DECORIN DEFICIENCY IN DIABETIC MICE: AGGRAVATION OF NEPHROPATHY DUE TO OVEREXPRESSION OF PROFIBROTIC FACTORS, ENHANCED APOPTOSIS AND MONONUCLEAR CELL INFILTRATION

Although deficiency of the small leucine-rich proteoglycan decorin aggravates diabetic nephropathy in mice, the precise mechanisms of action are not fully understood. In the present study we used decorin-deficient mice (Dcn−/−) to further elucidate the molecular mechanisms involved in the protective action of decorin in diabetes. We discovered that streptozotocin-induced diabetes in Dcn−/− mice led to increased proteinuria associated with enhanced cyclin-dependent kinase inhibitor p27Kip1 in podocytes and tubular epithelial cells. Furthermore, lack of decorin increased the rate of apoptosis and caused overexpression of the IGF-IR in tubular epithelial cells of diabetic kidneys. In vitro experiments using human proximal renal epithelial cells showed that recombinant decorin was bound to the IGF-IR and protected against high glucose-mediated apoptosis. Furthermore, overexpression of TGFβ1 and CTGF triggered by decorin deficiency resulted in enhanced accumulation of extracellular matrix in diabetic kidneys. Notably, diabetic Dcn−/− kidneys revealed marked upregulation of the proinflammatory proteoglycan biglycan and enhanced infiltration of mononuclear cells. Collectively, our results indicate that decorin is a protective agent during the development of diabetic nephropathy. Future therapeutic approaches that would either enhance the endogenous production of decorin or deliver recombinant decorin to the diseased kidney might improve the outcome of patients with diabetic nephropathy.

Key words: small leucine-rich proteoglycan, kidney, apoptosis, insulin-like growth factor-I receptor, mononuclear infiltrating cells

INTRODUCTION

Diabetic kidney disease is the leading cause of end stage renal disease (ESRD) in the Western world; it results in reduced life expectancy and is responsible for nearly half of all new ESRD cases in the United States (1). Loss of podocytes, enhanced glomerular and tubulointerstitial accumulation of extracellular matrix, apoptosis of tubular epithelial cells, infiltration of blood mononuclear cells and proliferation of interstitial mesenchymal cells are hallmark features of diabetic nephropathy (2, 3). The clinical manifestations are characterized by hyperfiltration, renal hypertrophy, progressively increasing proteinuria and deterioration of renal function (4). Despite the relevance of diabetic nephropathy, the mechanisms of its development, in particular in early stages are not fully understood. Both in vivo and in vitro studies strongly implicate transforming growth factor β (TGFβ) and its downstream mediator connective tissue growth factor (CTGF) as key players in the pathogenesis of this disease (5, 6). Recent evidence underscores the role of the cyclin-dependent kinase inhibitors p21Cip1/Waf1 and p27Kip1 as important mediators of early structural and functional renal abnormalities in diabetic nephropathy, including podocyte injury with subsequent proteinuria and renal hypertrophy (7, 8).

Decorin belongs to the family of small leucine-rich proteoglycans (SLRPs) and is characterised by a core protein with leucine-rich repeat motifs flanked by cysteine clusters and one chondroitin/dermatan-sulphate side chain (9). It is overexpressed in diabetic kidneys (10) and has been suggested to act as a protective factor (11). In vitro experiments using human proximal renal epithelial cells showed that recombinant decorin was bound to the IGF-IR and protected against high glucose-mediated apoptosis. Furthermore, overexpression of TGFβ1 and CTGF triggered by decorin deficiency resulted in enhanced accumulation of extracellular matrix in diabetic kidneys. Notably, diabetic Dcn−/− kidneys revealed marked upregulation of the proinflammatory proteoglycan biglycan and enhanced infiltration of mononuclear cells. Collectively, our results indicate that decorin is a protective agent during the development of diabetic nephropathy. Future therapeutic approaches that would either enhance the endogenous production of decorin or deliver recombinant decorin to the diseased kidney might improve the outcome of patients with diabetic nephropathy.
kinase as well as p21^{Cip1/Waf1} and p27^{Kip1} downstream of their cognate receptors (9). In contrast, biglycan, the closest SLRP for the Toll-like receptors 2 and 4 in macrophages. Upon its release from the extracellular matrix, it signals through the MAP kinases p38 and ERK and through the NF-κB pathways (21) resulting in enhanced infiltration of mononuclear cells into the lung in experimental sepsis and into the kidney in unilateral ureteral obstruction (21, 22).

In this study, we used the model of streptozotocin-induced diabetes type 1 in wild-type (Dcn+/+) and decorin-deficient (Dcn−/−) mice to further elucidate the role of decorin in diabetic nephropathy. Here, we show that in the diabetic kidney decorin deficiency is associated with: i) podocytopathy overexpression of p27^{Kip1} and enhanced proteinuria, ii) enhanced expression of TGFβ1 and CTGF resulting in increased accumulation of ECM, iii) overexpression of biglycan and elevated infiltration of mononuclear cells, iv) enhanced apoptosis of tubular epithelial cells despite overexpression of tubular IGF-IR. We further discovered that decorin binds to the IGF-IR in tubular epithelial cells and conveys protection against high glucose-mediated apoptosis. Collectively, our results provide strong evidence for a protective role of decorin during diabetic nephropathy development and suggest that the enhanced expression of decorin, either from endogenous or exogenous sources, might improve the prognosis of this devastating chronic disease.

MATERIALS AND METHODS

Animals

All animal experiments were conducted in accordance with the German Animal Protection Act and were approved by the Ethics Review Committee for laboratory animals of the District Governments of Muenster and Darmstadt, Germany. To induce type 1 diabetes, two-month-old male Dcn−/− and Dcn−/− mice were intraperitoneally injected with streptozotocin (STZ) (Sigma-Aldrich, Deisenhofen, Germany) (45 mg per kg of body weight) dissolved in 100 mmol/l sodium citrate buffer (pH 4.5) for four consecutive days (20). Control animals were injected only with citrate buffer. Mice that developed glucosuria received a consecutive days (20). Antibodies used for Western blots were rabbit-anti-human decorin (20), rabbit anti-β-chain of the IGF-IR (sc-713) and rabbit anti-β-tubulin (both from Santa Cruz Biotechnologies). Protein bands were visualized using the ECL Western blot detection reagents (GE Healthcare, Buckinghamshire, UK). Results from kidney samples are expressed as optical density normalized by β-tubulin. For quantification, the results of 3 samples per group were averaged.

Northern blot analysis

Total RNA extraction from whole kidneys and the 32P-labelled cDNA probe used for decorin has been described before (24). cDNAs for TGFβ1, CTGF and GAPDH were from American Type Culture Collection (ATCC) (Rockville, MD). Northern blots were performed and analyzed as described earlier (20). Quantification was performed with a STORM860 Phosphor Imager using IQ Solutions Image Quant software (both from Molecular Dynamics, Uppsala, Sweden). Each individual mRNA band was normalized to GAPDH. Values are given as means SEM from three Northern blots.

Production and purification of decorin

Expression of recombinant human decorin and purification of the native proteoglycan was performed as described earlier (25). The purity of decorin was checked by SDS-PAGE and silver staining. For some experiments the proteoglycans were digested with chondroitinase ABC (Seikagaku Kogyo, Tokyo, Japan) to remove the chondroitin sulfate and dermatan sulfate chains (26).

Cell culture and stimulation

Normal human proximal renal epithelial cells (HK-2), obtained from the American Type Culture Collection (ATCC) were cultured in Dulbecco’s modified eagles medium (DMEM/F12) (ScienCell Research Laboratories, Carlsbad, CA) supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 U/ml penicillin, 100 g/ml streptomycin (both from GibCO BRL Life Technologies, Grand Island, N.Y) and Insulin-Transferrin-Selenite Supplement (Roche Applied Science). HK-2 cells were maintained in serum-free DMEM/F12 supplemented with 0.1 mg/ml of fatty acid-free bovine serum albumin for 24 hours, to obtain quiescence. After changing media, cells were cultured with either 5 nM or 30 nM D-glucose for 24 hours. With the next replenishment of culture media, intact decorin at a concentration of 4 μg/ml was added and the cells were incubated for 24 hours. For osmotic controls, cells were cultured in equiosmolar concentrations of L-glucose assayed by TUNEL-staining (Roche Applied Science) as per the manufacturer’s instructions. Counterstaining was with methyl green or hematoxylin. The specificity of immunostaining was confirmed using negative controls by omitting the primary antibody and by using non-immune serum/unspecific IgG.

To evaluate individual kidneys, 10 randomly selected non-overlapping fields of renal sections were examined under high-power field (HPF) magnification as indicated and the extent of extracellular matrix accumulation, number of apoptotic nuclei, infiltrating mononuclear cells, Ki67−, p21^{Cip1/Waf1}−, and p27^{Kip1}− positive cells were evaluated using an image analysing system (Soft Imaging System; Olympus, Tokyo, Japan). Mean values of at least 5 kidneys per group were averaged.

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and mannitol (Sigma-Aldrich), respectively. Viability of cells was not altered under these conditions, as determined by lactate dehydrogenase release into the culture supernatant using a cytoxicity detection kit (Roche Applied Science). The levels of histone-associated DNA fragments were assayed and quantified using the Cell Death Enzyme-Linked Immunosorbent Assay kit (Roche Applied Science), as per the manufacturers instructions.

**Immunoprecipitation**

The IGF-IR from HK-2 cells was immunoprecipitated as described previously (20). Briefly, HK-2 cells (10^6) were incubated with 8 µg of intact human decorin or 8 µg of decorin protein core for 2 hours at 4°C, followed by incubation with 1 mM 3,3’-Dithiobis(sulfosuccinimidylpropionate), a thiol-cleavable, primary amino-reactive cross-linker (Pierce Biotechnology, Rockford, IL) used for cross-linking of decorin with cell surface proteins. Cells were harvested in lysis-buffer and immunoprecipitated with 10 µg of the rabbit anti-IGF-IR (sc-713, Santa Cruz). As control, the same amount of protein A-Sepharose (GE Healthcare) was incubated with the antibody in the absence of cell lysate. Additional controls included samples incubated without cross-linker and/or without decorin respectively. The eluted samples were analyzed by Western blot for the presence of decorin and IGF-IR.

**Statistics**

Data are given as means ± SD analyzed by one-way analysis of variance (ANOVA), with Dunnett’s significance correction test (SPSS software). Differences were considered significant at P-values <0.05.

**RESULTS**

Enhanced p27Kip1 in podocytes is associated with increased urinary protein excretion in Dcn−/− diabetic mice

STZ-injected Dcn+/+ and Dcn−/− mice were monitored for clinical signs at early (30 days) and advanced (9 weeks) stages of disease progression. Diabetic mice of both genotypes showed increased hyperglycemia, proteinuria, and decreased renal and body weight compared to non-diabetic mice. The proteinuria levels were significantly higher in Dcn−/− diabetic mice compared to Dcn+/+ diabetic mice within 30 days of STZ injection (Table 1). These observations indicate a more severe diabetic phenotype in the absence of decorin. The expression of p27Kip1, a cell cycle regulatory protein, was increased in podocytes from Dcn−/− diabetic kidneys (Fig. 1).

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**Table 1. Effects of STZ-induced diabetes (30 days, 9 weeks) on kidney weight, blood glucose levels and proteinuria in Dcn+/+ and Dcn−/− mice**

<table>
<thead>
<tr>
<th></th>
<th>30 days</th>
<th>9 weeks</th>
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<tr>
<td></td>
<td>Dcn+/+</td>
<td>Dcn−/−</td>
</tr>
<tr>
<td>Body weight, start (g)</td>
<td>26.1 ± 1.8</td>
<td>25.3 ± 1.6</td>
</tr>
<tr>
<td>Body weight, end (g)</td>
<td>27.6 ± 1.7</td>
<td>27.1 ± 1.1</td>
</tr>
<tr>
<td>Kidney weight (mg)</td>
<td>220 ± 20</td>
<td>212 ± 12</td>
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<tr>
<td>(Kidney weight/ Body weight) x 10^7</td>
<td>8.1 ± 0.8</td>
<td>7.8 ± 0.7</td>
</tr>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>94 ± 9</td>
<td>95 ± 8</td>
</tr>
<tr>
<td>Proteinuria (mg/mg creatinine)</td>
<td>0.41 ± 0.13</td>
<td>0.38 ± 0.14</td>
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p<0.05; *, for diabetic Dcn−/− versus non-diabetic Dcn+/+ mice; **, for diabetic Dcn−/− versus non-diabetic Dcn−/− mice; †, for diabetic Dcn−/+ versus diabetic Dcn−/− mice.

Data are given as means ± SEM.

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*Fig. 1. Enhanced p27Kip1 in Dcn−/− diabetic kidneys. (A) Immunostaining for p27Kip1 in glomeruli of non-diabetic and diabetic Dcn−/− and Dcn+/+ kidneys after 9 weeks of STZ-diabetes. (B) Quantification of p27Kip1 positive nuclei in glomeruli and (C) tubular epithelial cells (TEC). Results estimated by counting average number of p27Kip1 stained nuclei per glomerulus and for tubular epithelial cells from 10 randomly selected high power fields (HPF). (Original magnification, 400x). Arrows and insert (magnification 1000x) indicate that p27Kip1 positive nuclei are localized in podocytes. The asterisk between the bars indicates statistical differences between Dcn−/− and Dcn−− diabetic animals. P<0.05 (data given as mean ± SD, n=5).*
developed hyperglycemia with increased kidney to body weight ratio at day 30 and week 9 compared to non-diabetic mice with no distinguishable changes among the diabetic groups, irrespective of the decorin gene dose (Table 1). The only genotype-specific difference observed as a sign of progressive nephropathy was a sustained increase in proteinuria (p<0.05) at day 30 and week 9 in Dcn-/ diabetic mice relative to their Dcn+/+ diabetic counterparts. p27 Kip1 has been shown to be associated with podocyte damage resulting in proteinuria in experimental diabetic nephropathy (2, 7). Since decorin regulates both p21Cip1/Waf1 and p27 Kip1 in various cell types (22, 28), we analysed the expression of these cyclin-dependent kinase inhibitors as a potential cause of enhanced proteinuria in Dcn-/ diabetic mice. Enhanced p27 Kip1 both in tubular epithelial cells and glomeruli was revealed by immunohistochemical analysis in Dcn-/- diabetic renal sections at week 9 of diabetic nephropathy (Fig. 1A, B and C). In diabetic glomeruli, p27 Kip1 was mostly localized in podocytes (Fig. 1A), suggesting a potential role in podocyte injury. No difference was observed in the expression of p21Cip1/Waf1 in both diabetic groups (data not shown). Thus, in experimentally-induced diabetes, decorin deficiency resulted in enhanced expression of podocytic p27Kip1 associated with increased urinary protein excretion.

Overexpression of decorin in Dcn-/- diabetic kidneys

Next, the renal expression of decorin was analysed over a period of up to 9 weeks in Dcn+/+ diabetic kidneys. Northern blots of homogenates of diabetic kidneys revealed higher levels of decorin mRNA in Dcn+/+ diabetic mice compared to non-diabetic controls at 30 days and 9 weeks after STZ-injection (Fig. 2A). However, at 9 weeks of diabetes the expression of decorin had declined considerably compared to day 30, but was still higher than in non-diabetic kidneys (Fig. 2B). In Dcn-/- diabetic and non-diabetic kidneys no decorin mRNA was detectable. Immunostaining for decorin revealed enhanced expression of decorin in the mesangial matrix of glomeruli and in the peritubular space in Dcn+/+ diabetic kidney sections vs. non-diabetic controls at day 30 (Fig. 2C). These findings indicate a relative increase of decorin expression in the early stages of diabetic nephropathy which declines as the disease further progresses and supports the concept that decorin might act as a part of a natural response to hyperglycemia and to damage caused there from.
Decorin-deficiency is associated with enhanced apoptosis of tubular epithelial cells in diabetic kidneys

Decorin is known to be involved in the regulation of tumour cell proliferation (29, 30). Ki67 immunostaining revealed no genotype-specific differences neither in glomeruli nor in tubular epithelial cells of diabetic mice (data not shown). Previously, decorin has been reported to protect apoptosis of endothelial cells (19, 28). In contrast, recent evidence indicates that overexpression of endogenous decorin induces apoptosis and cell growth arrest of mesangial cells (31). In general, decorin causes apoptosis of squamous carcinoma cells (52) and rat mammary carcinoma cells (33) via caspase 3 activation. Accordingly, we tested whether lack of decorin would influence apoptosis of renal cells as well. In fact, a higher number of apoptosis-positive nuclei were found in tubular epithelial cells in kidney sections from Dcn−/− diabetic animals compared to Dcn+/+ diabetic counterparts at week 9 of diabetes (Fig. 3A and B). No significant differences were seen in the number of TUNEL-positive nuclei in glomeruli between diabetic mice (data not shown).

Decorin regulates apoptosis of tubular epithelial cells through the IGF-IR

As the genotype-specific differences in apoptosis were found to be more pronounced in tubular epithelial cells in vivo, we examined in human proximal tubular epithelial (HK-2) cells, whether the anti-apoptotic mechanism observed in diabetic nephropathy was a direct effect mediated by decorin. HK-2 cells, cultured in media with varying glucose concentrations, were analyzed for cell death in the presence and absence of decorin (4 µg/ml, ~40 nM). Addition of decorin to HK-2 cells cultured in a high glucose concentrations (30 mM), resulted in significantly reduced apoptosis (Fig. 4A). Viability of cells was not altered under equiosmolar concentrations of L-glucose or by mannitol used as osmotic controls (data not shown). Because decorin has been shown to act as a signaling molecule via the IGF-IR in endothelial cells and renal fibroblasts (19, 20), we further investigated whether the decorin-mediated anti-apoptotic effects in HK-2 cells involved a similar mechanism. Immunoprecipitation of HK-2 cell lysates cultured in high glucose media, to which decorin (intact or the protein core alone) had been added, showed co-immunoprecipitation of decorin with the IGF-IR (Fig. 4B). The decreased amount of IGF-IR immunoprecipitated in the complex when cells were incubated with intact decorin compared to the immunoprecipitate obtained from cells incubated with the same amount of core protein, suggests some interference from the side chains with the binding of decorin to the receptor.

Lack of decorin is associated with tubular overexpression of IGF-IR in diabetic kidney

The in vivo relevance of the decorin-mediated anti-apoptotic signaling through IGF-IR was further analyzed. Western blot lysates of diabetic kidneys (day 30) showed increased expression of the IGF-IR in Dcn−/− diabetic mice and an even more pronounced overexpression in Dcn−/− diabetic mice, probably as a compensatory mechanism for the lack of decorin (Fig. 5A and B). Non-diabetic mice of both genotypes did not show any upregulation of IGF-IR (data not shown). Similar results were observed after 9 weeks of diabetes as published by us earlier (20). These data were further confirmed by immunostaining that showed a more pronounced expression of the IGF-IR in tubular epithelial cells from Dcn−/− diabetic kidneys at day 30 compared to their Dcn+/+ diabetic counterparts (Fig. 5C and D). No difference was found in the IGF-IR score in control non-diabetic animals of both genotypes. Overall, these results indicate a likely involvement of IGF-IR in the decorin mediated anti-apoptotic mechanism in diabetic nephropathy.

Increased expression of TGFβ and CTGF in Dcn−/− diabetic kidneys is associated with mesangial matrix accumulation

Besides protection of tubular epithelial cells from apoptosis, decorin is also known to exhibit antifibrotic effects due to its interactions with TGFβ (14, 22). Quantification of Northern blots from kidney homogenates revealed increased mRNA expression of TGFβ1 (Fig. 6A and B) and CTGF (Fig. 6C) in both genotypes of diabetic mice after 9 weeks. Interestingly, TGFβ1 was found to be still further elevated in Dcn−/− diabetic kidneys compared to their Dcn+/+ diabetic counterparts. Similar results were observed in terms of CTGF expression (Fig. 6C), another profibrotic factor downstream to TGFβ involved in the progression of diabetic nephropathy and extracellular matrix accumulation (6).
STZ-diabetic mice do not show major morphological differences at early stages of nephropathy, quantification of PAS staining after 9 weeks revealed a slight, but significant expansion of the mesangial matrix in glomeruli from Dcn−/− diabetic mice compared to Dcn+/+ diabetic mice (Fig. 6D).

Increased glomerular expression of biglycan and mononuclear cell infiltration in Dcn−/− diabetic kidneys

Mononuclear cell infiltration is considered as one of the hallmarks of progressive renal disease in diabetes (27). Assessment of renal pathology by light microscopy of PAS-stained kidney sections revealed excessive mononuclear cell infiltration in Dcn−/− diabetic mice (86 ± 14 / high power field, n = 7) compared to their Dcn+/+ diabetic counterparts (13 ± 7 / high power field, n = 6, P<0.05), especially in perivascular regions at 9 weeks of diabetic nephropathy (Fig. 7A). As decorin deficiency has been associated with overexpression of the proinflammatory proteoglycan biglycan (21, 22, 34) and TGFβ (also a trigger of biglycan expression) (35), we further looked into the expression of biglycan in Dcn−/− diabetic mice. Quantification of Northern blots from diabetic kidney homogenates (at week 9) revealed increased biglycan mRNA expression in Dcn−/− diabetic mice compared to Dcn+/+ diabetic mice (Fig. 7B and C). This was confirmed by immunohistochemistry, showing significantly enhanced staining for biglycan in Dcn−/− diabetic kidney sections (Fig. 7D) which mainly co-localized with infiltrating cells. Thus, the compensatory increase in biglycan might be responsible for the increased mononuclear cell infiltration observed in Dcn−/− diabetic kidneys.

DISCUSSION

The present study underlines the importance of decorin as a factor regulating the progression of STZ-induced diabetic nephropathy. In this model, decorin is overexpressed in the mesangial matrix of the glomerulus and in the renal tubulointerstitium both at the mRNA (36) and protein level. These observations correlate well with the data obtained in renal biopsies from patients at various stages of diabetic nephropathy (10), suggesting clinical relevance of our findings for the human disease. This striking result that endogenous decorin expression increases in diabetic nephropathy, indicates that under normal, non-transgenic conditions there is a positive feedback loop that enhances decorin expression, likely in an attempt to counteract the damage caused by high glucose levels. The potential involvement of the insulin receptor will be addressed in the follow-up studies.

The overexpression of decorin in diabetic nephropathy might also represent a compensatory mechanism for increased TGFβ1 levels in the kidney. Decorin is capable of forming complexes with TGFβ1 (12), which then are removed via the vasculature and/or the urinary tract (10), thereby reducing the renal TGFβ1 burden. The simultaneous loss of decorin with the excreted complexes might explain the previously described discrepancy between elevated glomerular mRNA and low protein levels at early stages of diabetic nephropathy. The loss of decorin might have driven the expression of decorin mRNA higher (10). In advanced stages of diabetic nephropathy, when there is enhanced accumulation of extracellular matrix components that can bind to decorin, such as collagen type I, decorin probably forms a ternary complex with TGFβ1 and collagen I (10, 37), and hence may play a protective role by sequestering TGFβ1 in the matrix. On the other hand, it might also increase renal extracellular matrix expansion and fibrosis.
Besides regulation of TGFβ activity, enhanced levels of unsequestered decorin in diabetic kidneys could increase the availability of this proteoglycan for binding to its receptor(s), thereby regulating a host of cellular processes (9). In general, decorin causes apoptosis of carcinoma cells (32, 33) via caspase 3 activation. In contrast, in our study tubular epithelial cells from wild-type diabetic mice were protected from apoptosis. As decorin deficiency had previously been associated with

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**Fig. 6.** Decorin deficiency accelerates overexpression of TGFβ1 and CTGF mRNA and increases mesangial matrix expansion in diabetic kidneys. (A) Northern blots for TGFβ1 mRNA and (B) its quantification in non-diabetic and diabetic Dcn+/+ and Dcn−/− kidneys at week 9 of STZ-diabetes normalized to GAPDH (given as means ± SD). The asterisk indicates statistical significance; n=3, P<0.05. (C) Quantification of CTGF mRNA expression as indicated in (B). (D) Quantification of mesangial matrix expansion in PAS-stained kidney sections from Dcn+/+ and Dcn−/− non-diabetic and diabetic mice at week 9 of STZ-diabetes. Results are expressed as average percentage of glomerular area occupied by the mesangial matrix. The asterisk indicates statistical significance; n=5, P<0.05.

**Fig. 7.** Increased mononuclear cell infiltration associated with biglycan overexpression in diabetic kidneys is more pronounced in Dcn−/− mice. (A) PAS-stained kidney sections of Dcn+/+ and Dcn−/− diabetic mice at week 9 of STZ-diabetes. (Original magnification, 400x). (B) Northern blot for biglycan mRNA and (C) its quantification in non-diabetic and diabetic Dcn+/+ and Dcn−/− kidneys over 9 weeks of STZ-injection after normalization to GAPDH (given as means ± SD). The asterisk indicates statistical significance; n=3, P<0.05. (D) Immunostaining of biglycan (alkaline phosphatase anti-alkaline phosphatase, red colour) in Dcn+/+ and Dcn−/− diabetic kidney sections after 9 weeks of STZ-injection. Counterstaining was with haematoxylin. (Original magnification, 400x). Arrows indicate biglycan-positive mononuclear infiltrating cells.
enhanced apoptosis of tubular epithelial cells in obstructive nephropathy (22), it appears most unlikely that enhanced apoptosis in our Den−/− mice was due to the toxic effects of STZ. Both in STZ-diabetes and in obstructive nephropathy, the IGF-IR, previously described as a signaling receptor for decorin in endothelial cells and renal fibroblasts (19, 20), was up-regulated in kidneys from decorin-null mice (20, 22, 28). However, this could not compensate for the lack of decorin, resulting ultimately in increased apoptosis of tubular epithelial cells. Here we show direct evidence for decorin-mediated anti-apoptotic effects in tubular epithelial cells (using HK-2 cells) based on its interaction with the IGF-IR. Besides regulation of apoptosis, decorin also stimulates the synthesis of fibrillin-1 via the IGF-IR in renal fibroblasts (20) and mediates inhibition of proliferation and migration of trophoblasts by interacting with IGF-IR and vascular endothelial growth factor-receptor-2 (38). This indicates that the decorin/IGF-IR interaction is a crucial mechanism regulating various cellular processes of non-carcinoma cells.

Enhanced p27kip1 in podocytes and tubular epithelial cells of diabetic decorin-null kidneys probably represents another consequence of decorin signaling (22, 28) and might contribute to proteinuria in Den−/− diabetic mice as reported previously (11, 20). The importance of p27kip1 in high glucose-mediated injury of renal cells has been shown earlier (8, 39, 40). Furthermore, kidneys from diabetic p27kip1 knockout mice develop less podocyte injury and are protected from diabetic nephropathy (7). Therefore, it is conceivable that enhanced p27kip1 in podocytes of Den−/− diabetic mice was responsible for the increased proteinuria, the clinical signature of podocyte injury (41). Also enhanced p27kip1 in tubular epithelial cells of diabetic decorin-null mice might give rise to renal hypertrophy (42). This together with overexpressed TGF-β1 and CTGF, crucial mediators of renal hypertrophy (43) and enhanced extracellular matrix accumulation, should impact on kidney enlargement in Den−/− diabetic mice. However, in our hands there was an equal increment in kidney weights in diabetic mice of both genotypes which might have been due to enhanced apoptosis of tubular epithelial cells balancing the effects of enhanced TGF-β1, CTGF and p27kip1 in Den−/− diabetic kidneys.

An increased number of infiltrating mononuclear cells could be another factor accelerating progression of diabetic nephropathy in Den−/− diabetic mice (27, 44). Although the mechanisms of inflammation in diabetic nephropathy are not completely understood, there is a growing evidence for the role of Toll-like receptors in non-immune kidney diseases (21, 45). Biglycan, an endogenous ligand of the Toll-like receptors-2 and -4 and trigger of TNF-α and macrophage inflammatory protein-2 in macrophages, has been shown to drive an inflammatory response in an autocrine and paracrine manner (21). Therefore, it is conceivable that the overexpression of biglycan, triggered by the lack of decorin (22, 34) and by up-regulated TGF-β1 (35), accounts to a large degree for the enhanced mononuclear cell infiltration and progressive diabetic nephropathy in Den−/− mice (11). Recent report indicates a 3-fold enhanced expression of the ECM component hyaluronan in diabetic rats to be associated with hyperglycaemia, glycosuria and proteinuria (46). Also hyaluronan could activate TLR2 and TLR4, initiating an inflammatory response in the absence of pathogens and infiltrating immune cells (47), further emphasizing the role of proteoglycans in the course of diabetic nephropathy. Collectively, besides regulating TGF-β activity, decorin evokes multiple signaling pathways by direct interactions with its cognate receptors thereby retarding the progress of diabetic nephropathy. Future therapeutic approaches by manipulating and amplifying decorin signaling should be considered to slow down renal manifestations of diabetes mellitus.

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