The American Journal of Pathology, Vol. 187, No. 1, January 2017

The American Journal of PATHOLOGY
ajp.amjpathol.org

See related article on page 70

# COMMENTARY

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

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

*From the Einthoven Laboratory for Experimental Vascular Medicine, Department of Internal Medicine (Nephrology), Leiden University Medical Center, Leiden, the Netherlands*

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[1] 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,[2,3] 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).[4] 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.[2,5] It is increasingly appreciated that AKI is a major contributor to progressive fibrotic kidney disease that could eventually result in end-stage renal disease.[2]

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.[6] Despite significant improvements in kidney allograft survival in the first year, the rate of long-term graft loss did virtually not improve during the

Supported by a European Foundation for the Study of Diabetes grant (A.J.v.Z. and R.B.).

Accepted for publication October 20, 2016.

Disclosures: None declared.

Address correspondence to Roel Bijkerk, Ph.D., Department of Internal Medicine (Nephrology), Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, the Netherlands. E-mail: r.bijkerk@lumc.nl


http://dx.doi.org/10.1016/j.ajpath.2016.10.017

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 Coordinately Target Biological Pathways

Exploring a novel therapeutic strategy for AKI, Wilflingseder et al[1] 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 functionallyrelated biological pathways and consequently, cell fate.[7] 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.[8] 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.[9] 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.[10] 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.[11]

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

The observation reported in this issue by Wilflingseder et al[1] 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.[12] 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 $G_1$/S cell cycle control checkpoint genes in kidney disease (cyclin D).[13,14] 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 $G_2$/M mediates kidney fibrosis after injury, thereby linking acute kidney injury to the development of chronic kidney disease.[15] In multiple mouse models, it was demonstrated that AKI leads to $G_2$/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 $G_2$ phase of the cell cycle and subsequently release these signals to the interstitium to promote fibrogenesis.[16] Interestingly, this epithelial-mesenchymal transition process could be targeted to reverse established fibrotic disease.[17]

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

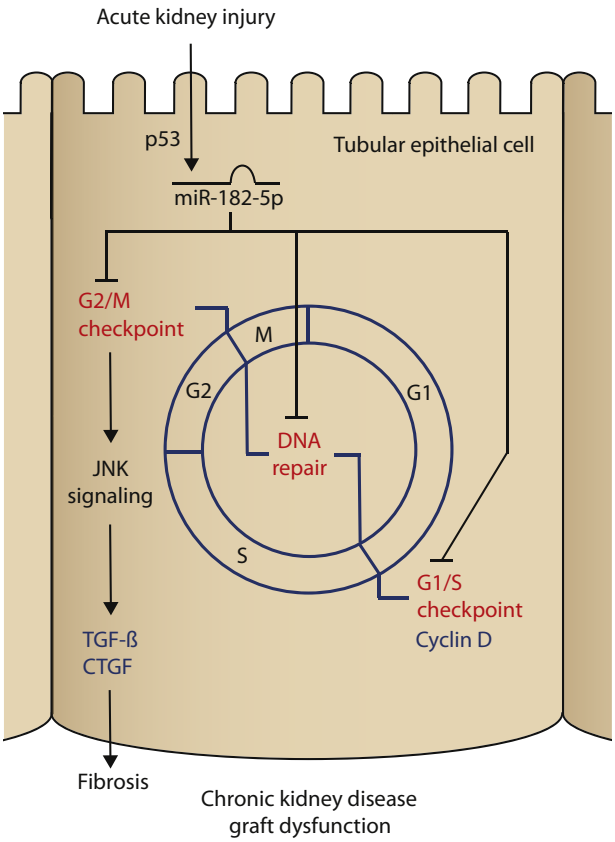


**Figure 1** The emerging concept that miR-182-5p is a potent network regulator of cell cycle control in the cellular response to acute kidney injury (AKI). During AKI, levels of the p53-induced miR-182-5p are increased. miR-182-5p can target multiple genes that regulate cell cycle control at different levels. Among those, cyclin D is directly targeted, whereby the $G_1$/S checkpoint is affected. Also, a network of genes involved in DNA repair is directly targeted by miR-182-5p, and as such regulates multiple checkpoints in the progression of the cell cycle. Consequently, also, genes that control the $G_2$/M checkpoint are affected by miR-182-5p. Interestingly, $G_2$/M cell cycle arrest has been demonstrated to be a central feature of AKI and the subsequent development of chronic kidney disease because this arrest in tubular epithelial cells results in increased c-jun NH2-terminal kinase (JNK) signaling that stimulates the release of profibrotic cytokines like transforming growth factor (TGF)-β and connective tissue growth factor (CTGF), eventually causing kidney fibrosis.

major enrichment for genes in DNA repair pathways and cell cycle arrest at the $G_2$/M phase DNA damage checkpoint.[18] In addition, another article demonstrated that miR-182 is induced by p53,[19] which also has a key role for activation of the $G_2$/M checkpoint in response to stress and DNA damage.[20] In AKI, the reported elevated $G_2$/M arrest in the mitochondria-rich epithelial cells may be caused by an increase in oxidative stress that has been reported to result in reactive oxygen species–dependent DNA modifications and damage. As such, miR-182-5p may serve as a central coordinator of the cellular response to kidney injury and may well be rate limiting in the transition from AKI to chronic kidney disease. Before drawing firm mechanistic conclusions, however, detailed information of the *in vivo* tubular specificity of expression of the miRNA and its putative relative targets is necessary. Our recent observation that silencing of miR-132 in a murine unilateral urether obstruction–induced fibrosis model reduced the proliferation of perivascular derived myofibroblasts, whereas the proliferation of tubular epithelial cells was not affected, further corroborates this notion.[21]

## 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.[22] 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).[23] miRNA sponge strategies are based on the expression of mRNAs containing multiple artificial miRNA-binding sites, which act as decoys or sponges.[24] Overexpression of these sponges could selectively sequester endogenous miRNAs and thus allows expression of the target mRNAs. 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[25]). 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 mRNA 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.[23]

Nevertheless, both miRNA inhibitors and mimics are successfully being explored and developed, targeting a variety of diseases and entering in clinical trials.[26] 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[27] involved in cell cycle control.[28] Furthermore, single-stranded oligonucleotides complementary to miR-21

are being applied to treat the chronic kidney disease Alport nephropathy,[29] and oligonucleotides complementary to miR-122 are being developed to treat hepatitis C virus.[30] 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,[30] 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 pleotropically 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,[18] provides an interesting opportunity for targeting kidney-related injuries, especially those that originate in these cells. Moreover, Wilflingseder et al[1] 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


1. Wilflingseder J, Jelencsis K, Bergmeister H, Sunzenauer J, Regele H, Eskandary F, Reindl-Schwaighofer R, Kainz A, Oberbauer R: miR-182-5p inhibition ameliorates ischmic acute kidney injury. Am J Pathol 2017, 187:70–79.
2. Zuk A, Bonventre JV: Acute kidney injury. Annu Rev Med 2016, 67: 293–307
3. Mehta RL, Cerda J, Burdmann EA, Tonelli M, Garcia-Garcia G, Jha V, Susantitaphong P, Rocco M, Vanholder R, Sever MS, Cruz D, Jaber B, Lameire NH, Lombardi R, Lewington A, Feehally J, Finkelstein F, Levin N, Pannu N, Thomas B, Aronoff-Spencer E, Remuzzi G: International Society of Nephrology's 0by25 initiative for acute kidney injury (zero preventable deaths by 2025): a human rights case for nephrology. Lancet 2015, 385:2616–2643
4. Torio CM, Andrews RM: National Inpatient Hospital Costs: The Most Expensive Conditions by Payer, 2011: Statistical Brief #160. Healthcare Cost and Utilization Project (HCUP) Statistical Briefs. Rockville, MD: Agency for Healthcare Research and Quality, 2013. Available at https://www.hcup-us.ahrq.gov/reports/statbriefs/sb160.jsp (accessed October 10, 2016)
5. Bonventre JV, Yang L: Cellular pathophysiology of ischemic acute kidney injury. J Clin Invest 2011, 121:4210–4221
6. Heinze G, Collins S, Benedict MA, Nguyen LL, Kramar R, Winkelmayer WC, Haas M, Kainz A, Oberbauer R: The association between angiotensin converting enzyme inhibitor or angiotensin receptor blocker use during postischemic acute transplant failure and renal allograft survival. Transplantation 2006, 82:1441–1448
7. Bartel DP: MicroRNAs: target recognition and regulatory functions. Cell 2009, 136:215–233
8. van Solingen C, Bijkerk R, de Boer HC, Rabelink TJ, van Zonneveld AJ: The role of microRNA-126 in vascular homeostasis. Curr Vasc Pharmacol 2015, 13:341–351
9. Cantaluppi V, Gatti S, Medica D, Figliolini F, Bruno S, Deregibus MC, Sordi A, Biancone L, Tetta C, Camussi G: Microvesicles derived from endothelial progenitor cells protect the kidney from ischemia-reperfusion injury by microRNA-dependent reprogramming of resident renal cells. Kidney Int 2012, 82:412–427
10. Bijkerk R, van Solingen C, de Boer HC, van der Pol P, Khairoun M, de Bruin RG, van Oeveren-Rietdijk AM, Lievers E, Schlagwein N, van Gijlswijk DJ, Roeten MK, Neshati Z, de Vries AA, Rodijk M, Pike-Overzet K, van den Berg YW, van der Veer EP, Versteeg HH, Reinders ME, Staal FJ, van Kooten C, Rabelink TJ, van Zonneveld AJ: Hematopoietic microRNA-126 protects against renal ischemia/reperfusion injury by promoting vascular integrity. J Am Soc Nephrol 2014, 25:1710–1722
11. Meng S, Cao JT, Zhang B, Zhou Q, Shen CX, Wang CQ: Downregulation of microRNA-126 in endothelial progenitor cells from diabetes patients, impairs their functional properties, via target gene Spred-1. J Mol Cell Cardiol 2012, 53:64–72
12. Wilflingseder J, Sunzenauer J, Toronyi E, Heinzel A, Kainz A, Mayer B, Perco P, Telkes G, Langer RM, Oberbauer R: Molecular pathogenesis of post transplant acute kidney injury: assessment of whole-genome mRNA and miRNA profiles. PloS One 2014, 9:e104164
13. Garcia MA, Meca R, Leite D, Boim MA: Effect of renal ischemia/reperfusion on gene expression of a pH-sensitive K+ channel. Nephron Physiol 2007, 106:1–7
14. Liu J, Qin J, Mei W, Zhang H, Yuan Q, Peng Z, Luo R, Yuan X, Huang L, Tao L: Expression of niban in renal interstitial fibrosis. Nephrology (Carlton) 2014, 19:479–489
15. Yang L, Besschetnova TY, Brooks CR, Shah JV, Bonventre JV: Epithelial cell cycle arrest in G2/M mediates kidney fibrosis after injury. Nat Med 2010, 16:535–543. 531p following 143
16. Lovisa S, LeBleu VS, Tampe B, Sugimoto H, Vadnagara K, Carstens JL, Wu CC, Hagos Y, Burckhardt BC, Pentcheva-Hoang T, Nischal H, Allison JP, Zeisberg M, Kalluri R: Epithelial-to-mesenchymal transition induces cell cycle arrest and parenchymal damage in renal fibrosis. Nat Med 2015, 21:998–1009
17. Grande MT, Sanchez-Laorden B, Lopez-Blau C, De Frutos CA, Boutet A, Arevalo M, Rowe RG, Weiss SJ, Lopez-Novoa JM, Nieto MA: Snail1-induced partial epithelial-to-mesenchymal transition drives renal fibrosis in mice and can be targeted to reverse established disease. Nat Med 2015, 21:989–997

18. Krishnan K, Steptoe AL, Martin HC, Wani S, Nones K, Waddell N, Mariasegaram M, Simpson PT, Lakhani SR, Gabrielli B, Vlassov A, Cloonan N, Grimmond SM: MicroRNA-182-5p targets a network of genes involved in DNA repair. RNA 2013, 19:230−242
19. Kouri FM, Hurley LA, Daniel WL, Day ES, Hua Y, Hao L, Peng CY, Merkel TJ, Queisser MA, Ritner C, Zhang H, James CD, Sznajder JI, Chin L, Giljohann DA, Kessler JA, Peter ME, Mirkin CA, Stegh AH: miR-182 integrates apoptosis, growth, and differentiation programs in glioblastoma. Genes Dev 2015, 29:732−745
20. Taylor WR, Stark GR: Regulation of the G2/M transition by p53. Oncogene 2001, 20:1803−1815
21. Bijkerk R, de Bruin RG, van Solingen C, van Gils JM, Duijs JM, van der Veer EP, Rabelink TJ, Humphreys BD, van Zonneveld AJ: Silencing of microRNA-132 reduces renal fibrosis by selectively inhibiting myofibroblast proliferation. Kidney Int 2016, 89:1268−1280
22. Fan PC, Chen CC, Chen YC, Chang YS, Chu PH: MicroRNAs in acute kidney injury. Hum Genomics 2016, 10:29
23. Li Z, Rana TM: Therapeutic targeting of microRNAs: current status and future challenges. Nat Rev Drug Discov 2014, 13:622−638
24. Ebert MS, Neilson JR, Sharp PA: MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. Nat Methods 2007, 4:721−726
25. Gumireddy K, Young DD, Xiong X, Hogenesch JB, Huang Q, Deiters A: Small-molecule inhibitors of microrna miR-21 function. Angew Chem Int Ed Engl 2008, 47:7482−7484
26. Matsui M, Corey DR: Non-coding RNAs as drug targets. Nat Rev Drug Discov 2016, [Epub ahead of print] doi:10.1038/nrd.2016.117
27. Bouchie A: First microRNA mimic enters clinic. Nat Biotechnol 2013, 31:577
28. Agostini M, Knight RA: miR-34: from bench to bedside. Oncotarget 2014, 5:872−881
29. Gomez IG, MacKenna DA, Johnson BG, Kaimal V, Roach AM, Ren S, Nakagawa N, Xin C, Newitt R, Pandya S, Xia TH, Liu X, Borza DB, Grafals M, Shankland SJ, Himmelfarb J, Portilla D, Liu S, Chau BN, Duffield JS: Anti-microRNA-21 oligonucleotides prevent Alport nephropathy progression by stimulating metabolic pathways. J Clin Invest 2015, 125:141−156
30. Lindow M, Kauppinen S: Discovering the first microRNA-targeted drug. J Cell Biol 2012, 199:407−412