MicroRNAs Grab Scientific Spotlight in Kidney Disease Research

They conducted gene expression profiles in tissue samples from kidney transplant patients with nephropathy. In new research, investigators found that gene expression for one miRNA—miRNA-21—was greater in renal fibrosis than in normal kidneys. The hope is that one day anti-miRNA-21 therapy could benefit patients with chronic kidney disease.

The research, conducted with laboratory animal models of kidney disease and with human tissue samples, was published in the February 15, 2012, issue of Science Translational Medicine (STM). The article is titled "MicroRNA-21 promotes fibrosis of the kidney by silencing metabolic pathways.”

Since the 2000 discovery of miRNAs in the human genome, scientists have uncovered evidence of their contribution to the pathophysiology of diseases ranging from cancer to kidney disease. About 1000 miRNAs have been identified in the human genome thus far.

In kidney research, miRNAs are being pursued in areas as diverse as transplant immunology, podocyte development, polycystic kidney disease, renal failure, and fibrosis. In the STM study, scientists at the University of Washington and Regulus Therapeutics, Inc., found that although miRNA-21 does not cause renal fibrosis, it amplifies the kidney’s responses to injury, resulting in the development of fibrosis.

“MicroRNA-21 of itself does not create injury and fibrosis, but it worsens it,” said Jeremy S. Duffield, MD, PhD, coauthor of the STM article and associate professor of medicine in the division of nephrology at the University of Washington.

A total of 24 miRNAs were initially identified by the University of California–Regulus research group when they conducted gene expression profiles to determine the most commonly upregulated regulatory elements in kidney fibrosis. They decided to focus on miRNA-21 because a previous study at another laboratory had linked it to cardiac fibrosis, and it was upregulated consistently in the researchers' animal models for kidney fibrosis and in tissue samples from kidney transplant patients with nephropyathy.

In the animal models, kidney injury linked to miRNA-21. In normal kidneys of laboratory animals, Duffield and his colleagues detected miRNA-21 expression in the medulla and papilla and in some perivascular cells. However, miRNA-21 expression becomes widespread in kidneys with experimentally induced injuries. The researchers also found that miRNA-21 was markedly upregulated in the pericyte precursors of the scar-forming myofibroblasts in the kidney, and miRNA-21 expression was much higher in inflammatory macrophages than in resident macrophages.

The researchers also found that miRNA-21 was upregulated in the kidneys of laboratory animals soon after experimentally induced renal injury but before the development of fibrosis. Levels of miRNA-21 were much higher in prospectively collected tissue samples from the transplanted kidneys of patients with nephropyathy than in tissue samples from healthy individuals. These results suggest that miRNA-21’s upregulation is an early response to injury.

To define the precise role of miRNA-21 in kidney fibrosis, the researchers knocked out the gene that codes for miRNA-21 in laboratory animal models and then induced kidney injury. Less interstitial fibrosis occurred in the miRNA-21 knockout mice than in their miRNA-21 intact litter mates.

The miRNA-21 knockout mice were healthy, with normal fertility, body weight, and life span of at least 6 months of age. Although surprising, the finding perhaps can be explained by the activity of miRNA-21 in laboratory animals with normal kidney functioning. “Our work shows that miRNA-21 is not active despite being highly expressed in normal kidney,” said Duffield.

The animal’s surprisingly good health is consistent with the “lack of activity” of the miRNA-21 in normal healthy mice, Duffield said, on the basis of observations that the molecular signature of normal unstressed kidney does not indicate miRNA-21 deficiency. The molecular signature of miRNA-21 deficiency is only apparent in response to stress, he pointed out.

In the laboratory animals with experimentally induced kidney fibrosis, the scientists systemically administered proprietary oligonucleotide drugs targeting miRNA-21. The experimental anti-miRNA-21 therapy also reduced the extracellular matrix proteins that contribute to fibrosis, as well as reducing protein leakage into the urine, a marker of chronic kidney disease.

“Genetic deletion of miRNA-21 in preclinical models protected kidneys from fibrosis, and treatment with anti-miRNA targeting miRNA-21 also blocked fibrosis in preclinical models,” said Duffield. “Taken together, these data suggest that anti–miRNA-21 could have a therapeutic benefit in patients with chronic kidney disease.”

Compounds are now being screened to identify potential candidates for clinical studies. Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are currently the main therapies for kidney fibrosis.

Daniel R. Salomon, MD, who was not involved in the research, said that apart from the University of Washington-Regulus work as “good science... done using cutting-edge methods.”

However, because the expression of miRNA-21 is not limited to the kidney, the development of an anti-miRNA-21 therapy—and any miRNA-targeted treatment—must consider the systems-wide effects of blocking an endogenous factor "which is a normal response to injury” in every single tissue in the body, said Salomon, who is program medical director of the Scripps Health Center for Organ Transplantation and professor and director of the Laboratory for Functional Genomics of The Scripps Research Institute in La Jolla.

Because human biology is so complex, one factor rarely is responsible for a biological process as central to health—renal tissue integrity as fibrosis, added Salomon, who heads a study on miRNA expression in human immunity sponsored by the National Institutes of Health.

In addition to avoiding systemicwide effects, anti-miRNA therapies must use safe and reliable delivery methods specific to the kidney and avoid toxicity derived from off-target effects and from activation of the innate and adaptive immune response, wrote Jordan Yz Li, PhD, and colleagues in a review article, “The role of microRNAs in kidney disease,” published in 2010 in Nephrology.

Avenues for drug discovery
Duffield and his colleagues identified other possible avenues for drug discovery in kidney disease by performing gene expression profiles on the miRNA-1 knockout mice. Upregulated in the knockout laboratory animals were groups of genes involved in metabolic pathways, including lipid metabolism and enhanced oxygen radical production. The analysis revealed that peroxisome proliferator-activated receptor-α (PPARα) and Mpv171 metabolic pathways are critical miRNA-21 targets, the researchers reported.

Peroxisome proliferator-activated receptors are a group of nuclear receptor proteins that act as transcription factors regulating the expression of genes. The researchers determined that PPARα is a major upstream regulator of lipid metabolism. MicroRNA-21 repressed Mpv171, a mitochondrial inhibitor of reactive oxygen species generation, correlating closely with enhanced oxidative kidney damage.

“It is likely that regulating metabolic pathways including lipid and fatty acid oxidation will become new targets for therapeutics in kidney disease,” Duffield said, noting that the University of Washington-Regulus study was the first to show that metabolic pathways contribute to the development of kidney disease.

“These are important pathways that prevent damage in the kidney,” Duffield said. “If we can target metabolic path- ways. The repression of these networks of genes leads to further injury to the kidney epithelium.”

The gene expression profiles produced another unexpected finding: before experimentally induced injury, the kidneys of both miRNA-21 knockout mice and miRNA-21 intact mice shared similar genetic profiles. Only in the injured kidney was miRNA-21 able to repress the critical genes that drive kidney disease. Duffield and his colleagues predict that the therapeutic strategies that target miRNA-21 will be specific because in healthy cells of other organs, miRNA-21 is likely to be inactive.

In the animal models, kidney injury was induced by either unilateral ureteral obstruction of the flow of urine or unilateral ischemia reperfusion injury. The slow initial injury led to a delayed ureteral obstruction accelerates with time. In ischemia reperfusion injury, a temporary occlusive clamp is placed on the renal ar-tery for about 30 minutes, followed by restoration of the flow before surgical closure. Severe injury accompanies the reperfusion, with only partial repair.