control vs. 4.676 ± 0.176 decorin-transfected cells; mean $\pm$ SD; Fig. 1).

Immunodetection of Decorin in Transfected HMC Cultures

Analysis of protein extracts by immunoblotting using anti-decorin IgG revealed a positive signal of about 52 MDa for the three HMC cultures transfected with pCR3.1-Deco, while no decorin signal was detected in control cell cultures. Representative immunoblots of transfected and control HMC are shown in Fig. 2.

Expression of Fibrosis-Related Genes in HMC With Increased Decorin Production

To investigate mRNA expression of the growth factors TGF-β_1 and PGDF-β, and the extracellular matrix components fibronectin, α_1 collagen type I, α_1 collagen type IV, and tenascin in HMC with increased decorin synthesis, we designed primers specific for these mRNA transcripts (Table 1). The specificity of the amplification products was confirmed by the correct size of the expected amplification product and by hybridization (data not shown). Total RNA was extracted from cells with increased decorin synthesis, and from control cells, and was used for RT-PCR analysis. Determination of TGF-β_1 mRNA levels revealed a more than twofold decrease in HMCs with increased decorin synthesis ($TGF\text{-}\beta_{128}/G_3PDH_{22}$ OD ratio 7.873 ± 0.88 control vs. 2.972 ± 0.491 decorin-producing cells; mean $\pm$SD; Fig. 3), showing that induced decorin synthesis down-regulates TGF-β_1 transcription in HMC. There was a statistically significant decrease of α_1 collagen type IV mRNA ($\alpha_1 coll.IV_{26}/G_3PDH_{24}$ OD ratio 0.433 ± 0.048 control vs. 0.126 ± 0.037 decorin-producing HMC; mean $\pm$ SD; Fig. 4) and fibronectin mRNA ($fibronectin_{26}/G_3PDH_{24}$ OD ratio 1.647 ± 0.336 control vs. 1.150 ± 0.029 decorin-producing cells; mean$\pm$SD; Fig. 4), although there was no significant difference in PDGF-β and tenascin mRNA levels in HMC cultures with increased decorin production. In addition, α_1 collagen type I transcripts were hardly detected in the HMCs, even after 32 cycles of amplification. This is in agreement with studies reporting the absence of α_1 collagen type I in normal HMCs (26).

DISCUSSION

Evidence of a pathogenetic role for TGF-β in mediating kidney diseases has been demonstrated in

many experimental models. In particular, this cytokine is overexpressed in glomeruli undergoing diabetic sclerosis, and in proximal tubules during progressive chronic failure. In fact, blocking TGF-β_1 activity with specific anti-TGF-β_1 antibodies (27), or antisense RNA therapy against TGF-β_1 (28), delayed the progression of kidney scarring in several animal models, resulting in a reduced synthesis of fibronectin and collagen fibrils. Repressing TGF-β activity by administration of the proteoglycan decorin may be preferable because decorin is a natural inhibitor of TGF-β and has a broader activity against TGF-β isoforms.

Since continuous administration of decorin is not feasible for therapeutic purposes, decorin gene transfer is a valid alternative to obtain in situ, continuous self-production of this therapeutic protein. Until now there have been few, if any, applications of gene therapy to directly treat diseases in the kidney. Due to the microscopic size and the large number of glomeruli, conventional gene therapy techniques have

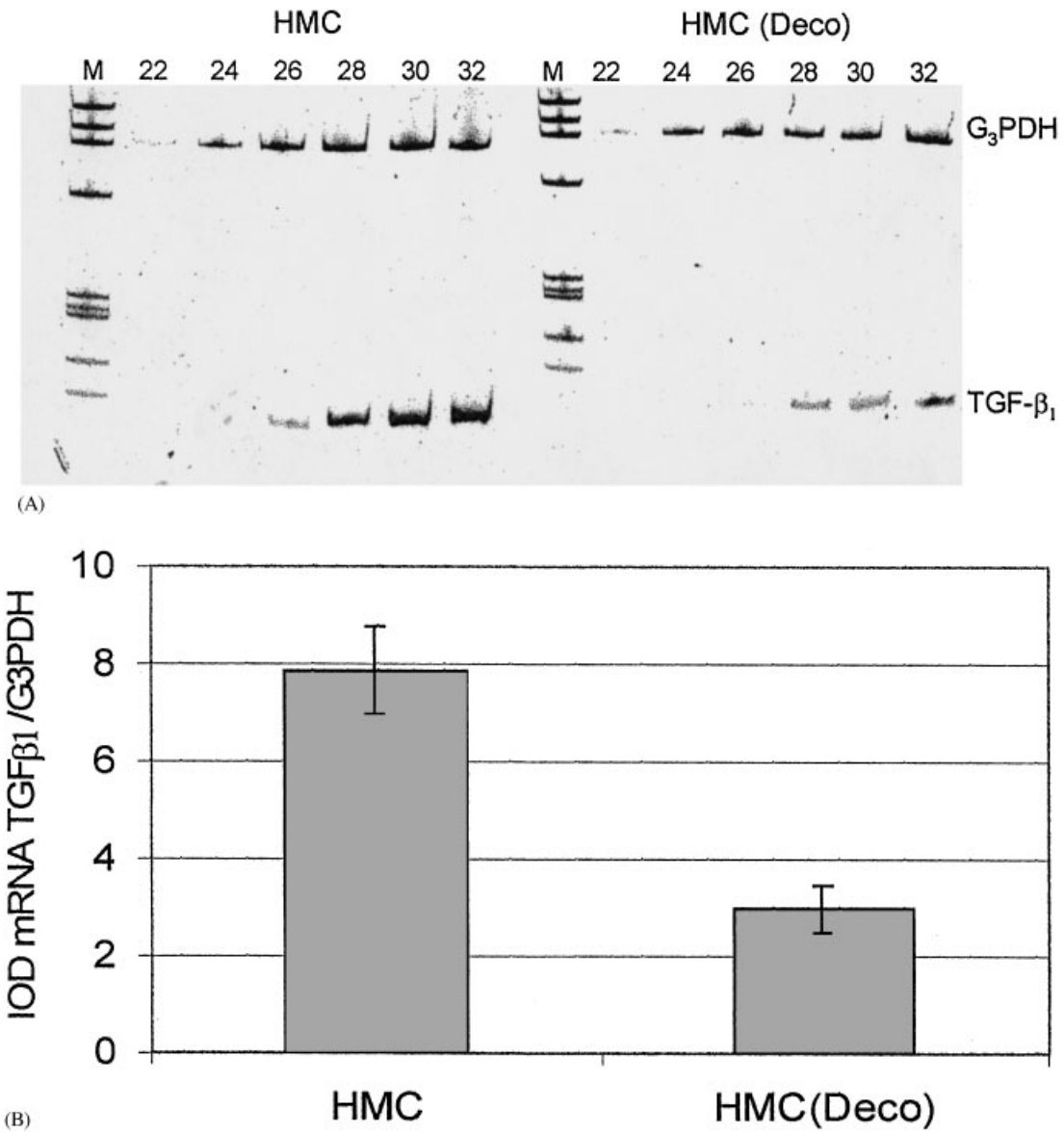

Fig. 3. Expression of TGFβ_1 mRNA in HCM cultures. After RT, samples were amplified by quantitative PCR with primers for TGF-β_1 and G_3PDH. **A:** Typical 161 bp TGF-β_1 and 983 bp G_3PDH PCR products after 22, 24, 26, 28, 30, and 32 cycles of amplification. **B:** Histogram of the TGF-β_1/G_3PDH mRNA ratio, measured as OD obtained from densitometric scanning of silver-stained PCR products. Data are expressed as the mean $\pm$ SD of three experiments using three different primary HMC cultures.

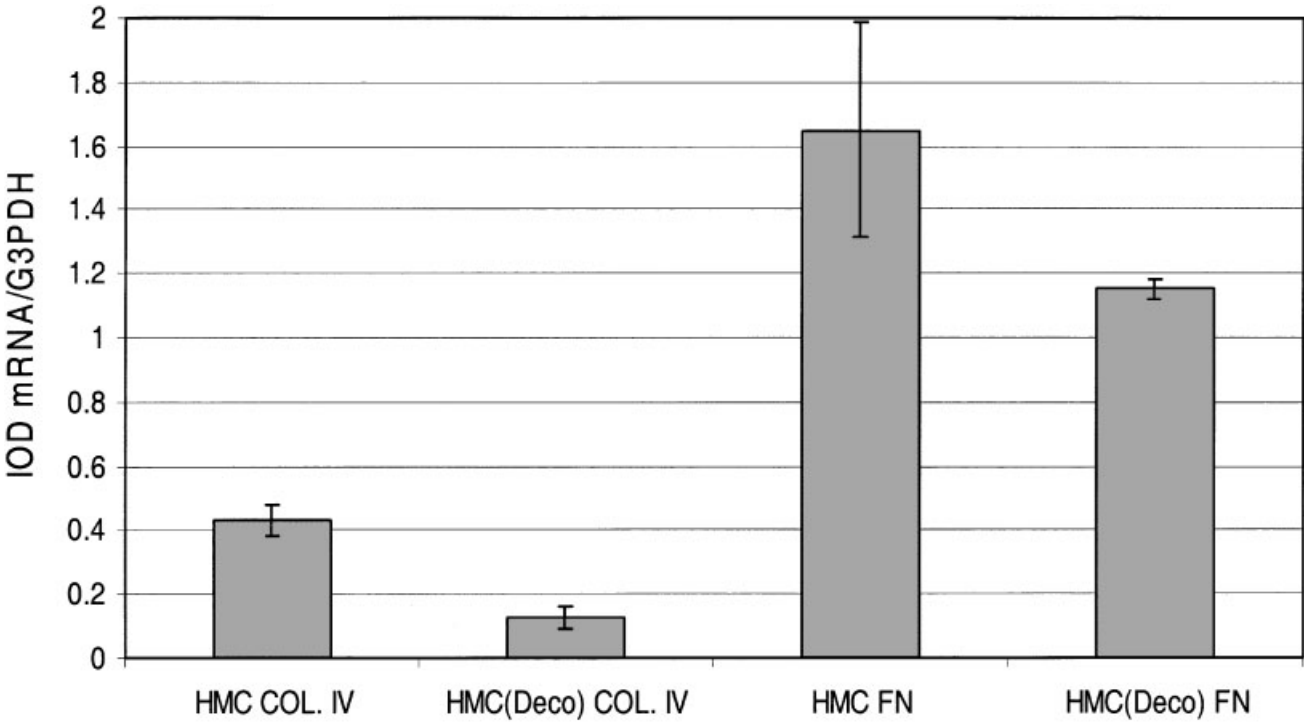

Fig. 4. Expression of α_1 collagen type IV and fibronectin mRNA. α_1 collagen type IV/G_3PDH mRNA ratio, and fibronectin/ G_3PDH mRNA ratio measured as OD obtained from densitometric scanning of silver-stained PCR products. PCR products were analyzed as described in Fig. 3.

been inefficient in delivering genes site-selectively into glomeruli. Recently, Kitamura (22) and colleagues (21) showed, using an animal model, that is possible to deliver genes into glomeruli by engineering ex vivo cultured mesangial cells, which can be subsequently veiculated to the kidney through the renal artery. Since the diameter of glomerular capillaries is smaller than that of mesangial cells, newly introduced cells are entrapped within the glomerulus.

HMCs can be easily established, propagated, and manipulated in vitro. Thus HMCs cultured from biopsy specimens can easily be transfected with exogenous genes and re-implanted into the original organ.

This study is the first to investigate the effect of decorin production in primary HMC cultures. The results demonstrate that increased production of decorin in HMCs, obtained by transfection with the decorin gene, downregulates the expression of TGF-β_1, which is a key mediator of fibrosis. Furthermore, in the HMC model α_1 collagen type IV and fibronectin gene expression was reduced, indicating an effect on matrix accumulation and retardation of fibrosis.

Fibronectin is an abundant protein in the normal mesangial matrix and is greatly enhanced in humans with mesangial proliferative glomerulonephritis. Since fibronectin is strongly induced by TGF-β_1 (29) the observed fibronectin downregulation is probably mediated by TGF-β_1.

Observed TGF-β_1 mRNA reduction, 24 hr after transfection, confirms that decorin does not only repress TGF-β_1 bioactivity by complex formation, but also by repressing TGF-β_1 mRNA transcription.

Recent evidence has shown that in several cell lines TGF-β directly suppress the expression of decorin mRNA and protein (30), and that a TGF-β inhibitor element is present in the P2 promoter region of the decorin gene (15). The absence of decorin promoter in our construct, in which decorin transcription was driven from the CMV promoter, should account for the absence of decorin feedback regulation by TGF-β, and the observed high expression level of the newly introduced decorin gene.

Some studies have reported a surfeit rather than a deficit of decorin in non-inflammatory kidney disease, and have questioned the idea that decorin upregulation may play a role in the progression of the human form of diabetic nephropathy. Nevertheless, increased decorin production in diabetic kidney disease may be a mechanism by which renal cells counteract injury produced by hyperglycemia-stimulated TGF-β (31).

This study is the first to investigate the effect of decorin production in primary HMC cultures, and the

results suggest that decorin gene therapy against renal fibrosis is a promising approach that deserves further investigation.

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