Published in final edited form as:

Drug Discov Today Dis Models. 2013 ; 10(3): e127–e135. doi:10.1016/j.ddmod.2012.08.004.

MicroRNAs are potential therapeutic targets in fibrosing kidney disease: lessons from animal models

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Abstract

Chronic disease of the kidneys has reached epidemic proportions in industrialized nations. New therapies are urgently sought. Using a combination of animal models of kidney disease and human biopsy samples, a pattern of dysregulated microRNA expression has emerged which is common to chronic diseases. A number of these dysregulated microRNA have recently been shown to have functional consequences for the disease process and therefore may be potential therapeutic targets. We highlight microRNA-21, the most comprehensively studied microRNA in the kidney so far. MicroRNA-21 is expressed widely in healthy kidney but studies from knockout mice indicate it is largely inert. Although microRNA-21 is upregulated in many cell compartments including leukocytes, epithelial cells and myofibroblasts, the inert microRNA-21 also appears to become activated, by unclear mechanisms. Mice lacking microRNA-21 are protected from kidney injury and fibrosis in several distinct models of kidney disease, and systemically administered oligonucleotides that specifically bind to the active site in microRNA-21, inhibiting its function, recapitulate the genetic deletion of microRNA-21, suggesting inhibitory oligonucleotides may have therapeutic potential. Recent studies of microRNA-21 targets in kidney indicate that it normally functions to silence metabolic pathways including fatty acid metabolism and pathways that prevent Reactive Oxygen Species generation in peroxisomes and mitochondria in epithelial

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Conflict of Interest

JSD serves on the Scientific Advisory Board of Regulus therapeutics and has a Research Grant sponsored by Regulus Therapeutics.

cells and myofibroblasts. Targeting specific pathogenic microRNAs in a specific manner is feasible in vivo and may be a new therapeutic target in disease of the kidney

Keywords

microRNA; fibrosis; PPAR α ; ROS; chronic kidney disease; chronic allograft dysfunction

Introduction

Chronic kidney disease (CKD) is a growing epidemic in the Industrialized Nations. In the USA it affects over 26 million or 8% of the population, and its prevalence is predicted to continue to rise [1]. The kidneys are particularly susceptible to the development of fibrosis due to their high vascularity and predisposition to ischemia [2], and fibrogenesis is increasingly held to be a central pathological mechanism in CKD [3]. CKD is defined as loss of glomerular filtration rate (a measure of kidney function) and/or signs of chronic kidney damage, including the abnormal leakage of plasma proteins in the urine.

Pathologically it is characterized by fibrosis of the glomeruli in combination with loss of glomerular capillaries (glomerulosclerosis) often accompanied by inflammation, and in the remainder of the kidney, it is characterized by interstitial fibrosis, inflammation (recruitment of leukocytes), injury with flattening (atrophy) and loss of the tubule epithelium, and loss of the peritubular capillaries [4] (Figure 1). Recent studies from animal models indicate that the myofibroblast, the principal cell in the kidney that deposits fibrosis, may be central to many of the features of CKD, including fibrosis, loss of capillaries and inflammation, and is therefore a new target for therapeutics [5] [3].

A wide array of diseases/conditions may affect renal function leading to CKD including Diabetes mellitus, Cardiovascular Disease, Hypertension, and Cancers, particularly of the bone marrow. Recipients of heart, liver and lung transplants are also predisposed to develop progressive CKD, and many drugs that are used to treat life threatening diseases elsewhere are toxic to the kidney with long lasting consequences. There is currently a large unmet need for new therapies to counteract the progression of CKD and also the equivalent process affecting kidney transplants, known as chronic allograft dysfunction (CAD). CKD and CAD often follow a relentless course, which progresses to organ failure, even if the initiating factors have been adequately treated. Furthermore episodes of acute kidney damage that occur during a number of illnesses or as a consequence of medical treatments, have been shown to accelerate the progression of established CKD [6]. Currently, only therapies which target the angiotensin receptor (angiotensin receptor-1 blockers [ARB] or angiotensin converting enzyme [ACE] inhibitors) are used in clinical practice to retard the progression of CKD or CAD. There is therefore a pressing need for new therapies designed to treat or protect the damaged kidney.

MicroRNAs (miRNA) are a family of small, non-coding RNAs that control gene expression by inhibiting translation of their complementary, 'target' messenger RNAs (mRNA) [7–9] They regulate gene expression predominantly by facilitating degradation of target mRNAs. They also inhibit protein translation of target mRNAs (translational suppression)[9,10].

MiRNAs bind by sequence complementarity to short sequences usually located in the 3' untranslated regions of messenger RNA (mRNA). The sequence is frequently found in many (>50) functionally-related but distinct mRNAs, therefore a single miRNA silences a number of functionally related genes. Frequently, one or more of these genes is functionally critical, and suppression of its function gives the miRNA itself, function. Dysregulated miRNA expression has been identified in human diseases and is also readily observed in animal models [11]. Modulation of dysregulated miRNAs *in vivo* can attenuate the manifestation of disease, suggesting that aberrant miRNA can contribute to disease pathogenesis[9,12,13]. Recent findings from several independent laboratories have identified a number of miRNAs that are dysregulated in human CKD with fibrosis, and also in animal models of human CKD. We will explore the evidence that by blocking that activity of one or more of such dysregulated miRNA we may develop new therapeutics to treat the progression of CKD or CAD in patients [14].

The role of fibrosis in disease progression

Recent studies, primarily from animal models of kidney disease, have identified a separate lineage of cells that in the normal kidney are called 'pericytes' or 'resident fibroblasts' and are of mesenchymal origin. These cells represent >5% of normal kidney cells and perform critical homeostatic and regenerative functions, particularly with respect to microvascular homeostasis [15–17]. In CKD these pericytes and resident fibroblasts become activated and are then known as myofibroblasts (Figure 1). Myofibroblasts are the contractile cells that deposit fibrillar pathological matrix, known as fibrosis or scar tissue in the kidney cortex and medulla. Myofibroblasts in the glomerulus deposit fibrillar pathological matrix known as mesangial matrix expansion or mesangial nodules, or referred to as glomerulosclerosis when combined with loss of glomerular capillaries. Fibrosis, is pathological fibrillar matrix which accumulates in the virtual space between capillaries and tubules of the nephron or around capillaries of the glomerulus, and obliterates local structures (Figure 1). In the glomerulus it frequently accumulates initially in the mesangial area, along the capillary loop itself, or in the proliferation of cells that occupies the urinary space, often known as a 'crescent'. The fibrotic material encroaches on capillaries and prevents them from functioning. Fibrosis *per se* reduces nephron function and promotes tissue ischemia, and distorts normal tissue architecture. However, the activated pericyte (known as myofibroblast) is contractile, and can distort tissue architecture, and the myofibroblast is also an inflammatory cell secreting innate immune cytokines and chemokines and oxygen radicals. Furthermore, the myofibroblast no longer performs normal pericyte functions (Figure 1). Pericytes normally nurse capillaries and support microvascular stability, but when they become myofibroblasts they no longer perform these functions, leaving unstable capillaries that are prone to ineffective angiogenesis, increased permeability and often capillary demise, which is seen in the kidney as capillary rarefaction. Therefore myofibroblasts cause fibrosis, are a source of inflammation, and promote loss of capillaries. The myofibroblast is therefore a major new target for therapeutics in kidney diseases.

Epithelial cell injury as a stimulus for fibrosis

Numerous studies have identified cell stress or cell injury in the tubule epithelial compartment, particularly the proximal tubule, as a stimulus for fibrosis [3]. Increasingly it is recognized that damaged or stressed epithelial cells exhibit a number of stereotyped responses: endoplasmic reticulum (ER) stress and the unfolded protein response; apoptosis and necrosis; activation of epithelial to mesenchymal transition genes; activation of Transforming growth factor β , and cell-cycle arrest [3]. Proximal tubule cells are particularly dependent on aerobic generation of high levels of ATP for survival and health. Therefore factors that affect or compromise ATP generation have profound impact on epithelial cell function. Not only do injured and stressed epithelial cells fail to perform normal functions, which are vital to kidney function, but they also generate a wide array of profibrotic and inflammatory factors that can, of themselves, drive the manifestations of CKD by cell to cell signaling mechanisms (Figure 1).

Animal Models of Chronic Kidney Diseases

Although there are no perfect models of CKD in rodents there are a number of validated models that recapitulate many or all of the features of human CKD, albeit over a shorter time scale than is seen in humans (Table I). Several of these models have been used to validate bioactive compounds and antibodies at the preclinical stage of development, that are now in human clinical trials. Although there is no perfect model of diabetic nephropathy, there are now a number of models of type-I Diabetes mellitus or type-II Diabetes mellitus, in mice and rats. The Type-II diabetes mellitus represents >95% of human diabetic kidney disease, but the mouse models are all based around the mutations in the leptin or leptin receptor genes [18,19], and the degree of nephropathy has been variable. The models of type I diabetes mellitus with nephropathy reproduce human disease reasonably well in rats, but the presence of hypertension is required, and the models take many months to manifest (Table I) [20]. Recent human outcomes data have indicated that acute kidney diseases, which were historically thought to recover fully, in fact frequently recover incompletely, leading therefore to CKD [6]. Therefore, a newer model of CKD can be generated by inducing acute kidney injury in a single kidney while leaving the other kidney intact (Unilateral Ischemia Reperfusion Injury [U-IRI]) [21]. This model is very similar to ischemic acute kidney injury in humans and leads to all the hallmarks of human chronic kidney disease in rodents over a few weeks.

Evidence for dysregulation of microRNAs in animal models of kidney disease

Although there do not appear to be any kidney specific microRNAs, a kidney signature has been reported by a number of recent studies using microarray approaches [22]. In addition, a consistent pattern of up-regulated and down-regulated microRNA has been described in response to acute and chronic kidney injuries [10,22,23] (Table II). Some of these miRNA changes will reflect recruitment of inflammatory cells, but many changes reflect abnormal regulation of genes by miRNA, and is known as dysregulation. In transplanted human kidneys, miRNA profiles from kidney biopsies have been shown to distinguish patients with

acute immunological rejection of the kidney transplant (allograft) from patients with a kidney transplant but no organ rejection. Acute rejection can be diagnosed with a high degree of accuracy with the use of levels of miRNA. Among the miRNA that were identified and indicative of organ rejection were 17 miRNAs, 10 (let-7c, miR-10a, miR-10b, miR-125a, miR-200a, miR-30a-3p, miR-30b, miR-30c, miR-30e-3p, and miR-32) that were downregulated in acute rejection biopsies compared to normal allograft biopsies, and 7 miRNAs (miR-142-5p, miR-142-3p, miR-155, miR-223, miR-146b, miR-146a, and miR-342) were overexpressed[24].

MicroRNA-21 in kidney disease

MicroRNA-21 (miR-21) when transgenically over-expressed in lymphocytes appears to function as an oncogenic miRNA (also known as an oncomir) [12]. In these lymphocytic cancer cells it appears to function by preventing apoptotic cell death. MiR-21 is however widely expressed in many tissues and is expressed quite highly in the normal kidney. It is also upregulated in a range of kidney diseases, and is one of the most highly expressed miRNAs in kidney disease (Table II) [10]. Recent studies reported that miR-21 levels were increased in cardiac fibroblasts of failing hearts and it was shown that miR-21 results in over-activation of the P42/P44 mitogen activated protein (MAP) kinase signaling pathway in cardiac fibroblasts, but not in cardiomyocytes [9]. Silencing of miR-21 by a specific modified RNA oligonucleotide complementary to the miR-21 sequence, which was conjugated to cholesterol and known as ‘antagomir’, reduced cardiac P42/P44 MAP kinase activity, interstitial fibrosis and attenuated cardiac dysfunction in models of cardiac failure in mice. Although follow-up studies failed to replicate these findings [25], these cardiac studies suggested that miR-21 was potentially a miRNA that played a role in amplifying the fibrogenic process, and therefore a potential target in other organs.[8]

Chau and colleagues generated a mouse in which the locus for miR-21 was successfully mutated by germline deletion [10]. The mice were essentially normal at 8 months of age in sterile animal housing facility, exhibited normal fertility, and the kidneys developed normally. However, in several models of CKD, the *miR21*^{-/-} kidneys suffered less tubule injury/atrophy, less fibrosis, less capillary destruction, and reduced P42/P44 MAP kinase cell activation pathway activation in response to the same degree of injury (Figure 2) [10]. The investigators developed and synthesized modified oligonucleotides based molecularly around ribose nucleotides, that are complementary to miR-21, freely enter cells in the kidney if injected subcutaneously, bind to intracellular miR-21 and stimulate degradation of miR-21, effectively silencing miR-21. Administration of these oligonucleotides (anti-miR21) to mice recapitulated the results of *miR21*^{-/-} in kidney disease, and in addition, reversed evolving disease in kidneys. When the investigators analyzed all the genes in a microarray of the kidneys from the *miR21*^{-/-} mouse, they found that in non-diseased kidneys there were no genes that were normally silenced by miR-21 even though it is expressed at quite high levels. In response to kidney injury, however, a characteristic pattern of genes, with the complementary sequence in their 3'UTRs to miR-21, was silenced in the *miR21*^{+/+} kidneys compared with the *miR21*^{-/-} kidneys. These observations suggested that, unlike many other miRNAs, miR-21 is sequestered in an intracellular compartment and released into the cytoplasm in response to cell stress where it is active. The genes (more than

80) that were silenced in diseased kidneys by miR-21 were surprising. Rather than inflammatory, innate immunity or fibrotic/matrix turnover genes, the genes were all involved in cell metabolism functions. In particular, there were genes that play critical roles in lipid metabolism and fatty acid oxidation, and Redox regulation in mitochondria.

The investigators identified the peroxisomal and mitochondrial fatty acid oxidation metabolic pathway regulated by the transcription factor Peroxisome proliferator activated receptor- α (PPAR α) as a major target for miR-21; in fact 9 distinct enzymes induced by PPAR α in this catalytic pathway, in addition to PPAR α itself, were all specifically silenced by miR-21 in the kidney (Figure 3). They went on to show that miR-21 engaged this pathway in kidney epithelial cells, particularly proximal tubule, but also in myofibroblasts, and that transgenic over-expression of PPAR α , 3-fold higher than normal in kidney epithelial cells alone, protected kidneys from the development of fibrosis. Finally they showed that while *Ppara*^{-/-} mice exhibited increased fibrogenesis and epithelial injury, oligonucleotides that block miR-21 action, were no longer able to inhibit epithelial injury and fibrogenesis, implicating the PPAR α fatty acid pathway as a major target of miR-21 in kidney disease. Importantly, PPAR α has been identified previously as a protective transcription factor in acute kidney injury [26,27]. PPAR α stimulates the proliferation of peroxisomes, and these organelles play a critical role in the oxidation of fatty acids with carbon chain longer than 22, and also provide detoxification and metabolism of oxygen radicals including H₂O₂. Detoxification of accumulated fatty acids in injured cells and metabolism of excess oxygen radicals are important factors that promote cell survival and function. In addition, peroxisomes are a major site of long chain fatty acid metabolism by the α -, and β -oxidation pathways. Epithelial cells rely heavily on fatty acids for ATP generation. While peroxisomal β -oxidation enzymes reduce very long chain fatty acids, mitochondrial β -oxidation enzymes oxidize fatty acids with less than 22 carbon atoms resulting in ATP generation. The proximal tubule nuclear receptor PPAR α plays a pivotal role in regulating peroxisomal and mitochondrial fatty acid oxidation in kidney tissue. Moreover many of the enzymes including Acyl-CoA oxidase 1 and Acyl-CoA Dehydrogenases (ACAD-M, ACAD-VL) and Carnitine Palmitoyl Transferase, that are critical in this pathway are also silenced by miR-21.

In addition to the fatty acid metabolic pathway Chau *et al* identified enzymes and cofactors involved in: intracellular redox state; and inhibition of reactive oxygen species (ROS) generation, as miR-21 targets, particularly in epithelial cells. Consistent with miR-21 stimulating ROS generation in kidney epithelium, they showed that in diseased kidneys from *miR21*^{-/-} mice there was reduced evidence of ROS generation, in interstitial cells as well as epithelial cells. One of the ROS proteins silenced by miR-21 was Mpv17-like (Figure 3). This, obscurely named protein was identified in kidney epithelial cell mitochondrial membranes and has recently been reported to interact via its PDZ domain with an serine protease enzyme, HTRA2, which prevents mitochondrial ROS generation and prevents mitochondrial triggered apoptosis [28]. Therefore it is likely that miR-21 also stimulates ROS generation in epithelial cells in response to cell stress.

These studies implicate impaired fatty acid metabolism and enhanced mitochondrial ROS generation in CKD development and progression [10]. It is interesting therefore that

excessive production of ROS by mitochondria has been strongly implicated in CKD progression in a number of independent studies and in development of kidney abnormalities that occur with normal aging [29,30] [31]. The gene, *Mpv17*, an orthologue of *Mpv17*-like, was identified more than 20 years ago as an epithelial and neuronal restricted protein located in the mitochondrial inner membrane and implicated in metabolism of ROS. Mice lacking *Mpv17* spontaneously develop proteinuric kidney disease with glomerulosclerosis and interstitial diseases similar to CKD [32–35]. In addition to these links between mitochondrial ROS generation, kidney disease progression, and miR-21, the family of PPAR transcription factors including PPAR γ and PPAR α have been drug targets for a number of years using a class of agents known as glitazones as ligands for PPAR γ and fibrates as ligands for PPAR α . Glitazones have been used to treat diabetes mellitus, by enhancing insulin sensitivity, but have been documented in animals, at least, to exhibit anti-inflammatory and anti-fibrotic effects in kidney disease. Moreover, PPAR γ has been suggested as a transcription factor that inhibits myofibroblast activation in a number of tissues including lung, liver and skin [36]. Fibrates on the other hand were used in patients with diabetes and the *Fenofibrate Intervention and Event Lowering in Diabetes* (FIELD) Study which was a 5-year trial of fenofibrate versus placebo demonstrated that end points of Cardiovascular Disease events and nonfatal myocardial infarction were significantly reduced, as were hospitalizations for acute coronary syndromes and coronary and carotid revascularization procedures. Fenofibrate also significantly reduced the microvascular complications of type 2 diabetes, including nephropathy [37]. Although glitazones are no longer used in kidney disease due to side effects in patients with reduced kidney function these clinical studies suggest that stimulation of peroxisome functions and fatty acid metabolism, potentially by antimiR21, are desirable strategies in kidney disease (Figure 3).

Studies from independent investigators have also identified that miR-21 is upregulated in models of kidney disease, and is upregulated in human CKD in kidney transplants or in native kidney disease [10,22,23]. Moreover, independent groups have shown that inhibition of microRNA-21 has therapeutic benefits [23]. These independent findings add weight to the significance of miR-21 as a candidate target for kidney disease.

Is microRNA-21 a viable therapeutic target in kidney disease?

Because microRNAs are located in cytoplasm of cells and because they control cell functions they are obvious candidates for therapy. In addition, because of sequence complementarity, drugs can be designed to highly specifically target a single miRNA, potential avoiding conventional small molecule drug side-effects. Recent advances in the development of oligonucleotides that can bind to mRNA and silence the translation of those mRNA (known as RNA silencing), heralded the development of small (22nt) oligonucleotides that are stable in the circulation, can freely enter cells and because of their sequence complementarity, bind specifically to miRNAs causing them to be silenced [10]. Such anti-microRNA oligonucleotides are already in clinical trials as therapeutics in cancer in hyperlipidemia. The animal studies reported by Chau et al showed that anti-miR21 oligonucleotides, accumulate in the kidney and effectively block miR-21 functions [10]. This type of oligonucleotide accumulates in kidney and liver at higher concentrations than other organs. Although miR-21 is widely expressed normally, it appears that it is not active

in healthy cells, but becomes active only in stressed or injured cells [10]. The fact that *miR21*^{-/-} mice are healthy attests to a dormant role for miR-21 in cell physiology. It is quite likely therefore that anti-miR21 oligonucleotides will only block miR-21 actions in areas of tissue injury or inflammation. Finally, the current anti-miR-21 oligonucleotides have been administered to animals for several months without any toxicity. An additional question that arises from these studies is what is the functional role for miR-21 since it appears to be only detrimental in during disease. Does it have a physiological role? At this time no clear physiological role has been apportioned to miR-21.

Evidence of dysregulation of other microRNAs playing roles in kidney disease

There are more than 20 dysregulated miRNAs identified in kidney disease (Table 2). It is likely therefore that in addition to miR-21 others will play pathological roles. In a mouse model of diabetic nephropathy, Putta and colleagues [38] showed that reduction of miR-192 retards renal fibrosis and improves proteinuria. Administration of modified RNA oligonucleotides, that are complementary to the miR-192 sequence and result in miR-192 degradation, attenuated histological evidence of glomerular expansion and renal interstitial fibrosis, as well as conferring improvement in renal function in the diabetic mice. MiR-192 was thought to promote diabetic kidney disease by binding to and silencing the E-box repressors Zeb1/2.

In another study, Qin *et al*[39] demonstrated that miR-29 negatively regulated fibrosis by targeting the process of collagen matrix synthesis rather than by inhibiting myofibroblast accumulation in a mouse model of unilateral ureteral obstruction nephropathy. MiR-29 was negatively regulated by TGFβ when it signals via the Smad3 dependent pathway. By using miRNA microarray and real-time PCR the investigators found that miR-29a,-b,-c family members were substantially reduced in the fibrotic kidney of UUO nephropathy in wild type mice but significantly increased in *Smad3*^{-/-} mice in which renal fibrosis was reduced as a result of absence of Smad3. These findings implicate miR-29 in TGFβ-dependent fibrosis mechanism. From a therapeutic perspective, the identification of miRNAs that are increased in disease and contribute to pathogenesis by silencing genes the design and successful delivery of mimetics of miR-29 is some way from a practical reality at this time.

Does Release of miRNAs into the Circulation or Urine Identify Disease Activity or Predict Kidney Disease Progression?

Recent studies have shown that miRNAs are found in the circulation in exosomes or stably bound to the assembly protein Argonaut. In addition to the blood stream, miRNAs have been detected in urine and other secreted fluids in a stable form[40–42]. An increasing number of investigations suggest that several circulating miRNAs are biomarkers for cancer growth, or for organ injuries, such as cardiac ischemia. It is likely therefore that miRNAs will be released into urine or blood in kidney disease. MiR-21 was shown recently to be upregulated in both the medulla and cortex of a rat model after gentamicin-induced nephrotoxicity [43]. The research team went further and investigated urine samples from

patients with acute kidney injury (AKI) in the intensive care unit and compared those samples to healthy subjects. They found that urinary levels of miR-21 were increased by a significant but modest 1.2 fold in the AKI patients over healthy patients. Although there does not appear to be a miRNA restricted to kidney in disease or development, future studies should be focused on the identification of patterns of miRNA that are released into the urine or blood by damaged kidneys.

Conclusions

Recent studies have identified dysregulated miRNA in animal models and human tissue samples of kidney disease. Evidence from knockout mice and miRNA-silencing oligonucleotides in rodents indicates that miR-21 is an important pathological miRNA in chronic kidney disease. miR-21 silences metabolic pathways involved in fatty acid metabolism and in removal of reactive oxygen species in peroxisomes and mitochondria. With recent advances in oligonucleotide technology a potential new type of therapeutic that specifically targets and degrades miRNAs, including miR-21 may soon be available to test in human kidney disease.

Acknowledgments

Duffield Laboratory is supported by NIH grants (DK84077, DK87389, DK93493) University of Washington, Institute for Stem Cell & Regenerative Medicine, Genzyme Research in Progress Grant, the Nephcure Foundation and Regulix Therapeutics. The Portilla laboratory is supported by NIH grant DK075976, and a VA Merit Award.

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Figure 1. Characteristic manifestations and model of disease mechanisms of chronic kidney disease in glomeruli and interstitium of human kidney cortex

(A) Normal human glomerulus and surrounding tubules and peritubular capillaries (PTCs) filled with erythrocytes (* placed above examples of PTCs) stained with Silver methenamine combined with PAS (Jones) stain which highlights collagens. Arteriole (a) is shown. Note back to back tubules with cuboidal or columnar epithelium. (B) Sclerotic glomerulus showing wedge shaped sclerotic region showing dense pink material on PAS stained section (arrowhead) and rather acellular weaker pink stained material more

peripherally (arrow) and obliteration of capillary loops. Also note the sclerotic region is fused to Bowman's capsule where there is local destruction of the basement membrane and periglomerular fibrosis (thick arrow). At the lower pole note a combination of increased cellularity and fibrosis in the mesangium, and basement membrane thickening in glomerular loops. **(C)** Jones stained image of cortex from diabetic nephropathy, showing injured tubules (tubule atrophy and tubule cell vacuolization, apoptotic cells, arrow), marked reduction in capillary density (* placed adjacent to examples of PTCs), expansion of the interstitial space with fibrotic material (fine black stain), and an increase in inflammatory cells. Note also thickening of the tubule basement membrane (black) **(D)** Trichrome stained image of kidney cortex from ischemic kidney disease showing marked expansion of interstitial fibrosis (cyan color) which has overtaken all of the tubules. The fibrosis is cellular showing inflammatory cells and myofibroblasts. The remaining tubules all show tubular atrophy with intraluminal debris. **(E)** Schema showing cellular mechanisms of CKD development in the kidney cortical interstitium.

Sirius red

PAS

Figure 2. Images of kidney disease after in mice that lack miR-21 compare with WT mice following kidney injury

Sirius red stained low power images showing red stained interstitial fibrosis following kidney injury, and medium power images showing PAS stain of kidney cortex after injury. Not reduced fibrosis in kidneys lacking miR-21. Note also that kidney epithelial cells are more injured in WT showing increased flattening and loss of typical purple brush border, whereas these features are more preserved in *miR21*^{-/-} kidneys.

Figure 3. Schema showing major gene products and pathways silenced by miR-21 in animal models of kidney disease

MiR-21 silences the transcriptional regulator PPARα and many of the downstream enzymes in fatty acid metabolism that are also regulated by PPARα, including transporters and enzymes (shown in grey) of the β-oxidation metabolic pathway of fatty acids that occurs in peroxisomes and mitochondria. The consequence of miR-21 activity is to reduce metabolism of fatty acids. In addition miR-21 increases reactive oxygen species and toxin formation/accumulation. Firstly by suppressing peroxisome formation and activity, the metabolism of H₂O₂ is retarded by miR-21 and secondly miR-21 silences genes that inhibit ROS generation in mitochondria, including MPV17-like which exerts its inhibitory function by binding to the mitochondrial protease HtrA2. In this configuration HtrA2 is also anti-apoptotic.

Table I**Animal Models of chronic kidney disease**

Animal Models of glomerulosclerosis		
Model	Timecourse	Notes
<i>Immunological</i>		
Nephrotoxic serum nephritis	3–4 wks	Patchy
Passive Heyman Nephritis		
<i>Basement membrane</i>		
Col4a3 ^{-/-} mouse	<3 mths	Mouse strain dependent
<i>Toxin</i>		
Adriamycin	4 mths	
Puromycin aminonucleoside		
Thy1.1 nephritis (repeated)	3–4wks	Rats only
5/6 Nephrectomy	3 mths	Rats only
HIV proteins (TG26)	8 wks	Collapsing GN first
<i>Diabetic models</i>		
Db/Db eNOS ^{-/-}	>4 mths	Mild/moderate interstitial disease
BTBR Ob/Ob	>3 mths	Predominantly glomerular disease
Akita	5 mths	mesangial disease only
Streptozocin + inducible hypertension	4 mths	Rats only
<i>Podocyte mutations</i>		
CD2 associated protein knockout	6 wks	Death at 6 wks
Nephrin knockout	6wks	
Podocin knockout	6wks	
α-actinin-4 knockout	6 mths	Slow progression
Animal Models of Interstitial Kidney Disease		
Models	Timecourse	Notes
<i>Obstructive</i>		
UUO	2 wks	Surgical procedure
<i>Ischemic</i>		
Unilateral IRI	3 wks	Surgical procedure
Unilateral IRI + uninephrectomy	4wks–4mths	
Angiotensin infusion		mild
<i>Hyperfiltration</i>		
5/6 nephrectomy	3 mths	rats only
<i>Toxin</i>		
Folic acid	3 mths	strain-dependent
Aristolochic Acid		
Cisplatin		high death rate

Animal Models of glomerulosclerosis

Model	Timecourse	Notes
Adriamycin nephropathy	>4 mths	
<i>Basement membrane</i>		
Col4a3 ^{-/-} mouse	2–3 mths	
<i>Immunological</i>		
Nephrotoxic Serum Nephritis	3 wks	
<i>Diabetic</i>		
Db/Db eNOS ^{-/-}	4–10 mths	
Streptozocin + uninephrectomy	4–8 mths	Rats + Mice

Table II

Commonly dysregulated microRNA in two mouse models of chronic kidney disease

miRNA	UUO		U-IRI	
	Fold change over normal kidney	P value	Fold change over normal kidney	P value
miR-214	55	5×10^{-5}	8	6×10^{-5}
miR-199a5p	29	3×10^{-3}	6	6×10^{-5}
miR-142-3p	20	2×10^{-3}	8	3×10^{-3}
miR-199a-3p	17	3×10^{-3}	6	1×10^{-4}
miR-342-3p	14	2×10^{-3}	5	2×10^{-3}
miR-18a	14	4×10^{-4}	5	2×10^{-3}
miR-21	14	1×10^{-3}	6	1×10^{-3}
miR-93	13	8×10^{-4}	2	4×10^{-3}
miR-183	12	4×10^{-4}	3	7×10^{-3}
miR-99b	12	2×10^{-3}	2	8×10^{-3}
miR-223	10	5×10^{-4}	9	3×10^{-3}
miR-146a	8	4×10^{-4}	5	9×10^{-3}
miR-199b	8	3×10^{-3}	4	1×10^{-3}
let7i	7	3×10^{-3}	3	3×10^{-3}
miR-19a	7	4×10^{-3}	2	7×10^{-3}
miR-15b	6	7×10^{-3}	3	4×10^{-3}
miR-20a	6	8×10^{-3}	2	6×10^{-3}
miR-25	5	9×10^{-3}	2	6×10^{-3}
miR-106b	5	8×10^{-3}	2	4×10^{-3}
miR-350	5	3×10^{-3}	3	4×10^{-3}
miR-674	5	2×10^{-3}	3	2×10^{-3}
miR-132	5	4×10^{-4}	7	4×10^{-3}
miR-92a	4	1×10^{-4}	2	7×10^{-3}
miR-15a	4	7×10^{-3}	2	3×10^{-3}