Epigenetic Unsilencing Reverses Renal Fibrosis

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In CKD, the development of fibrosis is directly related to progressive loss of renal function. Several recent reports indicate a critical role for epigenetics in the development of fibrotic pathways leading to CKD,[1–3] opening new avenues for the identification of biomarkers and novel therapeutics. Epigenetic changes are essential for kidney development and function, yet little is known about the epigenome of renal disease or indeed of any human disease other than cancer. It is now becoming apparent that epigenetic silencing of gene expression may be an important contributing factor to fibrogenesis in CKD. This concept is consistent with findings from epidemiologic studies indicating that adverse environments early in life and in adulthood (e.g., metabolic memory) have a major effect on CKD development.[4]

Epigenetic modification (methylation) by DNA methyltransferases at the 5-position of cytosine (5-methylcytosine [5mC]), sometimes called the “fifth base,” is associated with transcriptional silencing. 5mC-associated transcriptional silencing may be maintained in the long term, such as in genomic imprinting and X-chromosome inactivation.[5] Promoter methylation, a covalent but reversible epigenetic process, has been implicated in the control of gene expression in disease, primarily in cancer, but more recently in the development of fibrosis in the kidney and other organs. Whereas CpG island promoter methylation causes transcriptional silencing, active demethylation resulting in formation of 5-hydroxymethylcytosine (5hmC) is associated with increased gene expression.[6] In the elegant study by Tampe et al.[7] in this issue of JASN, endogenous DNA demethylation mechanisms and their role in the homeostasis of epigenetic modifications are described for the first time in the adult kidney. Identification of demethylation processes of specific genes and subsequent reversal of fibrogenesis opens the attractive possibilities of using this information both to identify specific pharmacologic agents to transiently target these processes and to identify the patients that will benefit from such therapies.

This research group previously established hypermethylation of the Rasal1 promoter and consequential gene silencing of Rasal1 as a critical component of experimental renal fibrogenesis after AKI leading to CKD.[3] In the study by Tampe et al.,[7] Rasal1 hypermethylation is found in experimental renal fibrosis, irrespective of the underlying disease model. Interestingly, treatment with the well characterized antifibrotic bone morphogenetic protein 7 (BMP7) resulted in hydroxymethylation of the Rasal1 promoter, indicating that BMP7 may mediate its antifibrotic actions in the kidney at least in part through enhancing active demethylation mechanisms.

The most prominent active demethylation mechanism involves enzymatic oxidation (hydroxymethylation) of 5mC to 5hmC.[8] 5hmC accumulates in most cell types, suggesting the possibility that this “sixth base”[9] in the genome may have a distinctive epigenetic role. The recently discovered ten-eleven translocation (Tet) proteins catalyze 5mC oxidation and generate 5mC derivatives, including 5hmC. Tet enzymes are thought to regulate the rapid disappearance of 5mC and the resulting increase in 5hmC.[10] This concept is supported by studies showing that Tet-mediated hydroxylation is critical for resetting methylation at imprinted domains.[10] Interestingly, hydroxylation of 5mC to 5hmC catalyzed by Tet enzymes not only reverses gene silencing, but also enhances gene expression from covalently modified promoters even compared with unmethylated promoters.[6] Tampe et al.[7] for the first time describe the role of Tet-mediated endogenous DNA demethylation mechanisms in kidney fibrosis and show antifibrotic BMP7-induced Rasal1 hydroxymethylation in mice that had been challenged with experimental renal fibrosis via a variety of independent models, including unilateral ureteral obstruction, streptozotocin-induced diabetic nephropathy in CD-1 mice, Col4A3-deficient Alport mice, and 5/6 nephrectomy, further supporting their data reported in the folic-acid induced nephropathy model and human fibroblast cultures in vitro.[3]

Because physiologic hydroxymethylation is mediated by Tet1, Tdet2, and Tet3, Tampe et al.[7] examined the expression of these genes in disease models and found reduced expression of Tet3, but not Tet1 or Tet2, to be associated with experimental renal fibrosis. The impact of this discovery is considerably strengthened with the finding that Tet3 expression is also reduced in human CKD (i.e., patients with diabetic nephropathy, hypertensive nephrosclerosis, IgA nephropathy, or lupus nephritis). The critical role of Tet3 in renal fibrosis is further strengthened by the observation that BMP7 reduced experimental renal fibrosis by normalizing Tet3 expression. Indeed, the antifibrotic action of BMP7 was considerably hampered when Tet3 was no longer expressed. Interestingly, Tet3 is also the most abundant Tet enzyme in zygotes and...
is critical in embryogenesis, whereas BMP7 is critical in kidney development regulating the differentiation of metanephric mesenchymal cells required for ureteric bud branching. It is tempting to speculate that BMP7 regulation of Tet3 expression and the resulting effect on the epigenome play an important role in nephrogenesis.

The study by Tampe et al. indicates that Rasal1 promoter hypermethylation and reduced Tet3 expression are features of renal fibrosis. BMP7 treatment resulted in normalization of Tet3 expression and consequently hydroxymethylation of the Rasal1 promoter. BMP7-mediated hydroxymethylation of the Rasal1 promoter was impaired when Tet3 expression was lost. Electrophoretic mobility shift assays confirmed binding of Tet3 to its CXXC binding motif flanking the Rasal1 CpG island promoter, further indicating the role of Tet3 in Rasal1 hydroxymethylation.

Preclinical data from this study indicate that aberrant methylation or perhaps active demethylation processes catalyzed by Tet3 determine the balance between health and CKD. These findings suggest that transient therapies targeted at amplifying or restoring Tet3 activity may prove beneficial to patients with CKD. Furthermore, analysis of methylation status (e.g., using Rasal1 as a methylation biomarker) to determine the individual risk of a patient to develop fibrosis or suitability for demethylation-based therapy appears both plausible and attractive. However, further studies in larger patient cohorts are warranted to establish these targets for both diagnosis and therapy.

Demethylation agents, such as 5-azacytidine, have already been described to rescue kidney fibrosis in experimental models and demethylation agents are in clinical trials for treatment of cancers; however, these drugs are cytotoxic and the long-term effects of genome-wide demethylation are unknown. It therefore seems more attractive to transiently and specifically reactivate an endogenous demethylation process and thereby readdress the imbalance that results in CKD. The identification of Tet3 as a critical player in both the development and reversal of renal fibrogenesis described in the study by Tampe et al. may be the first step toward achieving this attractive possibility.

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DISCLOSURES

None.

REFERENCES


All of the Twos, 22—Bingo!

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IL-22 is currently a hot topic, with researchers generating approximately 400–500 related articles per year. However,