

microRNA cascade in diabetic kidney disease

Big impact initiated by a small RNA

Mitsuo Kato and Rama Natarajan

Gonda Diabetes Center; Beckman Research Institute of City of Hope; Duarte, CA USA

Key words: microRNA, TGF β , diabetes, kidney disease, Akt, PTEN, fibrosis, hypertrophy, p53

microRNAs and Diabetic Kidney Diseases

MicroRNAs (miRNAs) are short non-coding RNAs that regulate gene expression at the post-transcriptional level by blocking translation or promoting cleavage of their target mRNAs.¹ Almost 1,000 human miRNAs have been identified that target and downregulate at least 60% of human protein-coding genes expressed in the genome.¹ Accumulating evidence suggests that miRNAs play important roles in various diseases including cancers,² diabetes and kidney dysfunction.³ In particular, key miRNAs were upregulated in the kidneys of diabetic mice.⁴⁻⁶ Furthermore, podocyte-specific deletion of Dicer, an essential enzyme involved in the processing of miRNAs, led to progressive renal glomerular and tubular damage in mice.⁷ Diabetic nephropathy (DN) is a progressive kidney disease and one of most common complications in diabetes. It is characterized by glomerular basement membrane thickening, mesangial expansion (hypertrophy), extracellular matrix (ECM) accumulation and podocyte dysfunction.³ Transforming growth factor- β 1 (TGF β) is increased in renal cells during DN progression and has been implicated in these events.³ miR-192, a miRNA highly expressed in the kidney, was increased in the renal glomeruli of diabetic mice and in glomerular mesangial cells (MC) treated with TGF β , and could induce type I collagen alpha2 chain (Col1a2) gene by inhibiting ZEB1 and ZEB2 (E-box repressors).³⁻⁵ In another study, miR-377 could increase fibronectin in MCs by targeting PAK1 and MnSOD.⁶

TGF β also activates the Phosphatidylinositol-3-kinase (PI3K)/Akt kinase pathway in MC^{4,8-10} and this signaling has been implicated in its cell survival and fibrotic responses such as collagen and fibronectin expression. Akt kinase activated by TGF β phosphorylates several downstream proteins, including mTOR, GSK3- β and Forkhead (FoxO) transcription factors to control cell growth, survival, oxidant stress and protein synthesis.^{4,8-10} One mechanism by which TGF β activates Akt is via direct interaction of TGF β receptor and PI3K.¹¹ This explains the quick (within 5 min) modest activation. Very recently another miRNA-dependent mechanism was described. This involved the upregulation of a miRNA cluster (miR-216a and miR-217) targeting PTEN (phosphatase and tensin homologue)⁴ which can explain the late (6-24 hr) robust activation of Akt by TGF β (Fig. 1A). These TGF β triggered miRNA circuits and downstream signaling could result in enhanced ECM accumulation, hypertrophy, cell survival and oxidant stress related to the pathogenesis of kidney diseases such as DN.

miRNA Cascade in Diabetic Kidney Diseases

Multiple miRNAs induced in the kidney under diabetic conditions might cooperate to promote renal dysfunction. Interestingly, miR-216a and miR-217 lie in the second intron of a non-coding RNA (RP23-298H6.1-001, RP23) located in mouse chromosome 11.⁴ miR-216a and miR-217 were expressed along with RP23 and induced by diabetic conditions or TGF β . The RP23 (miR-216a and miR-

217) promoter was activated by TGF β and also by miR-192 through E-box-regulated mechanisms as shown in Col1a2 gene regulation.⁵ Since E-boxes are also present in the upstream genomic regions of the miR-200 family (miR-141, -200a, -200b, -200c, -429), miR-200 family members may also themselves be regulated by ZEB1 and ZEB2.¹² It is possible that the miR-200 family upregulated by TGF β or in diabetic glomeruli under early stages of diabetes can also regulate collagen expression by targeting and downregulating E-box repressors.³ miR-192 as initiator might transmit signaling from TGF β to upregulate miR-200 family members, which subsequently could amplify the signaling by further regulating themselves and also the miR-216a and miR-217 cluster and Col1a2 gene through downregulation of E-box repressors (Fig. 1A).

Reports show that miR-192 and the tumor suppressor p53 enhance each other and induce cyclin-dependent kinase inhibitor p21-mediated cell cycle arrest, but not apoptosis in cancer cells.¹³ miR-192 can activate the promoter of the anti-apoptotic Survivin gene,¹⁴ and also inhibit apoptosis through Akt activation via inhibition of PTEN targeted by miR-216a and miR-217.⁴ miR-192 might also promote glomerular hypertrophy by activating p21 and p53 pathways (Fig. 1A). Therefore, the signaling cascade initiated by relatively small changes in a small RNA (miR-192) may have profound consequences in the diabetic kidney.

Balance and Fine Tuning

As mentioned above, miR-192 seems to regulate two opposite cellular processes.

Correspondence to: Mitsuo Kato and Rama Natarajan; Email: mkato@coh.org and RNatarajan@coh.org
Submitted: 08/13/09; Accepted: 08/16/09
Previously published online: www.landesbioscience.com/journals/cc/article/9816
Comment on: Kato M, et al. Nat Cell Biol 2009; 11:881-9.

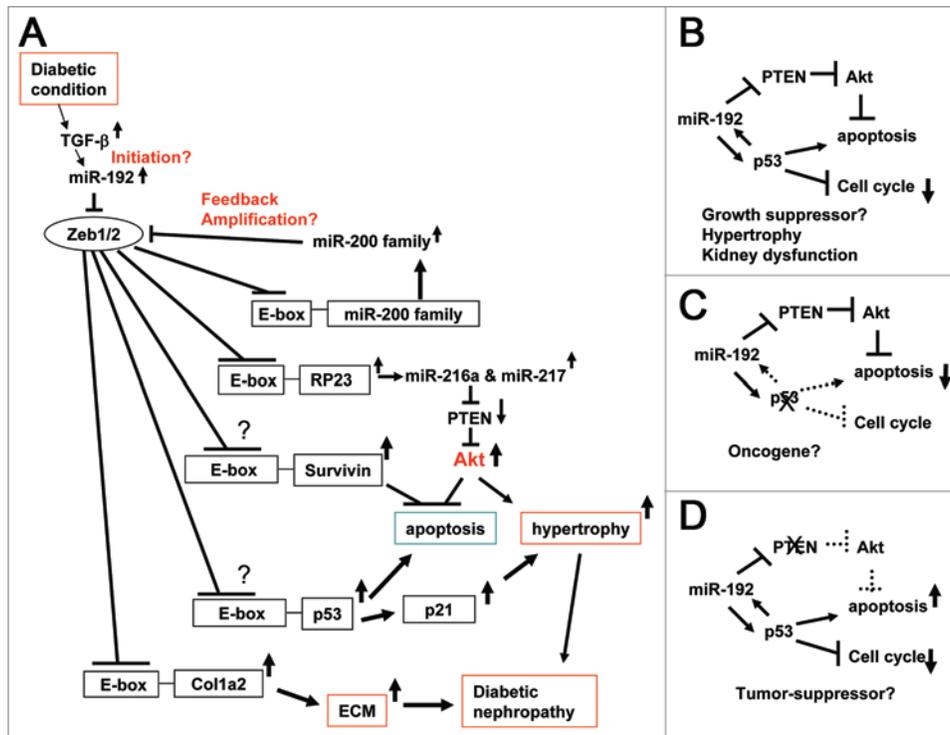


Figure 1. Signaling pathways initiated by miRNAs and TGFβ in diabetic kidney diseases. (A) miRNA cascade initiated by miR-192 upregulated by TGFβ in the diabetic kidney. (B) miR-192 as a double-edged sword. (C) miR-192 as an oncogene in p53 deficient cells. (D) miR-192 as a tumor-suppressor in PTEN deficient cells.

One is inhibition of PTEN which would promote Akt activation and cell survival, while the other is p53 activation which might promote apoptosis and cell cycle arrest (Fig. 1B). Since Akt inhibits apoptosis, while p53 induces apoptosis as well as cell cycle arrest, miR-192 probably induces only cell cycle arrest. This could be a defense (anti-apoptotic) mechanism to protect kidney cells from stress signals. However, in cancer cells which have mutations of p53 (Fig. 1C), miR-192 might only inhibit PTEN and thus act as an oncogene. On the other hand, in cancer cells with PTEN mutations (Fig. 1D), miR-192 might induce only p53 and thus act as a tumor-suppressor. This may explain why some cancer cells depict higher expression of miR-192, while others depict lower.^{2,13} Therefore, miR-192 may be a double-edged sword, with properties that might also induce kidney dysfunction. Extremely high or low levels of these miRNAs might worsen the situation. As such, a regulated balance in the expression of this miRNA (and possibly others) could be important to maintain MCs in the normal healthy

state, especially since miRNAs are implicated in the fine tuning of target genes.¹ Precise control of key miRNAs (small initiators) might be the next step for the treatment or prevention of human diseases.

Hope for the Treatment of Kidney Diseases by Targeting miRNAs

miRNAs are now being evaluated as therapeutic targets. Since miR-192 actions seem to enhance other renal miRNAs, miR-192 could be one such molecular target for preventing DN. Recently, LNA (locked nucleic acid) modified antisense nucleotides targeting key miRs (LNA-anti-miRs)¹⁵ were shown to be efficient inhibitors of miR actions in vivo. LNA-anti-miR-122 improved hypercholesterolemia in animal models without any toxicity.¹⁵ LNA-anti-miR-192 efficiently inhibited miR-192 and its downstream signaling in mouse kidney cortex.⁴ Such LNA modified oligonucleotides targeting specific miRNAs could be tested in animal models for potential use in similar human kidney disorders.³

Acknowledgements

The authors gratefully acknowledge grant support from the National Institutes of Health and the Juvenile Diabetes Research Foundation.

References

1. Bartel DP. *Cell* 2009; 136:215-33.
2. Visone R, et al. *Am J Pathol* 2009; 174:1131-8.
3. Kato M, et al. *Clin J Am Soc Nephrol* 2009; 4:1255-66.
4. Kato M, et al. *Nat Cell Biol* 2009; 11:881-9.
5. Kato M, et al. *Proc Natl Acad Sci USA* 2007; 104:3432-7.
6. Wang Q, et al. *FASEB J* 2008; 22:4126-35.
7. Ho JJ, et al. *J Am Soc Nephrol* 2008; 19:2043-6.
8. Kato M, Yer al. *J Am Soc Nephrol* 2006; 17:3325-35.
9. Mahaimanathan L, et al. *Diabetes* 2006; 55:2115-25.
10. Wu L, et al. *Dev Cell* 2009; 17:35-48.
11. Yi JY, et al. *J Biol Chem* 2005; 280:10870-6.
12. Gregory PA, et al. *Cell Cycle* 2008; 7:3112-8.
13. Georges SA, et al. *Cell Cycle* 2009; 8:680-1.
14. Gou D, et al. *Physiol Genomics* 2007; 31:554-62.
15. Elmén J, et al. *Nature* 2008; 452:896-9.