miRNA-Coordinated Networks as Promising Therapeutic Targets for Acute Kidney Injury

Anton Jan van Zonneveld, Ton J. Rabelink, and Roel Bijkerk

Since their discovery two decades ago, miRNAs have become established players in biological research, and their relevance in health and disease is undisputed. It is no more the question if miRNAs are involved in a particular biological pathway, but rather which miRNAs are involved.

Single miRNAs can target tens to hundreds of genes and thereby coordinate the control of multiple distinct cellular pathways that together drive cellular functions, such as those involved in the response to tissue injury. The notion that miRNAs can act as upstream post-transcriptional regulators of cellular function explains the wide interest of biomedical researchers in assessing the potential of miRNAs as therapeutic targets in disease. Because clinically proved antisense-RNA oligonucleotide (ASO) approaches to silence miRNAs are available and because, on intravenous injection, these ASOs readily accumulate in the kidney, the role of miRNAs in kidney pathophysiology is increasingly being explored.

In this issue of The American Journal of Pathology, Wilflingseder et al demonstrated a rate-limiting role for miR-182-5p in acute kidney injury (AKI). Although the expression of miR-185-5p is markedly increased in the affected kidney in a rodent model of kidney ischemia-reperfusion injury, silencing of miR-182-5p using ASOs resulted in preservation of kidney function and reduced tissue damage. They found miR-182-5p to simultaneously target several key genes important in the progression of kidney injury and fibrosis. Moreover, they showed that ex vivo perfusion of a pig kidney with the ASO efficiently reduced miR-182-5p expression and derepression of its targets, providing proof of principle that applying an ASO to silence a target miRNA is feasible in the clinical setting of kidney transplantation. In this commentary, we discuss the implications of these findings and the potential of miRNA-based therapeutics in the quest for effective clinical approaches to counteract acute kidney injury.

AKI Causes Fibrotic Chronic Kidney Disease and Needs New Therapies

AKI is a common complication in patients who are hospitalized with acute illness and is associated with high morbidity and mortality. It is a global public health concern affecting approximately 13.3 million patients per year, with a concomitant financial burden to society. In the United States, AKI was reported to be among the most expensive conditions treated, with a cost of 4.7 billion dollars (2011). The pathophysiology of AKI involves inflammation, loss of microvascular integrity, and epithelial cell injury, followed by a repair phase that can either restore epithelial cell integrity and function or result in a maladaptive development into chronic kidney disease. It is increasingly appreciated that AKI is a major contributor to progressive fibrotic kidney disease that could eventually result in end-stage renal disease.

In addition to the intensive care unit, AKI is also highly prevalent in renal allograft recipients, in whom post-ischemic acute transplant failure is directly related to reduced allograft survival. Despite significant improvements in kidney allograft survival in the first year, the rate of long-term graft loss did virtually not improve during the
past two decades (US Renal Data System, https://www.usrds.org/2015/view/v2_07.aspx, last accessed October 10, 2016). Therapeutic approaches that effectively counteract AKI could therefore also potentially have a major beneficial impact on long-term kidney graft function after transplantation. However, although animal models have provided detailed mechanistic insights into the pathophysiology of AKI, to date, no protective clinically applicable therapies to counteract AKI are available.

miRNAs Coordinate Target Biological Pathways

Exploring a novel therapeutic strategy for AKI, Willflingseder et al. used an established rat model of kidney ischemia-reperfusion injury to test the efficacy of silencing the injury-induced miR-182-5p. Assessing miRNAs follows the increased awareness that the cellular responses to injury are predominantly regulated at the post-transcriptional level, involving an intricate interplay between noncoding RNAs, such as miRNAs and long noncoding RNAs, and RNA-binding proteins. miRNAs are currently the most widely studied class of noncoding RNAs, control cell fate via temporal and spatial gene regulation, and can simultaneously repress multiple genes to directly influence the output of functionally related biological pathways and consequently, cell fate. So, miRNAs are not simply down-regulating individual target genes, but rather facilitate the coordination of signaling networks.

miR-126 provides an example of such an upstream coordinator. This miRNA, highly enriched in endothelial cells and essential for embryonic vascular development, was shown to play multiple roles in the control of the vascular response to injury. In the healthy endothelium, shear-stress–induced miR-126 facilitates cell survival and a proangiogenic phenotype by controlling the expression of genes that allow vascular endothelial growth factor– and AKT-dependent signaling. At the same time, miR-126 down-regulates key inflammatory genes, such as leukocyte adhesion receptor vascular cell adhesion molecule-1. However, in conditions that associated with endothelial injury or senescence, miR-126 expression decreases, allowing the expression of genes such as stromal cell-derived factor-1 (SDF-1) that drive a reparative vasculogenic response by the mobilization of vascular progenitor cells. The generation of these hematopoietic vascular progenitor cells is also facilitated by miR-126, and microvesicles that circulate in the bloodstream were shown to contain vasculogenic activity that augmented vascular repair. Two recent studies demonstrated that miR-126–mediated vascular healing can protect kidney function from ischemia-reperfusion injury. First, direct injection of miR-126 containing microvesicles secreted by endothelial progenitor cells was shown to protect kidney function by reprogramming of resident renal cells. Second, hematopoietic overexpression of miR-126 augmented the differentiation of bone marrow–derived progenitor cells into endothelial cells and pericytes, thereby strongly augmenting vasculogenesis and protecting the kidney microvasculature and function in a model of ischemia-reperfusion injury. Although these studies are performed in mice, circumstantial evidence suggests that the role of miR-126 in vascular homeostasis is conserved in humans because circulating miR-126 levels were found to be decreased in patients with impaired vascular integrity, such as patients with diabetes mellitus.

miR-182-5p, Regulating Cell-Cycle Control in Kidney Ischemia-Reperfusion Injury and Repair

The observation reported in this issue by Willflingseder et al. may provide yet another example of a miRNA that coordinates a regulatory network important for the response to kidney injury. Following up on a previous finding that miR-182-5p was consistently up-regulated after an ischemic insult in both human kidneys as well as in animal models, the group now demonstrated that selective in vivo inhibition of the miRNA preserved kidney function and counteracted the fibrotic response in a rat model of ischemia-reperfusion injury. In addition, they show that these protective actions were associated with derepression of several genes previously related to kidney injury (K+ channel protein Kcnj10), fibrosis (Fam129a), and G1/S cell cycle control checkpoint genes in kidney disease (cyclin D). Moreover, using pathway analysis in kidneys from ASO-treated rats and untreated controls, they identified cell proliferation to be among the most likely affected processes by miR-182-5p.

Although the true causal roles of these genes remain to be proved, together with recently reported actions of miR-182-5p, an image emerges that miR-182-5p may well provide another example of a potent network regulator of cell cycle control in the cellular response to injury (Figure 1). This topic is particularly relevant for AKI because it was shown that epithelial cell cycle arrest in G2/M mediates kidney fibrosis after injury, thereby linking acute kidney injury to the development of chronic kidney disease. In multiple mouse models, it was demonstrated that AKI leads to G2/M arrest of the proximal tubular cells with concomitant activation of c-jun NH2-terminal kinase signaling and subsequent up-regulation of hallmark fibrogenic cytokines, such as transforming growth factor-β1 and connective tissue growth factor. Also, Twist1 and Snail-induced epithelial-mesenchymal transition of tubular epithelial cells, a hallmark of kidney fibrosis, was found to induce an arrest in the G2 phase of the cell cycle and subsequently release these signals to the interstitium to promote fibrogenesis. Interestingly, this epithelial-mesenchymal transition process could be targeted to reverse established fibrotic disease.

A detailed assessment of the miR-182-5p target genes in a human embryonic kidney cell line (HEK293T) revealed a...
Acute kidney injury

The emerging concept that miR-182-5p is a potent network regulator of cell cycle control in the cellular response to acute kidney injury (AKI). During AKI, levels of the p53-induced miR-182-5p are increased. miR-182-5p can target multiple genes that regulate cell cycle control at different levels. Among those, cyclin D is directly targeted, whereby the miR-182-5p can target multiple genes that regulate cell cycle control at AKI. During AKI, levels of the p53-induced miR-182-5p are increased.

Figure 1

Fibrosis

Chronic kidney disease
graft dysfunction

The hypothesis of miR-182-5p as a regulator of cell cycle control in the cellular response to kidney injury. The miRNA targets multiple genes that regulate cell cycle control at different levels. Among those, cyclin D is directly targeted, whereby the miR-182-5p targets multiple genes that regulate cell cycle control at AKI. During AKI, levels of the p53-induced miR-182-5p are increased. miR-182-5p can target multiple genes that regulate cell cycle control at different levels. Among those, cyclin D is directly targeted, whereby the miR-182-5p targets multiple genes that regulate cell cycle control at AKI. During AKI, levels of the p53-induced miR-182-5p are increased.

Targeting miRNAs to Counteract AKI: Options and Hurdles for Clinical Application

In recent years, several miRNAs have already been identified to serve a putative role in the pathophysiology of AKI and several of these miRNAs were demonstrated to have potential as a therapeutic target in animal models for AKI. So, can we also expect to see these being developed for clinical use in AKI? Current technology to inhibit in vivo miRNA function involves the use of expression vectors (miRNA sponges), small-molecule inhibitors, and antisense oligonucleotides (ASOs). miRNA sponge strategies are based on the expression of miRNAs containing multiple artificial miRNA-binding sites, which act as decoys or sponges. Overexpression of these sponges could selectively sequester endogenous miRNAs and thus allows expression of the target miRNAs. Small molecule–based approaches rely on compound library screenings that mainly act through transcriptional regulation of targeted miRNAs rather than inhibition of target recognition (eg, azobenzene, which affects miR-21 expression). The most promising approach, however, seems to involve ASO technology, particularly ASOs that target miRNAs directly (anti-miRNAs) and specifically inhibit miRNA function, thereby blocking their binding to natural miRNA targets. For in vivo application, these oligonucleotides are chemically modified to increase resistance to nucleases to enhance binding affinity for targeted miRNA, and to improve delivery. Most of the chemically modified ASOs show limited tissue distribution when administered in the absence of a carrier, and are predominantly absorbed by the liver and kidney and rapidly excreted in urine. In addition, relatively high doses are necessary for sufficient inhibition, which increases the risk of off-target effects. Thus, an efficient delivery system will mostly be a necessity for the therapeutic use of ASOs outside the liver and kidney. Several sophisticated delivery methods are currently being explored, including conjugation-based methods, liposome-based methods, nanoparticle (polymer)-based methods, and antibody-based methods.

Nevertheless, both miRNA inhibitors and mimics are successfully being explored and developed, targeting a variety of diseases and entering in clinical trials. For example, miR-34-mimics has been designed to repress oncogene expression and block tumor growth, with miR-34 inhibiting cell growth by directly targeting a group of at least 24 genes involved in cell cycle control. Furthermore, single-stranded oligonucleotides complementary to miR-21
are being applied to treat the chronic kidney disease Alport nephropathy, and oligonucleotides complementary to mir-122 are being developed to treat hepatitis C virus. One of those mir-122 inhibitors, miravirsen, was demonstrated in a phase 2 study, in patients with chronic hepatitis C virus infection who received five weekly subcutaneous injections of the drug, to decrease serum hepatitis C virus RNA titers on average 2 to 3 log, whereas hepatitis C virus RNA was completely undetectable in four of nine patients who received the highest dose tested. More important, no serious adverse effects were reported, making miravirsen probably the first miRNA-based therapeutic drug to enter the market.

However, irrespective of the high potential of these approaches, some restrictions of the use of miRNA inhibitors have to be considered. A major limitation is that only few miRNAs are expressed in a tissue-specific manner, such as mir-122 in the hepatocytes or mir-126 in the endothelial cells. Most miRNAs are highly pleiotropically functioning in different tissues in different functional networks. For instance, mir-182-5p has been reported to be expressed in a wide range of tissues, including cells of the hematopoietic compartment, bone, muscle, eyes, or liver. Because most of the target genes related to cell cycle control and DNA damage repair will be expressed in virtually all of these cells, for preventing AKI, selective targeting of the mir-182-5p inhibitors will be required to avoid off-target adverse effects. The default distribution of intravenously injected ASOs in the kidney, and in particular in proximal tubular epithelial cells, provides an interesting opportunity for targeting kidney-related injuries, especially those that originate in these cells. Moreover, Willfingseder et al herein show that mir-182-5p can be efficiently targeted in ex vivo perfused pig kidneys, a next step toward using miRNA targeting for preconditioning of human donor renal allografts to counteract AKI.

Summary and Future Directions

miRNAs have been established as key regulators of kidney injury, and the concept emerged that they are coordinators of regulatory networks. It is therefore important to identify and elucidate these networks that might yield novel therapeutic targets. More important, this knowledge, when coupled with the recent development of sophisticated delivery methods for RNA-based therapeutics, provides the possibility to selectively block miRNA expression, allowing for the simultaneous modulation of related molecular pathways that are involved in disease pathogenesis.

References


