Galectin-3 preserves renal tubules and modulates extracellular matrix remodeling in progressive fibrosis

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Galectin-3 (Gal-3) is a 32- to 35-kDa multifunctional lectin protein expressed by epithelial cells, endothelial cells, and macrophages that regulates numerous biological processes through interactions between its carbohydrate recognition domain and via carbohydrate-independent mechanisms. Although it is predominantly located in the cytoplasm, Gal-3 can be secreted extracellularly and it can also shuttle into the nucleus. Intracellular Gal-3 is an important protein for cell survival due to its ability to block the intrinsic apoptotic pathway, while in the nucleus Gal-3 promotes cell proliferation—both via carbohydrate-independent mechanisms (10, 18, 26). Extracellular Gal-3 modulates important interactions between epithelial cells and extracellular matrix through its carbohydrate domain and it plays an important role during embryonic development of collecting ducts (5, 34). Several studies also suggest that Gal-3 is important in alternative macrophage activation (28), macrophage phagocytosis (42), and clearance of advanced glycation end-products (40). Due to its functional diversity, the role of Gal-3 in progressive renal disease remains controversial and is likely context dependent (17, 21, 22). The present study investigates the primary functions of Gal-3 in an experimental model of progressive renal fibrosis by comparing Gal-3-deficient and wild-type mice. Our findings suggest that Gal-3 attenuates renal fibrosis by limiting renal tubular apoptosis and modulating extracellular matrix remodeling.

METHODS

Experimental Design

Breeding pairs of Gal-3-deficient (Gal-3−/−) mice on a C57BL/6 background obtained from Dr. Fu-Tong Liu’s colony were bred in our animal facility in Seattle. In brief, Gal-3-deficient mice were generated in Dr. Liu’s laboratory using blastocysts from C57BL/6 mice and sent to us after nine generations (19). Wild-type C57BL/6 male
mice, 8–10 wk of age, were purchased from Jackson Laboratory (Maine, CT). Unilateral ureteral obstruction (UUO) surgery was performed on Gal-3+/− and wild-type male mice, 8–10 wk of age (n = 6–10 each), and they were killed at 3, 7, 14, or 21 days after surgery. For mice in the UUO group, the left ureter was exposed through a midabdominal incision and ligated using 4–0 silk. All surgeries were performed under general anesthesia with isoflurane. All procedures were performed in accordance with the guidelines established by National Research Council Guide for the Care and Use of Laboratory Animals and approval of our Institute Animal Care and Use Committee (IACUC). Contralateral and UUO kidneys were harvested and processed for RNA and protein extraction and histological studies as previously described (32, 37, 38). Frozen tissue samples were stored at −80°C.

Genotyping

Genotyping was performed by PCR using genomic DNA isolated from tails. PCR primer sequences were obtained from Dr. Liu and genotyping was performed as described previously (19). Primers for the wild-type Gal-3 allele are 5′-GTAGGTGAGAGTCACAAGCTGGAGGCC; 3′-CACCCTCAAAAGGGGAAGGCTGACTGTC (band size ~450 bp). The primers for the Gal-3-deficient allele include the 5′-GGCTGACCGCTTCCTCGTGCTTTACGG; and the 3′ wild-type Gal-3 primer (band size ~300 bp).

Collagen Content

Hydroxyproline content of kidney tissue (μg of hydroxyproline per mg of wet wt kidney section) was measured by acid hydrolysis of the tissue section using procedures established in our laboratory (32, 37, 38).

Histological Examination

Immunohistochemical staining was performed on sections of paraffin-embedded tissue or cryosections of snap-frozen tissue using procedures established in our laboratory with VECTASTAIN Elite ABC Kits (Vector Laboratories, Burlington, CA) and AEC Substrate Chromogen K3464 (Dako, Carpinteria, CA). Sections were blocked with avidin/biotin blocking kit (Vector Laboratories). Confocal microscopy was performed on 5-μm cryosections fixed with 4% paraformaldehyde and imaged with the Zeiss LSM 5 Pascal confocal microscope with LSM software (Thornwood, NY). Confocal z-stack images were analyzed with Imaris 7.0 software (Bitplane, St. Paul, MN). In some cases, tyramide signal amplification was utilized (TSA kit #3–488 tyramide and TSA kit #4–546 tyramide; Invitrogen, Carlsbad, CA). Nuclei were stained with TO-PRO-3 iodide. Primary antibodies used were reactive with Gal-3 (mouse anti-mouse monoclonal-FITC; Cedarlane Laboratories, Burlington, NC), F4/80/80 (rat anti-mouse F4/80 monoclonal; AbD Serotec, Raleigh, NC), α-smooth muscle actin (α-SMA; mouse anti-mouse monoclonal α-SMA-Cy3; Sigma, St. Louis, MO), Endo180 (sheep anti-mouse Mrc2; R&D Systems, Minneapolis, MN), E cadherin (goat anti-mouse E cadherin; R&D Systems), and IκB-α and phosphorylated IκB-α (rabbit anti-human IκBα polyclonal and rabbit anti-human phospho IκB-α polyclonal; Cell Signaling Technology, Danvers, MA). Interstitial myofibroblasts were quantified by staining using peroxidase-conjugated marine anti-human α-SMA 1A4 monoclonal antibody (Dako) as described previously (32, 35, 49). TdT-mediated dUTP nick end labeling (TUNEL) and Picosirius red staining was performed as previously described (32, 49). Secondary antibodies were shown to be nonreactive with tissue sections stained without the primary antibody. Semi-quantitative computer-assisted image analysis of tubulointerstitial proteins was performed on six randomly selected ×400 magnified images of slides from individual animals with Image-Pro Plus software (Media tech). Glomerular areas and space not occupied by tissue were subtracted in the analysis. Interstitial macrophage density was expressed as percent F4/80-positive interstitial area on fluorescent-stained cryosections. The percent macrophage density was determined from results of six randomly selected ×400 magnified images of slides from individual animals with assistance from Image-Pro Plus software (Mediatech). The investigator was blinded to the experimental groups at the time of analysis. Analysis of fluorescent dual stained kidney sections by confocal microscopy was performed using the histogram function in the LSM software with thresholds set by staining specificity.

Western Blotting

Protein was isolated from homogenized frozen kidney and Western blotting was performed as previously described (37). The primary

![Image](http://ajprenal.physiology.org/Downloadedfromhttp://ajprenal.physiology.org/)

Fig. 1. Galectin-3 (Gal-3) expression is increased after unilateral ureteral obstruction (UUO) in tubular and interstitial cells. A: graph summarizes analysis of relative Gal-3 mRNA expression normalized to 18S, UUO relative to contralateral, by semi-quantitative real-time qPCR (†P < 0.01; n = 6) in wild-type mice. Representative confocal photomicrographs of Gal-3 (green) localization demonstrated how basal expression in tubular cells of normal kidneys (B) that is upregulated in tubular cells (*) at early time points (C) and transitioned to interstitial cells (arrows) by day 14 (D). A small subpopulation of α-smooth muscle actin (α-SMA +; red) myofibroblasts expressed Gal-3 (yellow merged, indicated by arrow; E) while the majority of the Gal-3 interstitial cells are F4/80+ (†) macrophages (F; arrows indicate an aggregated subpopulation of dual stained Gal-3+F4/80+ cells). Bar = 20 μm. Magnification ×400. G, glomeruli.
antibodies are described above. Bands were normalized using beta actin (anti-mouse beta actin; Sigma). The secondary antibodies were anti-rabbit, anti-sheep, anti-goat, and anti-mouse IR700Dye and IR800Dye (Rockland Immunochemicals, Gilbertsville, PA). Protein bands were visualized and quantified using the Odyssey (Li-Cor Biosciences, Lincoln, NE).

Semiquantitative Real-Time qPCR

Total RNA from frozen kidney tissue homogenate was obtained using the Maxwell 16 instrument (Promega, Madison, WI). RNA samples were loaded on a Agilent RNA 6000 Nano Chip and analyzed in the Agilent 2100 Bioanalyzer (Agilent Technologies, Foster City, CA) for RNA concentration and quality; samples with RNA integrity numbers greater than 8.0 were utilized for cDNA synthesis. First-strand cDNA was prepared from 1 μg of total RNA using the Bio-Rad iScript cDNA Synthesis kit (Bio-Rad Laboratories, Hercules, CA). Semiquantitative real-time qPCR was performed according to the IQ SYBR Green Supermix (Bio-Rad Laboratories, Lincoln, NE). Bands were visualized and quantified using the Odyssey (Li-Cor IR800Dye (Rockland Immunochemicals, Gilbertsville, PA). Protein antibodies are described above. Bands were normalized using beta actin (anti-mouse beta actin; Sigma). The secondary antibodies were anti-rabbit, anti-sheep, anti-goat, and anti-mouse IR700Dye and IR800Dye (Rockland Immunochemicals, Gilbertsville, PA). Protein bands were visualized and quantified using the Odyssey (Li-Cor Biosciences, Lincoln, NE).

Fig. 2. A: total kidney collagen content measured by the hydroxyproline assay is significantly increased in obstructed kidneys from Gal-3-deficient mice compared with wild-type mice. B and C: representative polarized light microscopy photomicrographs (×200) illustrate increased collagen deposition by Picrosirius red staining at day 14 in Gal-3-deficient mice. D: graph summarizes the results of polarized Picrosirius red quantification by computer-assisted image analysis, expressed as the percent positive area. E: renal E-cadherin Western blot illustrates significantly lower levels in Gal-3-deficient mice at day 14 after UUO reflecting the loss of intact tubules. The graph below summarizes the results of single-band density measurements, expressed as beta-actin-normalized E-cadherin band levels.

RESULTS

Gal-3 Is Protective During Chronic Kidney Injury

Gal-3 expression increases during UUO. In normal kidneys, basal Gal-3 expression level is low. In response to chronic injury induced by obstruction, Gal-3 steady-state mRNA levels dramatically increased with a peak relative expression (95-fold increase, 2 log = 6.6) observed 7 days after UUO in wild-type mice measured by semiquantitative real-time qPCR (normal-
ized to 18S, UUO relative to contralateral kidney, \( n = 6/\text{group}, P = 0.001; \) Fig. 1A). By immunolocalization, Gal-3 expression was upregulated predominantly in renal tubular epithelial cells at early time points (days 3 and 7). As the kidney injury progressed over time, there was a shift in Gal-3 expression from tubular cells to interstitial cells (Fig. 1, B, C, D). By dual-staining confocal microscopy, macrophages were identified as the primary interstitial cells expressing Gal-3 during later time points, while only a small proportion of myofibroblasts were Gal-3-positive (Fig. 1, E and F).

**Fibrosis severity worse in gal-3-deficient mice.** To investigate whether Gal-3 modulated fibrosis severity after UUO, total collagen levels were measured as hydroxyproline content per wet weight from obstructed kidneys. Fibrosis severity was significantly increased by 35 and 21% in Gal-3-deficient compared with wild-type mice at 14 and 21 days after UUO, respectively (\( n = 5–12/\text{group}, P < 0.01; \) Fig. 2A). Picrosirius red staining provided histological confirmation that there was significantly more interstitial collagen deposited at day 14 in Gal-3-deficient compared with wild-type mice (\( n = 7–8/\text{group}, F = 19.6, P = 0.0007; \) Fig. 2, B-D). There was no difference in baseline levels of interstitial collagen in normal kidneys of Gal-3-deficient mice compared with wild-type mice measured by Picrosirius red staining (wild-type vs. Gal-3-deficient, \( n = 5/\text{group}, \) contralateral: 0.3 ± 0.04 vs. 0.4 ± 0.04%, \( P = 0.2; \) Supplemental Fig. 1; the online version of this article contains supplemental data). A limitation of the UUO model is the inability to measure the functional consequences of fibrosis.

**Intracellular Gal-3 is primarily localized to the cytoplasm.** Intracellular Gal-3 is primarily localized to the cytoplasm where it functions as an anti-apoptotic factor by inhibiting caspase activity similar to Bcl2 in the intrinsic pathway (47). However, extracellular Gal-3 can form oligomers and bind to cell surface receptors in a carbohydrate-dependent manner and promote apoptosis (16, 46, 54). Since Gal-3 expression was initially prominent in tubular epithelial cells and tubular E-cadherin levels were significantly lower in Gal-3-deficient mice, its effects on apoptosis were investigated. The number of TUNEL-positive cells was significantly increased by 123% at day 14 after UUO in Gal-3-deficient compared with wild-type mice (\( n = 8–10/\text{group}, z = 3.1, P = 0.002; \) Fig. 3, A–C). Cytochrome c release from mitochondria represents an important step in activating the intrinsic pathway of apoptosis (24, 43). Semiquantitative analysis of kidney sections stained for cytochrome c showed significantly more positive tubules (29%) at day 14 after UUO in Gal-3-deficient compared with wild-type mice (\( n = 8/\text{group}, F = 6.6, P = 0.02; \) Fig. 3, D and E). In addition to the increase in apoptotic cells, there was a significant decrease in cell proliferation with a 62% decrease in the number of BrdU-positive tubulointerstitial cells at day 14 compared with wild-type mice (7–8/\text{group}, \( z = 2.47, P = 0.01, \) wild-type vs. Gal-3-deficient).

**Gal-3 Attenuates Apoptosis in Damaged Renal Tubular Cells.**

**Fig. 3.** A and B: representative photomicrographs (×400) and the graph (C) demonstrate increased TdT-mediated dUTP nick end labeling (TUNEL+) cells in Gal-3-deficient mice on UUO day 14. The arrows highlight TUNEL+ tubular cells and the (*) interstitial cells. D and E: representative photomicrographs illustrate more cytochrome c-positive tubules in Gal-3-deficient mice and the graph (F) summarizes total cytochrome c levels on UUO day 14. G and H: representative photomicrographs illustrate more proliferating BrdU+ tubular and (*) interstitial cells in wild-type mice and the graph (I) summarizes the differences in proliferating BrdU+ cells on UUO day 14. All results are expressed as means ± SE. NS, not significant. † \( P < 0.05, \) ‡ \( P < 0.01, \) wild-type vs. Gal-3-deficient.
Paradoxical decreased interstitial myofibroblasts despite increased fibrosis. Since fibrosis severity was significantly increased in Gal-3-deficient mice, studies were performed to examine its potential effect on interstitial myofibroblast accumulation. Despite more severe fibrosis, the number of interstitial α-SMA-positive interstitial cells was significantly decreased by 58% in Gal-3-deficient compared with wild-type mice at day 14 after UUO (n = 7–8/group, F = 49.6, P = 0.00001; Fig. 5). Fibronectin and interstitial collagens are primary components of the extracellular matrix in kidney fibrosis. Since α-SMA-positive myofibroblasts are the primary source of these extracellular matrix proteins during kidney injury, matrix gene transcription was examined by real-time semiquantitative qPCR. Consistent with the decrease in α-SMA-positive myofibroblasts, there was a significant decrease by more than 80% in steady-state mRNA levels of fibronectin and procollagen I in Gal-3-deficient compared with wild-type mice by real-time semiquantitative qPCR at day 14 after UUO (normalized to 18S and GAPDH, n = 6/group, P < 0.01; Table 1). These findings suggested that myofibroblast accumulation and matrix synthesis rates cannot explain the worse fibrosis that developed in Gal-3-deficient mice.

Altering matrix remodeling. Polarized light microscopy of tissues stained with Picrosirius red can be used qualitatively to evaluate matrix maturity: the closely packed thick fibrils of type I-like collagen stain an intense red-orange, as seen in advanced scar, while faint yellow-green staining of

Gal-3 Does Not Affect Indicators of Inflammation

Gal-3 has been reported to be an important factor in alternative macrophage activation (28) and interstitial macrophage infiltration is a requisite step in kidney fibrosis. Therefore, since Gal-3 affected later time points in collagen accumulation, interstitial macrophage infiltration was examined at days 7 and 14 after UUO. Significant differences were not observed in F4/80+ interstitial area at either time points examined (wild-type vs. Gal-3-deficient, n = 6/group, P = 0.3; Fig. 4). By dual fluorescent confocal microscopy, only a small subpopulation of the interstitial F4/80+ macrophages expressed Gal-3; these numbers did not change as fibrosis advanced (day 7 vs. day 14, n = 6/group: 11 ± 3.7% vs. 14 ± 2.7%, P = 0.7). To consider the possibility that proinflammatory signaling might differ between Gal-3-deficient and wild-type macrophages despite similar recruitment levels, phosphorylated IkB-α (pIkB-α)-to-total IkB-α ratios were measured by Western blotting. There was no difference in NF-κB activation between Gal-3-deficient and wild-type mice at day 3 or day 7 after UUO (pIkB-α total IkB-α wild-type vs. Gal-3-deficient, n = 5–6/group: day 3: 0.8 ± 0.02 vs. 0.8 ± 0.04, P = 0.7; day 7: 0.7 ± 0.002 vs. 0.7 ± 0.009, P = 0.6).

Gal-3 Modulates Matrix Turnover

Polarized light microscopy of tissues stained with Picrosirius red can be used qualitatively to evaluate matrix maturity: the closely packed thick fibrils of type I-like collagen stain an intense red-orange, as seen in advanced scar, while faint yellow-green staining of

Fig. 4. Gal-3 deficiency did not alter macrophage infiltration after ureteral obstruction. Representative confocal photomicrographs (×400) are shown at top and the graph at the bottom summarizes the quantitative results of F4/80-positive (red) interstitial staining. Results are expressed as means ± SE. NS, wild-type vs. Gal-3-deficient.

Fig. 5. Interstitial α-SMA+ myofibroblast numbers were significantly lower in Gal-3-deficient mice compared with wild-type mice at day 14 after UUO despite an increase in fibrosis severity. Representative α-SMA-stained immunohistochemical photomicrographs (×400) are shown at top and the graph at the bottom summarizes the quantitative results of α-SMA interstitial staining. Results are expressed as means ± SE. †P < 0.01, wild-type vs. Gal-3-deficient.
Matrix metalloproteinases, such as MMP9, MMP12, and MMP13, can also degrade interstitial collagen matrices. There was no difference in steady-state mRNA levels of MMP9, MMP12, and MMP13 between Gal-3-deficient mice and wild-

Table 1. Relative expression of extracellular matrix genes in Gal-3-deficient mice

<table>
<thead>
<tr>
<th>Genes</th>
<th>Day 7 UUO</th>
<th>Day 14 UUO</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibronectin</td>
<td>1.2 (0.4–7.8)</td>
<td>0.77</td>
<td>0.2 (0.07–0.7)</td>
</tr>
<tr>
<td>Collagen I</td>
<td>0.4 (0.1–1.2)</td>
<td>0.11</td>
<td>0.2 (0.05–0.6)</td>
</tr>
<tr>
<td>Collagen III</td>
<td>0.9 (0.5–1.4)</td>
<td>0.61</td>
<td>0.8 (0.4–2.0)</td>
</tr>
</tbody>
</table>

Semi-quantitative real-time qPCR of galectin-3 (Gal-3)-deficient compared with wild-type mice. Data were analyzed with REST software using both GAPDH and 18S as reference housekeeping genes. Each sample was performed in triplicate (n = 6/group, 95% tile confidence interval in parentheses). UUO, unilateral ureteral obstruction. *P < 0.01.

Fig. 6. Gal-3 expression alters matrix remodeling. A and B: representative Picrosirius red photomicrographs (×1,000) and corresponding polarized images (C, D) demonstrate increased intensity of red-orange collagen matrix in Gal-3-deficient mice on UUO day 14. E and F: representative confocal photomicrographs of Picrosirius red staining illustrates disordered interstitial matrix patterns (arrows) in Gal-3-deficient mice at day 14. G and H: representative 3-dimensional confocal image using Imaris 7.0 software illustrates vesicle-like structures containing Picrosirius red-positive matrix (arrows, G) in wild-type mice and confirmed disorganized interstitial matrix patterns in Gal-3-deficient mice (arrows, H). G, glomerulus. Magnifications are ×1,000 (A–D). Bar = 10 µm (E–H).

Endo180, also known as urokinase receptor-associated protein, UPARAP, and mannose receptor 2, Mrc2, has recently been identified as a transmembrane endocytic receptor for several collagens and it appears to function as an intracellular pathway for collagen degradation (7). Endo180 is expressed by interstitial cells during renal fibrogenesis (Fig. 7B). It is known to be expressed by both macrophages and fibroblasts. There was a 58% decrease in steady-state Endo180 mRNA levels at day 14 after UUO in Gal-3-deficient compared with wild-type mice as measured by semiquantitative real-time qPCR (normalized to 18S and GAPDH, n = 6/group, Gal-3-deficient relative to wild-type: day 7: expression ratio 0.98, 95% CI 0.77–1.27, P = 0.9; day 14: expression ratio 0.42, 95% CI 0.15–1.84, P = 0.03; Fig. 7D). In the Gal-3-deficient mice, Endo180 protein expression also decreased by Western blot at day 14 after UUO compared with wild-type mice (n = 6–7/group, P = 0.03; Fig. 7E and F). α2β1 Integrin is both a potential binding partner for Gal-3 and another known receptor for collagen (15). During UUO, interstitial cells were shown to express α2 integrin at day 14 (Fig. 7C), raising the possibility of a link with Gal-3 and Endo180.
types at day 7 and day 14 as measured by quantitative real-time qPCR (normalized to 18S and GAPDH; Gal-3-deficient relative to wild-type, n = 5/group; day 14 UUO, MMP9: 0.9, 95% CI 0.4–2.2, P = 0.8; MMP12: 1.0, 95% CI 0.4–2.8, P = 1.0; MMP13: 1.6, 95% CI 0.4–15.4, P = 0.6).

DISCUSSION

The results of the present study demonstrated that Gal-3 protects the kidney from progressive damage due to chronic injury by modulating two important pathways: tubular apoptosis and extracellular matrix remodeling. Renal tubular epithelial cell apoptosis and subsequent tubular atrophy are an important cause of nephron loss that causes progressive functional deterioration. Whether a cell is committed to a fate of apoptosis or recovery following injury or severe stress is determined by the balance between proapoptotic factors, such as BH3-only proteins, and anti-apoptotic factors, such as Bcl-2 (4). BH3-only proteins can lead to the proapoptotic factors, such as BH3-only proteins, and anti-apoptotic factor by blocking apoptosis and promoting tubular cell proliferation.

In the present study, there were significantly increased cytosolic levels of cytochrome c and greater numbers of tubular apoptotic cells in Gal-3-deficient compared with wild-type mice after UUO consistent with an anti-apoptotic role during chronic kidney injury. In addition, Gal-3 may enhance tubular cell proliferation which is an important component of the adaptive response during obstructive kidney injury. Nuclear Gal-3 is known to be an important regulator of cell proliferation via its ability to regulate the cell cycle and to serve as a pre-mRNA splicing factor (9, 23). Significantly fewer proliferating tubular cells were detected in Gal-3-deficient compared with wild-type mice after UUO, suggesting that Gal-3 facilitates tubular cell regeneration during chronic injury. Taken together, these data establish that intracellular Gal-3 expression is upregulated in tubular cells during chronic kidney injury where it appears to function as an important survival factor by blocking apoptosis and promoting tubular cell proliferation.

Our data also suggest that a second major pathway is modulated by Gal-3 during chronic kidney injury that has important effects on extracellular matrix remodeling. In contrast to the study by Henderson and colleagues (17) that focused on the initial phase of UUO-induced kidney injury and reported less fibrosis at 7 days, the present study demonstrated that fibrosis severity was increased at day 14 and persisted through day 21 in Gal-3-deficient compared with wild-type mice. In the present study, fibrosis severity was measured both biochemically (hydroxyproline content) and histologically (Picosirius red) and extended to structural consequences on renal tubular integrity (E-cadherin Western). Consistent with the findings of Henderson and colleagues, we confirmed significantly fewer α-SMA-positive interstitial myofibroblasts...
which likely accounted for lower rates of extracellular matrix gene transcription in Gal-3-deficient compared with wild-type mice. These findings suggested the possibility that the anti-fibrotic effects of Gal-3 might be due to enhanced extracellular matrix turnover.

The histoarchitecture of the interstitial matrix in the Gal-3-deficient mice was more disorganized and characterized by thicker, more intensely red-orange fibrils compared with wild-type mice. Wound remodeling studies suggest that this pattern indicates the predominance of collagen I-type fibrils (8). A recent study by Oliveria and colleagues (39) demonstrated that collagen fibers surrounding acute and chronic granulomas in Gal-3-deficient mice were loosely packed and oriented in a disorganized pattern in a schistosomiasis model of liver fibrosis. Extracellular matrix remodeling is a critical process that involves three components: synthesis, deposition, and degradation. Despite intense interest in the contribution of matrix degradation to renal fibrogenesis, the specific pathways that are involved remain elusive. It had been assumed that matrix remodeling is an extracellular process mediated by MMPs, but this hypothesis has yet to be definitively established. Previous studies on certain serine protease and gelatinase systems in chronic kidney injury suggest that these proteases do not mediate significant matrix degradation but likely have a more prominent role in fibrogenic signaling that may actually enhance fibrosis (6, 12, 13, 32, 52). Endo180 has recently been identified as an endocytic receptor for collagen I and IV and is likely a key receptor for matrix degradation during wound remodeling (7, 29). Studies in our lab showed that Endo180 protein levels increased steadily after the onset of obstruction and that renal fibrosis is significantly worse in Endo180-deficient mice (27). In the present study, Endo180 mRNA and protein levels were significantly lower in Gal-3-deficient compared with wild-type mice. Since Gal-3 is not a transmembrane receptor, it is not yet clear how Gal-3 might regulate Endo180 expression.

Gal-3 may also play a more direct role in intracellular matrix turnover through extracellular interactions with one of its known binding partners, the α2β1 integrin. Gal-3 binds α2β1 integrin through its carbohydrate recognition domain (54) and a recent study reported that Gal-3 was an important regulator of α2β1 integrin-mediated adhesion to collagen I and collagen IV (15). In addition to promoting adhesion, functional β1 integrins also stabilize fibronectin matrix fibrils and promote endocytosis of matrix fibronectin (45). Shi and colleagues (44) recently demonstrated that endocytosis of collagen I from extracellular matrix is dependent on both β1 integrin and Endo180 and the authors further speculated that Endo180 may interact with β1 integrin to modulate collagen I endocytosis. However, the specific mechanisms of this interaction remain unknown. We speculate that extracellular Gal-3 may form oligomers to direct β1 integrin/Endo180-mediated collagen endocytosis and is the subject of future investigations.

Both macrophages and fibroblasts can express Endo180 and are capable of performing Endo180-mediated collagen endocytosis (29, 30); whether this process resides within myofibroblasts and/or macrophages during chronic kidney injury remains to be determined. In the present study, macrophages were identified as the primary Gal-3-positive interstitial cell population, representing ~10 to 14% of all F4/80-positive macrophages. The importance of macrophage functional heterogeneity is increasingly recognized, although the specific phenotype and function of fibrosis-associated macrophages are not yet clear (41). Although classically associated with matrix production, fibroblasts are also known to be functionally heterogeneous and may contribute to intracellular matrix degradation (3, 14). In the present study, a small subpopulation of interstitial α-SMA-positive myofibroblasts was shown to express Gal-3. Future studies are needed to determine whether Gal-3 expression identifies subpopulations of macrophages and/or fibroblasts that phenotypically define cells that promote matrix degradation.

In summary, the present study demonstrated that Gal-3 not only protects renal tubules from chronic injury by limiting apoptosis but that it may be an important factor in matrix remodeling and fibrosis attenuation. Based on our findings and previously published studies (15, 26, 34, 54), they further suggest that intracellular Gal-3 through carbohydrate-independent mechanism may preserve renal tubules while extracellular Gal-3 through its carbohydrate domain may direct Endo180-mediated collagen degradation.

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DISCLOSURES

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