Renal Ureteral Obstruction and Alpha-smooth Muscle Actin (alpha-SMA) Research Literatures

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Abstract: Acute unilateral obstructive uropathy urology a unilateral block of urine flow through the ureter of 1 kidney, resulting in a backup of urine, distension of the renal pelvis and calyces, and hydronephrosis etiology kidney stone, trauma, stricture in children–of one ureter causes acute unilateral obstructive uropathy; slow, progressive blockage causes chronic unilateral obstructive uropathy; either may result in permanent damage and failure of one kidney and HTN, resulting in the so-called Goldblatt kidney, which is usually asymptomic–the remaining kidney usually has functional reserve. Ureteral obstruction results in renal fibrosis in part due to inflammatory injury. This article introduces recent research reports as references in the related studies.
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Key words: renal; ureteral obstruction; alpha-smooth muscle actin (alpha-SMA); research; literatures; life; cell

Introduction

Renal fibrosis is the final common pathway of most progressive renal diseases. Acute unilateral obstructive uropathy urology a unilateral block of urine flow through the ureter of 1 kidney, resulting in a backup of urine, distension of the renal pelvis and calyces, and hydronephrosis etiology kidney stone, trauma, stricture in children–of one ureter causes acute unilateral obstructive uropathy; slow, progressive blockage causes chronic unilateral obstructive uropathy; either may result in permanent damage and failure of one kidney and HTN, resulting in the so-called Goldblatt kidney, which is usually asymptomic–the remaining kidney usually has functional reserve. Ureteral obstruction results in renal fibrosis in part due to inflammatory injury.

The following introduces recent reports as references in the related studies.

Akin, M., S. Demirbilek, et al. "Attenuation of ureteral obstruction-induced renal injury by polyenylphosphatidylcholine." Int J Urol. 2007 Apr;14(4):350-6.

BACKGROUND: The cytoprotective, antioxidant and antifibrotic effects of polyenylphosphatidylcholine (lecithin, PPC) have been demonstrated both experimentally and clinically. The present study investigated whether PPC treatment has any beneficial effect on renal injury in unilateral partial ureteral obstruction (UUO) in rats. METHODS: Forty Wistar-Albino rats were split into three groups (sham-operated controls, untreated and treated rats). Rats of the untreated and treated groups (n = 15) underwent UUO with two-thirds of the left ureter embedded in the psoas muscle. In group 3, PPC was given orally at a dose of 100 mg/day for 30 days. At the end of the 30th day of the experimental period, obstructed kidneys and blood samples were harvested. To investigate the therapeutic efficacy of PPC treatment in UUO kidneys, oxidant and antioxidant enzyme levels, lipid peroxidation, proinflammatory cytokines (interleukin-1, interleukin-6, tumor necrosis factor alpha), transforming growth factor beta-1 (TGFbeta-1), alpha smooth muscle actin (alpha-SMA) and nuclear factor kappa beta (NF-kappabeta) expression, leukocyte infiltration (ED1, ED2, CD4 and CD8 immunohistochemistry), and tubulointerstitial damage in the obstructed kidneys were studied. RESULTS: Oxidative stress, neutrophil infiltration, release of cytotoxic mediators, TGFbeta-1 levels, tubulointerstitial damage, alpha-SMA and NF-KB expressions in kidney tissue were significantly increased in the UUO rats. PPC treatment attenuated oxidative stress, leukocyte infiltration, cytotoxic mediator, and TGFbeta-1 levels and also decreased expressions of alpha-SMA and NF-kappabeta. It was associated with decreased tubulointerstitial damage, compared with UUO alone. CONCLUSIONS: These results indicate that PPC treatment protects against UUO-induced renal injury in rats possibly through its antioxidant, anti-inflammatory and antifibrotic actions.

Ardura, J. A., R. Berruguete, et al. "Parathyroid hormone-related protein interacts with vascular endothelial growth factor to promote fibrogenesis in the obstructed mouse kidney." Am J Physiol Renal Physiol. 2008 Aug;295(2):F415-25. doi: 10.1152/ajprenal.00018.2008. Epub 2008 Jun 11.

Parathyroid hormone-related protein (PTHrP) interacts with vascular endothelial growth factor (VEGF) in osteoblasts. Since both PTHrP and VEGF have both proinflammatory and profibrogenic features,

we assessed here whether these factors might act in concert to promote fibrogenesis in the obstructed kidney. VEGF receptor (VEGFR)-1 was upregulated, while VEGFR-2 was downregulated (at both mRNA and protein levels) in the mouse kidney within 2-6 days after ureteral obstruction. VEGF protein levels also increased in the obstructed kidney at the latter time. Moreover, this VEGF and VEGFR-1 upregulation was higher in mice overexpressing PTHrP in the proximal tubule than in control littermates. These changes were associated with higher fibronectin mRNA expression and alpha-smooth muscle actin (alpha-SMA) and integrin-linked kinase (ILK) immunostaining and lower apoptotic tubulointerstitial cells in the mouse obstructed kidney than in control littermates. Pretreatment with a neutralizing anti-VEGF antibody reversed these responses in the obstructed kidney of both types of mice. In vitro, PTHrP-(1-36) increased (maximal 2-fold vs. basal, at 100 nM) alpha-SMA and ILK protein expression and decreased E-cadherin protein levels in renal tubuloepithelial mouse cortical tubule and normal rat kidney (NRK) 52E cells. PTHrP-(1-36) also decreased cyclosporine A- and/or osmotic stress-induced apoptosis in these cells and in renal fibroblastic NRK 49F cells. These effects elicited by PTHrP-(1-36) were associated with both VEGF and VEGFR-1 upregulation, and abolished by the anti-VEGF antibody. Collectively, these findings strongly suggest that VEGF acts as an important mediator of PTHrP to promote fibrogenesis in the obstructed kidney.

Ardura, J. A., S. Rayego-Mateos, et al. "Parathyroid hormone-related protein promotes epithelial-mesenchymal transition." J Am Soc Nephrol. 2010 Feb;21(2):237-48. doi: 10.1681/ASN.2009050462. Epub 2009 Dec 3.

Epithelial-mesenchymal transition (EMT) is an important process that contributes to renal fibrogenesis. TGF-beta1 and EGF stimulate EMT. Recent studies suggested that parathyroid hormone-related protein (PTHrP) promotes fibrogenesis in the damaged kidney, apparently dependent on its interaction with vascular endothelial growth factor (VEGF), but whether it also interacts with TGF-beta and EGF to modulate EMT is unknown. Here, PTHrP(1-36) increased TGF-beta1 in cultured tubuloepithelial cells and TGF-beta blockade inhibited PTHrP-induced EMT-related changes, including upregulation of alpha-smooth muscle actin and integrin-linked kinase, nuclear translocation of Snail, and downregulation of E-cadherin and zonula occludens-1. PTHrP(1-36) also induced EGF receptor (EGFR) activation; inhibition of protein kinase C and metalloproteases abrogated this activation. Inhibition of EGFR activation abolished these EMT-related changes, the activation of ERK1/2, and upregulation of TGF-beta1 and VEGF by PTHrP(1-36). Moreover, inhibition of ERK1/2 blocked EMT induced by either PTHrP(1-36), TGF-beta1, EGF, or VEGF. In vivo, obstruction of mouse kidneys led to changes consistent with EMT and upregulation of TGF-beta1 mRNA, p-EGFR protein, and PTHrP. Taken together, these data suggest that PTHrP, TGF-beta, EGF, and VEGF might cooperate through activation of ERK1/2 to induce EMT in renal tubuloepithelial cells.

Asanuma, H., B. A. Vanderbrink, et al. "Arterially delivered mesenchymal stem cells prevent obstruction-induced renal fibrosis." J Surg Res. 2011 Jun 1;168(1):e51-9. doi: 10.1016/j.jss.2010.06.022. Epub 2010 Jul 8.

BACKGROUND: Mesenchymal stem cells (MSCs) hold promise for the treatment of renal disease. While MSCs have been shown to accelerate recovery and prevent acute renal failure in multiple disease models, the effect of MSC therapy on chronic obstruction-induced renal fibrosis has not previously been evaluated. MATERIALS AND METHODS: Male Sprague-Dawley rats underwent renal artery injection of vehicle or fluorescent-labeled human bone marrow-derived MSCs immediately prior to sham operation or induction of left ureteral obstruction (UUO). One or 4 wk later, the kidneys were harvested and the renal cortex analyzed for evidence of stem cell infiltration, epithelial-mesenchymal transition (EMT) as evidenced by E-cadherin/alpha-smooth muscle actin (alpha-SMA) expression and fibroblast specific protein (FSP+) staining, renal fibrosis (collagen content, Masson's trichrome staining), and cytokine and growth factor activity (ELISA and real time RT-PCR). RESULTS: Fluorescent-labeled MSCs were detected in the interstitium of the kidney up to 4 wk post-obstruction. Arterially delivered MSCs significantly reduced obstruction-induced alpha-SMA expression, FSP+ cell accumulation, total collagen content, and tubulointerstitial fibrosis, while simultaneously preserving E-cadherin expression, suggesting that MSCs prevent obstruction-induced EMT and renal fibrosis. Exogenous MSCs reduced obstruction-induced tumor necrosis factor-alpha (TNF-alpha) levels, but did not alter transforming growth factor-beta1 (TGF-beta1), vascular endothelial growth factor (VEGF), interleukin-10 (IL-10), fibroblast growth factor (FGF), or hepatocyte growth factor (HGF) expression. CONCLUSIONS: Human bone marrow-derived MSCs remain viable several weeks after delivery into the kidney and provide protection against obstruction-induced EMT and chronic renal fibrosis. While the mechanism of MSCs-induced renal protection during obstruction remains unclear, our

results demonstrate that alterations in TNF-alpha production may be involved.

Bani-Hani, A. H., J. A. Leslie, et al. "IL-18 neutralization ameliorates obstruction-induced epithelial-mesenchymal transition and renal fibrosis." Kidney Int. 2009 Sep;76(5):500-11. doi: 10.1038/ki.2009.216. Epub 2009 Jun 17.

Ureteral obstruction results in renal fibrosis in part due to inflammatory injury. The role of interleukin-18 (IL-18), an important mediator of inflammation, in the genesis of renal fibrosis was studied using transgenic mice overexpressing human IL-18-binding protein. In addition, HK-2 cells were analyzed following direct exposure to IL-18 compared to control media. Two weeks after ureteral obstruction, the kidneys of wild-type mice had a significant increase in IL-18 production, collagen deposition, alpha-smooth muscle actin and RhoA expression, fibroblast and macrophage accumulation, chemokine expression, and transforming growth factor-beta1 (TGF-beta1) and tumor necrosis factor-alpha (TNF-alpha) production, whereas E-cadherin expression was simultaneously decreased. The transgenic mice with neutralized IL-18 activity exhibited significant reductions in these indicators of obstruction-induced renal fibrosis and epithelial- mesenchymal transition, without demonstrating alterations in TGF-beta1 or TNF-alpha activity. Similarly, the HK-2 cells exhibited increased alpha-smooth muscle actin expression and collagen production, and decreased E-cadherin expression in response to IL-18 stimulation without alterations in TNF-alpha or TGF-beta1 activity. Our study demonstrates that IL-18 is a significant mediator of obstruction-induced renal fibrosis and epithelial- mesenchymal transition independent of downstream TGF-beta1 or TNF-alpha production.

Beghdadi, W., L. C. Madjene, et al. "Mast cell chymase protects against renal fibrosis in murine unilateral ureteral obstruction." Kidney Int. 2013 Aug;84(2):317-26. doi: 10.1038/ki.2013.98. Epub 2013 Mar 20.

Mast cell release of chymase is important in tissue remodeling and may participate in inflammation leading to fibrosis and organ failure. Here we analyzed the function of chymase in unilateral ureteral obstruction, an established accelerated model of renal tubulointerstitial fibrosis. Mice deficient in mouse mast cell protease 4 (mMCP4), the functional counterpart of human chymase, had increased obstruction-induced fibrosis when compared to wild-type mice indicating a protective effect of mMCP4. Engraftment of mast cell-deficient Kit(Wsh/Wsh) mice with wild type, but not mMCP4-deficient mast cells, restored protection confirming the role of mMCP4. Kidneys of mMCP4-deficient mice had higher levels of renal tubular damage, interstitial fibrosis, collagen deposition, increased alpha-smooth muscle actin, and decreased E-cadherin expression compared to the kidneys of wild-type mice. Further analysis showed an elevated inflammatory response in mMCP4-deficient mice with increased levels of kidney-infiltrating macrophages and T cells and local profibrotic TGF-beta1 and CCL2. Granulated and degranulated mast cells and mMCP4 were mainly found in the kidney capsule, respectively, before and after ureteral obstruction. Analysis of mMCP4 substrates showed that it mediates its anti-fibrotic actions by degrading interstitial deposits of fibronectin, a known promoter of inflammatory cell infiltration and adhesion. Thus, mast cell released mMCP4 has anti-fibrotic potential in acutely induced obstructive nephropathy.

Boor, P., A. Konieczny, et al. "Complement C5 mediates experimental tubulointerstitial fibrosis." J Am Soc Nephrol. 2007 May;18(5):1508-15. Epub 2007 Mar 27.

Renal fibrosis is the final common pathway of most progressive renal diseases. C5 was recently identified as a risk factor for liver fibrosis. This study investigated the role of C5 in the development of renal tubulointerstitial fibrosis by (1) induction of renal fibrosis in wild-type and C5(-/-) mice by unilateral ureteral ligation (UUO) and (2) investigation of the effects of a C5a receptor antagonist (C5aRA) in UUO. In C5(-/-) mice, when compared with wild-type controls, markers of renal fibrosis (Sirius Red, type I collagen, fibronectin, alpha-smooth muscle actin, vimentin, and infiltrating macrophages) were significantly reduced on day 5 of UUO. On day 10, fibronectin mRNA and protein expression were still reduced in the C5(-/-) mice. Cortical mRNA of all PDGF isoforms and of TGF-beta(1) (i.e., central mediators of renal disease) were significantly reduced in C5(-/-) mice when compared with controls. Renal tubular cell expression of the C5aR was sparse in normal cortex but markedly upregulated after UUO. Treatment of wild-type UUO mice with C5aRA also led to a significant reduction of cortical Sirius Red staining, fibronectin protein expression, and PDGF-B mRNA expression on day 5. Neither genetic C5 deficiency nor C5aRA treatment caused any histologic changes in the nonobstructed kidneys. In cultured murine cortical tubular cells, C5a stimulated production of TGF-beta(1), and this was inhibited by C5aRA. Using a combined genetic and pharmacologic approach, C5, in particular C5a, is identified as a novel profibrotic factor in renal disease and as a potential new therapeutic target.

Campbell, M. T., K. L. Hile, et al. "Toll-like receptor 4: a novel signaling pathway during renal fibrogenesis." J Surg Res. 2011 Jun 1;168(1):e61-9. doi: 10.1016/j.jss.2009.09.053. Epub 2009 Oct 23.

BACKGROUND: The toll-like receptor (TLR) family serves an important regulatory role in the innate immune system, and recent evidence has implicated TLR signaling in the pro-inflammatory response of a variety of endogenous and exogenous stimuli within the kidney. The role of TLR signaling in fibrotic renal injury, however, remains unknown. MATERIALS AND METHODS: C3H/HeJ TLR4 hyporesponsive mice (TLR4(Lps-d)) or WT controls (C3H/HeOu/J) underwent either sham operation or 1 wk of unilateral ureteral obstruction (UUO). The kidneys were harvested and tissues were analyzed for TLR4 expression (Western blot; RTPCR), E-cadherin and alpha smooth muscle actin (alpha-SMA) expression (Western blot), fibroblast accumulation (fibroblast specific protein (FSP-1+) staining), renal fibrosis (collagen I RTPCR, total collagen assay, Masson's trichrome staining), cytokine gene expression (tumor necrosis factor-alpha (TNF-alpha) and transforming growth factor-beta1 (TGF-beta1) RTPCR), and pSMAD2 and integrin alpha1 expression (Western blot). RESULTS: Mice with intact TLR4 signaling demonstrate a significant increase in TLR4 expression, alpha-SMA expression, fibroblast accumulation, collagen deposition, and interstitial fibrosis, and a significant decrease in E-cadherin expression in response to UUO. TLR4 deficient mice, however, exhibit a significant reduction in obstruction-induced alpha-SMA expression, fibroblast accumulation, and renal fibrosis, with preservation of E-cadherin expression. TLR4's influence on fibroblast accumulation and renal fibrosis occurred independent of any alterations in TNF-alpha, TGF-beta1, or pSMAD2 expression, but did involve alterations integrin alpha1 expression. CONCLUSION: TLR4 appears to be a significant mediator of fibrotic renal injury. While TLR4 signaling is recognized as a critical component of the innate immune response, this is the first study to demonstrate a novel role for TLR4 in renal fibroblast accumulation and tubulointerstitial fibrosis.

Forbes, M. S., B. A. Thornhill, et al. "Fight-or-flight: murine unilateral ureteral obstruction causes extensive proximal tubular degeneration, collecting duct dilatation, and minimal fibrosis." Am J Physiol Renal Physiol. 2012 Jul 1;303(1):F120-9. doi: 10.1152/ajprenal.00110.2012. Epub 2012 Apr 25.

Unilateral ureteral obstruction (UUO) is the most widely used animal model of progressive renal disease. Although renal interstitial fibrosis is commonly used as an end point, recent studies reveal that obstructive injury to the glomerulotubular junction leads to the formation of atubular glomeruli. To quantitate the effects of UUO on the remainder of the nephron, renal tubular and interstitial responses were characterized in mice 7 and 14 days after UUO or sham operation under anesthesia. Fractional proximal tubular mass, cell proliferation, and cell death were measured by morphometry. Superoxide formation was identified by nitro blue tetrazolium, and oxidant injury was localized by 4-hydroxynonenol and 8-hydroxydeoxyguanosine. Fractional areas of renal vasculature, interstitial collagen, alpha-smooth muscle actin, and fibronectin were also measured. After 14 days of UUO, the obstructed kidney loses 19% of parenchymal mass, with a 65% reduction in proximal tubular mass. Superoxide formation is localized to proximal tubules, which undergo oxidant injury, apoptosis, necrosis, and autophagy, with widespread mitochondrial loss, resulting in tubular collapse. In contrast, mitosis and apoptosis increase in dilated collecting ducts, which remain patent through epithelial cell remodeling. Relative vascular volume fraction does not change, and interstitial matrix components do not exceed 15% of total volume fraction of the obstructed kidney. These unique proximal and distal nephron cellular responses reflect differential "fight-or-flight" responses to obstructive injury and provide earlier indexes of renal injury than do interstitial compartment responses. Therapies to prevent or retard progression of renal disease should include targeting proximal tubule injury as well as interstitial fibrosis.

Kato, N., T. Kosugi, et al. "Basigin/CD147 promotes renal fibrosis after unilateral ureteral obstruction." Am J Pathol. 2011 Feb;178(2):572-9. doi: 10.1016/j.ajpath.2010.10.009.

Regardless of their primary causes, progressive renal fibrosis and tubular atrophy are the main predictors of progression to end-stage renal disease. Basigin/CD147 is a multifunctional molecule-it induces matrix metalloproteinases and hyaluronan, for example-and has been implicated in organ fibrosis. However, the relationship between basigin and organ fibrosis has been poorly studied. We investigated basigin's role in renal fibrosis using a unilateral ureteral obstruction model. Basigin-deficient mice (Bsg(-/-)) demonstrated significantly less fibrosis after surgery than Bsg(+/+) mice. Fewer macrophages had infiltrated in Bsg(-/-) kidneys. Consistent with these in vivo data, primary cultured tubular epithelial cells from Bsg(-/-) mice produced less matrix metalloproteinase and exhibited less motility on stimulation with transforming growth factor beta. Furthermore, Bsg(-/-) embryonic fibro blasts produced less hyaluronan and alpha-smooth muscle actin after

transforming growth factor beta stimulation. Together, these results demonstrate for the first time that basigin is a key regulator of renal fibrosis. Basigin could be a candidate target molecule for the prevention of organ fibrosis.

Kawai, T., T. Masaki, et al. "PPAR-gamma agonist attenuates renal interstitial fibrosis and inflammation through reduction of TGF-beta." Lab Invest. 2009 Jan;89(1):47-58. doi: 10.1038/labinvest.2008.104. Epub 2008 Nov 10.

Thiazolidinediones (TZDs), synthetic peroxisome proliferator-activated receptor (PPAR)-gamma ligands, have a central role in insulin sensitization and adipogenesis. It has been reported that TZDs exert protective effects in both diabetic and nondiabetic models of renal disease, although the exact mechanism is not well understood. In particular, only a few studies have reported the renoprotective effects of TZDs in nondiabetic models of tubulointerstitial fibrosis and inflammation. Therefore, we investigated the anti-fibrotic and anti-inflammatory effects of the TZD troglitazone in the mouse model of unilateral ureteral obstruction (UUO). C57BL/6J mice underwent UUO and were studied after 3 and 7 days. Animals were divided into three groups and received control vehicle, troglitazone (150 mg/kg per day) or troglitazone (300 mg/kg per day) by gavage. Kidneys were harvested for morphological, mRNA and protein analysis. Reverse-transcriptase-PCR was used to assess the expression of transforming growth factor-beta1 (TGF-beta1) and the TGF-beta1 type I receptor (TGF beta R-I). Protein expression was assessed by western blotting (TGF beta R-I) and immunostaining (TGF beta R-I, alpha-smooth muscle actin (alpha-SMA), type I collagen (collagen I), F4/80, and proliferating cell nuclear antigen (PCNA)). The expression of alpha-SMA, collagen I, and F4/80 was decreased in mice treated with troglitazone compared with the control group. The numbers of PCNA-positive interstitial cells were decreased in mice treated with troglitazone. TGF-beta1 mRNA and TGF beta R-I mRNA and protein expression were decreased in the group treated with troglitazone compared with the control group. The beneficial effects of troglitazone treatment were also dose dependent. PPAR-gamma agonist significantly reduced TGF-beta and attenuated renal interstitial fibrosis and inflammation in the model of UUO.

Kida, Y., K. Asahina, et al. "Characterization of vitamin A-storing cells in mouse fibrous kidneys using Cygb/STAP as a marker of activated stellate cells." Arch Histol Cytol. 2007 Jul;70(2):95-106.

The expression of the cytoglobin/stellate cell activation-associated protein (Cygb/STAP) was recently confirmed in all splanchnic vitamin A-storing cells--including hepatic stellate cells (HSCs)--in normal conditions. In the hepatic fibrous lesion, the expression of Cygb/STAP has been shown to be upregulated in activated HSCs and myofibroblasts (MFs), which have synthesized extracellular matrices. Furthermore, splanchnic vitamin A-storing cells have been reported to be distributed in the kidney. In this study, we clarify the contribution of vitamin A-storing cells to renal fibrosis by focusing on Cygb/ STAP. Adult mice were subjected to unilateral ureteral obstruction (UUO) and kidneys were harvested 1, 3, 7, and 10 days after UUO. Numbers of Cygb/STAP-immunopositive cells as well as Cygb/STAP mRNA 3 days after UUO (UUO day 3 kidney) increased. Vitamin A-autofluorescence was observed in intertubular spaces of controls but gradually declined in a time-dependent manner after UUO. Cygb/STAP+ cells were not completely identical with alpha-smooth muscle actin (alphaSMA)-positive cells in the control or UUO day 7 kidneys. Immunohistochemical analysis for Cygb/STAP and fibulin-2 (Fib), a specific marker for distinguishing MFs from activated HSCs, revealed that the number of Fib+STAP+ cells (MFs) and Fib-STAP+ cells (splanchnic vitamin A-storing cells) significantly increased in UUO day 3 and UUO day 7 kidneys compared with the controls. Our present findings support the concept that Cygb/STAP can be a unique marker for splanchnic fibroblast-like cells, namely the vitamin A-storing cell lineage, and suggest that splanchnic vitamin A-storing cells contribute to renal fibrogenesis in the obstructed kidney.

Kishimoto, K., K. Kinoshita, et al. "Therapeutic effect of retinoic acid on unilateral ureteral obstruction model." Nephron Exp Nephrol. 2011;118(3):e69-78. doi: 10.1159/000322409. Epub 2011 Jan 13.

BACKGROUND: Retinoic acids, a group of natural and synthetic vitamin A derivatives, have potent anti-proliferative, anti-inflammatory and anti-fibrotic properties. We investigated the therapeutic effect of all-trans-retinoic acid (ATRA) on unilateral ureteral obstruction (UUO) model mice. METHODS: First, to evaluate the prophylactic effect, we administered 0.5 mg of ATRA for 3 days before UUO (UUO ATRA). Then, to evaluate the therapeutic effects, we administered 0.5 mg of ATRA 3 days after UUO (Day 3 ATRA). We compared the histological changes and immunostaining of macrophages, alpha-smooth muscle actin (alpha-SMA) and collagen I, and mRNA expression of monocyte chemotactic protein-1 (MCP-1), transforming growth factor (TGF)-beta(1) and TGF-beta R-II by RT-PCR 7 days after UUO. RESULTS: In the UUO ATRA and Day 3 ATRA groups, we observed a significant improvement in histological and immunological findings, including

macrophage infiltration and improved expression of MCP-1, TGF-beta(1), alpha-SMA and collagen I compared with the UUO Day 7 group. CONCLUSION: ATRA treatment is not only an effective prophylactic strategy, but also a therapeutic strategy for the treatment of progressive renal fibrosis in diseased kidneys.

Kitagawa, H., K. C. Pringle, et al. "Early bladder wall changes after creation of obstructive uropathy in the fetal lamb." Pediatr Surg Int. 2006 Nov;22(11):875-9.

Vesico-amniotic shunting of obstructive uropathy in fetal lambs produced a thick-walled, poorly compliant bladder. We report the early histological changes in the obstructed bladder wall. We created an obstructive uropathy in fetal lambs at 60 days gestation by ligating the urethra and urachus. Vesicostomy or vesico-amniotic shunt tube insertion and biopsy of the bladder wall were performed 21 days later. The fetuses were delivered at term (145 days) and the kidneys and bladder sampled for histology. Colloidal iron (Col Fe), and alpha-smooth muscle actin (alpha-SMA) immunohistochemical stains were used for these samples. Seventeen fetuses were shunted with 15 biopsies taken at that time. Six (shunt failure or missed urachal ligation) were excluded. All biopsies taken at shunting had positive Col Fe and alpha-SMA. Term lambs had mild multicystic dysplastic kidney (MCDK) in five, severe MCDK in two, and hydronephrosis in four. All bladders had small volume and were severely fibrotic. Fetal shunt operations 3 weeks after the creation of obstructive uropathy provided partial preservation of renal histology but did not preserve normal bladder histology. We suggest that the high hyaluronic acid synthesis activity or hyperplasia of the myofibroblasts in the dilated fetal bladder wall at the time of shunting results in irreversible damage to the developing bladder muscle and fibrosis.

Kuratsune, M., T. Masaki, et al. "Signal transducer and activator of transcription 3 involvement in the development of renal interstitial fibrosis after unilateral ureteral obstruction." Nephrology (Carlton). 2007 Dec;12(6):565-71.

BACKGROUND: In vitro studies suggest that the signal transducer and activator of transcription (STAT) plays a critical role in renal fibrosis. However, the process of STAT activation in vivo remains unclear. This study in rats aimed to localize STAT3 activation within the kidney and examine the in vivo relationship between STAT3 activation and renal fibrosis. METHODS: Unilateral ureteral obstruction (UUO) was induced in the rats and the kidneys examined 3 or 7 days after obstruction. Activation of STAT3 in western blot and immunohistochemical analyses was identified by the phosphorylated form of STAT3 (pSTAT3). RESULTS: Myofibroblasts were identified by alpha-smooth muscle actin expression and were upregulated in obstructed kidneys. pSTAT3 was localized mainly in tubular epithelial cells of collecting ducts in normal and obstructed kidneys and interstitial cells in obstructed kidneys. After UUO, western blotting showed a fourfold increase in pSTAT3, with a peak at day 7. Immunostaining showed a sixfold increase in pSTAT3 at day 7 in tubular epithelial cells and a 2500-fold increase at day 7 in interstitial cells. CONCLUSION: STAT3 was activated in rat tubular epithelial cells and myofibroblasts after UUO, suggesting that STAT3 may contribute to the progression of interstitial fibrosis.

Kushibiki, T., N. Nagata-Nakajima, et al. "Delivery of plasmid DNA expressing small interference RNA for TGF-beta type II receptor by cationized gelatin to prevent interstitial renal fibrosis." J Control Release. 2005 Jul 20;105(3):318-31.

Renal interstitial fibrosis is the common pathway of chronic renal disease, while it causes end-stage renal failure. Transforming growth factor-beta (TGF-beta) is well recognized to be one of the primary mediators to induce accumulation of extracellular matrix (ECM) in the fibrotic area. Therefore, it is expected that local suppression of TGF-beta receptor (TGF-betaR) is one of the crucial strategies for anti-fibrotic therapy. The objective of this study is to investigate feasibility of small interference RNA (siRNA) for TGF-betaR in the selective degradation of TGF-betaR mRNAs, resulting in fibrotic inhibition. A plasmid DNA of TGF-betaR siRNA expression vector with or without complexation of a cationized gelatin was injected to the left kidney of mice via the ureter. Unilateral ureteral obstruction (UUO) was performed for the injected mice to evaluate the anti-fibrotic effect. The injection of plasmid DNA-cationized gelatin complex significantly decreased the level of TGF-betaR and alpha-smooth muscle actin (alpha-SMA) over-expression, the collagen content of mice kidney, and the fibrotic area of renal cortex, in contrast to free plasmid DNA injection. It is concluded that retrograde injection of TGF-betaR siRNA expression vector plasmid DNA complexed with the cationized gelatin is available to suppress progression of renal interstitial fibrosis.

Kushiyama, T., T. Oda, et al. "Alteration in the phenotype of macrophages in the repair of renal interstitial fibrosis in mice." Nephrology (Carlton). 2011 Jul;16(5):522-35. doi: 10.1111/j.1440-1797.2010.01439.x.

AIM: Renal interstitial fibrosis is the final common pathway determining long-term prognosis of chronic kidney diseases, but its repair process is scarcely understood. Because recent reports indicate that M2 macrophages play important roles in the repair of various tissues, special attention was paid to the phenotypes of infiltrating macrophages in the present study when the histological changes occurring in mouse kidneys after the release of unilateral ureteral obstruction (UUO) inducing renal fibrosis were analyzed. METHODS: The left ureter of male mice was obstructed for 10 days by using a vascular clamp, and that kidney was removed for analysis either on the day when the clamp was removed or after the kidney had been allowed to recover for 3, 7 or 21 days. RESULTS: Interstitial fibrosis assessed by picrosirius red staining decreased with time after the release, and this decrease was paralleled by a decrease in the interstitial area positive for alpha-smooth muscle actin. Macrophage infiltration assessed by F4/80 staining also significantly decreased from day 3. In contrast, real-time reverse transcription polymerase chain reaction revealed that the ratios of mRNA for the macrophage scavenger receptor (CD204) and the mannose receptor (CD206), both of which are preferentially expressed on M2 macrophages, to CD68 (a general macrophage marker) were significantly greater on day 7 than on day 0 in the UUO-released mice. CONCLUSION: Although the total number of infiltrating myofibroblasts and macrophages decreased after UUO release, the ratios of macrophages expressing CD204 and CD206 increased, suggesting that M2 macrophages play an important role in the repair of renal fibrosis.

Machida, Y., K. Kitamoto, et al. "Renal fibrosis in murine obstructive nephropathy is attenuated by depletion of monocyte lineage, not dendritic cells." J Pharmacol Sci. 2010;114(4):464-73. Epub 2010 Nov 26.

The role of renal dendritic cells (DCs) in renal fibrosis is unknown. The present study was conducted to examine the relative role of renal DCs and macrophages in the development of renal fibrosis in murine obstructive nephropathy. CD11c-diphtheria toxin receptor (DTR) transgenic mice and CD11b-DTR transgenic mice were subjected to unilateral ureteral obstruction. To conditionally and selectively deplete DCs or macrophages, DT was given to these mice and kidneys were harvested on day 5. Ureteral obstruction elicited renal fibrosis characterized by tubulointerstitial collagen III deposition and accumulation of alpha-smooth muscle actin-positive cells. Flow cytometric analysis revealed a marked increase in cell counts of F4/80(+) macrophages, F4/80(+) DCs, as well as neutrophils and T cells in the obstructed kidney. DT administration to CD11c-DTR mice led to selective depletion of renal CD11c(+) DCs, but did not affect renal fibrosis. In contrast, administration of DT to CD11b-DTR mice resulted in ablation of all monocyte lineages including macrophages and DCs and attenuated renal fibrosis. Our results do not support the role of renal DCs, but confirm the importance of monocyte lineage cells other than DCs in the development of the early phase of renal fibrosis following ureteral obstruction in mice.

Manson, S. R., J. B. Song, et al. "HDAC dependent transcriptional repression of Bmp-7 potentiates TGF-beta mediated renal fibrosis in obstructive uropathy." J Urol. 2014 Jan;191(1):242-52. doi: 10.1016/j.juro.2013.06.110. Epub 2013 Jun 29.

PURPOSE: Recombinant BMP-7 inhibits the pathogenesis of renal injury in response to various stimuli. However, little is known about the molecular regulation of endogenous BMP-7 and its renal protective functions. We examined transcriptional regulation of Bmp-7 and its role in the pathogenesis of renal injury resulting from urinary tract dysfunction. MATERIALS AND METHODS: Obstruction induced renal injury was modeled in vivo in mice by unilateral ureteral obstruction and in vitro in primary kidney cells by treatment with transforming growth factor-beta, a profibrotic cytokine that is increased in the obstructed kidney. RESULTS: Unilateral ureteral obstruction resulted in the loss of BMP-7 expression in conjunction with histone deacetylation and transcriptional repression of the Bmp-7 promoter. The histone deacetylase inhibitor trichostatin A stimulated Bmp-7 expression in primary kidney cells. Trichostatin A also inhibited the expression of transforming growth factor-beta dependent profibrotic genes in a manner that depended on BMP receptor signaling. These findings extended to the obstructed kidney in vivo, in which trichostatin A treatment restored the expression of Bmp-7 along with BMP-7 mediated suppression of transforming growth factor-beta dependent signaling pathways. Finally, trichostatin A stimulated activation of the BMP-7 pathway the ameliorated obstruction induced renal injury by preventing disruption of the renal architecture and the development of renal fibrosis. CONCLUSIONS: These findings show that histone deacetylase dependent repression of Bmp-7 transcription is a critical event during the pathogenesis of renal injury in obstructive uropathy. Accordingly, treatment with histone deacetylase inhibitors represents a potentially effective strategy to restore BMP-7 expression and its renal protective functions during treatment of obstructive uropathy.

Meldrum, K. K., R. Misseri, et al. "TNF-alpha neutralization ameliorates obstruction-induced renal fibrosis and dysfunction." Am J Physiol Regul Integr Comp Physiol. 2007 Apr;292(4):R1456-64. Epub 2006 Dec 14.

Upper urinary tract obstruction results in tubulointerstitial fibrosis and a progressive decline in renal function. Although several inflammatory mediators have been implicated in the pathophysiology of renal obstruction, the contribution of TNF-alpha to obstruction-induced fibrosis and renal dysfunction has not been thoroughly evaluated. To study this, male Sprague-Dawley rats were subjected to left unilateral ureteral obstruction vs. sham operation. Rats received either vehicle or a pegylated form of soluble TNF receptor type 1 (PEG-sTNFR1) every 84 h. The kidneys were harvested 1, 3, or 7 days postoperatively, and tissue samples were analyzed for TNF-alpha expression (ELISA), macrophage infiltration (ED-1 staining), transforming growth factor-beta(1) expression (ELISA, RT-PCR), collagen I and IV activity (Western Blot, immunohistochemistry), alpha-smooth muscle actin accumulation (immunohistochemistry, Western blot analysis), and angiotensinogen expression (Western blot). In a separate arm, the glomerular filtration rate (inulin clearance) of rats subjected to unilateral ureteral obstruction in the presence of either vehicle or PEG-sTNFR1 was determined. Renal obstruction induced increased tissue TNF-alpha and transforming growth factor-beta(1) levels, collagen I and IV activity, interstitial volume, alpha-smooth muscle actin accumulation, angiotensinogen expression, and renal dysfunction, whereas treatment with PEG-sTNFR1 significantly reduced each of these markers of renal fibrosis. These results demonstrate that TNF-alpha mediates obstruction-induced renal fibrosis and identify TNF-alpha neutralization as a potential therapeutic option for the amelioration of obstruction-induced renal injury.

Meldrum, K. K., H. Zhang, et al. "Profibrotic effect of interleukin-18 in HK-2 cells is dependent on stimulation of the Toll-like receptor 4 (TLR4) promoter and increased TLR4 expression." J Biol Chem. 2012 Nov 23;287(48):40391-9. doi: 10.1074/jbc.M112.402420. Epub 2012 Oct 1.

BACKGROUND: IL-18 induces profibrotic changes in TECs independent of TGF-beta1 activity. RESULTS: IL-18 stimulates the TLR4 promoter via AP-1 activation to increase TLR4 expression in TECs and stimulates profibrotic changes in TECs through increased TLR4 expression/signaling. CONCLUSION: The profibrotic effect of IL-18 in TECs is mediated through stimulation of TLR4 expression via activation of AP-1. SIGNIFICANCE: This represents a novel fibrotic signaling pathway in TECs independent of TGF-beta1. IL-18 is an important mediator of obstruction-induced renal fibrosis and tubular epithelial cell injury independent of TGF-beta1 activity. We sought to determine whether the profibrotic effect of IL-18 is mediated through Toll-like receptor 4 (TLR4). Male C57BL6 wild type and mice transgenic for human IL-18-binding protein were subjected to left unilateral ureteral obstruction versus sham operation. The kidneys were harvested 1 week postoperatively and analyzed for IL-18 production and TLR4 expression. In a separate arm, renal tubular epithelial cells (HK-2) were directly stimulated with IL-18 in the presence or absence of a TLR4 agonist, TLR4 antagonist, or TLR4 siRNA knockdown. Cell lysates were analyzed for TLR4, alpha-smooth muscle actin, and E-cadherin expression. TLR4 promotor activity, as well as AP-1 activation and the effect of AP-1 knockdown on TLR4 expression, was evaluated in HK-2 cells in response to IL-18 stimulation. The results demonstrate that IL-18 induces TLR4 expression during unilateral ureteral obstruction and induces TLR4 expression in HK-2 cells via AP-1 activation. Inhibition of TLR4 or knockdown of TLR4 gene expression in turn prevents IL-18-induced profibrotic changes in HK-2 cells. These results suggest that IL-18 induces profibrotic changes in tubular epithelial cells via increased TLR4 expression/signaling.

Metcalfe, P. D., J. A. Leslie, et al. "Testosterone exacerbates obstructive renal injury by stimulating TNF-alpha production and increasing proapoptotic and profibrotic signaling." Am J Physiol Endocrinol Metab. 2008 Feb;294(2):E435-43. Epub 2007 Dec 11.

Upper urinary tract obstruction is a common cause of renal dysfunction in children and adults. While there is clinical evidence of an increased male incidence and mortality rate with acute renal failure, the effect of gender and testosterone on obstructive renal injury has not previously been evaluated. We hypothesized that testosterone exacerbates proinflammatory TNF-alpha production and proapoptotic and profibrotic signaling during renal obstruction, resulting in increased apoptotic cell death and tubulointerstitial fibrosis. To study this, male, female, castrated male, and testosterone-treated oophorectomized female rats were subjected to sham operation or 3 days of unilateral ureteral obstruction (UUO). Renal cortical tissue was then analyzed for TNF-alpha production; proapoptotic caspase-8, -9, and -3 activity; apoptotic cell death; profibrotic transforming growth factor-beta1 production; and alpha-smooth muscle actin expression. In a separate arm, glomerular filtration rate (inulin clearance) was measured in rats pre- and post-UUO. Male and

testosterone-treated oophorectomized female rats demonstrated a significant increase in TNF-alpha production, caspase activity, apoptotic cell death, tubulointerstitial fibrosis, and renal dysfunction during UUO compared with castrated males and normal female rats subjected to the same time course of obstruction. These results demonstrate that endogenous testosterone production in normal male rats and testosterone exogenously administered to oophorectomized females significantly increases TNF production and proapoptotic and profibrotic signaling during renal obstruction, resulting in increased apoptotic cell death, tubulointerstitial fibrosis, and renal dysfunction.

Mishima, K., A. Maeshima, et al. "Involvement of N-type Ca(2+) channels in the fibrotic process of the kidney in rats." Am J Physiol Renal Physiol. 2013 Mar 15;304(6):F665-73. doi: 10.1152/ajprenal.00561.2012. Epub 2013 Jan 16.

N-type Ca(2+) channels are densely distributed in sympathetic nerves that innervate renal tubules. However, the role of N-type Ca(2+) channels in renal fibrosis remains unknown. To address this issue, we examined the difference between the effects of amlodipine (an L-type Ca(2+) channel blocker) and cilnidipine (a dual L/N-type Ca(2+) channel blocker) on fibrotic changes using a rat unilateral ureteral obstruction (UUO) model. The expression of both L-type and N-type Ca(2+) channels was significantly upregulated in UUO kidneys compared with that in contralateral kidneys. There were no significant differences in mean blood pressure among the rats tested. Both amlodipine and cilnidipine significantly attenuated fibrotic changes in UUO kidneys. The antifibrotic effect of cilnidipine was more potent than that of amlodipine. Amlodipine as well as cilnidipine reduced type III collagen deposition, alpha-smooth muscle actin (alpha-SMA) expression, and interstitial cell proliferation. In addition, cilnidipine significantly reduced deposition of type I collagen and macrophage infiltration in UUO kidneys. With the use of in vivo bromodeoxyuridine labeling, label-retaining cells (LRCs) were identified as a population of tubular cells that participate in epithelial-mesenchymal transition after UUO. Some LRCs migrated into the interstitium, expressed alpha-SMA and vimentin, and produced several extracellular matrixes in UUO kidneys. The number of interstitial LRCs was significantly decreased by cilnidipine but not amlodipine. These data suggest that N-type Ca(2+) channels contribute to multiple steps of renal fibrosis, and its blockade may thus be a useful therapeutic approach for prevention of renal fibrosis.

Moon, J. A., H. T. Kim, et al. "IN-1130, a novel transforming growth factor-beta type I receptor kinase (ALK5) inhibitor, suppresses renal fibrosis in obstructive nephropathy." Kidney Int. 2006 Oct;70(7):1234-43. Epub 2006 Aug 23.

The transforming growth factor-beta (TGF-beta) plays a central role in the progression of renal fibrosis. TGF-beta transduces its signal through the activin receptor-like kinase (ALK)5. IN-1130, a novel small molecule ALK5 inhibitor, inhibited the purified kinase domain of ALK5-mediated Smad3 phosphorylation with an IC(50) value of 5.3 nM. IN-1130 proved to be highly selective in a panel of 27 serine/threonine and tyrosine kinases including p38alpha mitogen-activated protein kinase. We evaluated the efficacy of IN-1130 to block renal fibrogenesis induced by unilateral ureteral obstruction (UUO) in rats. Either vehicle (saline) or IN-1130 (10 and 20 mg/kg/day) was intraperitoneally administered to UUO rats for 7 and 14 days. Phosphorylated Smad2 (pSmad2) and markers of fibrosis were analyzed in kidney tissues. In UUO control kidneys, interstitial fibrosis including tubular atrophy, loss and dilation, inflammatory cell infiltration, and fibroblast cell proliferation was prominent. These morphological changes were notably reduced by IN-1130 treatment. IN-1130 decreased levels of TGF-beta1 messenger RNA (mRNA), type I collagen mRNA, and pSmad2, compared to UUO control rats. As determined by measuring the hydroxyproline content, total kidney collagen amount was increased in UUO control kidneys, but significantly reduced by IN-1130 treatment, which was comparable to results of histochemical staining for collagen. IN-1130 also suppressed the expression of alpha-smooth muscle actin (alpha-SMA) and fibronectin in UUO kidneys. Our results show that IN-1130 suppressed the fibrogenic process of UUO, further underscoring the potential clinical benefits of IN-1130 in the treatment of renal fibrosis.

Morinaga, J., Y. Kakizoe, et al. "The antifibrotic effect of a serine protease inhibitor in the kidney." Am J Physiol Renal Physiol. 2013 Jul 15;305(2):F173-81. doi: 10.1152/ajprenal.00586.2012. Epub 2013 May 22.

Interstitial fibrosis is a final common pathway for the progression of chronic kidney diseases. Activated fibroblasts have an extremely important role in the progression of renal fibrosis, and transforming growth factor (TGF)-beta(1) is a major activator of fibroblasts. Since previous reports have indicated that serine protease inhibitors have a potential to inhibit TGF-beta(1) signaling in vitro, we hypothesized that a synthetic serine protease inhibitor, camostat mesilate (CM), could slow the progression of renal fibrosis. TGF-beta(1) markedly increased the phosphorylation

of TGF-beta type I receptor, ERK 1/2, and Smad2/3 and the levels of profibrotic markers, such as alpha-smooth muscle actin (alpha-SMA), connective tissue growth factor (CTGF), and plasminogen activator inhibitor-1, in renal fibroblasts (NRK-49F cells), and they were all significantly reduced by CM. In protocol 1, 8-wk-old male Sprague-Dawley rats were subjected to unilateral ureteral obstruction (UUO) and were concurrently treated with a slow-release pellet of CM or vehicle for 14 days. Protocol 2 was similar to protocol 1 except that CM was administered 7 days after UUO. CM substantially improved renal fibrosis as determined by sirius red staining, collagen expression, and hydroxyproline levels. The phosphorylation of ERK1/2 and Smad2/3 and the levels of alpha-SMA, CTGF, promatrix metalloproteinase-2, and matrix metalloproteinase-2 were substantially increased by UUO, and they were all significantly attenuated by CM. These antifibrotic effects of CM were also observed in protocol 2. Our present results suggest the possibility that CM might represent a new class of therapeutic drugs for the treatment of renal fibrosis through the suppression of TGF-beta(1) signaling.

Munoz-Felix, J. M., J. M. Lopez-Novoa, et al. "Heterozygous disruption of activin receptor-like kinase 1 is associated with increased renal fibrosis in a mouse model of obstructive nephropathy." Kidney Int. 2014 Feb;85(2):319-32. doi: 10.1038/ki.2013.292. Epub 2013 Aug 14.

Tubulointerstitial fibrosis is characterized by an accumulation of extracellular matrix in the renal interstitium, myofibroblast activation, cell infiltration, and tubular cell apoptosis, leading to chronic renal failure. Activin receptor-like kinase 1 (ALK1) is a transforming growth factor-beta1 type I receptor with a pivotal role in endothelial proliferation and migration, but its role in the development of renal fibrosis is unknown. To assess this we used the unilateral ureteral obstruction model of tubulointerstitial fibrosis in ALK1 haploinsufficient (ALK1(+/-)) and wild-type mice. After 15 days, there was an increase in extracellular matrix protein expression in the obstructed kidneys from both ALK1(+/+) and ALK1(+/-) mice, but obstructed kidneys from ALK1(+/-) mice showed significantly higher expression of type I collagen than those from wild-type mice. Ureteral obstruction increased kidney myofibroblasts markers (alpha-smooth muscle actin and S100A4), without differences between mouse genotypes. ALK1 expression was increased after ureteral obstruction, and this increased expression was located in myofibroblasts. Moreover, cultured renal fibroblasts from ALK1(+/-) mice expressed more collagen type I and fibronectin than fibroblasts derived from wild-type mice. Thus, ALK1 modulates obstruction-induced renal fibrosis by increased extracellular matrix synthesis in myofibroblasts, but without differences in myofibroblast number.

Mure, P. Y., T. Gelas, et al. "Complete unilateral ureteral obstruction in the fetal lamb. Part II: Long-term outcomes of renal tissue development." J Urol. 2006 Apr;175(4):1548-58.

PURPOSE: We analyzed the dynamics of the renal tissue response to experimental fetal urinary flow impairment concerning renal morphology, extracellular matrix composition, regulators of connective tissue degradation and PAX2 protein expression. MATERIALS AND METHODS: A total of 26 fetal lambs underwent surgical unilateral ureteral obstruction at 90 days of gestation and 14 twin matched animals served as controls. Kidneys were harvested 10, 20 and 40 days after the prior procedure in groups 1 to 3, respectively and in 1-month-old lambs (group 4). Morphological analysis was done using light microscopy. Picrosirius red staining was used to evaluate the area occupied by extracellular matrix components. Collagen I, III and IV, alpha-smooth muscle actin, MMP-1, 2 and 9, TIMP-1 and 2 and PAX2 protein were assessed using immunochemistry. RESULTS: All obstructed kidneys were hydronephrotic without dysplasia. Hypoplasia resulting from a decreased NGG was observed. The inflammatory response to obstruction was poor in fetal obstructed kidneys. From 10 days after obstruction interstitial fibrosis was noted and confirmed by an increase in picrosirius red staining. In obstructed kidneys immunochemistry showed an increase in collagen deposition beginning from the papillae and extending through the whole parenchyma. Aberrant interstitial collagen IV deposition was observed. The increase in alpha-smooth muscle actin staining was mainly localized in the blastema and interstitial cells in obstructed kidneys. MMP and TIMP immunostaining was mainly present in tubules throughout the whole nephrogenic period and persisted in mature kidneys. Beginning from 20 days after obstruction a progressive increase in MMP and TIMP expression was noted. This was associated with ectopic expression in the medullary tubules. PAX2 protein was highly expressed in the nephrogenic zone, decreasing progressively to being markedly decreased in control lamb kidneys. No difference was found in PAX2 expression during the fetal period when comparing unobstructed and obstructed kidneys, it but remained strongly expressed in the dilated collecting ducts of obstructed lambs. CONCLUSIONS: Complete unilateral ureteral obstruction performed in fetal lambs at 90 days of gestation led to pure hydronephrotic transformation, hypoplasia and a marked increase in connective tissue

deposition. Inflammatory infiltrates and PAX2 dysregulation were not seen as having a decisive role in these modifications.

Okamura, D. M., N. M. Bahrami, et al. "Cysteamine modulates oxidative stress and blocks myofibroblast activity in CKD." J Am Soc Nephrol. 2014 Jan;25(1):43-54. doi: 10.1681/ASN.2012090962. Epub 2013 Sep 5.

Therapy to slow the relentless expansion of interstitial extracellular matrix that leads to renal functional decline in patients with CKD is currently lacking. Because chronic kidney injury increases tissue oxidative stress, we evaluated the antifibrotic efficacy of cysteamine bitartrate, an antioxidant therapy for patients with nephropathic cystinosis, in a mouse model of unilateral ureteral obstruction. Fresh cysteamine (600 mg/kg) was added to drinking water daily beginning on the day of surgery, and outcomes were assessed on days 7, 14, and 21 after surgery. Plasma cysteamine levels showed diurnal variation, with peak levels similar to those observed in patients with cystinosis. In cysteamine-treated mice, fibrosis severity decreased significantly at 14 and 21 days after unilateral ureteral obstruction, and renal oxidized protein levels decreased at each time point, suggesting reduced oxidative stress. Consistent with these results, treatment of cultured macrophages with cysteamine reduced cellular generation of reactive oxygen species. Furthermore, treatment with cysteamine reduced alpha-smooth muscle actin-positive interstitial myofibroblast proliferation and mRNA levels of extracellular matrix proteins in mice and attenuated myofibroblast differentiation and proliferation in vitro, but did not augment TGF-beta signaling. In a study of renal ischemia reperfusion, cysteamine therapy initiated 10 days after injury and continued for 14 days decreased renal fibrosis by 40%. Taken together, these data suggest previously unrecognized antifibrotic actions of cysteamine via TGF-beta-independent mechanisms that include oxidative stress reduction and attenuation of the myofibroblast response to kidney injury and support further investigation into the potential benefit of cysteamine therapy in the treatment of CKD.

Oujo, B., J. M. Munoz-Felix, et al. "L-Endoglin overexpression increases renal fibrosis after unilateral ureteral obstruction." PLoS One. 2014 Oct 14;9(10):e110365. doi: 10.1371/journal.pone.0110365. eCollection 2014.

Transforming growth factor-beta (TGF-beta) plays a pivotal role in renal fibrosis. Endoglin, a 180 KDa membrane glycoprotein, is a TGF-beta co-receptor overexpressed in several models of chronic kidney disease, but its function in renal fibrosis remains uncertain. Two membrane isoforms generated by alternative splicing have been described, L-Endoglin (long) and S-Endoglin (short) that differ from each other in their cytoplasmic tails, being L-Endoglin the most abundant isoform. The aim of this study was to assess the effect of L-Endoglin overexpression in renal tubulo-interstitial fibrosis. For this purpose, a transgenic mouse which ubiquitously overexpresses human L-Endoglin (L-ENG+) was generated and unilateral ureteral obstruction (UUO) was performed in L-ENG+ mice and their wild type (WT) littermates. Obstructed kidneys from L-ENG+ mice showed higher amounts of type I collagen and fibronectin but similar levels of alpha-smooth muscle actin (alpha-SMA) than obstructed kidneys from WT mice. Smad1 and Smad3 phosphorylation were significantly higher in obstructed kidneys from L-ENG+ than in WT mice. Our results suggest that the higher increase of renal fibrosis observed in L-ENG+ mice is not due to a major abundance of myofibroblasts, as similar levels of alpha-SMA were observed in both L-ENG+ and WT mice, but to the higher collagen and fibronectin synthesis by these fibroblasts. Furthermore, in vivo L-Endoglin overexpression potentiates Smad1 and Smad3 pathways and this effect is associated with higher renal fibrosis development.

Park, S. A., M. J. Kim, et al. "EW-7197 inhibits hepatic, renal, and pulmonary fibrosis by blocking TGF-beta/Smad and ROS signaling." Cell Mol Life Sci. 2014 Dec 9.

Fibrosis is an inherent response to chronic damage upon immense apoptosis or necrosis. Transforming growth factor-beta1 (TGF-beta1) signaling plays a key role in the fibrotic response to chronic liver injury. To develop anti-fibrotic therapeutics, we synthesized a novel small-molecule inhibitor of the TGF-beta type I receptor kinase (ALK5), EW-7197, and evaluated its therapeutic potential in carbon tetrachloride (CCl4) mouse, bile duct ligation (BDL) rat, bleomycin (BLM) mouse, and unilateral ureteral obstruction (UUO) mouse models. Western blot, immunofluorescence, siRNA, and ChIP analysis were carried out to characterize EW-7197 as a TGF-beta/Smad signaling inhibitor in LX-2, Hepa1c1c7, NRK52E, and MRC5 cells. In vivo anti-fibrotic activities of EW-7197 were examined by microarray, immunohistochemistry, western blotting, and a survival study in the animal models. EW-7197 decreased the expression of collagen, alpha-smooth muscle actin (alpha-SMA), fibronectin, 4-hydroxy-2, 3-nonenal, and integrins in the livers of CCl4 mice and BDL rats, in the lungs of BLM mice, and in the kidneys of UUO mice. Furthermore, EW-7197 extended the lifespan of CCl4 mice, BDL rats, and

BLM mice. EW-7197 blocked the TGF-beta1-stimulated production of reactive oxygen species (ROS), collagen, and alpha-SMA in LX-2 cells and hepatic stellate cells (HSCs) isolated from mice. Moreover, EW-7197 attenuated TGF-beta- and ROS-induced HSCs activation to myofibroblasts as well as extracellular matrix accumulation. The mechanism of EW-7197 appeared to be blockade of both TGF-beta1/Smad2/3 and ROS signaling to exert an anti-fibrotic activity. This study shows that EW-7197 has a strong potential as an anti-fibrosis therapeutic agent via inhibition of TGF-beta-/Smad2/3 and ROS signaling.

Picard, N., O. Baum, et al. "Origin of renal myofibroblasts in the model of unilateral ureter obstruction in the rat." Histochem Cell Biol. 2008 Jul;130(1):141-55. doi: 10.1007/s00418-008-0433-8. Epub 2008 May 1.

Tubulo-interstitial fibrosis is a constant feature of chronic renal failure and it is suspected to contribute importantly to the deterioration of renal function. In the fibrotic kidney there exists, besides normal fibroblasts, a large population of myofibroblasts, which are supposedly responsible for the increased production of intercellular matrix. It has been proposed that myofibroblasts in chronic renal failure originate from the transformation of tubular cells via epithelial-mesenchymal transition (EMT) or from infiltration by bone marrow-derived precursors. Little attention has been paid to the possibility of a transformation of resident fibroblasts into myofibroblasts in renal fibrosis. Therefore we examined the fate of resident fibroblasts in the initial phase of renal fibrosis in the classical model of unilateral ureter obstruction (UUO) in the rat. Rats were perfusion-fixed on days 1, 2, 3 and 4 after ligature of the right ureter. Starting from 1 day of UUO an increasing expression of alpha-smooth muscle actin (alphaSMA) in resident fibroblasts was revealed by immunofluorescence and confirmed by the observation of bundles of microfilaments and webs of intermediate filaments in the electron microscope. Inversely, there was a decreased expression of 5'-nucleotidase (5'NT), a marker of renal cortical fibroblasts. The RER became more voluminous, suggesting an increased synthesis of matrix. Intercellular junctions, a characteristic feature of myofibroblasts, became more frequent. The mitotic activity in fibroblasts was strongly increased. Renal tubules underwent severe regressive changes but the cells retained their epithelial characteristics and there was no sign of EMT. In conclusion, after ureter ligature, resident peritubular fibroblasts proliferated and they showed progressive alterations, suggesting a transformation in myofibroblasts. Thus the resident fibroblasts likely play a central role in fibrosis in that model.

Poosti, F., R. Bansal, et al. "Selective delivery of IFN-gamma to renal interstitial myofibroblasts: a novel strategy for the treatment of renal fibrosis." FASEB J. 2015 Mar;29(3):1029-42. doi: 10.1096/fj.14-258459. Epub 2014 Dec 2.

Renal fibrosis leads to end-stage renal disease demanding renal replacement therapy because no adequate treatment exists. IFN-gamma is an antifibrotic cytokine that may attenuate renal fibrosis. Systemically administered IFN-gamma causes side effects that may be prevented by specific drug targeting. Interstitial myofibroblasts are the effector cells in renal fibrogenesis. Here, we tested the hypothesis that cell-specific delivery of IFN-gamma to platelet-derived growth factor receptor beta (PDGFRbeta)-expressing myofibroblasts attenuates fibrosis in an obstructive nephropathy [unilateral ureteral obstruction (UUO)] mouse model. PEGylated IFN-gamma conjugated to PDGFRbeta-recognizing peptide [(PPB)-polyethylene glycol (PEG)-IFN-gamma] was tested in vitro and in vivo for antifibrotic properties and compared with free IFN-gamma. PDGFRbeta expression was >3-fold increased ($P < 0.05$) in mouse fibrotic UUO kidneys and colocalized with alpha-smooth muscle actin-positive (SMA(+)) myofibroblasts. In vitro, PPB-PEG-IFN-gamma significantly inhibited col1a1, col1a2, and alpha-SMA mRNA expression in TGF-beta-activated NIH3T3 fibroblasts ($P < 0.05$). In vivo, PPB-PEG-IFN-gamma specifically accumulated in PDGFRbeta-positive myofibroblasts. PPB-PEG-IFN-gamma treatment significantly reduced renal collagen I, fibronectin, and alpha-SMA mRNA and protein expression. Compared with vehicle treatment, PPB-PEG-IFN-gamma preserved tubular morphology, reduced interstitial T-cell infiltration, and attenuated lymphangiogenesis (all $P < 0.05$) without affecting peritubular capillary density. PPB-PEG-IFN-gamma reduced IFN-gamma-related side effects as manifested by reduced major histocompatibility complex class II expression in brain tissue ($P < 0.05$ vs. free IFN-gamma). Our findings demonstrate that specific targeting of IFN-gamma to PDGFRbeta-expressing myofibroblasts attenuates renal fibrosis and reduces systemic adverse effects.- Poosti, F., Bansal, R., Yazdani, S., Prakash, J., Post, E., Klok, P., van den Born, J., de Borst, M. H., van Goor, H., Poelstra, K., Hillebrands, J.-L. Selective delivery of IFN-gamma to renal interstitial myofibroblasts: a novel strategy for the treatment of renal fibrosis.

Pradere, J. P., J. Klein, et al. "LPA1 receptor activation promotes renal interstitial fibrosis." J Am Soc Nephrol. 2007 Dec;18(12):3110-8. Epub 2007 Nov 14.

Tubulointerstitial fibrosis in chronic renal disease is strongly associated with progressive loss of renal function. We studied the potential involvement of lysophosphatidic acid (LPA), a growth factor-like phospholipid, and its receptors LPA(1-4) in the development of tubulointerstitial fibrosis (TIF). Renal fibrosis was induced in mice by unilateral ureteral obstruction (UUO) for up to 8 d, and kidney explants were prepared from the distal poles to measure LPA release into conditioned media. After obstruction, the extracellular release of LPA increased approximately 3-fold. Real-time reverse transcription PCR (RT-PCR) analysis demonstrated significant upregulation in the expression of the LPA(1) receptor subtype, downregulation of LPA3, and no change of LPA2 or LPA4. TIF was significantly attenuated in LPA1 (-/-) mice compared to wild-type littermates, as measured by expression of collagen III, alpha-smooth muscle actin (alpha-SMA), and F4/80. Furthermore, treatment of wild-type mice with the LPA1 antagonist Ki16425 similarly reduced fibrosis and significantly attenuated renal expression of the profibrotic cytokines connective tissue growth factor (CTGF) and transforming growth factor beta (TGFbeta). In vitro, LPA induced a rapid, dose-dependent increase in CTGF expression that was inhibited by Ki16425. In conclusion, LPA, likely acting through LPA1, is involved in obstruction-induced TIF. Therefore, the LPA1 receptor might be a pharmaceutical target to treat renal fibrosis.

Samarakoon, R., A. D. Dobberfuhl, et al. "Induction of renal fibrotic genes by TGF-beta1 requires EGFR activation, p53 and reactive oxygen species." Cell Signal. 2013 Nov;25(11):2198-209. doi: 10.1016/j.cellsig.2013.07.007. Epub 2013 Jul 18.

While transforming growth factor-beta (TGF-beta1)-induced SMAD2/3 signaling is a critical event in the progression of chronic kidney disease, the role of non-SMAD mechanisms in the orchestration of fibrotic gene changes remains largely unexplored. TGF-beta1/SMAD3 pathway activation in renal fibrosis (induced by ureteral ligation) correlated with epidermal growth factor receptor(Y845) (EGFR(Y845)) and p53(Ser15) phosphorylation and induction of disease causative target genes plasminogen activator inhibitor-1 (PAI-1) and connective tissue growth factor (CTGF) prompting an investigation of the mechanistic involvement of EGFR and tumor suppressor p53 in profibrotic signaling. TGF-beta1, PAI-1, CTGF, p53 and EGFR were co-expressed in the obstructed kidney localizing predominantly to the tubular and interstitial compartments. Indeed, TGF-beta1 activated EGFR and p53 as well as SMAD2/3. Genetic deficiency of either EGFR or p53 or functional blockade with AG1478 or Pifithrin-alpha, respectively, effectively inhibited PAI-1and CTGF induction and morphological transformation of renal fibroblasts as did SMAD3 knockdown or pretreatment with the SMAD3 inhibitor SIS3. Reactive oxygen species (ROS)-dependent mechanisms initiated by TGF-beta1 were critical for EGFR(Y845) and p53(Ser15) phosphorylation and target gene expression. The p22(Phox) subunit of NADPH oxidase was also elevated in the fibrotic kidney with an expression pattern similar to p53 and EGFR. EGF stimulation alone initiated, albeit delayed, c-terminal SMAD3 phosphorylation (that required the TGF-beta1 receptor) and rapid ERK2 activation both of which are necessary for PAI-1 and CTGF induction in renal fibroblasts. These data highlight the extensive cross-talk among SMAD2/3, EGFR and p53 pathways essential for expression of TGF-beta1-induced fibrotic target genes.

Shiohira, S., T. Yoshida, et al. "Sphingosine-1-phosphate acts as a key molecule in the direct mediation of renal fibrosis." Physiol Rep. 2013 Dec 5;1(7):e00172. doi: 10.1002/phy2.172. eCollection 2013 Dec 1.

The major sphingolipid metabolite, sphingosine-1-phosphate (S1P), has important biological functions. S1P serves as a ligand for a family of five G-protein-coupled receptors with distinct signaling pathways regulating important biological pathways. S1P induces renal fibrosis through an inflammatory pathway. However, its direct fibrosis-inducing effect on the kidney has not been shown. The role of S1P as a direct mediator of renal fibrosis was investigated in normal rat kidney interstitial fibroblast (NRK-49F) cells (in vitro) and kidneys of a unilateral ureteral obstruction (UUO) mouse model (in vivo). To clarify the role of S1P in renal fibrosis, we adopted nude UUO mice with immune response deficits. NRK-49F cells were stimulated with various concentrations of exogenous S1P and FTY720 (a S1P receptor agonist) or N,N-dimethylsphingosine (DMS; a sphingosine kinase inhibitor). C57BL6 and nude UUO mice were pretreated with FTY720, DMS, or saline. Expression levels of alpha-smooth muscle actin (a-SMA), E-cadherin, collagen type 1 (COL1), collagen type 4 (COL4), tissue inhibitor of matrix metalloproteinase-1 (TIMP1), and plasminogen activator inhibitor-1 (PAI1) were examined. S1P stimulated fibrosis in NRK-49F cells and UUO mice. Increased a-SMA, COL1, COL4, TIMP1, and PAI1 and decreased E-cadherin expression levels were observed in both the

S1P-stimulated cells and UUO mice. Nude UUO mouse kidneys expressed fibrotic markers. Fibrotic changes were successfully induced in both UUO and nude UUO mice, evident through prominent fibronectin and COL1 staining. These S1P-induced fibrotic changes were suppressed by FTY720 and DMS both in vitro and in vivo. Thus, S1P essentially and directly mediates renal fibrosis.

Sugiura, H., T. Yoshida, et al. "Reduced Klotho expression level in kidney aggravates renal interstitial fibrosis." Am J Physiol Renal Physiol. 2012 May 15;302(10):F1252-64. doi: 10.1152/ajprenal.00294.2011. Epub 2012 Feb 15.

Renal expression of the klotho gene is markedly suppressed in chronic kidney disease (CKD). Since renal fibrosis is the final common pathology of CKD, we tested whether decreased Klotho expression is a cause and/or a result of renal fibrosis in mice and cultured renal cell lines. We induced renal fibrosis by unilateral ureteral obstruction (UUO) in mice with reduced Klotho expression (kl/+ mice) and compared them with wild-type mice. The UUO kidneys from kl/+ mice expressed significantly higher levels of fibrosis markers such as alpha-smooth muscle actin (alpha-SMA), fibronectin, and transforming growth factor-beta(1) (TGF-beta(1)) than those from wild-type mice. In addition, in cultured renal fibroblast cells (NRK49F), the levels of alpha-SMA and PAI1 expression were significantly suppressed by addition of recombinant Klotho protein to the medium. The similar effects were observed by a TGF-beta(1) receptor inhibitor (ALK5 inhibitor). These observations suggest that low renal Klotho expression enhances TGF-beta(1) activity and is a cause of renal fibrosis. On the other hand, TGF-beta(1) reduced Klotho expression in renal cultured epithelial cells (inner medullary collecting duct and human renal proximal tubular epithelium), suggesting that low renal Klotho expression is a result of renal fibrosis. Taken together, renal fibrosis can trigger a deterioration spiral of Klotho expression, which may be involved in the pathophysiology of CKD progression.

Summers, S. A., P. Y. Gan, et al. "Mast cell activation and degranulation promotes renal fibrosis in experimental unilateral ureteric obstruction." Kidney Int. 2012 Sep;82(6):676-85. doi: 10.1038/ki.2012.211. Epub 2012 Jun 6.

Progressive renal fibrosis is the final common pathway leading to renal failure irrespective of the initiating cause. Clinical studies of renal fibrosis found that prominent mast cell accumulation correlated with worse outcomes. Mast cells are pluripotent innate immune cells that synthesize and secrete profibrotic mediators. Here we use mast cell-deficient (Kit(W-sh/W-sh)) mice to define a functional pathogenic role for these cells in the development of renal fibrosis. Intrarenal collagen deposition was significantly decreased in mast cell-deficient compared to wild-type mice 7 and 14 days after unilateral ureteric obstruction. The intrarenal expression of mRNAs for transforming growth factor-beta, alpha-smooth muscle actin, chemokines, and renal macrophages and CD4(+) T cells were also decreased in mast cell-deficient mice. Reconstitution of the mast cell population in mast cell-deficient mice with wild-type bone marrow-derived mast cells restored the pattern and intensity of renal fibrosis to levels seen in wild-type mice following ureteric ligation. Interestingly, the mast cells were recruited, activated, and degranulated within 6 h of ureteric ligation. A mast cell stabilizer that impairs degranulation, disodium chromoglycate, significantly attenuated renal fibrosis following ureteric ligation in wild-type mice. Thus, mast cells promote renal fibrosis and their targeting may offer therapeutic potential in the treatment of renal fibrosis.

Surendran, K., S. Schiavi, et al. "Wnt-dependent beta-catenin signaling is activated after unilateral ureteral obstruction, and recombinant secreted frizzled-related protein 4 alters the progression of renal fibrosis." J Am Soc Nephrol. 2005 Aug;16(8):2373-84. Epub 2005 Jun 8.

beta-Catenin functions as a transducer of Wnt signals to the nucleus, where it interacts with the T cell factor (TCF) family of DNA binding proteins to regulate gene expression. On the basis of the genes regulated by beta-catenin and TCF in various biologic settings, two predicted functions of beta-catenin/TCF-dependent transcription are to mediate the loss of epithelial polarity and to promote fibroblast activities, such as the increased synthesis of fibronectin during chronic renal disease. These predictions were tested by determination of the expression and function of an inhibitor of Wnt signaling, secreted frizzled-related protein 4 (sFRP4), during renal tubular epithelial injury initiated by unilateral ureteral obstruction (UUO). Despite increased sFRP4 gene expression in perivascular regions of injured kidneys, total sFRP4 protein levels decreased after injury. The decreased sFRP4 protein levels after UUO accompanied increased Wnt-dependent beta-catenin signaling in tubular epithelial and interstitial cells, along with increased expression of markers of fibrosis. Administration of recombinant sFRP4 protein caused a reduction in tubular epithelial beta-catenin signaling and suppressed the progression of renal fibrosis, as evidenced by a partial maintenance of E-cadherin mRNA expression and a reduction in the amount of fibronectin and alpha-smooth muscle actin proteins. Furthermore, recombinant sFRP4 reduced the number

of myofibroblasts, a central mediator of fibrosis. It is concluded that beta-catenin signaling is activated in tubular epithelial and interstitial cells after renal injury, and recombinant sFRP4 can interfere with epithelial de-differentiation and with fibroblast differentiation and function during progression of renal fibrosis.

Suzuki, S., H. Fukasawa, et al. "Renal damage in obstructive nephropathy is decreased in Skp2-deficient mice." Am J Pathol. 2007 Aug;171(2):473-83. Epub 2007 Jul 9.

Ubiquitin-dependent degradation of the cyclin-dependent kinase inhibitor p27 mediated by SCF-Skp2 ubiquitin ligase is involved in cell cycle regulation. Proliferation of tubular cells is a characteristic feature in obstructed kidneys of unilateral ureteral obstruction. Comparing Skp2(+/+) mice with Skp2(-/-) mice, we investigated the involvement of Skp2, a component of SCF-Skp2 ubiquitin ligase for p27, in the progression of renal lesions in unilateral ureteral obstructed kidneys. mRNA expression of Skp2 was markedly increased in the obstructed kidneys from Skp2(+/+) mice and peaked 3 days after unilateral ureteral obstruction. Renal atrophy, tubular dilatation, tubulointerstitial fibrosis, and increases in alpha-smooth muscle actin expression, the number of tubular cells, and proliferating tubular cells positive for Ki67 were observed in the obstructed kidneys from Skp2(+/+) mice; however, these findings were significantly attenuated in Skp2(-/-) mice. The p27 protein level was increased in the obstructed kidneys but was significantly greater in Skp2(-/-) mice. The number of Ki67-positive p27-negative cells was lower in obstructed kidneys from Skp2(-/-) mice than Skp2(+/+) mice, whereas that of Ki67-negative p27-positive cells was greater in Skp2(-/-) mice. These findings suggest that p27 accumulation, which results from SCF-Skp2 ubiquitin ligase deficiency in Skp2(-/-) mice, is involved in the amelioration of renal damage induced by obstructive nephropathy.

Takahashi, S., Y. Taniguchi, et al. "Mizoribine suppresses the progression of experimental peritoneal fibrosis in a rat model." Nephron Exp Nephrol. 2009;112(2):e59-69. doi: 10.1159/000213896. Epub 2009 Apr 23.

BACKGROUND/AIMS: Peritoneal fibrosis is a serious complication of peritoneal dialysis (PD). It has been reported that administration of mizoribine, an effective immunosuppressant, ameliorated renal fibrosis in a rat model of unilateral ureteral obstruction. We therefore examined the effects of mizoribine in an experimental model of peritoneal fibrosis. METHODS: 24 rats were given a daily intraperitoneal injection of chlorhexidine gluconate and ethanol dissolved in saline. The rats were divided into three groups (n = 8 per group) that received either vehicle or mizoribine at a dose of 2 or 8 mg/kg once a day. 28 days after the start of the treatments the rats were sacrificed and peritoneal tissue samples collected. Macrophage infiltration (ED1), myofibroblast accumulation (alpha-smooth muscle actin (SMA)) and expression of type III collagen, transforming growth factor (TGF)-beta and monocyte chemotactic protein-1 (MCP-1) were examined by immunohistochemistry. RESULTS: Mizoribine significantly suppressed submesothelial zone thickening and reduced macrophage infiltration. Mizoribine also reduced collagen III(+) area and decreased the number of alpha-SMA(+), TGF-beta(+) and MCP-1(+) cells. The magnitude of the changes observed was dose-dependent. CONCLUSION: The administration of mizoribine prevented the progression of peritoneal fibrosis in this rat model. Mizoribine may represent a novel therapy for peritoneal sclerosis in patients undergoing long-term PD.

Takeda, Y., T. Nishikimi, et al. "Beneficial effects of a combination of Rho-kinase inhibitor and ACE inhibitor on tubulointerstitial fibrosis induced by unilateral ureteral obstruction." Hypertens Res. 2010 Sep;33(9):965-73. doi: 10.1038/hr.2010.112. Epub 2010 Jul 22.

We and others recently reported that long-term Rho-kinase inhibition has renoprotective effects. This study was designed to compare the effects of an angiotensin-converting enzyme (ACE) inhibitor (imidapril), a Rho-kinase inhibitor (fasudil) and a combination of them both on renal interstitial fibrosis induced by unilateral ureteral obstruction (UUO). We also attempted to elucidate the mechanism involved. Imidapril (50 mg l(-1)), fasudil (1 g l(-1)) or a combination of them both was given in drinking water to mice, and their effects were compared on renal interstitial fibrosis induced by UUO. We assessed histological findings, monocyte/macrophage infiltration, myofibroblast differentiation, oxidative stress and the expression of various mRNA in the kidney by UUO. Eleven days after UUO, wild-type kidney was characterized by increased fibrotic area, dihydroethidium (DHE)-positive area, alpha-smooth muscle actin (SMA)-positive area, F4/80-positive area and the increased expression of various mRNA. Fasudil and imidapril similarly improved fibrotic area (-23%, -15%), DHE-positive area (-13%, -11%), alpha-SMA-positive area (-22%, -15%), F4/80-positive area (-42%, -34%) and the expression of various mRNA, most of which were significant ($P<0.05$). The combination of imidapril and fasudil further improved fibrotic area (-52%), DHE-positive

area (-26%), alpha-SMA-positive area (-33%), F4/80-positive area (-62%) and the expression of various mRNA (all P<0.05 vs. monotherapy). Compared with either agent alone, the combination of an ACE inhibitor and a Rho-kinase inhibitor was more effective for the prevention of renal interstitial fibrosis because of the inhibition of transforming growth factor-beta/collagen, monocyte/macrophage infiltration, myofibroblast differentiation, inflammation and the oxidative stress pathway.

Terashima, H., M. Kato, et al. "A sensitive short-term evaluation of antifibrotic effects using newly established type I collagen reporter transgenic rats." Am J Physiol Renal Physiol. 2010 Oct;299(4):F792-801. doi: 10.1152/ajprenal.00141.2009. Epub 2010 Jul 21.

Fibrosis is the final common pathway for various tissue lesions that lead to chronic progressive organ failure, and consequently effective antifibrotic drugs are strongly desired. However, there are few animal models in which it is possible to evaluate fibrosis sensitively in a short period of time. We therefore generated two transgenic rats harboring a firefly luciferase reporter gene under the control of the 5'-flanking region of rat alpha(1)(I) collagen (Col1a1-Luc Tg rats) and alpha(2)(I) collagen (Col1a2-Luc Tg rats). The luciferase activities of these transgenic rats were highly correlated with the hydroxyproline content in various organs. In unilateral ureteral obstruction (UUO), a well-characterized model of renal fibrosis, the luciferase activity in obstructed kidneys showed a significant increase after even 3 days of UUO, while the hydroxyproline content showed little increase. In addition, the renal hydroxyproline content had a higher correlation with the luciferase activity than alpha(1)(I) collagen mRNA level for over 2 wk after UUO. Although both an ANG II type 1 receptor blocker (ARB), olmesartan, and a transforming growth factor-beta (TGF-beta) type I receptor kinase (ALK5) inhibitor, SB-431542, inhibited renal luciferase activities in UUO, only SB-431542 inhibited luciferase activity induced by TGF-beta1 in isolated glomeruli. Double immunostaining for luciferase and alpha-smooth muscle actin (alpha-SMA) revealed that some alpha-SMA-positive tubular epithelial cells and tubular interstitial cells produced type I collagen, which would lead to renal fibrosis. Thus collagen reporter transgenic rats would be very useful for the evaluation of antifibrotic effects and analysis of their mechanisms.

Togao, O., S. Doi, et al. "Assessment of renal fibrosis with diffusion-weighted MR imaging: study with murine model of unilateral ureteral obstruction." Radiology. 2010 Jun;255(3):772-80. doi: 10.1148/radiol.10091735. Epub 2010 Apr 20.

PURPOSE: To test, in a murine model of unilateral ureteral obstruction (UUO), whether the magnetic resonance (MR) imaging-derived apparent diffusion coefficient (ADC) changes during the progression of renal fibrosis and correlates with the histopathologic changes observed in renal fibrogenesis. MATERIALS AND METHODS: This study was approved by the institutional animal care and use committee. A UUO was created in each of 14 mice. In five mice, longitudinal diffusion-weighted (DW) imaging was performed before the UUO (day 0) and on days 3 and 7 after the UUO and was followed by histopathologic analysis. The nine remaining mice were examined with cross-sectional studies on days 0 (n = 4) and 3 (n = 5). ADCs were measured with a spin-echo echo-planar sequence at five b values ranging from 350 to 1200 sec/mm(2). Differences in ADC among the time points and between the sides were assessed by using Tukey-Kramer and Student t tests, respectively. ADC was correlated with cell density and alpha-smooth muscle actin (alpha-SMA, a marker of myofibroblasts) expression at linear regression analysis. RESULTS: Histopathologic examination revealed typical renal fibrosis on the side with UUO. The ADC decreased over time on the UUO side, from (1.02 +/- 0.06 [standard deviation]) x 10(-3) mm(2)/sec on day 0 to (0.70 +/- 0.08) x 10(-3) mm(2)/sec on day 3 (P < .001) and (0.57 +/- 0.10) x 10(-3) mm(2)/sec on day 7 (P < .001). The percentage change in ADC was greater on the UUO side than on the contralateral side on days 3 (29% +/- 9, P = .05) and 7 (44% +/- 11, P < .01). ADC correlated with both increased cell density and increased alpha-SMA expression (P < .001 for both correlations). CONCLUSION: An ADC decrease in renal fibrosis is associated with an increased number of cells, including fibroblasts. ADC has the potential to serve as a sensitive noninvasive biomarker of renal fibrosis.

Vidyasagar, A., S. Reese, et al. "HSP27 is involved in the pathogenesis of kidney tubulointerstitial fibrosis." Am J Physiol Renal Physiol. 2008 Sep;295(3):F707-16. doi: 10.1152/ajprenal.90240.2008. Epub 2008 Jul 2.

We hypothesized that heat shock protein 27 (HSP27), a small heat shock protein with actin-remodeling properties, is involved in the pathogenesis of kidney tubulointerstitial fibrosis. We first examined its expression in the rat unilateral ureteral obstruction (UUO) model of kidney fibrosis and epithelial-to-mesenchymal transition (EMT). Immunoblot analyses showed that UUO resulted in significant upregulation of TGF-beta1, alpha-smooth muscle actin (alpha-SMA), total and phosphorylated HSP27, and

phosphorylated p38MAPK. Immunofluorescence studies showed that HSP27 costained with TGF-beta1, alpha-SMA, and E-cadherin in areas of tubulointerstitial injury. We next attempted to translate these studies in an in vitro model of EMT using rat proximal tubular epithelial cells (NRK52E). TGF-beta1 (20 ng/ml) treatment resulted in EMT (upregulation of alpha-SMA and downregulation of E-cadherin) and significant upregulation of total and phosphorylated HSP27 and p38MAPK after 3 days. Real-time PCR analyses showed that HSP27, vimentin, and fibronectin increased whereas E-cadherin mRNA levels decreased. Double-staining immunofluorescence studies showed intracytoplasmic colocalization of HSP27 with both F-actin and E-cadherin in cells undergoing EMT. HSP27 overexpression by transient transfection significantly increased E-cadherin while decreasing E-cadherin repressor Snail levels. In aggregate, these studies show that HSP27 is involved in the pathogenesis of TGF-beta1-induced EMT and chronic tubulointerstitial fibrosis. HSP27 overexpression may delay injury by upregulating E-cadherin through downregulation of Snail.

Vieira, J. M., Jr., E. Mantovani, et al. "Simvastatin attenuates renal inflammation, tubular transdifferentiation and interstitial fibrosis in rats with unilateral ureteral obstruction." Nephrol Dial Transplant. 2005 Aug;20(8):1582-91. Epub 2005 Apr 26.

BACKGROUND: The pleiotropic actions of statins have been largely explored. These drugs have been tested in several models of progressive renal disease, most of them accompanied by hypertension. We sought to investigate more closely the effects of simvastatin on renal interstitial fibrosis due to unilateral ureteral obstruction (UUO). METHODS: Munich-Wistar rats were submitted to UUO and studied after 14 days. Animals were divided into two groups: vehicle (VH) or simvastatin (SIMV) 2 mg/kg b.i.d. by gavage. At sacrifice kidneys were harvested for morphology, mRNA and protein analysis. RT-PCR was done to assess expression of collagen I and III, fibronectin, MCP-1, TGF-beta1 and bFGF. Protein expression was assessed by western blot (TGF-beta) and immunostaining (macrophage, lymphocyte, PCNA, vimentin and alpha-smooth muscle actin). Contralateral kidneys (CL) were used as controls. RESULTS: SIMV-treated animals had less severe renal inflammation. MCP-1 was markedly expressed in obstructed kidneys and diminished with SIMV (48.9+/- 2.5 vs 64.3+/-3.1 OD in VH, P<0.01). Interstitial fibrosis (IF) was significantly attenuated with SIMV (8.2+/-1.3 vs 13.2+/-0.6%, P<0.01 SIMV vs VH), which was confirmed by a decrease in collagen I and fibronectin renal expression. Vimentin, a marker of dedifferentiation, was expressed in tubular cells of VH and decreased with SIMV treatment. alpha-SMA, a marker of myofibroblast-type cells, was increased in renal interstitium of VH rats and SIMV significantly reduced its expression. PCNA was increased in the UUO kidneys, but SIMV did not decrease tubular or interstitial proliferating cells. TGF-beta1, which was highly induced in the obstructed kidneys, decreased at the post-transcriptional level with SIMV treatment (5.35+/-0.75 vs 13.10+/-2.9 OD in VH, P<0.05). bFGF mRNA was also overexpressed in the obstructed kidneys, although SIMV treatment did not significantly decrease its expression. CONCLUSIONS: SIMV had an evident protective effect on renal interstitial inflammation and fibrosis. It is conceivable that by attenuating inflammation, SIMV prevented tubular activation and transdifferentiation, two processes largely involved in the renal fibrosis of the UUO model.

Voelkl, J., S. Mia, et al. "PKB/SGK-resistant GSK-3 signaling following unilateral ureteral obstruction." Kidney Blood Press Res. 2013;38(1):156-64. doi: 10.1159/000355763. Epub 2014 Mar 15.

BACKGROUND/AIMS: Renal tissue fibrosis contributes to the development of end-stage renal disease. Causes for renal tissue fibrosis include obstructive nephropathy. The development of renal fibrosis following unilateral ureteral obstruction (UUO) is blunted in gene-targeted mice lacking functional serum- and glucocorticoid-inducible kinase SGK1. Similar to Akt isoforms, SGK1 phosphorylates and thus inactivates glycogen synthase kinase GSK-3. The present study explored whether PKB/SGK-dependent phoshorylation of GSK-3alpha/beta impacts on pro-fibrotic signaling following UUO. METHODS: UUO was induced in mice carrying a PKB/SGK-resistant GSK-3alpha/beta (gsk-3(KI)) and corresponding wild-type mice (gsk-3(WT)). Three days after the obstructive injury, expression of fibrosis markers in kidney tissues was analyzed by quantitative RT-PCR and western blotting. RESULTS: GSK-3alpha and GSK-3beta phosphorylation was absent in both, the non-obstructed and the obstructed kidney tissues from gsk-3(KI) mice but was increased by UUO in kidney tissues from gsk-3(WT) mice. Expression of alpha-smooth muscle actin, type I collagen and type III collagen in the non-obstructed kidney tissues was not significantly different between gsk-3(KI) mice and gsk-3(WT) mice but was significantly less increased in the obstructed kidney tissues from gsk-3(KI) mice than from gsk-3(WT) mice. After UUO treatment, renal beta-catenin protein abundance and renal expression of the beta-catenin sensitive genes: c-Myc, Dkk1, Twist and Lef1 were

again significantly less increased in kidney tissues from gsk-3(KI) mice than from gsk-3(WT) mice. CONCLUSIONS: PKB/SGK-dependent phosphorylation of glycogen synthase kinase GSK-3 contributes to the pro-fibrotic signaling leading to renal tissue fibrosis in obstructive nephropathy.

White, L. R., J. B. Blanchette, et al. "The characterization of alpha5-integrin expression on tubular epithelium during renal injury." Am J Physiol Renal Physiol. 2007 Feb;292(2):F567-76. Epub 2006 Oct 3.

The hallmark of progressive chronic kidney disease is the deposition of extracellular matrix proteins and tubulointerstitial fibrosis. Integrins mediate cell-extracellular matrix interaction and may play a role tubular epithelial injury. Murine primary tubular epithelial cells (TECs) express alpha(5)-integrin, a fibroblast marker and the natural receptor for fibronectin. Microscopy localized alpha(5)-integrin on E-cadherin-positive cells, confirming epithelial expression. The expression of alpha(5)-integrin increased in TECs grown on fibronectin and occurred in parallel with an upregulation of alpha-smooth muscle actin (alphaSMA), a marker of epithelial-mesenchymal transition (EMT). Exposure of TECs to transforming growth factor (TGF)-beta also increased TEC alpha(5)-integrin expression in association with alphaSMA and EMT. Knock-down of alpha5-integrin expression with short interfering RNA attenuated the TGF-beta induction of alphaSMA but did not alter morphologic EMT. Rather, alpha5-integrin was necessary for epithelial cell migration on fibronectin but not type IV collagen during cell spreading and epithelial wound healing in vitro. Immunohistochemistry revealed basolateral tubular epithelial alpha(5)-integrin expression in mouse kidneys after unilateral ureteric obstruction but not in contralateral control kidneys. In patient biopsies of nondiabetic kidney disease, alpha(5)-integrin expression was increased significantly in the renal interstitium. Focal basolateral staining was also detected in injured, but not in normal, tubular epithelium. In summary, these data show that TECs are induced to express alpha(5)-integrin during EMT and tubular epithelial injury in vitro and in vivo. These results increase our understanding of the biology of integrins during EMT and tubular injury in chronic kidney disease.

Winbanks, C. E., L. Grimwood, et al. "Role of the phosphatidylinositol 3-kinase and mTOR pathways in the regulation of renal fibroblast function and differentiation." Int J Biochem Cell Biol. 2007;39(1):206-19. Epub 2006 Aug 18.

Tubulointerstitial fibrosis is largely mediated by (myo)fibroblasts present in the interstitium. In this study, we investigated the role of mTOR and phosphatidylinositol 3-kinase in the regulation of fibroblast kinetics, fibroblast differentiation, and collagen synthesis. Rat renal fibroblasts were propagated from kidneys 3 days post-ureteric obstruction and specific inhibitors of mTOR (RAD) and phosphatidylinositol 3-kinase (LY294002) were used to examine the regulation of fibrogenesis. LY294002 but not RAD completely inhibited phosphorylation of Akt, while both inhibitors decreased phosphorylation of the S6 ribosomal protein. RAD and LY decreased foetal calf serum stimulated proliferation and DNA synthesis. In addition to their individual effects, treatment with both RAD and LY294002 decreased serum-induced fibroblast proliferation and DNA synthesis significantly more than either drug alone. TUNEL positive cells (apoptosis) in RAD and LY294002 treated groups were not different from control groups. In addition to their effect on proliferation, both inhibitors also reduced total collagen synthesis. Differentiation studies indicated an increase in alpha-smooth muscle actin expression relative to beta-actin (western blotting), with cytochemistry confirming that all doses of RAD and LY294002 increased the proportion of alpha-smooth muscle actin positive cells, and hence myofibroblasts. Effects were independent of cell toxicity. These results highlight the potential significance of PI3K and mTOR, in the regulation of renal (myo)fibroblast activity. The synergistic effects of LY and RAD on proliferation suggest that mTOR signalling involves pathways other than phosphatidylinositol 3-kinase. These results provide a novel insight into the mechanisms of fibroblast regulation during fibrogenesis.

Wolak, T., H. Kim, et al. "Osteopontin modulates angiotensin II-induced inflammation, oxidative stress, and fibrosis of the kidney." Kidney Int. 2009 Jul;76(1):32-43. doi: 10.1038/ki.2009.90. Epub 2009 Apr 8.

Osteopontin, a secreted glycoprotein has been implicated in several renal pathological conditions such as those due to ureteral obstruction, ischemia, and cyclosporine toxicity. We studied its possible role in angiotensin II-mediated renal injury by infusing wild-type and osteopontin knockout mice with angiotensin II and found that it raised blood pressure and increased urinary albumin/creatinine ratios in both strains of mice. However, while wild-type mice responded to the infusion by macrophage infiltration and increased expression of alpha-smooth muscle actin, fibronectin, and transforming growth factor-beta; the osteopontin knockout mice developed none of these. Further, the

knockout mice had increased expression of monocyte chemoattractant protein-1; NADPH oxidase subunits such as NOX2, gp47phox, and NOX4; and plasminogen activator inhibitor-1 compared to the wild type animals. Proximal tubule epithelial cells in culture treated with recombinant osteopontin and angiotensin II had increased alpha-smooth muscle actin and transforming growth factor-beta expression. The effect of angiotensin II was blocked by an antibody to osteopontin. In addition, osteopontin attenuated angiotensin II-induced plasminogen activator inhibitor-1 expression. These studies show that osteopontin is a promoter and an inhibitor of inflammation, oxidative stress, and fibrosis that is capable of modulating angiotensin II-induced renal damage.

Yamaguchi, I., J. M. Lopez-Guisa, et al. "Endogenous urokinase lacks antifibrotic activity during progressive renal injury." Am J Physiol Renal Physiol. 2007 Jul;293(1):F12-9. Epub 2007 Mar 13.

Interstitial fibrosis is a universal feature of progressive kidney disease. Urokinase-type plasminogen activator (uPA) is thought to participate for several reasons: 1) uPA is produced predominantly in kidney, 2) its inhibitor plasminogen activator inhibitor-1 (PAI-1) is a strong promoter of interstitial fibrosis, whereas its receptor (uPAR) attenuates renal fibrosis, 3) uPA reduces fibrosis in liver and lung, and 4) uPA can activate hepatocyte growth factor (HGF), a potent antifibrotic growth factor. The present study tested the hypothesis that endogenous uPA reduces fibrosis severity by investigating the unilateral ureteral obstruction (UUO) model in wild-type (WT) and uPA-/- mice. Several outcomes were measured: renal collagen 3-21 days after UUO, macrophage accumulation (F4/80 Western blotting), interstitial myofibroblast density (alpha-smooth muscle actin immunostaining), and tubular injury (E-cadherin and Ksp-cadherin Western blotting). None of these measures differed significantly between WT and uPA-/- mice. uPA genetic deficiency was not associated with compensatory changes in renal uPAR mRNA levels, PAI-1 protein levels, or tissue plasminogen activator activity levels after UUO. Despite the known ability of uPA to activate latent HGF, immunoblotting failed to detect significant differences in levels of the active HGF alpha-chain and phosphorylated cMET (the activated HGF receptor) between the WT and uPA-/- groups. These findings suggest that the profibrotic actions of PAI-1 are uPA independent and that an alternative pathway must activate HGF in kidney. Finally, these results highlight a significant organ-specific difference in basic fibrogenic pathways, as enhanced uPA activity has been reported to attenuate pulmonary and hepatic fibrosis.

Yamashita, S., A. Maeshima, et al. "Involvement of renal progenitor tubular cells in epithelial-to-mesenchymal transition in fibrotic rat kidneys." J Am Soc Nephrol. 2005 Jul;16(7):2044-51. Epub 2005 May 11.

Renal progenitor tubular cells (label-retaining cells [LRC]) were recently identified in normal kidneys by in vivo bromodeoxyuridine (BrdU) labeling. This study was conducted to examine the behavior of LRC in renal fibrosis. BrdU was injected intraperitoneally into normal rats daily for 7 d. After a 2-wk chase period, unilateral ureteral obstruction (UUO) was induced in these rats. In normal and contralateral kidneys, LRC were observed scattering among tubular epithelial cells. After UUO, the number of the LRC significantly increased, and most of them were positive for proliferating cell nuclear antigen (PCNA). In contrast, PCNA+ cells lacking BrdU label were rarely observed. It is interesting that LRC were detected not only in tubules but also in the interstitium after UUO. Laminin staining showed that a number of the LRC were adjacent to the destroyed tubular basement membrane. Some tubules, including LRC, lost the expression of E-cadherin after UUO. A large number of cell populations expressed vimentin, heat shock protein 47, or alpha-smooth muscle actin in the UUO kidneys, and each population contained LRC. None of the LRC was positive for these fibroblastic markers in contralateral kidneys. When renal tubules from BrdU-treated rats were cultured in the gel, some cells protruded from the periphery of the tubules and migrated into the gel. Most of these cells were BrdU+. Neither the total content of BrdU in the kidneys nor the number of LRC in bone marrow significantly changed after UUO. Collectively, these results suggest that LRC is a cell population that proliferates, migrates, and transdifferentiates into fibroblast-like cells during renal fibrosis.

Yamate, J., M. Kuribayashi, et al. "Differential immunoexpressions of cytoskeletons in renal epithelial and interstitial cells in rat and canine fibrotic kidneys, and in kidney-related cell lines under fibrogenic stimuli." Exp Toxicol Pathol. 2005 Nov;57(2):135-47. Epub 2005 Aug 15.

Myofibroblasts play an important role in chronic renal interstitial fibrosis. However, the origin and developmental mechanisms remain to be elucidated. The myofibroblasts may express various cytoskeletons during the development. Immunoexpressions of vimentin, desmin and alpha-smooth muscle actin (alpha-SMA) were analyzed using experimentally (cisplatin and unilateral ureteral obstruction) induced rat and spontaneous canine fibrotic kidneys or kidney-related cell lines incubated

with transforming growth factor-beta1 (TGF-beta1), platelet-derived growth factor-BB (PDGF-BB) or their combination at various concentrations. In rat fibrotic kidneys, both renal epithelia and interstitial cells showed positive reactions to alpha-SMA and vimentin, supporting epithelial-mesenchymal transition (EMT) theory; however, renal epithelia did not react to desmin, though interstitial cells were reactive. Renal epithelia in canine fibrotic kidneys did not show a positive reaction to alpha-SMA, whereas interstitial cells reacted strongly to alpha-SMA; conversely, renal epithelia reacted strongly to desmin, but interstitial cells did not; vimentin expression was infrequently seen in renal epithelia and interstitial cells of canine kidneys. Exposure of TGF-beta1 to porcine renal epithelial cells (LLC-PK1), rat renal interstitial cells (NRK-49F), and rat immature mesenchymal cells (MT-9) dose-dependently increased selectively alpha-SMA-positive cell numbers. Moreover, PDGF-BB exhibited an additive effect on TGF-beta1-induced alpha-SMA expression in these cell lines when simultaneously added. alpha-SMA was the most plastic cytoskeleton under fibrogenic stimuli. This study shows that there are interspecies differences in cytoskeletal immunoexpressions of renal epithelia or interstitial cells between rat and canine fibrotic kidneys, and that the derivation of renal myofibroblasts may be heterogeneous, such as renal epithelia, interstitial cells or immature mesenchymal cells.

Yoon, H. E., S. J. Kim, et al. "Tempol attenuates renal fibrosis in mice with unilateral ureteral obstruction: the role of PI3K-Akt-FoxO3a signaling." J Korean Med Sci. 2014 Feb;29(2):230-7. doi: 10.3346/jkms.2014.29.2.230. Epub 2014 Jan 28.

This study investigated whether tempol, an anti-oxidant, protects against renal injury by modulating phosphatidylinositol 3-kinase (PI3K)-Akt-Forkhead homeobox O (FoxO) signaling. Mice received unilateral ureteral obstruction (UUO) surgery with or without administration of tempol. We evaluated renal damage, oxidative stress and the expression of PI3K, Akt, FoxO3a and their target molecules including manganese superoxide dismutase (MnSOD), catalase, Bax, and Bcl-2 on day 3 and day 7 after UUO. Tubulointerstitial fibrosis, collagen deposition, alpha-smooth muscle actin-positive area, and F4/80-positive macrophage infiltration were significantly lower in tempol-treated mice compared with control mice. The expression of PI3K, phosphorylated Akt, and phosphorylated FoxO3a markedly decreased in tempol-treated mice compared with control mice. Tempol prominently increased the expressions of MnSOD and catalase, and decreased the production of hydrogen peroxide and lipid peroxidation in the obstructed kidneys. Significantly less apoptosis, a lower ratio of Bax to Bcl-2 expression and fewer apoptotic cells in TUNEL staining, and decreased expression of transforming growth factor-beta1 were observed in the obstructed kidneys from tempol-treated mice compared with those from control mice. Tempol attenuates oxidative stress, inflammation, and fibrosis in the obstructed kidneys of UUO mice, and the modulation of PI3K-Akt-FoxO3a signaling may be involved in this pathogenesis.

Zimmerman, D. L., J. Zimpelmann, et al. "The effect of angiotensin-(1-7) in mouse unilateral ureteral obstruction." Am J Pathol. 2015 Mar;185(3):729-40. doi: 10.1016/j.ajpath.2014.11.013. Epub 2015 Jan 24.

Angiotensin-(1-7) is a ligand for the Mas receptor and may protect against tissue injury associated with renin-angiotensin system activation. We determined the effects of endogenous or exogenous angiotensin-(1-7) in mice with unilateral ureteral obstruction (UUO). Mice with UUO were treated with or without the angiotensin-(1-7) antagonist A779 or with 6, 24, or 62 mug/kg per hour exogenous angiotensin-(1-7). After 10 days, kidneys were harvested for histology, immunoblots, and measurement of NADPH oxidase. Compared with controls, A779 treatment significantly increased fibronectin, transforming growth factor-beta, and alpha-smooth muscle actin expression in obstructed kidneys and enhanced tubulointerstitial injury, apoptosis, and NADPH oxidase. Unexpectedly, administration of angiotensin-(1-7) to mice with UUO caused injury in obstructed kidneys compared with controls and increased macrophage infiltration. In obstructed kidneys from mice with gene deletion of Mas (Mas(-/-)), apoptosis and macrophage infiltration were increased compared with wild-type mice. Angiotensin-(1-7) (but not A779) further increased apoptosis and macrophage influx in obstructed kidneys from Mas(-/-) mice, compared with untreated Mas(-/-) mice. These data indicate that endogenous angiotensin-(1-7) protects against kidney injury in UUO. In mice with or without the Mas receptor, however, delivery of exogenous angiotensin-(1-7) worsens kidney damage. The results suggest dose-dependent effects of angiotensin-(1-7) in the kidney in UUO, with endogenous angiotensin-(1-7) promoting repair pathways via interaction with Mas and higher amounts exacerbating injury.

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