

 FIBROSIS

## Targeting methylation

Aberrant methylation of gene promoters leads to the transcriptional silencing of genes and has been linked to pathologies including renal fibrosis. Such DNA methylation can be inhibited through the use of demethylating drugs, but the nonspecific nature of these agents limits their clinical utility. Michael Zeisberg and colleagues now show that delivery of a gene-specific system can be used to specifically reverse methylation of two genes — *Rasa1* and *Kl* (which encodes Klotho) — and attenuate kidney fibrosis in mice.

Previous work had shown associations between hypermethylation of *Rasa1* and *Kl* with renal fibrosis, indicating that demethylation of these genes might inhibit the fibrotic process. In addition to demethylating drugs, reversal of hypermethylation can be achieved by ten-eleven translocation (TET) family members through a process called hydroxymethylation. To demethylate specific genes, the researchers designed constructs that directed TET3 — the main TET protein in the kidney — to specific promoter sites using CRISPR–Cas9 technology. “We fused the sequence encoding the catalytic domain of TET3 to an endonuclease-deactivated high-fidelity Cas9, and targeted this to *Rasa1* and *Kl* promoters using guide RNAs,” says Zeisberg. “The endonuclease mutation enables Cas9 to maintain its site specificity, but it no longer cleaves its target. It therefore no longer functions as a molecular scissor but rather as a molecular eraser that removes unwanted methylation marks from DNA in a site-specific manner.”

Following confirmation that these constructs could rescue expression of hypermethylated *Rasa1* and *Kl* in vitro, the researchers demonstrated that lentiviral delivery of the *Rasa1*-targeted fusion protein to interstitial cells and of the *Kl*-targeted fusion protein to tubular epithelial cells resulted in reduced promoter methylation, reactivation of the specific gene and attenuation of renal fibrosis in mice with unilateral ureteral obstruction. “This study opens the possibility of using a molecularly informed specific epigenetic therapy for fibrosis, in which specific demethylation therapy is based on the detection of aberrant DNA methylation,” says Zeisberg.

Susan J. Allison

**ORIGINAL ARTICLE** Xu, X. et al. High-fidelity CRISPR/Cas9-based gene-specific hydroxymethylation rescues gene expression and attenuates renal fibrosis. *Nat. Commun.* **9**, 3509 (2018)

 KIDNEY CANCER

## Sweet success for ccRCC isotope tracing

Human clear cell renal cell carcinoma (ccRCC) tumours display extensive metabolic alterations, mainly driven by the pseudo-hypoxic state associated with loss of the *VHL* (von Hippel–Lindau) tumour suppressor gene — the principal genetic cause of ccRCC. Although mechanistic and metabolomic studies had already suggested that ccRCC cells produce energy mainly from aerobic glycolysis, also termed the Warburg effect, isotope tracing studies recently published in *Cell Press* represent the first convincing in vivo evidence of this effect in human tumours.

Courtney et al. show that glycolysis is increased and glucose oxidation is suppressed, a phenotype consistent with the Warburg effect, in ccRCC tumours compared with matched adjacent healthy kidney tissue. The researchers administered  $^{13}\text{C}$ -glucose (in which all six carbon atoms were substituted with  $^{13}\text{C}$ ) to five patients with ccRCC who were undergoing nephrectomy or partial nephrectomy (this infusion increased the patients' plasma glucose levels by 30–50%). The presence of this isotope in metabolites was traced by

proton-decoupled  $^{13}\text{C}$ -NMR, with values normalized to the patients' steady-state plasma  $^{13}\text{C}$ -glucose levels.

The ccRCC tumours exhibited minimal glucose oxidation and minimal activity of the tricarboxylic acid (TCA) cycle. Moreover, the alterations in levels of TCA cycle intermediates were consistent with decreased activity of pyruvate dehydrogenase, a key enzyme controlling entry into the TCA cycle.

Interestingly, although the Warburg effect was initially hypothesized to be common to all aggressive tumours, Courtney et al. also report that ccRCC tumours seem to rely more heavily on aerobic glycolysis than do brain or lung tumours — in which similar isotope tracing studies found surprisingly high levels of oxidation of glucose and other fuels, despite evidence of aerobic glycolysis. These observations further highlight the distinctive metabolic profile of ccRCC tumours.

Caroline Barranco

**ORIGINAL ARTICLE** Courtney, K. D. et al. Isotope tracing of human clear cell renal cell carcinomas demonstrates suppressed glucose oxidation in vivo. *Cell Metab.* **28**, 1–8 (2018)

 DIABETIC KIDNEY DISEASE

## ASK1 inhibition shows potential in DKD

Inhibition of apoptosis signal-regulating kinase 1 (ASK1) might be useful in patients with diabetic kidney disease (DKD), according to the results of a new study.

“Previous studies in ASK1 knockout mice demonstrated that ASK1 is a critical mediator of tissue injury and progressive fibrosis, particularly in settings of oxidative stress,” says John Liles, an author on the new paper. “Oxidative stress is a key common driver of tissue injury and fibrosis in diseases such as DKD and nonalcoholic steatohepatitis (NASH), suggesting that ASK1 may be a therapeutic target to halt or reverse fibrosis in these diseases.”

The researchers used high-throughput screening to identify ASK1 inhibitors from a library of approximately 100,000 compounds. Using medicinal chemistry techniques to optimize compounds for potency, selectivity, adsorption, distribution, metabolism and excretion, GS-444217 was established as a potent and highly selective ATP-competitive inhibitor of ASK1.

Liles et al. showed that GS-444217 reduced oxidative-stress-induced ASK1 signalling in rat

models of acute kidney injury, preserving renal function by reducing fibrosis, inflammation and tubulointerstitial cell death. They also showed that ASK1 pathway activation was increased in renal biopsies from patients with DKD compared with non-DKD kidneys. A mouse model of DKD showed that GS-444217 attenuated progressive loss of renal function, blocked increases in albuminuria and reduced other pathological features of DKD such as glomerulosclerosis, loss of podocytes and tubulointerstitial fibrosis and apoptosis.

Combining GS-444217 with enalapril, an angiotensin-converting enzyme inhibitor, resulted in greater improvements in glomerulosclerosis and albuminuria than either monotherapy alone in a rodent model of chronic glomerular injury. “Our results show that ASK1 inhibition could be a promising new approach to reduce GFR decline in this high unmet need DKD patient population,” concludes Liles.

Rebecca Kelsey

**ORIGINAL ARTICLE** Liles, J. T. et al. ASK1 contributes to fibrosis and dysfunction in models of kidney disease. *J. Clin. Invest.* <https://doi.org/10.1172/JCI99768> (2018)