

The TGF- β Route to Renal Fibrosis Is Not Linear: The miR-21 Viaduct

Allison A. Eddy

Seattle Children's Research Institute and Department of Pediatrics, University of Washington, Seattle, Washington

J Am Soc Nephrol 22: 1573–1575, 2011.
doi: 10.1681/ASN.2011070698

The chronic kidney disease (CKD) riddle appeared solved 20 years ago when TGF beta (TGF- β) was identified as a potent inducer of renal fibrosis.¹ It not only stimulated extracellular matrix production but also efficiently transformed the phenotype of critical cellular mediators into profibrotic activists and induced the expression of additional fibrogenic molecules (plasminogen activator inhibitor-1, connective tissue growth factor, for example) to amplify the response. A multitude of studies on fibrogenic mechanisms all appeared to converge on TGF- β , its receptors, and its dose—both upstream and downstream.²

The impetus to translate these landmark scientific discoveries into badly needed human therapeutics encountered a significant obstacle when it became evident that TGF- β is a multifunctional growth factor that includes a critical role in immune surveillance. Indeed, TGF- β null mice die in infancy due to an aggressive multisystem autoimmune inflammatory disorder.³ Hope for translational application resumed when a multitude of studies determined that several intracellular signaling pathways are potentially activated when TGF- β engages the type I plus type II receptor complex,⁴ and that one pathway in particular was a major effector of its fibrogenic actions—phosphorylation and nuclear import of the transcription factors mothers against decapentaplegic homologues (Smad 2/3). Smad7, another member of this family, attenuates fibrotic responses. These discoveries raised the possibility that cell-membrane-permeable therapeutic agents might selectively block the profibrotic effects of TGF- β . As a proof of the concept of this study, renal fibrosis is less severe in Smad3 null mice.⁵

In this issue of *JASN*, Zhong and colleagues⁶ identify microRNA-21 (miR-21) as another downstream target of

Smad3 that executes TGF- β -induced renal fibrosis. It was identified as a potential candidate on the basis of upregulated expression in tubular epithelia exposed to TGF- β and its fibrosis-promoting effects in other solid organs. Using specific knockout mice, they show that Smad3, but not Smad 2, is necessary for miR-21 expression. They provide evidence that Smad3 functions as a miR-21 transcription factor by interacting with Smad binding sites 1 and 2 within the promoter region of the miR-21 gene in the presence of TGF- β .

Although miRNAs were first discovered in *Caenorhabditis elegans* in 1993, their role in fibrotic events has only recently become clear.^{7,8} Now approximately 1000 miRNAs have been identified in humans, yet, together, they may influence the post-transcriptional control of the majority of the protein-encoding genes. miRNAs—small, genetically encoded but protein noncoding RNAs (18 to 24 nucleotides)—act in the cytoplasm to disrupt the translation of their target RNAs. They do so within the RNA-induced silencing complex by binding to a short region (7 nucleotides) in the 3' untranslated region (UTR), which blocks translation or destabilizes the RNA, leading to its degradation. It is thought that each miRNA has as many as 10 to 100 target genes. Enhanced miR-21 expression was first reported in 2005 in human glioblastomas.⁹ Antagomir-based inhibition studies now establish its role in cardiac and pulmonary fibrosis. In the Zhong study, ultrasound-microbubble-mediated gene transfer of a miR-21 knockdown plasmid attenuates fibrosis in mice with chronic kidney disease (CKD) induced by unilateral ureteral obstruction (UUO). Increased miR-21 is also reported in hypertensive nephropathy, anti-Thy1.1 glomerulonephritis, and diabetic nephropathy.^{10,11}

What is remarkable is the fact that TGF- β regulates the expression of numerous miRNAs—inducing some and suppressing others—such that TGF- β is one of the most extensively investigated pathways in miRNA biology. TGF- β signaling may initiate the transcription of genes that encode the precursor (primary) nuclear miRNA (pri-miRNA). In the study by Zhong *et al.*, the finding of increased levels of kidney tissue pri-miR-21 is consistent with its transcriptional upregulation. However, TGF- β may also enhance the rate of processing of certain premature miRNAs, including miR-21, through a unique mechanism that employs Smad proteins. Two essential pri-miRNA processing steps involve cleavage by RNAase III enzymes. The enzyme, Drosha, mediates the initial nuclear step, and the cytoplasmic enzyme, Dicer, performs the second. Acting on the first step, TGF- β promotes the formation of an active microprocessor complex containing SMAD proteins, pri-miRNAs, and Drosha to generate premiRNA, the product that is exported into the cytoplasm by exportin-5 nuclear channels.¹²

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Allison A. Eddy, Seattle Children's Research Institute, Tissue and Cell Sciences Center, C9S-5, 1900 Ninth Avenue, Seattle, WA 98101-1309. Phone: 206-987-3030; Fax: 206-884-1407; Email: allison.eddy@seattlechildrens.org

Copyright © 2011 by the American Society of Nephrology

Initially selected on the basis of differential expression, miR-21 is now one of the most extensively investigated miRNAs thus far. It appears to be a ubiquitous oncogene, given its upregulated expression in the majority of cancers profiled, to date, and its biologic actions of enhanced proliferation and reduced apoptosis.¹³ An emerging theme is that miR-21 has cell type-specific and unique disease-state effects. These variable responses may explain why the outcome of miRNA inhibition may not be the reverse of the effects of overexpression, and why differences in interrogation strategies (varied chemical methods to engineer miRNA inhibitory oligonucleotide antagomirs or use of genetically engineered mice) may yield conflicting results. For example, in a model of cardiac-stress-induced fibrosis, miR-21 is upregulated in fibroblasts, where it enhances fibrosis by stimulating MAP kinase signaling due to *Sprouty1* inhibition.¹⁴ miR-21 inhibition significantly reduces cardiac fibrosis, yet when the same model is induced in miR-21 null mice, no differences are observed.¹⁵ In a kidney model of ischemia-reperfusion injury, miR-21 was identified as one of nine differentially expressed miRNAs, upregulated primarily within proliferating tubular cells.¹⁶ Although miR-21 improves cell survival *in vitro*, overexpression *in vivo* fails to prevent tubular cell death.

In the study by Zhong *et al.*, miR-21 knockdown significantly reduces fibrosis in association with fewer α SMA⁺ interstitial myofibroblasts, interstitial collagen I, and fibronectin, and lowers mRNA levels encoding TGF- β 1. A key question that deserves further consideration is the nature of the cell-specific effects of miR-21 during renal fibrosis. While the TGF- β /Smad/miR-21 axis is clearly associated with enhanced matrix production by tubular cells, it also served a beneficial function—enhancing tubular cell survival. This is important because tubular cell death, leading to nephron atrophy, is the root cause of declining renal function in fibrosis-mediated CKD.¹ Although not specific to miR-21, mice with a renal proximal tubular cell-specific genetic deficiency of the primary miRNA processing enzyme, Dicer, develop less tubular damage after ischemia-reperfusion, suggesting the global effect of miRNA expression in this model is detrimental.¹⁷ Future studies will need to differentiate tubular cell-specific effects of miR-21 from effects on interstitial fibroblasts, macrophages (miR-21 is increased during monocyte differentiation),¹⁸ and capillaries (miR-21 induces endothelial cell inflammatory adhesion molecules and inhibits angiogenesis)^{19,20}, and include experiments in animal model systems that make it possible to measure effects of miR-21 inhibition on tubular cell density and renal function. Since miRNAs do not encode proteins, their localization in tissue requires *in situ* hybridization techniques. Studies in the UUO model performed by Zhong *et al.* indicate both tubular and interstitial cells express miR-21. Although their work emphasizes effects on the former, modulating interstitial myofibroblast activities would be consistent with the important role shown for fibroblasts in models of miR-21-induced cardiac¹⁴ and pulmonary fibrosis,²¹ and the fact that interstitial myofibroblasts may be a

primary source of interstitial scar-forming extracellular matrix proteins in CKD.

A key step to elucidating the molecular basis of cell-specific and context-dependent effects of miR-21 and other miRNAs will be the identification of their target genes. This is not a trivial undertaking, as their bp binding only requires partial complementarity—between its *seed nucleotides* (2–8) and the binding site within the 3'UTR of the target gene. This minimal requirement likely explains why a single miRNA can interfere with the translation of a multitude of genes. Many of their potential targets are hypothetical candidates, selected by computational analysis of the 3'UTR region of potential target genes. Relatively few studies provide evidence of direct interactions. Presently, more targets are known for miR-21 than most miRNAs; many involve the TGF- β family, extracellular matrix-receptor interactions, mitochondrial apoptosis, and the p53 tumor suppressor protein.

While a growing body of evidence suggests that miRNAs participate in fibrotic processes, the current miR-21 story is almost certainly the first chapter of a large novel that has yet to be written. Although the highlighted paper focuses on Smad3, other TGF- β signaling pathways are likely involved, and miR-21 may be induced by TGF- β -independent mechanisms (for example, a STAT3 mechanism has been reported).²² A more global version of the miRNA CKD molecular signature will eventually emerge. This will be a complicated story; miR-192, for example, is both downregulated²³ or upregulated^{24,25} by TGF- β , and, in both directions, has been implicated in the pathogenesis of CKD. Still other miRNAs, such as miR-29a and miR-200a, appear to be constitutively expressed in the kidney and downregulation by TGF- β , with detrimental effects, presumably due to the depression of harmful genes.^{26,27} To complicate matters further, the repressive effects of miRNAs are generally modest (< 50% reduction in protein expression), which can make it challenging to detect clear biologic effects in complex *in vivo* model systems. Furthermore, it has been suggested that some miRNAs may even enhance the post-transcription expression of target genes. Much of the effect of these changes may depend on context and timing.

Will miR-21 emerge as a serum or urine biomarker for chronic kidney disease? miRNAs are known to circulate in a stable soluble form in serum, and profiling studies have recently been reported for patients with acute kidney injury.²⁸ Genetically determined polymorphisms in the 3' UTR of genes will become of greater interest if they modify miRNA binding, as recently reported for the +1166A/C variant in the human angiotensin II type 1 receptor gene.²⁹ For now, miR-21 is high on the list of noncoding, small, regulatory RNAs that promote kidney fibrosis, but many questions await answers.

DISCLOSURES

None.

REFERENCES

1. Zeisberg M, Neilson EG: Mechanisms of tubulointerstitial fibrosis. *J Am Soc Nephrol* 21: 1819–34, 2010
2. Kopp JB: TGF-beta signaling and the renal tubular epithelial cell: Too much, too little, and just right. *J Am Soc Nephrol* 21: 1241–1243, 2010
3. Kulkarni AB, Huh CG, Becker D, Geiser A, Lyght M, Flanders KC, Roberts AB, Sporn MB, Ward JM, Karlsson S: Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. *Proc Natl Acad Sci U S A* 90: 770–774, 1993
4. Xavier S, Niranjana T, Krick S, Zhang T, Ju W, Shaw AS, Schiffer M, Bottinger EP: TbetaRI independently activates Smad- and CD2AP-dependent pathways in podocytes. *J Am Soc Nephrol* 20: 2127–2137, 2009
5. Sato M, Muragaki Y, Saika S, Roberts AB, Ooshima A: Targeted disruption of TGF-beta1/Smad3 signaling protects against renal tubulointerstitial fibrosis induced by unilateral ureteral obstruction. *J Clin Invest* 112: 1486–1494, 2003
6. Zhong X, Chung ACK, Chen H-Y, Meng X-M, Lan HY: Smad3-mediated upregulation of miR-21 promotes renal fibrosis. *J Am Soc Nephrol* 22: 1668–1681, 2011
7. Lee RC, Feinbaum RL, Ambros V: The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 75: 843–854, 1993
8. Wightman B, Ha I, Ruvkun G: Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*. *Cell* 75: 855–862, 1993
9. Chan JA, Krichevsky AM, Kosik KS: MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res* 65: 6029–6033, 2005
10. Denby L, Ramdas V, McBride MW, Wang J, Robinson H, McClure J, Crawford W, Hillyard DZ, Khanin R, Agami R, Dominiczak AF, Sharpe CC, Baker AH: miR-21 and miR-214 are consistently modulated during renal injury in rodent models. *Am J Pathol* 179: 661–672, 2011
11. Dey N, Das F, Mariappan MM, Mandal CC, Ghosh-Choudhury N, Kasinath BS, Ghosh Choudhury G: microRNA-21 orchestrates high glucose-induced signals to TORC1 for renal cell pathology in diabetes. *J Biol Chem* <http://www.jbc.org/content/early/2011/05/25/jbc.M110.208066>
12. Davis BN, Hilyard AC, Lagna G, Hata A: SMAD proteins control DROSHA-mediated microRNA maturation. *Nature* 454: 56–61, 2008
13. Krichevsky AM, Gabrieli G: miR-21: A small multi-faceted RNA. *J Cell Mol Med* 13: 39–53, 2009
14. Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M, Galuppo P, Just S, Rottbauer W, Frantz S, Castoldi M, Soutschek J, Kotliarsky V, Rosenwald A, Basson MA, Licht JD, Pena JT, Rouhanifard SH, Muckenthaler MU, Tuschl T, Martin GR, Bauersachs J, Engelhardt S: MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. *Nature* 456: 980–984, 2008
15. Patrick DM, Montgomery RL, Qi X, Obad S, Kauppinen S, Hill JA, van Rooij E, Olson EN: Stress-dependent cardiac remodeling occurs in the absence of microRNA-21 in mice. *J Clin Invest* 120: 3912–3916, 2010
16. Godwin JG, Ge X, Stephan K, Jurisch A, Tullius SG, Iacomini J: Identification of a microRNA signature of renal ischemia reperfusion injury. *Proc Natl Acad Sci U S A* 107: 14339–14344, 2010
17. Wei Q, Bhatt K, He HZ, Mi QS, Haase VH, Dong Z: Targeted deletion of Dicer from proximal tubules protects against renal ischemia-reperfusion injury. *J Am Soc Nephrol* 21: 756–761, 2010
18. Cekaite L, Clancy T, Sioud M: Increased miR-21 expression during human monocyte differentiation into DCs. *Front Biosci (Elite Ed)* 2: 818–828, 2010
19. Zhou J, Wang KC, Wu W, Subramaniam S, Shyy JY, Chiu JJ, Li JY, Chien S: MicroRNA-21 targets peroxisome proliferator-activated receptor- α in an autoregulatory loop to modulate flow-induced endothelial inflammation. *Proc Natl Acad Sci U S A* 108: 10355–10360, 2011
20. Sabatell C, Malvaux L, Bovy N, Deroanne C, Lambert V, Gonzalez ML, Colige A, Rakic JM, Noel A, Martial JA, Struman I: MicroRNA-21 exhibits antiangiogenic function by targeting RhoB expression in endothelial cells. *PLoS One* 6: e16979, 2011
21. Liu G, Friggeri A, Yang Y, Milosevic J, Ding Q, Thannickal VJ, Kaminski N, Abraham E: miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. *J Exp Med* 207: 1589–1597, 2010
22. Iliopoulos D, Jaeger SA, Hirsch HA, Bulyk ML, Struhl K: STAT3 activation of miR-21 and miR-181b-1 via PTEN and CYLD are part of the epigenetic switch linking inflammation to cancer. *Mol Cell* 39: 493–506, 2010
23. Krupa A, Jenkins R, Luo DD, Lewis A, Phillips A, Fraser D: Loss of MicroRNA-192 promotes fibrogenesis in diabetic nephropathy. *J Am Soc Nephrol* 21: 438–447, 2010
24. Kato M, Zhang J, Wang M, Lanting L, Yuan H, Rossi JJ, Natarajan R: MicroRNA-192 in diabetic kidney glomeruli and its function in TGF-beta-induced collagen expression via inhibition of E-box repressors. *Proc Natl Acad Sci U S A* 104: 3432–3437, 2007
25. Chung AC, Huang XR, Meng X, Lan HY: miR-192 mediates TGF-beta/Smad3-driven renal fibrosis. *J Am Soc Nephrol* 21: 1317–1325, 2010
26. Wang B, Koh P, Winbanks C, Coughlan MT, McClelland A, Watson A, Jandeleit-Dahm K, Burns WC, Thomas MC, Cooper ME, Kantharidis P: miR-200a prevents renal fibrogenesis through repression of TGF-beta2 expression. *Diabetes* 60: 280–287, 2011
27. Du B, Ma LM, Huang MB, Zhou H, Huang HL, Shao P, Chen YQ, Qu LH: High glucose down-regulates miR-29a to increase collagen IV production in HK-2 cells. *FEBS Lett* 584: 811–816, 2010
28. Lorenzen JM, Kielstein JT, Hafer C, Gupta SK, Kumpers P, Faulhaber-Walter R, Haller H, Fliser D, Thum T: Circulating miR-210 predicts survival in critically ill patients with acute kidney injury. *Clin J Am Soc Nephrol* 6: 1540–1546, 2011
29. Martin MM, Buckenberger JA, Jiang J, Malana GE, Nuovo GJ, Chotani M, Feldman DS, Schmittgen TD, Elton TS: The human angiotensin II type 1 receptor +1166 A/C polymorphism attenuates microRNA-155 binding. *J Biol Chem* 282: 24262–24269, 2007

See related article, “Smad3-Mediated Upregulation of miR-21 Promotes Renal Fibrosis,” on pages 1668–1681.

Making a Tubule the Noncanonical Way

Neil A. Hukriede* and Igor B. Dawid†

*Department of Developmental Biology, University of Pittsburgh, Pittsburgh, Pennsylvania; †Laboratory of Molecular Genetics, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland

J Am Soc Nephrol 22: 1575–1577, 2011.
doi: 10.1681/ASN.2011070710

Tubulogenesis is an essential feature during formation of multiple organ systems. The genitourinary, cardiovascular,

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Igor B. Dawid, Laboratory of Molecular Genetics, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892. Phone: 301-496-7940; Fax: 301-496-0243; E-mail: idawid@mail.nih.gov

Copyright © 2011 by the American Society of Nephrology