Chapter 6

Upregulation of decorin in minimal change disease may protect against TGF-β-induced glomerulosclerosis and interstitial fibrosis

Joris A. Aben
Martijn A. Verkade
Hans J. Baelde
Michael Eikmans
Jan A. Bruijn
Emile de Heer
Ingeborg M. Bajema

Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands

Submitted for publication
Abstract

Decorin, a proteoglycan with a low molecular weight, binds and counteracts TGF-β. We hypothesize that differences in decorin expression between minimal change disease (MCD) and focal and segmental glomerulosclerosis (FSGS) in the kidney may account for the differences between MCD and FSGS with respect to the development of glomerulosclerosis and tubulo-interstitial fibrosis. Cryostat sections of renal biopsies from patients with MCD, FSGS, normal controls, Wegener’s granulomatosis, lupus nephritis class III/IV, and IgA nephropathy were immunohistochemically stained for decorin and TGF-β. With real-time PCR and in situ hybridization, mRNA expression of decorin and TGF-β were investigated in renal tissue samples from patients with MCD, FSGS, and normal controls.

At the protein level, the lowest expression of decorin was found in FSGS, while the expression of decorin in MCD was significantly higher than in FSGS and in normal controls. These findings were mainly determined by a relatively low tubulo-interstitial expression of decorin in FSGS, and a relatively high glomerular and periglomerular expression of decorin in MCD. mRNA levels for decorin in FSGS were comparable to those in normal controls, whereas mRNA levels for decorin in MCD were significantly higher. At the protein level, total TGF-β was comparable among patient groups, but MCD and FSGS expressed significantly higher levels of TGF-β mRNA.

Our results suggest that in MCD, in which the histological alterations are minimal, the profibrogenic effect of TGF-β is counteracted by decorin.
Introduction

TGF-β has been identified as the main factor responsible for fibrotic changes in response to renal injury. TGF-β is present in normal glomeruli and in a variety of glomerular diseases. TGF-β may be the link between glomerular injury and interstitial fibrosis because, as it is released from the glomerulus into the tubular system, it most likely induces its own production in the interstitium. The finding that the amount of TGF-β in renal biopsies and in urine correlates with the grade of interstitial fibrosis and renal function impairment in various renal diseases is in accordance with this hypothesis. Apart from inducing its own production, TGF-β stimulates the synthesis of proteoglycans, in particular decorin, the natural antagonist of TGF-β. Binding of decorin to TGF-β may result in the inactivation of TGF-β bioactivity. The involvement of decorin in limiting TGF-β overexpression has been suggested on the basis of the therapeutic effects of decorin administered to diseased rats, either by skeletal muscle-delivered gene therapy, mesangial cell-delivered gene therapy, or recombinant protein. In addition, the absence of decorin deteriorates tubulointerstitial fibrosis of obstructed kidneys in decorin-deficient mice. De Cosmo et al. reported allelic variance in the human decorin gene which is related to slower progression of diabetic nephropathy in type 1 diabetic patients. In human fibrosing renal diseases decorin is present in glomeruli, tubulointerstitium and urine. Furthermore, decorin was shown to be the particular extracellular matrix (ECM)-molecule that predicted the severity of interstitial fibrosis and renal failure in a variety of glomerulonephritides.

Minimal change disease (MCD) is the main cause (90% of the cases) of the nephrotic syndrome in children. In adults, MCD accounts for 20-40% of the nephrotic syndrome. By means of light microscopy, MCD is defined by heavy proteinuria in continuation with normal glomeruli and the absence of interstitial fibrosis and by electron microscopy-confirmed obliteration of glomerular epithelial cell pedicles. Focal and segmental glomerulosclerosis (FSGS) is histomorphologically characterized by segmental areas of sclerosis in a minority of the total number of glomeruli in a renal biopsy. FSGS is regarded by some as the worse side of the spectrum of the nephrotic syndrome. There is evidence that in time, MCD may evolve into FSGS: in a study of 49 children with nephrotic syndrome and repeat renal biopsies, 25 originally had MCD which developed into FSGS in a later stage in 14. Despite extensive research, the pathogenesis of both MCD and FSGS is still largely unsolved. The question remains how both ends of the spectrum of nephrotic syndromes develop. The present study investigates the relation between both TGF-β and decorin mRNA and protein expression in MCD and FSGS in comparison to other renal diseases and normal controls.
Chapter 6

Materials and methods

Patients

Patient material consisted of tissue samples from patients with MCD (N=14) and FSGS (N=9). Criteria for MCD included the presence of a nephrotic syndrome (>3.5g/24h), the absence of light microscopic lesions, no deposits of IgG, IgA, IgM or complement components detected by immunofluorescence, and obliteration of glomerular epithelial cell pedicles in the absence of dense deposits confirmed by electron microscopy. Patients classified as MCD, but with macroscopic haematuria and hypertension were excluded. Only FSGS patients with biopsy-proven focal and segmental glomerulosclerosis were included. A disease control group consisted of patients with primary cellular or humoral involvement of the immune system in the pathogenesis of glomerulonephritis, including Wegener’s granulomatosis (N=7), Lupus Nephritis WHO class III/IV (N=6), and IgA nephropathy (N=6). Clinical information of all patients is summarized in Table 1. All biopsies were taken for diagnostic purposes, between the period of 1990 and 1998. Consecutive patients were included in this study, of whom the biopsies had at least 10 glomeruli. Patient studies were approved by the institutional ethical committee.

A normal control group consisted of tissues from 8 cadaveric donor kidneys considered inadequate for transplantation because of technical reasons. Clinical data of these cadaveric donors are unknown.

<table>
<thead>
<tr>
<th>Disease Control</th>
<th>No.</th>
<th>Age (yr)</th>
<th>Sex (F/M)</th>
<th>GFR (mL/min)</th>
<th>Serum creat (µM)</th>
<th>Proteinuria (mg/24h)</th>
<th>Serum albumin (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA nephropathy</td>
<td>6</td>
<td>28.3 ± 12.7 (1.9)</td>
<td>66.3 ± 32.9</td>
<td>114.3 ± 38.9</td>
<td>2.8 ± 3.1</td>
<td>41.0 ± 4.4</td>
<td></td>
</tr>
<tr>
<td>Wegener’s granulomatosis</td>
<td>7</td>
<td>50.4 ± 12.9 (3.4)</td>
<td>57.3 ± 15.3</td>
<td>210.3 ± 241.9</td>
<td>0.9 ± 0.6</td>
<td>30.6 ± 4.4</td>
<td></td>
</tr>
<tr>
<td>Lupus Nephritis</td>
<td>6</td>
<td>30.7 ± 18.0 (2.7)</td>
<td>67.1 ± 71.4</td>
<td>117.7 ± 98</td>
<td>3.8 ± 1.2</td>
<td>35.8 ± 9.4</td>
<td></td>
</tr>
</tbody>
</table>

Normal Control 8

*MCD: minimal change disease, FSGS: Focal and segmental glomerulosclerosis*  
*Data are presented as means ± SD* 
*Normal control group consisted of tissues from 8 cadaveric donor kidneys considered inadequate for transplantation because of technical reasons. Clinical data of these cadaveric donors are unknown.*
Clinical Parameters
Proteinuria (g/24 hours), serum creatinine (µmol/L), glomerular filtration rate (GFR) and serum albumin (g/L) at time of biopsy were included in the final analysis which relates these data to those of the results obtained with immunohistochemistry and Real-Time PCR (Q-PCR).

Antibodies
Rabbit anti-human TGF-ß (DAKO, Glostrup, Denmark) and rabbit anti-human decorin (Telios, San Diego, CA, USA) polyclonal antibodies were used as primary antibodies, with peroxidase-labeled swine anti-rabbit IgG (DAKO, Glostrup, Denmark) as secondary antibodies. All antibodies were diluted by a factor 1:100 in bovine serum albumin (Boseral, Organon, Oss, The Netherlands). Embryonic rat kidney tissue was used as positive control for rabbit anti-human TGF-ß, while normal renal tissue was used as a positive control for rabbit anti-human decorin. Phosphate-buffered saline (PBS) was used as a negative control for the primary antibodies.

Immunohistochemical Staining
After the standard tests required for diagnostic purposes were performed, the snap frozen biopsy material was stored at -70ºC. The tissue samples were cut into sections of four micrometers thick and mounted on Starfrost glass slides. Sections were air dried at room temperature for one hour, and fixed in acetone for 5 minutes. Immediately after pre-incubation for 15 minutes with normal goat serum (NGS) that had been diluted by a factor 1:10 in bovine serum albumin (Boseral, Organon, Oss, The Netherlands), incubation with anti-human TGF-ß or anti-human decorin was performed in a dark, moisturized box for 60 minutes at room temperature. To exclude inter-assay variation, staining with anti-human TGF-ß and anti-human decorin was performed on all sections in one experiment simultaneously. After three washings with PBS of 5 minutes each, the secondary antibody, containing 1% normal human serum (NHS), was incubated for 30 minutes. After the second step, the sections were washed three times with PBS again. They were incubated with 2.5 mg 3,3-diamino-benzidine (Fluka, Zwijndrecht, The Netherlands) in 50 ml of 0.5 M TRIS-HCL (pH=7.6), rinsed in PBS, counterstained by Mayer’s haematoxylin for 15 seconds, and rinsed in running tap water for 5 minutes. The sections were mounted in mannitol.

Image-analysis
An image-analysis system (Zeiss Axioscope) equipped with a Sony color video CCD camera (model DXC 950p) and connected to a computer with Kontron Vision KS400 software.
(version 3.0) was used to analyze the stained sections. To exclude variations in the intensity of light between series of recordings, light-standardization was performed by the program. Immunohistochemically stained sections were included for image analysis if at least three whole glomeruli were measurable and at least two recordings of different areas of the tubulo-interstitium could be made of the section at a 10-fold magnification factor. We investigated TGF-ß and decorin in three cortical areas: the periglomerular area, the glomerular area, and the tubulo-interstitium. From the inside of Bowman’s capsule, an area with a diameter similar to that of one tubule (average tubular diameter derived from 25 randomly selected samples) was defined as the periglomerular area. The amount of staining was measured and expressed as a percentage of the area (0-100%).

**Tubulo-interstitial Fibrosis**

Silver-stained sections were used to score the presence of tubulo-interstitial fibrosis. Biopsies either showed mild interstitial fibrosis or no interstitial fibrosis. Extensive interstitial fibrosis was never present. It was therefore scored semi-quantitatively as – or +.

**Real-Time Polymerase Chain Reaction (Q-PCR)**

Cortical mRNA levels for TGF-ß, decorin, and the housekeeping gene GAPDH were quantitated with real-time PCR (Perkin-Elmer Prism 7700™ Sequence Detector System) in renal biopsies from patients with MCD (N=12), FSGS (N=4), and normal controls (N=8). The primers (In Vitrogen, Gaithersburg, MD, USA) and TaqMan™ probe (Perkin-Elmer Life Science, Hounslow, UK) for each molecule were, respectively: 5’–CCC AGC ATC TGC AAA GCT C-3’, 5’–GTC AA T GT A CAG CTG CCG CA-3’, and 5’–ACA CCA ACT ATT GCT TCA GCT CCA CGG A-3’ (TGF-ß); 5’–ACA TCC GCA TTG CTG A TA CCA-3’, 5’–AGT CCT TTC AGG CTA GCT GCA TC-3’, and 5’–TCA CCA GCA TTC CTC AAG GTC TTC CTC C-3’ (decorin); 5’–TTC CAG GAG CGA GA T CCC T-3’, 5’–CAC CCA TGA CGA ACA TGG G-3’, and 5’–CCC AGC CTT CTC CAT GGT GGT GAA-3’ (GAPDH). For each transcript, the probe sequence was chosen over an exon-intron junction in order to prevent amplification of genomic DNA. The 5’ ends of the decorin and GAPDH probe were labeled by the reporter dye 6-carboxyfluorescein (FAM) and the 5’ end of the TGF-ß probe by tetrachlore-6-carboxyfluorescein (TET). The 3’ ends of the probes were labeled by the quencher dye 6-carboxy-tetramethylrhodamine (TAMRA). The PCR reaction contained 100 nM (GAPDH) or 80 nM (TGF-ß and decorin) of the probe, 300 nM of both primers, and TaqMan Universal PCR Master Mix (Perkin-Elmer Life Science, Hounslow, UK) including 1.25 U of AmpliTaq Gold DNA polymerase, 300 mM dNTP, 0.5 U of AmpErase UNG, and 2.5 mM MgCl₂. Optical 96-well reaction plates covered with optical caps (Perkin-Elmer Life Science,
Hounslow, UK) were used to carry out the reactions. Amplification cycles were 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and at 60°C for 1 minute. The point at which the intensity of fluorescence exceeded the standard deviation of the baseline fluorescence was a measure for the amount of cDNA, and thus for mRNA, in the sample. All measurements were performed in duplicate.

In situ hybridization (ISH) for TGF-β and Decorin mRNA
Renal biopsies from patients with MCD (N=14), FSGS (N=8), and normal controls (N=5) were fixed in 4% buffered formaldehyde for at least 24 hours and embedded in paraffin. Sections of four micrometers thick were placed on Superfrost® Plus slides and dried overnight at 37°C. For TGF-β, a 484 bp riboprobe was transcribed from a cDNA construct as described elsewhere (36). A 349 bp PCR product of decorin cDNA was cloned into a pGEM 3Zf- vector (Promega, Madison, USA) and used as a template for decorin. Sense and anti-sense digoxigenin (DIG)-labeled RNA probes were produced by in vitro transcription with T7 and T3 (TGF-β) or SP6 and T7 (decorin) RNA polymerase (Roche Diagnostics, Mannheim, Germany). Prior to hybridization, sections were pretreated with 5 µg/µl proteinase K for 15 min. at 37°C. To block non-specific binding sites, sections were then incubated with 10 mM dithiothreitol (DTT), which was followed by incubation with acetic anhydride in triethanolamine. Hybridization was performed at 62°C for 16 hours with 100 ng/ml riboprobe in 50% formamide, 250 µg/ml salmon sperm DNA, 1 mg/ml tRNA, 10 mM DTT, 1x Denhardt’s solution, 10% dextran sulphate, and 2x SSC. Sections were washed with 50% formamide in 2x SSC at 37°C for 30 min. and 20 mM β-mercaptoethanol in 0.1 SSC, which was followed by treatment with 1U/ml RNase T1 (Roche Diagnostics, Mannheim, Germany). DIG-labeled hybrids were detected with mouse anti-DIG, rabbit anti-mouse and APAAP complex (DAKO Denmark) in 1% blocking buffer (Roche Diagnostics, Mannheim, Germany) with 2% normal rabbit serum. This procedure was followed by incubation with nitroblue tetrazolium, bicholylindolyl and 1% levamisole (Vector, Burlingame, USA).

Statistical Analyses
Differences in decorin and TGF-β protein expression and mRNA levels among patient groups were analyzed by one-way analysis of variance (ANOVA) making use of the Bonferroni correction. Correlations between image-analyzed, Q-PCR derived data and clinical parameters were evaluated with Pearson’s correlation coefficient and Spearman’s correlation coefficient respectively. The presence of tubulo-interstitial fibrosis was correlated with the data that had been obtained through image analysis with Spearman’s correlation coefficient. P< 0.05 was considered statistically significant.
Figure 1. Total (A), periglomerular (B), glomerular (C), and tubulo-interstitial (D) immunohistochemical scores of decorin in minimal change disease (MCD), focal and segmental glomerulosclerosis (FSGS), normal controls (norm), IgA nephropathy (IgA), Wegener’s granulomatosis (WG), and lupus nephritis (LN). Scored by means of computer controlled image-analysis, expressed as the area of percentage (%) on the Y-axis. In MCD, total decorin levels (A) are significantly higher than those in FSGS (p<0.03) and to those in NC (p<0.0001). The latter is reflected both in the periglomerular (B; p<0.001) and in the glomerular area (C; p<0.03). Only in FSGS, tubulo-interstitial decorin (D) was at a significantly lower level than in normal controls (p<0.003). In LN we found similar immunohistochemical staining of decorin as in MCD.

Results

Decorin protein expression

Although the total expression of decorin (glomerular, periglomerular and interstitial areas) tended to be higher in all patient groups than in normal controls, this difference only reached a statistical significance for MCD (Fig. 1A). This was reflected in both a difference in the percentage of staining in the periglomerular area (Fig. 1B) and in the glomeruli (Fig. 1C). In the tubulointerstitial area there was no difference in decorin expression between MCD and normal controls (Fig. 1D). In MCD, total decorin expression was significantly higher than in FSGS (Fig. 1A), although this difference did not reach statistical significance in the separate
Figure 2. Representative illustrations of decorin staining in MCD and FSGS renal biopsies. A relatively high glomerular and periglomerular expression of decorin in MCD (A), and a relatively low tubulo-interstitial expression of decorin in FSGS (B).

histological areas (Fig. 1B to D). FSGS showed the lowest total decorin expression of all disease groups, which was comparable to that of normal controls (Fig. 1A). The tubulo-interstitial expression of decorin in FSGS was even significantly lower than in normal controls, in contrast to all other diseases (Fig. 1D). Typical examples of decorin expression in MCD and FSGS are reflected in Figures 2A and 2B, respectively.

Patients with IgA nephropathy and Wegener’s granulomatosis had a significantly higher tubulo-interstitial expression of decorin than patients in the other disease groups (Fig. 1D). Only in lupus nephritis we found a similar immunohistochemical expression of glomerular and periglomerular decorin as in MCD (Fig. 1B and C).

TGF-β protein expression
Total TGF-β expression was essentially the same in all disease groups and normal controls, with the exception of lupus nephritis which had a relatively high TGF-β expression in comparison to normal controls (Fig. 3), reflected by a significantly higher expression of TGF-β1 in the glomerular and periglomerular areas (data not shown). MCD and FSGS did not differ significantly in their expression of TGF-β. They did not differ significantly from normal controls either (Fig. 3).

TGF-β and decorin cortical mRNA
Cortical mRNA levels for TGF-β were significantly higher in MCD and FSGS than in normal controls (Fig. 4A). The mRNA levels for decorin in MCD were significantly higher than in normal controls. Cortical mRNA levels for decorin in FSGS were comparable to those in normal controls (Fig. 4B). The cortical mRNA ratio of TGF-β:decorin differed significantly between MCD and FSGS (Fig. 4C) and between MCD and normal controls (Fig. 4C). The TGF-β:decorin ratio in FSGS did not differ from that in normal controls.
Figure 3. Total immunohistochemical staining of TGF-ß scored by means of image-analysis in minimal change disease (MCD), focal and segmental glomerulosclerosis (FSGS), normal controls (norm), IgA nephropathy (IgA), Wegener’s granulomatosis (WG), and lupus nephritis (LN). Y-axis presents the area of percentage (%). Similar results were found between various disease groups with the exception of LN which had a relatively high TGF-ß expression in comparison to that in normal controls (p<0.007), which was reflected by a significantly increased glomerular (p<0.013) and periglomerular (p<0.002) TGF-ß (not illustrated).

Figure 4. Cortical mRNA levels of TGF-ß/GAPDH in minimal change disease (MCD), focal and segmental glomerulosclerosis (FSGS), and normal controls (norm). TGF-ß/GAPDH mRNA ratio is 2-fold (p<0.01) increased in MCD and 3-fold increased (p<0.001) in FSGS compared to that in NC (A). Cortical mRNA levels of decorin/GAPDH in minimal change disease (MCD), focal and segmental glomerulosclerosis (FSGS), and normal controls (norm). Decorin/GAPDH mRNA ratio is 6.5-fold (p<0.001) increased in MCD in comparison to normal controls (B). TGF-ß/decorin ratio in minimal change disease (MCD), focal and segmental glomerulosclerosis (FSGS), and normal controls (norm). TGF-ß/decorin mRNA ratio is 2-fold (p<0.05) decreased in MCD in comparison to that in FSGS and 2.5-fold decreased (p<0.01) compared to that in normal controls (C).
Clinical data

In MCD, there was a significant correlation between decorin mRNA levels and serum albumin (Fig. 5A) and between the TGF-β:decorin mRNA ratio and serum albumin (Fig. 5B). At the protein level, decorin and TGF-β did not correlate with any of the studied clinical parameters, including creatinine clearance (GFR). However, in the disease- and disease control group the tubulo-interstitial TGF-β:decorin protein ratio correlated significantly with the presence of tubulo-interstitial fibrosis (Fig. 5C).

In situ hybridization

In normal controls, in situ hybridization with a probe for TGF-β demonstrated TGF-β mRNA in mesangial cells, parietal epithelial cells and tubular epithelial cells. In distal tubular
Figure 6. *In situ* hybridization analysis of TGF-β mRNA expression in normal controls (B), minimal change disease (C), and focal and segmental glomerulosclerosis (D). There are no differences between the observed groups in localization of TGF-β transcripts. The TGF-β antisense probe binds to mesangial cells, parietal epithelial cells and tubular epithelial cells. Distal epithelial cells seem to have a higher transcription level than proximal tubular epithelial cells (B). No binding is observed with the TGF-β sense probe (A).

Figure 7. *In situ* hybridization analysis of decorin mRNA expression in normal controls (B), minimal change disease (C), and focal and segmental glomerulosclerosis (D). There are no differences in localization of decorin transcripts between the observed groups. The decorin antisense probe binds to mesangial cells, parietal epithelial cells and tubular epithelial cells. No binding is observed with the decorin sense probe (A).
epithelial cells more TGF-β mRNA was detectable than in proximal tubular epithelial cells (Fig. 6B). TGF-β mRNA varied in intensity between normal controls. However, no difference in localization of TGF-β transcripts was found between normal controls, MCD (Fig. 6C), and FSGS (Fig. 6D). In the tubulointerstitium, TGF-β mRNA was only observed in the presence of interstitial infiltrates (Fig. 6D).

*In situ* hybridization for decorin rendered results that were similar to those for TGF-β in normal controls, MCD, and FSGS (Fig. 7, B, C, and D, respectively). In all control tissues, decorin mRNA was confined to a few mesangial cells and parietal epithelial cells (Fig. 6B). Both in MCD and in FSGS, a higher number of mesangial cells, parietal epithelial cells and tubular epithelial cells expressed decorin mRNA than in normal controls. Within the interstitial compartment a few fibroblasts demonstrated decorin mRNA. Vessel walls (mainly intima) were strongly positive for decorin mRNA.

**Discussion**

This study shows that in MCD, decorin protein and mRNA expression are significantly upregulated in comparison to normal controls. In FSGS, a downregulation of decorin protein in the tubulointerstitium was found in comparison to normal controls. This finding help to further define the essential difference between MCD and FSGS. MCD and FSGS are clinically comparable in the presentation of a nephrotic syndrome and histomorphologically comparable to an extent that some authors regard them as two sides of a spectrum. The completely opposite patterns of decorin expression may be responsible for the phenomenon that FSGS, is more likely to progress to extensive glomerulosclerosis and interstitial and consequently to end stage renal failure.

Based on our results, there are various hypotheses as to why decorin is upregulated specifically in MCD. One possibility is that increased decorin levels in MCD patients may be a result of enhanced decorin transcription, as the results derived from real-time PCR and *in situ* hybridization appear to suggest. Decorin transcription may in its turn be influenced by cytokines, proteinuria, and corticosteroids, which is in accordance with the findings that proteinuria coincides with increased expression of genes that promote interstitial inflammation. It is known that dexamethason prevents the downregulation of decorin with TGF-β as an intermediating factor. Therefore, the final amount of decorin protein is most probably the result of the cumulative effects of many parameters, which may vary under different conditions. TGF-β, for instance, has been reported to inhibit decorin gene expression in fibroblast cultures, whereas in cultured mesangial cells, production of decorin was increased after treatment with TGF-β.
Alternatively, disregarding the possibility of enhanced transcription, there could be less decorin degradation in MCD than in FSGS. Imai et al., for instance, described proteolytic degradation of decorin by matrix metalloproteinase MMP-3 and MMP-7, which in turn are downregulated by TGF-β. If the MMP profiles of patients with MCD and FSGS differ, it is conceivable that in FSGS more cleaving of decorin would occur than in MCD. This might be an explanation for the fact that FSGS has the lowest overall expression of decorin, significantly lower than in MCD. This, however, has not been investigated so far. Another possibility would be that the proteolytic degradation of decorin is impeded when it is bound to TGF-β. In this scenario, more complexes would be present in MCD, and less TGF-β would be released from MCD.

Although there currently is little or no evidence for all possible pathogenetic mechanisms suggested above, it appears that TGF-β plays a pivotal role in most of them. Our observation that the amount of TGF-β protein did not significantly differ between FSGS patients and normal controls, is in contrast to previous observations showing that TGF-β expression was higher in patients with FSGS than in normal controls, and similar to the expression in patients with IgA nephropathy, lupus nephritis, and Wegener’s granulomatosis. However, we found higher TGF-β mRNA levels in FSGS and MCD than in normal controls. These findings may indicate that mRNA upregulation of TGF-β is a non-specific event in renal diseases in general, whereas the amount of decorin counteraction specifically determines renal outcome. Additional evidence for this hypothesis is provided by the large variation in mRNA levels for TGF-β in normal controls, which emphasizes its non-specific nature, and which is in sharp contrast to the more or less similar mRNA levels for decorin in the same controls.

The present findings have a relevant clinical purpose. In most centers, children with a nephrotic syndrome are primarily treated with corticosteroids without undergoing a renal biopsy, which categorizes them into a clinically responding and non-responding group. Renal biopsies are only taken in those who do not respond to therapy, after which in most cases they are diagnosed as either MCD or FSGS patients, with their different prognoses. If decorin levels would separate the responders from the non-responders in the initial stages of the disease, the non-responders could either be spared the possible harmful consequences of corticosteroid therapy or, alternatively, they might benefit from a more aggressive therapy from the beginning.

In conclusion, the present study has demonstrated that decorin protein expression is increased in the glomerular and periglomerular areas in MCD, whereas in FSGS, the expression of decorin in the tubulo-interstitium is low. The increased decorin protein expression partly seemed to be a result of increased renal cortical decorin mRNA transcription.
One of the limitations of our study is that within the expression of cortical decorin mRNA, we could not differentiate between glomerular and tubular decorin mRNA. Our results may indicate that in MCD, in which the histological alterations are minimal, the fibrotic effects of TGF-ß are successfully counteracted by decorin, which provides an explanation for the absence of glomerulosclerosis. In FSGS, the low expression of decorin in the tubulo-interstitium may represent a failure to respond to the presence of TGF-ß, and it may be responsible for the occurrence of interstitial fibrosis, which determines the poor renal outcome in these patients. Our finding that the presence of tubulo-interstitial fibrosis correlates significantly with the TGF-ß/decorin protein ratio emphasizes this. Furthermore, our results corroborate the potential role of decorin as a natural therapeutic or prophylactic agent in human renal diseases characterized by glomerulosclerosis and tubulo-interstitial fibrosis, as has been suggested previously. The predictive and diagnostic values of decorin levels in renal biopsies need to be investigated in a prospective study.

Acknowledgements


References