Vascular Endothelial Growth Factor (VEGF) and Renal Obstruction Literatures

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Abstract: Obstructive uropathy is a condition in which the flow of urine is blocked. This causes the urine to back up and injure one or both kidneys. Obstructive uropathy occurs when urine cannot drain through a ureter. Urine backs up into the kidney and causes it to become hydronephrosis. It can occur suddenly, or be a long-term problem. If the blockage comes on suddenly, kidney damage is less likely if the problem is detected and treated promptly, and the damage to the kidneys goes away normally. Long-term damage to the kidneys may occur if the blockage has been present for a long time. If the problem is caused by a blockage in the bladder, the bladder may have long-term damage, which may lead to problems emptying the bladder or leakage of urine. Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate. Serum concentration of VEGF is high in many diseases such as bronchial asthma and diabetes mellitus, etc. VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury, muscle following exercise, and new vessels to bypass blocked vessels. Overexpression of VEGF can cause disease. Solid cancers cannot grow beyond a limited size without an adequate blood supply, but are able to grow and metastasize with the overexpression of VEGF. Overexpression of VEGF can cause vascular disease in the retina of the eye and other parts of the body, and drugs such as bevacizumab and ranibizumab can inhibit VEGF and control or slow these diseases.
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Introduction

Obstructive uropathy is a condition in which the flow of urine is blocked. This causes the urine to back up and injure one or both kidneys. Obstructive uropathy occurs when urine cannot drain through a ureter. Urine backs up into the kidney and causes it to become hydronephrosis. It can occur suddenly, or be a long-term problem. If the blockage comes on suddenly, kidney damage is less likely if the problem is detected and treated promptly, and the damage to the kidneys goes away normally. Long-term damage to the kidneys may occur if the blockage has been present for a long time. If the problem is caused by a blockage in the bladder, the bladder may have long-term damage, which may lead to problems emptying the bladder or leakage of urine.

Animal partial ureteral obstruction (PUO) model is a useful research model in the ureteral obstruction studies which produces a well documented triphasic response in the renal hemodynamics. Urinary traction infection frequently occurs in the obstructed kidney. PUO produces a well model introducing renal injury and inflammation. The rat PUO surgery can be done as the following: The rats are anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and a low midline abdominal incision is made. Right nephrectomy is performed first. The right kidney is mobilized with minimal dissection. Two 2-0 silk ties are placed around the hilar vessels and the right kidney is removed. The left ureter is then traced to its insertion in the bladder, mobilized with minimal dissection to preserve the surrounding neurovasculature and retracted with vessel loops. The psoas muscle is split by blunt dissection to create a space which will accommodate two-thirds of the length of the ureter. The left ureter is moved into that interstice, after which the muscle is reapproximated with three interrupted 5-0 silk sutures. The abdominal wound is then closed with sutures (Figure 1). For the sham operation, the rats undergo the same surgical procedure, including right nephrectomy, but the left ureter is diverted into the psoas muscle. After recovery from anesthesia, the rats are housed individually in metal cages and given buprenorphine hydrochloride (0.02 mg/kg BW, i.m.) to relieve postoperative discomfort. One night before the surgery and clearance experiments, the rats fasted but had free access to water.

Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate. Serum concentration of VEGF is high in many diseases such as bronchial asthma and diabetes mellitus, etc. VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury, muscle following exercise, and new vessels to

bypass blocked vessels.

Overexpression of VEGF can cause disease. Solid cancers cannot grow beyond a limited size without an adequate blood supply, but are able to grow and metastasize with the overexpression of VEGF. Overexpression of VEGF can cause vascular disease in the retina of the eye and other parts of the body, and drugs such as bevacizumab and ranibizumab can inhibit VEGF and control or slow these diseases.

VEGF is a sub-family of the platelet-derived growth factor family of cystine-knot growth factors that are important signaling proteins involved in both vasculogenesis and angiogenesis. The VEGF family comprises in mammals five members: VEGF-A, placenta growth factor (PGF), VEGF-B, VEGF-C and VEGF-D. VEGF-A has been shown to stimulate endothelial cell mitogenesis and cell migration. VEGF-A is also a vasodilator and increases microvascular permeability and was originally referred to as vascular permeability factor.

There are multiple isoforms of VEGF-A that result from alternative splicing of mRNA from a single, 8-exon VEGFA gene. These are classified into two groups which are referred to according to their terminal exon (exon 8) splice site: the proximal splice site or distal splice site. In addition, alternate splicing of exon 6 and 7 alters their heparin-binding affinity and amino acid number (in humans: VEGF121, VEGF121b, VEGF145, VEGF165, VEGF165b, VEGF189, VEGF206). These domains have important functional consequences for the VEGF splice variants, as the terminal (exon 8) splice site determines whether the proteins are pro-angiogenic (proximal splice site, expressed during angiogenesis) or anti-angiogenic (distal splice site, expressed in normal tissues). In addition, inclusion or exclusion of exons 6 and 7 mediate interactions with heparan sulfate proteoglycans (HSPGs) and neuropilin co-receptors on the cell surface, enhancing their ability to bind and activate the VEGF receptors (VEGFRs). VEGF-C has been shown to be an important inducer of neurogenesis.

All members of the VEGF family stimulate cellular responses by binding to tyrosine kinase receptors on the cell surface, causing them to dimerize and become activated through transphosphorylation, although to different sites, times, and extents. The VEGF receptors have an extracellular portion consisting of 7 immunoglobulin-like domains, a single transmembrane spanning region, and an intracellular portion containing a split tyrosine-kinase domain.

VEGF-A production can be induced in cells that are not receiving enough oxygen. When a cell is deficient in oxygen, it produces hypoxia-inducible factor (HIF, a transcription factor). HIF stimulates the release of VEGF-A. Circulating VEGF-A then binds to VEGF receptors on endothelial cells, triggering a tyrosine kinase pathway leading to angiogenesis. The expression of angiopoietin-2 in the absence of VEGF leads to endothelial cell death and vascular regression. HIF1-alpha and HIF1-beta are constantly being produced but HIF1-alpha is highly O_2 labile and in aerobic conditions it is degraded. When the cell becomes hypoxic, HIF1-alpha persists and the HIF1alpha/beta complex stimulates VEGF release.

VEGF-A has been implicated with poor prognosis in breast cancer and there is a decreased overall survival and disease-free survival in those tumors overexpressing VEGF. The overexpression of VEGF-A may be an early step in the process of tumor metastasis. VEGF-A is also released in rheumatoid arthritis in response to TNF-α, increasing endothelial permeability and swelling and also stimulating angiogenesis. VEGF-A is also important in diabetic retinopathy (DR). The microcirculatory problems in the retina of people with diabetes can cause retinal ischaemia, which results in the release of VEGF-A, and a switch in the balance of pro-angiogenic VEGF isoforms over the normally expressed VEGF isoforms. VEGF may then cause the creation of new blood vessels in the retina and elsewhere in the eye, heralding changes that may threaten the sight. VEGF-A plays a role in the disease pathology of the wet form age-related macular degeneration (AMD), which is the leading cause of blindness for the elderly of the industrialized world. The vascular pathology of AMD shares certain similarities with diabetic retinopathy, although the cause of disease and the typical source of neovascularization differs between the two diseases. Patients suffering from pulmonary emphysema have been found to have decreased levels of VEGF in the pulmonary arteries. In the kidney, increased expression of VEGF-A in glomeruli directly causes the glomerular hypertrophy that is associated with proteinuria.

Anti-VEGF therapies are important in the treatment of certain cancers and in age-related macular degeneration. They can involve monoclonal antibodies such as bevacizumab (Avastin), antibody derivatives such as ranibizumab (Lucentis), or orally-available small molecules that inhibit the tyrosine kinases stimulated by VEGF: lapatinib (Tykerb), sunitinib (Sutent), sorafenib (Nexavar), axitinib, and pazopanib. THC and cannabidiol both inhibit VEGF and slow Glioma growth. Both antibody-based compounds are commercialized. The first three orally available compounds are commercialized, as well. Bergers and Hanahan concluded in 2008 that anti-VEGF drugs can show therapeutic efficacy in mouse models of cancer and in an increasing number of human cancers. VEGF is also inhibited by thiazolidinediones (used for diabetes mellitus type 2 and related disease), and this

effect on granulosa cells gives the potential of thiazolidinediones to be used in ovarian hyperstimulation syndrome. Ranibizumab, a monoclonal antibody fragment (Fab) derived from bevacizumab, has been developed by Genetech for intraocular use.

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

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, Ardura et al 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. Parathyroid hormone-related protein (PTHrP) promotes fibrogenesis in the damaged kidney dependent on its interaction with vascular endothelial growth factor (VEGF). 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.

Mesenchymal stem cells (MSCs) hold promise for the treatment of renal disease. 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). 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. As the conclusion, 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.

Bige, N., N. Shweke, et al. "Thrombospondin-1 plays a profibrotic and pro-inflammatory role during ureteric obstruction." Kidney Int. 2012 Jun;81(12):1226-38. doi: 10.1038/ki.2012.21. Epub 2012 Mar 14.

Thrombospondin-1 (TSP-1) is an endogenous activator of transforming growth factor-beta (TGF-beta), and an anti-angiogenic factor, which may prevent kidney repair. Here Bige et al investigated whether TSP-1 is involved in the development of chronic kidney disease using rats with unilateral ureteral obstruction, a well-known model to study renal fibrosis. Obstruction of 10 days duration induced inflammation, tubular cell atrophy, dilation, apoptosis, and proliferation, leading to interstitial fibrosis. TSP-1 expression was increased in parallel to that of collagen III and TGF-beta. Relief of the obstruction at day 10 produced a gradual improvement in renal structure and function, the reappearance of peritubular capillaries, and restoration of renal VEGF content over a 7- to 15-day post-relief period. TSP-1 expression decreased in parallel with that of TGF-beta1 and collagen III. Mice in which the TSP-1 gene was knocked out displayed less inflammation and had better preservation of renal tissue and the peritubular capillary network compared to wild-type mice. Additional studies showed that the inflammatory effect of TSP-1 was mediated, at least in part, by monocyte chemoattractant protein-1 and activation of the Th17 pathway. Thus, TSP-1 is an important profibrotic and inflammatory mediator of renal disease. Blockade of its action may be a treatment against the development of chronic kidney disease.

Burt, L. E., M. S. Forbes, et al. "Renal vascular endothelial growth factor in neonatal obstructive nephropathy. I. Endogenous VEGF." Am J Physiol Renal Physiol. 2007 Jan;292(1):F158-67. Epub 2006 Jun 20.

Obstructive nephropathy constitutes a major cause of renal impairment in children. Chronic unilateral ureteral obstruction (UUO) impairs maturation of the developing kidney and leads to tubular apoptosis and interstitial inflammation. Vascular endothelial growth factor (VEGF) is involved in recovery from various forms of renal injury. Burt et al questioned whether the renal expression of endogenous VEGF and its receptor (VEGFR2/Flk-1) is modified by UUO in early development. Neonatal rats were subjected to partial or complete UUO or sham operation. The distribution of immunoreactive VEGF in each kidney was examined after 7, 14, or 28 days. Adult rats were also subjected to sham operation or complete UUO. Tubular VEGF increased between 14 and 28 days in sham-operated rats and in some partially obstructed neonatal rats but decreased with complete UUO. Parallel changes were found by Western blotting, but not by RT-PCR. Immunoreactive VEGF colocalized with mitochondria in proximal and distal tubules and also appeared in type A intercalated cells, glomerular vascular endothelium, and podocytes. While neonatal microvascular renal VEGFR2 receptor staining was strongly positive regardless of UUO, staining was weak in sham-operated adults but increased following UUO. Parallel changes in VEGFR2 expression were verified by RT-PCR and Western blotting. We conclude that endogenous renal VEGF is developmentally regulated in the neonatal rat and is differentially regulated by partial and complete UUO. Following UUO in the adult, the VEGF receptor is upregulated. Endogenous VEGF may serve an adaptive role in responding to tubular injury caused by UUO and may modulate adaptation by the contralateral kidney.

Cancela, A. L., R. D. Santos, et al. "Phosphorus is associated with coronary artery disease in patients with preserved renal function." PLoS One. 2012;7(5):e36883. doi: 10.1371/journal.pone.0036883. Epub 2012 May 10.

High serum phosphorus levels have been associated with mortality and cardiovascular events in patients with chronic kidney disease and in the general population. In addition, high phosphorus levels have been shown to induce vascular calcification and endothelial dysfunction in vitro. The aim of this study was to evaluate the relation of phosphorus and coronary calcification and atherosclerosis in the setting of normal renal function. This was a cross-sectional study involving 290 patients with suspected coronary artery disease and undergoing elective coronary angiography, with a creatinine clearance >60 ml/min/1.73 m(2). Coronary artery obstruction was assessed by the Friesinger score and coronary artery

calcification by multislice computed tomography. Serum phosphorus was higher in patients with an Agatston score >10 than in those with an Agatston score </= 10 (3.63 +/- 0.55 versus 3.49 +/- 0.52 mg/dl; p = 0.02). In the patients with Friesinger scores >4, serum phosphorus was higher (3.6 +/- 0.5 versus 3.5 +/- 0.6 mg/dl, p = 0.04) and median intact fibroblast growth factor 23 was lower (40.3 pg/ml versus 45.7 pg/ml, p = 0.01). Each 0.1-mg/dl higher serum phosphate was associated with a 7.4% higher odds of having a Friesinger score >4 (p = 0.03) and a 6.1% greater risk of having an Agatston score >10 (p = 0.01). Fibroblast growth factor 23 was a negative predictor of Friesinger score (p = 0.002). In conclusion, phosphorus is positively associated with coronary artery calcification and obstruction in patients with suspected coronary artery disease and preserved renal function.

Chade, A. R. and S. Kelsen "Renal microvascular disease determines the responses to revascularization in experimental renovascular disease." Circ Cardiovasc Interv. 2010 Aug;3(4):376-83. doi: 10.1161/CIRCINTERVENTIONS.110.951277. Epub 2010 Jun 29.

Percutaneous transluminal renal angioplasty (PTRA) is the most frequent therapeutic approach to resolving renal artery stenosis (RAS). However, renal function recovers in only 30% of the cases. The causes of these poor outcomes are still unknown. We hypothesized that preserving the renal microcirculation distal to RAS will improve the responses to PTRA. RAS was induced in 28 pigs. In 14, vascular endothelial growth factor (VEGF)-165 0.05 microg/kg was infused intrarenally (RAS+VEGF). Single-kidney function was assessed in all pigs in vivo using ultrafast CT after 6 weeks. Observation of half of the RAS and RAS+VEGF pigs was completed. The other half underwent PTRA and repeated VEGF, and CT studies were repeated 4 weeks later. Pigs were then euthanized, the stenotic kidney removed, renal microvascular (MV) architecture reconstructed ex vivo using 3D micro-CT, and renal fibrosis quantified. The degree of RAS and hypertension were similar in RAS and RAS+VEGF. Renal function and MV density were decreased in RAS but improved in RAS+VEGF. PTRA largely resolved RAS, but the improvements of hypertension and renal function were greater in RAS+VEGF+PTRA than in RAS+PTRA, accompanied by a 34% increase in MV density and decreased fibrosis. Preservation of the MV architecture and function in the stenotic kidney improved the responses to PTRA, indicating that renal MV integrity plays a role in determining the responses to PTRA. This study indicates that damage and early loss of renal MV is an important determinant of the progression of renal injury in RAS and instigates often irreversible damage.

Chang, F. C., W. C. Chiang, et al. "Angiopoietin-2-induced arterial stiffness in CKD." J Am Soc Nephrol. 2014 Jun;25(6):1198-209. doi: 10.1681/ASN.2013050542. Epub 2014 Feb 7.

The mechanism of vascular calcification in CKD is not understood fully, but may involve collagen deposition in the arterial wall upon osteo/chondrocytic transformation of vascular smooth muscle cells (VSMCs). Increased levels of circulating angiopoietin-2 correlate with markers of CKD progression and angiopoietin-2 regulate inflammatory responses, including intercellular and vascular adhesion and recruitment of VSMCs. Here, we investigate the potential role of angiopoietin-2 in the pathogenesis of arterial stiffness associated with CKD. In a cohort of 416 patients with CKD, the plasma level of angiopoietin-2 correlated independently with the severity of arterial stiffness assessed by pulse wave velocity. In mice subjected to 5/6 subtotal nephrectomy or unilateral ureteral obstruction, plasma levels of angiopoietin-2 also increased. Angiopoietin-2 expression markedly increased in tubular epithelial cells of fibrotic kidneys but decreased in other tissues, including aorta and lung, after 5/6 subtotal nephrectomy. Expression of collagen and profibrotic genes in aortic VSMCs increased in mice after 5/6 subtotal nephrectomy and in mice producing human angiopoietin-2. Angiopoietin-2 stimulated endothelial expression of chemokines and adhesion molecules for monocytes, increased Ly6C(low) macrophages in aorta, and increased the expression of the profibrotic cytokine TGF-beta1 in aortic endothelial cells and Ly6C(low) macrophages. Angiopoietin-2 blockade attenuated expression of monocyte chemokines, profibrotic cytokines, and collagen in aorta of mice after 5/6 subtotal nephrectomy. This study identifies angiopoietin-2 as a link between kidney fibrosis and arterial stiffness. Targeting angiopoietin-2 to attenuate inflammation and collagen expression may provide a novel therapy for cardiovascular disease in CKD.

Cheung, C. M., C. Chrysochou, et al. "The management of renovascular disease: ASTRAL and beyond." Curr Opin Nephrol Hypertens. 2011 Jan;20(1):89-94. doi: 10.1097/MNH.0b013e328340ffe5.

This review concentrates on the new findings published in the atheromatous renovascular disease (ARVD) literature since the beginning of 2009, a period in which the results of two randomized control trials have been released. The key advances have arisen with respect to the epidemiology of ARVD, the

effects of revascularization as demonstrated by the results of randomized controlled trials, an understanding of the pathophysiology of the ischaemic kidney, and also there have been further insights regarding the selection of patients for revascularization utilizing structural and functional imaging. Optimal medical management (and not revascularization therapy) is the established cornerstone for all patients with ARVD. Future studies should be directed to identifying individuals who will significantly benefit from renal revascularization.

Ebrahimi, B., A. Eirin, et al. "Mesenchymal stem cells improve medullary inflammation and fibrosis after revascularization of swine atherosclerotic renal artery stenosis." PLoS One. 2013 Jul 3;8(7):e67474. doi: 10.1371/journal.pone.0067474. Print 2013.

Atherosclerotic renal artery stenosis (ARAS) raises blood pressure and can reduce kidney function. Revascularization of the stenotic renal artery alone does not restore renal medullary structure and function. This study tested the hypothesis that addition of mesenchymal stem cells (MSC) to percutaneous transluminal renal angioplasty (PTRA) can restore stenotic-kidney medullary tubular transport function and attenuate its remodeling. Twenty-seven swine were divided into three ARAS (high-cholesterol diet and renal artery stenosis) and a normal control group. Six weeks after ARAS induction, two groups were treated with PTRA alone or PTRA supplemented with adipose-tissue-derived MSC (10 x 10(6) cells intra-renal). Multi-detector computed tomography and blood-oxygenation-level-dependent (BOLD) MRI studies were performed 4 weeks later to assess kidney hemodynamics and function, and tissue collected a few days later for histology and micro-CT imaging. PTRA effectively decreased blood pressure, yet medullary vascular density remained low. Addition of MSC improved medullary vascularization in ARAS+PTRA+MSC and increased angiogenic signaling, including protein expression of vascular endothelial growth-factor, its receptor (FLK-1), and hypoxia-inducible factor-1alpha. ARAS+PTRA+MSC also showed attenuated inflammation, although oxidative-stress remained elevated. BOLD-MRI indicated that MSC normalized oxygen-dependent tubular response to furosemide (-4.3 +/- 0.9, -0.1 +/- 0.4, -1.6 +/- 0.9 and -3.6 +/- 1.0 s(-1) in Normal, ARAS, ARAS+PTRA and ARAS+PTRA+MSC, respectively, $p<0.05$), which correlated with a decrease in medullary tubular injury score ($R(2) = 0.33$, $p = 0.02$). Therefore, adjunctive MSC delivery in addition to PTRA reduces inflammation, fibrogenesis and vascular remodeling, and restores oxygen-dependent tubular function in the stenotic-kidney medulla, although additional interventions might be required to reduce oxidative-stress. This study supports development of cell-based strategies for renal protection in ARAS.

Ebrahimi, B., Z. Li, et al. "Addition of endothelial progenitor cells to renal revascularization restores medullary tubular oxygen consumption in swine renal artery stenosis." Am J Physiol Renal Physiol. 2012 Jun 1;302(11):F1478-85. doi: 10.1152/ajprenal.00563.2011. Epub 2012 Mar 14.

Renal artery stenosis (RAS) promotes microvascular rarefaction and fibrogenesis, which may eventuate in irreversible kidney injury. We have shown that percutaneous transluminal renal angioplasty (PTRA) or endothelial progenitor cells (EPC) improve renal cortical hemodynamics and function in the poststenotic kidney. The renal medulla is particularly sensitive to hypoxia, yet little is known about reversibility of medullary injury on restoration of renal blood flow. This study was designed to test the hypothesis that PTRA, with or without adjunct EPC delivery to the stenotic kidney, may improve medullary remodeling and tubular function. RAS was induced in 21 pigs using implantation of irritant coils, while another group served as normal controls ($n = 7$ each). Two RAS groups were then treated 6 wk later with PTRA or both PTRA and EPC. Four weeks later, medullary hemodynamics, microvascular architecture, and oxygen-dependent tubular function of the stenotic kidneys were examined using multidetector computed tomography, microcomputed tomography, and blood oxygenation level-dependent MRI, respectively. Medullary protein expression of vascular endothelial growth factor, endothelial nitric oxide synthase, hypoxia-inducible factor-1alpha, and NAD(P)H oxidase p47 were determined. All RAS groups showed decreased medullary vascular density and blood flow. However, in RAS+PTRA+EPC animals, EPC were engrafted in tubular structures, oxygen-dependent tubular function was normalized, and fibrosis attenuated, despite elevated expression of hypoxia-inducible factor-1alpha and sustained downregulation of vascular endothelial growth factor. In conclusion, EPC delivery, in addition to PTRA, restores medullary oxygen-dependent tubular function, despite impaired medullary blood and oxygen supply. These results support further development of cell-based therapy as an adjunct to revascularization of RAS.

Eirin, A., Z. Li, et al. "A mitochondrial permeability transition pore inhibitor improves renal outcomes after revascularization in experimental atherosclerotic renal artery stenosis." Hypertension. 2012 Nov;60(5):1242-9. doi: 10.1161/HYPERTENSIONAHA.112.199919. Epub 2012 Oct 8.

Revascularization improves blood pressure but not renal function in most patients with atherosclerotic renal artery stenosis (ARAS), possibly related to injury incurred during renal reperfusion. Bendavia, a novel tetrapeptide that inhibits mitochondrial permeability transition pore opening, reduces apoptosis, oxidative stress, and ischemia-reperfusion injury in experimental models. However, its potential for improving renal response to revascularization of chronic ARAS is unknown. We hypothesized that adjunct Bendavia would improve renal structure and function after percutaneous transluminal renal angioplasty (PTRA). Pigs were treated after 6 weeks of ARAS or control with PTRA+stenting (or sham), adjunct continuous 4-hour infusion of Bendavia (0.05 mg/kg IV) or vehicle (n=7 each) during PTRA. Single-kidney renal blood flow and glomerular filtration rate were studied 4 weeks later and renal mitochondrial biogenesis, microvascular architecture, and injurious pathways evaluated ex vivo. Monocyte chemoattractant protein-1 levels rose after PTRA, suggesting inflammatory injury. Bendavia did not immediately affect inflammatory cytokine levels, yet 4 weeks later, stenotic kidney renal blood flow and glomerular filtration rate both improved (44.00 +/- 0.21% and 36.40 +/- 10.21%, respectively) in ARAS+PTRA+Bendavia compared with ARAS+PTRA+vehicle. Renal mitochondrial biogenesis was restored after PTRA+Bendavia, and microvascular rarefaction, apoptosis, oxidative stress, tubular injury, and fibrosis decreased. Infusion of Bendavia during PTRA preserved mitochondrial biogenesis, renal hemodynamics, and function, and attenuated tissue injury in swine ARAS. Thus, functional mitochondrial injury during renal reperfusion may sustain renal inflammatory injury and limit kidney recovery after PTRA. Potent antiapoptotic and antioxidant effects provide Bendavia a novel therapeutic potential for improving kidney outcomes after PTRA in experimental ARAS.

Eirin, A., X. Y. Zhu, et al. "Endothelial outgrowth cells shift macrophage phenotype and improve kidney viability in swine renal artery stenosis." Arterioscler Thromb Vasc Biol. 2013 May;33(5):1006-13. doi: 10.1161/ATVBAHA.113.301164. Epub 2013 Feb 21.

Endothelial outgrowth cells (EOC) decrease inflammation and improve endothelial repair. Inflammation aggravates kidney injury in renal artery stenosis (RAS), and may account for its persistence upon revascularization. We hypothesized that EOC would decrease inflammatory (M1) macrophages and improve renal recovery in RAS. Pigs with 10 weeks of RAS were studied 4 weeks after percutaneous transluminal renal angioplasty (PTRA+stenting) or sham, with or without adjunct intrarenal delivery of autologous EOC (10x10(6)), and compared with similarly treated normal controls (n=7 each). Single-kidney function, microvascular and tissue remodeling, inflammation, oxidative stress, and fibrosis were evaluated. Four weeks after PTRA, EOC were engrafted in injected RAS-kidneys. Stenotic-kidney glomerular filtration rate was restored in RAS+EOC, RAS+PTRA, and RAS+PTRA+EOC pigs, whereas stenotic-kidney blood flow and angiogenesis were improved and fibrosis attenuated only in EOC-treated pigs. Furthermore, EOC increased cell proliferation and decreased the ratio of M1 (inflammatory)/M2 (reparative) macrophages, as well as circulating levels and stenotic-kidney release of inflammatory cytokines. Cultured-EOC released microvesicles in vitro and induced phenotypic switch (M1-to-M2) in cultured monocytes, which was inhibited by vascular endothelial growth factor blockade. Finally, a single intrarenal injection of rh-vascular endothelial growth factor (0.05 mug/kg) in 7 additional RAS pigs also restored M1/M2 ratio 4 weeks later. Intrarenal infusion of EOC after PTRA induced a vascular endothelial growth factor-mediated attenuation in macrophages inflammatory phenotype, preserved microvascular architecture and function, and decreased inflammation and fibrosis in the stenotic kidney, suggesting a novel mechanism and therapeutic potential for adjunctive EOC delivery in experimental RAS to improve PTRA outcomes.

Fenghua, W., S. Junjie, et al. "Does intervention in utero preserve the obstructed kidneys of fetal lambs? A histological, cytological, and molecular study." Pediatr Res. 2009 Aug;66(2):145-8. doi: 10.1203/PDR.0b013e3181aa42f6.

Ureteropelvic junction obstruction is a common cause of end-stage nephropathy in children. Our aim was to investigate whether relief of obstruction in utero can alleviate the development of nephropathy. A silastic tube was tied around the left superior segment ureter to induce unilateral partial ureteral obstruction in 22 fetal sheep at 75- 85 d of gestation. Three weeks later, the tubes were removed to relieve the obstruction in 10 of the 22 lambs. A sham operation was performed on four fetuses (the control). At birth, the lambs were killed, and their kidneys were removed to study the changes in histology, podocytes, and expression of paired-box 2 (PAX2) and VEGF. In the obstructed kidneys, we observed cysts of various sizes in the cortex, fibrosis in the interstitial tissue, much decreased number of glomeruli, severe podocyte foot process fusion, and markedly increased PAX2 and decreased VEGF expressions. However, relief of obstruction preserved the number of glomeruli, significantly increased

VEGF expression, reduced fusion of the podocyte foot processes, andrestored expression of PAX2 to some extent. Thus, relief ofobstruction in utero may prevent or attenuate the development ofnephropathy in lambs.

Grone, H. J., M. Simon, et al. "Expression of vascular endothelial growth factor in renal vascular disease and renal allografts." J Pathol. 1995 Nov;177(3):259-67.

Vascular endothelial growth factor (VEGF) is a dimeric glycoprotein that exerts a proliferative effect specifically on endothelial cells. VEGF can increase vascular permeability and collagenase activity, is chemotactic for monocytes, and may dilate blood vessels. It can be induced by phorbol ester and cAMP in both mesenchymal and epithelial cells. In vitro cell culture experiments suggest that VEGF is upregulated by oxygen deprivation. In this study we tested whether in vivo acute and/or chronic reduction of renal blood flow by vascular obstruction would result in increased expression of VEGF mRNA and protein. Three normal kidneys, five human kidneys with narrowing of preglomerular vessels by vascular rejection or by vasculitis, and eight kidneys with nephrosclerosis and/or diabetic nephropathy were examined. In situ hybridization with 35S-labelled riboprobes showed a pronounced expression of VEGF mRNA in acutely hypoxic proximal and distal tubules of both the cortex and medulla; VEGF protein was demonstrated in the epithelia of these tubules by immunohistochemistry. In kidneys with chronically reduced blood flow, the majority of atrophic tubules were negative for VEGF mRNA and protein, although interstitial cells expressed VEGF mRNA. In arcuate arteries showing intimal and adventitial fibrosis, some medial smooth muscle cells were positive for VEGF mRNA. In glomeruli with segmental sclerosis, viable podocytes showed a prominent signal for VEGF mRNA. Mesangial cells did not express VEGF in the cases studied. It is possible that hypoxia itself led to the upregulation of VEGF in tubular epithelia and vascular smooth muscle cells. The vasodilatory and permeability-promoting effects of the endothelial growth factor produced by damaged tubular epithelia may constitute a mechanism to alleviate a decrease in blood flow and substrate availability and to re-establish vascular integrity.

Hewitson, T. D., W. Y. Ho, et al. "Antifibrotic properties of relaxin: in vivo mechanism of action in experimental renal tubulointerstitial fibrosis." Endocrinology. 2010 Oct;151(10):4938-48. doi: 10.1210/en.2010-0286. Epub 2010 Sep 8.

This study examined the efficacy and in vivo mechanism of action of the antifibrotic hormone, relaxin, in a mouse model of unilateral ureteric obstruction (UUO). Kidney fibrosis was assessed in recombinant human gene-2 relaxin-treated animals maintained for 3 and 9 d after UUO. Results were compared with untreated and unoperated animals (d 0). Total collagen, collagen subtypes (I, IV), TGF-beta2 production, mothers against decapentaplegic homolog 2 (Smad2) phosphorylation, myofibroblast differentiation, mitosis, and apoptosis were all progressively increased by UUO (all $P<0.05$ vs. d 0 group at d 3 and d 9), whereas TGF-beta1 production was increased and vascular endothelial growth factor expression (angiogenesis) decreased at d 9 (both $P<0.05$ vs. d 0). A progressive increase in matrix metalloproteinase (MMP)-2 after UUO suggested that it was reactive to the increased fibrogenesis. Conversely, MMP-9 was decreased at d 9, whereas its inhibitor tissue inhibitor of metalloproteinase-1 progressively decreased after UUO. Human gene-2 relaxin pretreatment of animals from 4 d prior to UUO ameliorated the increase in total collagen, collagen IV, Smad2 phosphorylation, and myofibroblasts at both time points (all $P<0.05$ vs. untreated groups) and inhibited TGF-beta2 production and cell proliferation (both $P<0.05$ vs. untreated groups) with a trend toward normalizing vascular endothelial growth factor expression at d 9, with no effect on TGF-beta1 production or apoptosis. The relaxin-mediated regulation of MMPs and tissue inhibitor of metalloproteinases in this model was not consistent with its antifibrotic properties. The beneficial effects of relaxin were lost when treatment was stopped. These findings establish that relaxin can inhibit both early and established phases of tubulointerstitial fibrosis, primarily by suppressing cell proliferation, myofibroblast differentiation, and collagen production. Not all of these effects paralleled changes to TGF-beta-Smad signaling.

Iliescu, R., S. R. Fernandez, et al. "Role of renal microcirculation in experimental renovascular disease." Nephrol Dial Transplant. 2010 Apr;25(4):1079-87. doi: 10.1093/ndt/gfp605. Epub 2009 Nov 23.

Renal artery stenosis (RAS) causes renal injury partly via microvascular (MV) endothelial dysfunction and damage. Vascular endothelial growth factor (VEGF) is crucial for preservation of microvasculature and promotes vascular proliferation and endothelial repair. Iliescu et al have previously shown that MV rarefaction is associated with decreased VEGF in the kidney exposed to chronic RAS, accompanied by deteriorated renal function and fibrosis. Unilateral RAS was induced in 16 pigs. In eight, VEGF (0.05 micrograms/kg) was infused intra-renally at the onset of RAS. After 6 weeks, single-kidney haemodynamics and function were assessed using in vivo multi-detector computed tomography

(CT). Renal microvessels, angiogenic pathways and morphology were investigated ex vivo using micro-CT, real-time PCR and histology. RESULTS: Blood pressure and degree of RAS was similar in RAS and RAS + VEGF pigs. Single-kidney renal blood flow (RBF) and glomerular filtration rate (GFR) were reduced in RAS compared to Normal (221.1 +/- 46.5 and 29.9 +/- 3.8 vs. 522.5 +/- 60.9 and 49.3 +/- 3.4 mL/min, respectively, P < 0.05), accompanied by decreased cortical MV density and increased renal fibrosis. Pre-emptive administration of VEGF preserved MV architecture, attenuated fibrosis and normalized RBF and GFR (510.8 +/- 50.9 and 39.9.1 +/- 4.1 mL/min, P = not significant vs. Normal). This study underscores the importance of the renal microcirculation in renovascular disease. Intra-renal administration of VEGF preserved renal MV architecture and function of the stenotic kidney, which in turn preserved renal haemodynamics and function and decreased renal fibrosis. These observations suggest that preventing renal MV loss may be a potential target for therapeutic approaches for patients with chronic renovascular disease.

Kelsen, S., J. E. Hall, et al. "Endothelin-A receptor blockade slows the progression of renal injury in experimental renovascular disease." Am J Physiol Renal Physiol. 2011 Jul;301(1):F218-25. doi: 10.1152/ajprenal.00089.2011. Epub 2011 Apr 6.

Endothelin (ET)-1, a potent renal vasoconstrictor with mitogenic properties, is upregulated by ischemia and has been shown to induce renal injury via the ET-A receptor. The potential role of ET-A blockade in chronic renovascular disease (RVD) has not, to our knowledge, been previously reported. We hypothesized that chronic ET-A receptor blockade would preserve renal hemodynamics and slow the progression of injury of the stenotic kidney in experimental RVD. Renal artery stenosis, a major cause of chronic RVD, was induced in 14 pigs and observed for 6 wk. In half of the pigs, chronic ET-A blockade was initiated (RVD+ET-A, 0.75 mg.kg(-1).day(-1)) at the onset of RVD. Single-kidney renal blood flow, glomerular filtration rate, and perfusion were quantified in vivo after 6 wk using multidetector computer tomography. Renal microvascular density was quantified ex vivo using three-dimensional microcomputer tomography, and growth factors, inflammation, apoptosis, and fibrosis were determined in renal tissue. The degree of stenosis and increase in blood pressure were similar in RVD and RVD+ET-A pigs. Renal hemodynamics, function, and microvascular density were decreased in the stenotic kidney but preserved by ET-A blockade, accompanied by increased renal expression of vascular endothelial growth factor, hepatocyte growth factor, and downstream mediators such as phosphorilated-Akt, angiopoietins, and endothelial nitric oxide synthase. ET-A blockade also reduced renal apoptosis, inflammation, and glomerulosclerosis. This study shows that ET-A blockade slows the progression of renal injury in experimental RVD and preserves renal hemodynamics, function, and microvascular density in the stenotic kidney. These results support a role for ET-1/ET-A as a potential therapeutic target in chronic RVD.

Kelsen, S., X. He, et al. "Early superoxide scavenging accelerates renal microvascular rarefaction and damage in the stenotic kidney." Am J Physiol Renal Physiol. 2012 Aug 15;303(4):F576-83. doi: 10.1152/ajprenal.00154.2012. Epub 2012 May 23.

Renal artery stenosis (RAS), the main cause of chronic renovascular disease (RVD), is associated with significant oxidative stress. Chronic RVD induces renal injury partly by promoting renal microvascular (MV) damage and blunting MV repair in the stenotic kidney. We tested the hypothesis that superoxide anion plays a pivotal role in MV dysfunction, reduction of MV density, and progression of renal injury in the stenotic kidney. RAS was induced in 14 domestic pigs and observed for 6 wk. Seven RAS pigs were chronically treated with the superoxide dismutase mimetic tempol (RAS+T) to reduce oxidative stress. Single-kidney hemodynamics and function were quantified in vivo using multidetector computer tomography (CT) and renal MV density was quantified ex vivo using micro-CT. Expression of angiogenic, inflammatory, and apoptotic factors was measured in renal tissue, and renal apoptosis and fibrosis were quantified in tissue sections. The degree of RAS and blood pressure were similarly increased in RAS and RAS+T. Renal blood flow (RBF) and glomerular filtration rate (GFR) were reduced in the stenotic kidney (280.1 +/- 36.8 and 34.2 +/- 3.1 ml/min, P < 0.05 vs. control). RAS+T kidneys showed preserved GFR (58.5 +/- 6.3 ml/min, P = not significant vs. control) but a similar decreases in RBF (293.6 +/- 85.2 ml/min) and further decreases in MV density compared with RAS. These changes were accompanied by blunted angiogenic signaling and increased apoptosis and fibrosis in the stenotic kidney of RAS+T compared with RAS. The current study shows that tempol administration provided limited protection to the stenotic kidney. Despite preserved GFR, renal perfusion was not improved by tempol, and MV density was further reduced compared with untreated RAS, associated with increased renal apoptosis and fibrosis. These results suggest that a tight balance of the renal redox status is necessary for a normal MV repair response to injury, at least at the

early stage of RVD, and raise caution regarding antioxidant strategies in RAS.

Kitamoto, Y., M. Takeya, et al. "Glomerular endothelial cells are maintained by vascular endothelial growth factor in the adult kidney." Tohoku J Exp Med. 2001 Sep;195(1):43-54.

Vascular endothelial growth factor (VEGF) is known to maintain endothelial cells of immature vessels and is constitutively expressed in the kidney from the embryo to adult. We tested the hypothesis that VEGF activity is needed to maintain glomerular endothelial cells in the adult. Neutralizing antibody to VEGF165 was intraperitoneally administered to mice for 3 days to strongly suppress its intrinsic activity. On the fourth day, mice were sacrificed and tissues were examined by light and electron microscopies. Vascular casts of renal vessels were observed by a scanning electron microscopy. Distribution of the administered antibody and expressions of VEGF and Flk-1 were examined immunohistochemically. The suppression of endogenous VEGF activity caused swelling and vacuolation of endothelial cells and obstruction of capillaries in the glomerulus. Other tissues were not impaired significantly. The administered antibody was specifically localized to the glomerulus, and was found more predominantly in the juxta-medullary than in the cortical glomerulus. This pattern of antibody deposition was similar to that of Flk-1. VEGF expression in the glomerulus was compensatively elevated by the antibody treatment. These results show that demand for VEGF signaling in the glomerulus is much higher than in other tissues, probably to protect its endothelial cells against high tension for blood filtration. This demand may be fulfilled by enriched signaling through the Flk-1 in the glomerulus.

Lavoz, C., M. Alique, et al. "Gremlin regulates renal inflammation via vascular endothelial growth factor receptor 2 pathway." J Pathol. 2015 Mar 23. doi: 10.1002/path.4537.

Inflammation is a main feature of progressive kidney disease. Gremlin binds to bone morphogenetic proteins (BMPs), acting as an antagonist and regulating nephrogenesis and fibrosis among other processes. Gremlin also binds to vascular endothelial growth factor receptor-2 (VEGFR2) in endothelial cells to induce angiogenesis. In renal cells, Gremlin regulates proliferation and fibrosis, but there are no data about inflammatory-related events. We have investigated the direct effects of Gremlin in the kidney, evaluating whether VEGFR2 is a functional Gremlin receptor. Administration of recombinant Gremlin to murine kidneys induced rapid and sustained activation of VEGFR2 signalling, located in proximal tubular epithelial cells. Gremlin bound to VEGFR2 in these cells in vitro, activating this signalling pathway independently of its action as an antagonist of BMPs. In vivo, Gremlin caused early renal damage, characterized by activation of the nuclear factor-kappaB pathway linked to up-regulation of pro-inflammatory factors and infiltration of immune inflammatory cells. VEGFR2 blockade diminished Gremlin-induced renal inflammatory responses. The link between Gremlin/VEGFR2 and NF-kappaB/inflammation was confirmed in vitro. Gremlin overexpression was associated to VEGFR2 activation in human renal disease and in the unilateral ureteral obstruction experimental model, where VEGFR2 kinase inhibition diminished renal inflammation. Our data show that a Gremlin/VEGFR2 axis participates in renal inflammation and could be a novel target for kidney disease.

Lee, A. S., J. E. Lee, et al. "Vascular endothelial growth factor-C and -D are involved in lymphangiogenesis in mouse unilateral ureteral obstruction." Kidney Int. 2013 Jan;83(1):50-62. doi: 10.1038/ki.2012.312. Epub 2012 Aug 29.

Lymphatic remodeling in inflammation has been found in tracheal mycoplasma infection, human kidney transplant, skin inflammation, peritonitis, and corneal inflammation. Here we investigated lymphangiogenesis in fibrotic area in unilateral ureteral obstruction, a model of progressive renal fibrosis, and evaluated the roles of vascular endothelial growth factor (VEGF)-C and -D in the obstructed kidney. Compared to sham-operated mice, the number of LYVE-1-positive lymphatic vessels, the proliferation of LYVE-1-positive lymphatic endothelial cells, along with VEGF-C and -D mRNA expression were all significantly increased following ureteral obstruction. Depletion of macrophages with clodronate decreased lymphangiogenesis in the obstructed kidney. VEGF-C expression was higher in M2- than in M1-polarized macrophages from bone marrow-derived macrophages, and also increased in Raw 264.7 or renal proximal tubule cells by stimulation with TGF-beta1 or TNF-alpha. VEGF-D reversed the inhibitory effect of TGF-beta1 on VEGF-C-induced migration, capillary-like tube formation, and proliferation of human lymphatic endothelial cells. Additionally, the blockade of VEGF-C and VEGF-D signaling decreased obstruction-induced lymphangiogenesis. Thus, VEGF-C and VEGF-D are associated with lymphangiogenesis in the fibrotic kidney in a mouse model of ureteral obstruction.

Liang, M., L. E. Woodard, et al. "Protective Role of Insulin-Like Growth Factor-1 Receptor in Endothelial Cells against Unilateral Ureteral Obstruction-Induced

Renal Fibrosis." Am J Pathol. 2015 Mar 14. pii: S0002-9440(15)00126-1. doi: 10.1016/j.ajpath.2015.01.027.

Insulin-like growth factor-1 receptor (IGF-1R) can regulate vascular homeostasis and endothelial function. We studied the role of IGF-1R in oxidative stress-induced endothelial dysfunction. Unilateral ureteral obstruction (UUO) was performed in wild-type (WT) mice and mice with endothelial cell (EC)-specific IGF-1R knockout (KO). After UUO in endothelial IGF-1R KO mice, endothelial barrier dysfunction was more severe than in WT mice, as seen by increased inflammatory cell infiltration and vascular endothelial (VE)-cadherin phosphorylation. UUO in endothelial IGF-1R KO mice increased interstitial fibroblast accumulation and enhanced extracellular protein deposition as compared with the WT mice. Endothelial barrier function measured by transendothelial migration in response to hydrogen peroxide (H2O2) was impaired in ECs. Silencing IGF-1R enhanced the influence of H2O2 in disrupting the VE-protein tyrosine phosphatase/VE-cadherin interaction. Overexpression of IGF-1R suppressed H2O2-induced endothelial barrier dysfunction. Furthermore, by using the piggyBac transposon system, we expressed IGF-1R in VE cells in mice. The expression of IGF-1R in ECs also suppressed the inflammatory cell infiltration and renal fibrosis induced by UUO. IGF-1R KO in the VE-cadherin lineage of bone marrow cells had no significant effect on the UUO-induced fibrosis, as compared with control mice. Our results indicate that IGF-1R in the endothelium maintains the endothelial barrier function by stabilization of the VE-protein tyrosine phosphatase/VE-cadherin complex. Decreased expression of IGF-1R impairs endothelial function and increases the fibrosis of kidney disease.

Nlandu Khodo, S., E. Dizin, et al. "NADPH-oxidase 4 protects against kidney fibrosis during chronic renal injury." J Am Soc Nephrol. 2012 Dec;23(12):1967-76. doi: 10.1681/ASN.2012040373. Epub 2012 Oct 25.

NADPH oxidases synthesize reactive oxygen species that may participate in fibrosis progression. NOX4 and NOX2 are NADPH oxidases expressed in the kidneys, with the former being the major renal isoform, but their contribution to renal disease is not well understood. Here, we used the unilateral urinary obstruction model of chronic renal injury to decipher the role of these enzymes using wild-type, NOX4-, NOX2-, and NOX4/NOX2-deficient mice. Compared with wild-type mice, NOX4-deficient mice exhibited more interstitial fibrosis and tubular apoptosis after obstruction, with lower interstitial capillary density and reduced expression of hypoxia-inducible factor-1alpha and vascular endothelial growth factor in obstructed kidneys. Furthermore, NOX4-deficient kidneys exhibited increased oxidative stress. With NOX4 deficiency, renal expression of other NOX isoforms was not altered but NRF2 protein expression was reduced under both basal and obstructed conditions. Concomitant deficiency of NOX2 did not modify the phenotype exhibited by NOX4-deficient mice after obstruction. NOX4 silencing in a mouse collecting duct (mCCD(cl1)) cell line increased TGF-beta1-induced apoptosis and decreased NRF2 protein along with expression of its target genes. In addition, NOX4 silencing decreased hypoxia-inducible factor-1alpha and expression of its target genes in response to hypoxia. In summary, these results demonstrate that the absence of NOX4 promotes kidney fibrosis, independent of NOX2, through enhanced tubular cell apoptosis, decreased microvascularization, and enhanced oxidative stress. Thus, NOX4 is crucial for the survival of kidney tubular cells under injurious conditions.

Ohashi, R., A. Shimizu, et al. "Peritubular capillary regression during the progression of experimental obstructive nephropathy." J Am Soc Nephrol. 2002 Jul;13(7):1795-805.

Injury to the renal microvasculature may be a major factor contributing to the progression of renal disease. Although severe disruption of peritubular capillaries (PTC) could lead to marked tubulointerstitial scarring, elucidation of that process remains incomplete. This study investigated the morphologic changes in PTC and their likely regulation by vascular endothelial growth factor (VEGF) during the progression of tubulointerstitial injuries. Unilateral ureteral obstruction was induced in Wistar rats by ligation of the left ureter, and the kidneys were then collected at selected times. PTC lumina and the expression of VEGF and its receptor Flk-1 were immunohistochemically detected. Morphologic changes in PTC endothelial cells were examined by using Ki67 staining, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling, and electron-microscopic studies. In the first week of the disease period, immunohistochemical labeling of tubular VEGF intensified, with accompanying deformation and dilation of adjacent thrombomodulin (TM)-positive PTC lumina; an angiogenic response of endothelial cells was demonstrated with Ki67 and TM double-staining. During the subsequent 2 wk, tubular VEGF labeling decreased until it was virtually absent, an effect confirmed by Western blotting. Concomitantly, labeling of the VEGF receptor Flk-1 in PTC endothelial cells decreased and PTC lumina began to regress, demonstrating endothelial cell apoptosis (as detected in terminal deoxynucleotidyl transferase-

mediated dUTP-biotin nick end-labeling and electron-microscopic studies). By the end of week 4, the numbers of TM-positive PTC lumina were significantly decreased in areas of marked tubulointerstitial scarring. These results suggest that PTC regression, involving an early, unsustained, angiogenic response followed by progressive endothelial cell apoptosis, could be a potential factor contributing to tubulointerstitial scarring in this unilateral ureteral obstruction model.

Ozbek, E., G. Adas, et al. "Role of Mesenchymal Stem Cells Transfected With Vascular Endothelial Growth Factor in Maintaining Renal Structure and Function in Rats with Unilateral Ureteral Obstruction." Exp Clin Transplant. 2014 Dec 22. doi: 10.6002/ect.2014.0080.

Mesenchymal stem cells hold promise for renal disease treatment. Vascular endothelial growth factor may heal tubule-interstitial fibrosis in unilateral ureteral obstruction by inhibiting epithelial-mesenchymal transition. We investigated the protective effect of vascular endothelial growth factor in transfected mesenchymal stem cells in unilateral ureteral obstruction-induced renal injury in rats. Male Wistar Albino rats (32 rats; weight, 250-300 g) were divided into 4 equal groups: group 1, control; group 2, unilateral ureteral obstruction; group 3, unilateral ureteral obstruction and mesenchymal stem cells; and group 4, unilateral ureteral obstruction and vascular endothelial growth factor-transfected mesenchymal stem cells. Vascular endothelial growth factor-transfected mesenchymal stem cells were administered intravenously before onset of unilateral ureteral obstruction. On day 14, the rats were killed and kidneys were retrieved. Tubular necrosis, mononuclear cell infiltration, and interstitial fibrosis were evaluated in paraffin blocks. We evaluated green fluorescent protein-positive and vascular endothelial growth factor-positive cells; anti-inflammatory (Prostaglandin E2 Receptor) and interleukin 1 receptor antagonist), proinflammatory/anti-inflammatory (interleukin 6), and proinflammatory (MPO) cytokine expression levels; and levels of nitric oxide; transforming growth factor beta1, E-cadherin, and hydroxyproline. Green fluorescent protein-positive cells were negative in the renal parenchyma in groups 1 and 2 and positive in groups 3 and 4. Vascular endothelial growth factor levels were significantly higher in group 4. Transforming growth factor beta1, nitric oxide, and E-cadherin levels were significantly higher in the unilateral ureteral obstruction than control group; however, in the study groups, these values were not significantly different from the unilateral ureteral obstruction group. In stem cell-transplanted tissue samples, EP3, interleukin 1 receptor antagonist, and interleukin 6 levels were elevated, but MPO expression levels were low. Although there were significant differences for tubular necrosis and fibrosis in group 2, there were significant reductions in tubular injury and fibrosis in groups 3 and 4. Systemic stem cells transplanted into the kidney protected against unilateral ureteral obstruction-induced renal epithelial-mesenchymal transition and renal fibrosis.

Rajekar, H., R. K. Vasishta, et al. "Noncirrhotic portal hypertension." J Clin Exp Hepatol. 2011 Sep;1(2):94-108. doi: 10.1016/S0973-6883(11)60128-X. Epub 2011 Nov 9.

Portal hypertension is characterized by an increase in portal pressure (> 10 mmHg) and could be a result of cirrhosis of the liver or of noncirrhotic diseases. When portal hypertension occurs in the absence of liver cirrhosis, noncirrhotic portal hypertension (NCPH) must be considered. The prognosis of this disease is much better than that of cirrhosis. Noncirrhotic diseases are the common cause of portal hypertension in developing countries, especially in Asia. NCPH is a heterogeneous group of diseases that is due to intrahepatic or extrahepatic etiologies. In general, the lesions in NCPH are vascular in nature and can be classified based on the site of resistance to blood flow. In most cases, these disorders can be explained by endothelial cell lesions, intimal thickening, thrombotic obliterations, or scarring of the intrahepatic portal or hepatic venous circulation. Many different conditions can determine NCPH through the association of these various lesions in various degrees. Many clinical manifestations of NCPH result from the secondary effects of portal hypertension. Patients with NCPH present with upper gastrointestinal bleeding, splenomegaly, ascites after gastrointestinal bleeding, features of hypersplenism, growth retardation, and jaundice due to portal hypertensive biliopathy. Other sequelae include hyperdynamic circulation, pulmonary complications, and other effects of portosystemic collateral circulation like portosystemic encephalopathy. At present, pharmacologic and endoscopic treatments are the treatments of choice for portal hypertension. The therapy of all disorders causing NCPH involves the reduction of portal pressure by pharmacotherapy or portosystemic shunting, apart from prevention and treatment of complications of portal hypertension.

Rouschop, K. M., N. Claessen, et al. "CD44 disruption prevents degeneration of the capillary network in obstructive nephropathy via reduction of TGF-beta1-induced apoptosis." J Am Soc Nephrol. 2006 Mar;17(3):746-53. Epub 2006 Feb 1.

CD44 is a glycoprotein that is involved in inflammation and cell-cell/cell-matrix interactions, is upregulated in the kidney upon injury, and leads to fibrosis through enhancement of TGF-beta1 signaling. Absence of CD44 prevents development of renal fibrosis in unilateral ureteral obstruction (UUO). A hallmark of development of renal fibrosis is the degeneration of the capillary network. This study shows that CD44 is upregulated on capillary endothelial cells during UUO. For elucidation of the role of CD44 on peritubular endothelial cells in UUO, capillary network degeneration was compared in CD44+/+ and CD44-/- mice. As expected, degeneration of the capillary network was observed in CD44+/+ mice during UUO, associated with increased endothelial apoptosis. However, in the absence of CD44, degeneration of the network is prevented as a result of a decrease in the rate of apoptosis in endothelial cells. The divergence in endothelial apoptosis is not correlated to differential vascular endothelial growth factor or thrombospondin-1 expression.

Schrimpf, C., C. Xin, et al. "Pericyte TIMP3 and ADAMTS1 modulate vascular stability after kidney injury." J Am Soc Nephrol. 2012 May;23(5):868-83. doi: 10.1681/ASN.2011080851. Epub 2012 Mar 1.

Kidney pericytes are progenitors of scar-forming interstitial myofibroblasts that appear after injury. The function of kidney pericytes as microvascular cells and how these cells detach from peritubular capillaries and migrate to the interstitial space, however, are poorly understood. Here, we used an unbiased approach to identify genes in kidney pericytes relevant to detachment and differentiation in response to injury in vivo, with a particular focus on genes regulating proteolytic activity and angiogenesis. Kidney pericytes rapidly activated expression of a disintegrin and metalloprotease with thrombospondin motifs-1 (ADAMTS1) and downregulated its inhibitor, tissue inhibitor of metalloproteinase 3 (TIMP3) in response to injury. Similarly to brain pericytes, kidney pericytes bound to and stabilized capillary tube networks in three-dimensional gels and inhibited metalloproteolytic activity and angiogenic signaling in endothelial cells. In contrast, myofibroblasts did not have these vascular stabilizing functions despite their derivation from kidney pericytes. Pericyte-derived TIMP3 stabilized and ADAMTS1 destabilized the capillary tubular networks. Furthermore, mice deficient in Timp3 had a spontaneous microvascular phenotype in the kidney resulting from overactivated pericytes and were more susceptible to injury-stimulated microvascular rarefaction with an exuberant fibrotic response. Taken together, these data support functions for kidney pericytes in microvascular stability, highlight central roles for regulators of extracellular proteolytic activity in capillary homoeostasis, and identify ADAMTS1 as a marker of activation of kidney pericytes.

Soubrier, M. "[POEMS syndrome]." Presse Med. 2007 Nov;36(11 Pt 2):1676-82. Epub 2007 Jul 12.

The POEMS syndrome combines a constant polyneuropathy (P), organomegaly (O), endocrinopathy (E), monoclonal gammopathy (M) (or other plasma cell disorder) and skin changes (S). Other manifestations may be observed: anasarca, fever, sweating, clubbed fingers, renal damage, arterial obstruction, pulmonary hypertension, thrombocytosis, and polycythemia. Its pathogenesis is not well elucidated but elevated levels of vascular endothelial growth factor (VEGF) appear to characterize it. Consistent plasma cell disorders include a monoclonal component, often in small quantities with a lambda light chain isotype, and plasmacytoma, often solitary lesions. Treatment depends on specific characteristics of the disease and the patient (radiation therapy for plasmocytoma, autologous bone marrow transplantation in young subjects, corticosteroid therapy or chemotherapy in the elderly).

Sun, D., L. Bu, et al. "Therapeutic effects of human amniotic fluid-derived stem cells on renal interstitial fibrosis in a murine model of unilateral ureteral obstruction." PLoS One. 2013 May 28;8(5):e65042. doi: 10.1371/journal.pone.0065042. Print 2013.

Interstitial fibrosis is regarded as the main pathway for the progression of chronic kidney disease (CKD) and is often associated with severe renal dysfunction. Stem cell-based therapies may provide alternative approaches for the treatment of CKD. Human amniotic fluid-derived stem cells (hAFSCs) are a novel stem cell population, which exhibit both embryonic and mesenchymal stem cell characteristics. Herein, the present study investigated whether the transplantation of hAFSCs into renal tissues could improve renal interstitial fibrosis in a murine model of unilateral ureteral obstruction (UUO). We showed that hAFSCs provided a protective effect and alleviated interstitial fibrosis as reflected by an increase in microvascular density; additionally, hAFSCs treatment beneficially modulated protein levels of vascular endothelial growth factor (VEGF), hypoxia inducible factor-1alpha (HIF-1alpha) and transforming growth factor-beta1 (TGF-beta1). Therefore, we hypothesize that hAFSCs could represent an alternative, readily available source of stem cells that can be applied for the treatment of renal interstitial fibrosis.

Sun, Y. B., X. Qu, et al. "Endothelial dysfunction exacerbates renal interstitial fibrosis through enhancing fibroblast Smad3 linker phosphorylation in the mouse obstructed kidney." PLoS One. 2013 Dec 31;8(12):e84063. doi: 10.1371/journal.pone.0084063. eCollection 2013.

Endothelial dysfunction and enhanced transforming growth factor-beta (TGF-beta)/Smad3 signalling are common features of progressive renal fibrosis. This study investigated a potential link between these mechanisms. In unilateral ureteric obstruction (UUO) we observed an acute (6 hr) down-regulation of nitric oxide synthase 3 (NOS3/eNOS) levels and increased phosphorylation of the linker region of Smad3 at T179 and S208 in Smad3/JNK complexes. These events preceded Smad3 C-terminal domain phosphorylation and the induction of myofibroblast proliferation at 48 hrs. Mice deficient in NOS3 showed enhanced myofibroblast proliferation and collagen accumulation compared to wild type mice in a 7 day UUO model. This was associated with enhanced phosphorylation of Smad3 T179 and S208 by 92% and 88%, respectively, whereas Smad3-C-terminal phosphorylation was not affected. Resolvin D1 (RvD1) can suppress renal fibrosis in the UUO model, and further analysis herein showed that RvD1 protected against endothelial dysfunction and suppressed Smad3/JNK complex formation with a consequent reduction in phosphorylation of Smad3 T179 and S208 by 78% and 65%, respectively, while Smad3 C-terminal phosphorylation was unaltered. In vitro, conditioned media from mouse microvascular endothelial cells (MMEC) treated with a general inhibitor of nitric oxide synthase (L-NAME) augmented the proliferation and collagen production of renal fibroblasts (NRK49F cells) compared to control MMEC media and this was associated with increased phosphorylation of JNK and Smad3 T179 and S208, whereas Smad3-C-terminal domain phosphorylation was unaffected.

Suzuki, Y., Y. Ito, et al. "Transforming growth factor-beta induces vascular endothelial growth factor-C expression leading to lymphangiogenesis in rat unilateral ureteral obstruction." Kidney Int. 2012 May;81(9):865-79. doi: 10.1038/ki.2011.464. Epub 2012 Jan 18.

Inflammation is recognized as an important contributor to lymphangiogenesis; however, in tubulointerstitial lesions in human chronic kidney diseases, this process is better correlated with the presence of myofibroblasts rather than macrophages. As little is known about the interaction between lymphangiogenesis and renal fibrosis, we utilized the rat unilateral ureteral obstruction model to analyze inflammation, fibrosis, lymphangiogenesis, and growth factor expression. Additionally, we determined the relationship between vascular endothelial growth factor-C (VEGF-C), an inducer of lymphangiogenesis, and the profibrotic factor, transforming growth factor-beta1 (TGF-beta1). The expression of both TGF-beta1 and VEGF-C was detected in tubular epithelial and mononuclear cells, and gradually increased, peaking 14 days after ureteral obstruction. The kinetics and localization of VEGF-C were similar to those of TGF-beta1, and the expression of these growth factors and lymphangiogenesis were linked with the progression of fibrosis.

Taniguchi, Y., N. Yorioka, et al. "Transforming growth factor-beta1 may be involved in shunt obstruction in patients on chronic hemodialysis." Nephron. 1999 Jan;81(1):102-5.

Obstructed shunt vessels were studied immunohistochemically to clarify the mechanism of shunt obstruction in hemodialysis patients. The subjects were 12 hemodialysis patients with shunt obstruction, and 8 patients newly started on hemodialysis were used as the controls. Cryosections of shunt tissue were prepared and stained for thrombomodulin as well as transforming growth factor-beta1 using the enzyme antibody method. In the obstructed shunt group, the intima was significantly thicker than in the control group. In addition, staining of the intima for thrombomodulin was decreased in the obstructed shunt group when compared with the controls. Staining for transforming growth factor-beta1 was related to intimal thickening and cell proliferation. These results indicate that release of thrombomodulin occurs with vascular endothelial cell damage and that transforming growth factor-beta1 may be involved in intimal hypertrophic change and shunt obstruction.

Xie, X. S., H. C. Liu, et al. "Ginsenoside Rg1 modulation on thrombospondin-1 and vascular endothelial growth factor expression in early renal fibrogenesis in unilateral obstruction." Phytother Res. 2010 Nov;24(11):1581-7. doi: 10.1002/ptr.3190.

Renal interstitial fibrosis is the major histopathological change seen in a variety of renal disorders and is closely related to renal dysfunction. Progressive interstitial fibrosis accompanied by the loss of renal tubules and interstitial capillaries typifies all progressive renal disease. Thrombospondin-1 (TSP-1) is a major angiogenic inhibitor. It is demonstrated that TSP-1 levels were correlated with the loss of glomerular and peritubular capillaries and TSP-1 could promote renal scarring by effects on the endothelium. It has been reported that ginsenoside Rg1 inhibited renal interstitial fibrosis in rats via suppressing the expression of TSP-1. The present

study was designed to examine whether ginsenoside Rg1 could modulate the integrity of the microvasculature and hence affect the progression of renal fibrosis in a rat unilateral ureteral obstruction (UUO) model. In UUO control kidneys, associated with interstitial fibrosis, lower peritubular capillary densities were prominent. These changes were all improved by ginsenoside Rg1 treatment. Interestingly, ginsenoside Rg1 decreased the expression of TSP-1 and enhanced vascular endothelial growth factor (VEGF) expression. The results show for the first time that ginsenoside Rg1 can evidently inhibit renal interstitial fibrosis in rats with UUO. The mechanism might be related to suppression of the expression of TSP-1 and to repair of the peritubular capillary.

Yoo, K. H., B. A. Thornhill, et al. "Inducible nitric oxide synthase modulates hydronephrosis following partial or complete unilateral ureteral obstruction in the neonatal mouse." Am J Physiol Renal Physiol. 2010 Jan;298(1):F62-71. doi: 10.1152/ajprenal.00234.2009. Epub 2009 Nov 4.

To investigate the role of endogenous inducible nitric oxide synthase (iNOS) in the response of the developing kidney to unilateral ureteral obstruction (UUO), neonatal iNOS null mutant (-/-) and wild-type (WT) mice were subjected to partial or complete UUO. At 7 and 21 days of age, apoptosis, renin, vascular endothelial growth factor (VEGF), fibroblasts (anti-fibroblast-specific peptide 1), myofibroblasts (alpha-smooth muscle actin), macrophages (F4/80), and collagen were measured in kidney tissue. Compared with WT, renal parenchymal thickness was increased, with preservation of the papilla, in -/- mice with partial UUO, but decreased in -/- mice with complete UUO. Ureteral peristalsis increased with severity of pelvic dilatation in WT, and increased further in -/- mice with partial UUO. Apoptosis, fibroblasts, and macrophages were increased in -/- mice with complete UUO, but there was no effect of iNOS on other histological parameters following complete UUO.

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