Nature Reviews Nephrology | Published online 3 Jul 2017; doi:10.1038/nrneph.2017.99

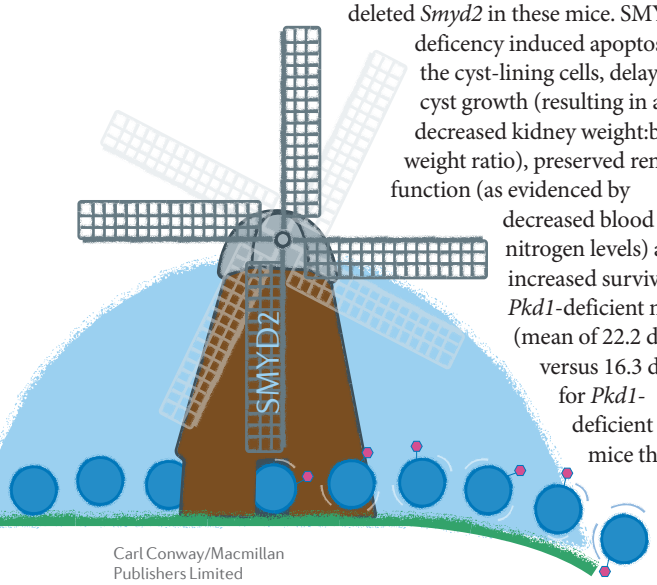
POLYCYSTIC KIDNEY DISEASE

SMYD2 is a novel epigenetic regulator of cyst growth

> pharmacological inhibition of SMYD2 using AZ505 delayed cyst growth

Autosomal dominant polycystic kidney disease (ADPKD), which is caused by mutations in *PKD1* and *PKD2*, is the most common inherited kidney disease, but no FDA-approved treatment currently exists. In a new study, Xiaogang Li and colleagues report that the methyltransferase SMYD2 is an epigenetic regulator of cyst growth in ADPKD. They also show that the SMYD2 inhibitor AZ505 delays cyst formation, improves renal function and extends the survival of ADPKD mice. "This is the first study that links the epigenetic regulator SMYD2 to ADPKD and identifies the key novel regulatory components that might serve as effective targets to slow disease progression," says Li.

The researchers report that SMYD2 levels were increased in renal cells from patients with ADPKD and from *Pkd1*-deficient mice. To investigate the contribution of SMYD2 overexpression to the pathogenesis of ADPKD *in vivo*, they deleted *Smyd2* in these mice. SMYD2 deficency induced apoptosis of the cyst-lining cells, delayed cyst growth (resulting in a decreased kidney weight:body weight ratio), preserved renal function (as evidenced by decreased blood urea nitrogen levels) and increased survival of *Pkd1*-deficient mice (mean of 22.2 days versus 16.3 days for *Pkd1*-deficient mice that expressed SMYD2). Similarly, in early or late-onset mouse models of ADPKD, pharmacological inhibition of SMYD2 using AZ505 delayed cyst growth through decreased proliferation and increased apoptosis of cystic epithelial cells.



Carl Conway/Macmillan Publishers Limited

To determine the molecular basis of the effects of SMYD2 on cyst growth, the researchers characterized the substrates and assessed the transcriptional activity of SMYD2 using co-immunoprecipitation assays, western blots and chromatin immunoprecipitation (ChIP)–quantitative PCR. In *Pkd1*-deficient cells, increased SMYD2-mediated methylation of the transcription factors STAT3 and p65 (a subunit of NF-κB) promoted their phosphorylation and expression of their transcriptional targets. Some of these targets, such as cyclin D1, TNF and c-Myc, control cell death and proliferation and have been implicated in cystogenesis. In addition, SMYD2 depletion weakened the interaction between p65 and STAT3, and inhibition of either STAT3 or p65 impaired the interaction of SMYD2 with p65 or STAT3, respectively, and hence hampered their SMYD2-mediated methylation. "We identified STAT3 and p65 as novel non-histone substrates of SMYD2; these new substrates and SMYD2 itself might form a complex that regulates gene expression synergistically," explains Li. "A STAT3 inhibitor has already been used in ADPKD animal models so combining it with AZ505 could lead to a more effective therapeutic strategy."

In addition to regulating gene expression through direct promoter binding, STAT3 and NF-κB can alter signalling pathways indirectly through cytokines such as IL-6 and TNF. Administration of these cytokines stimulated SMYD2 expression in healthy renal cells, whereas inhibition of their activity decreased SMYD2 levels in *Pkd1*-deficient cells, indicating the existence of a regulatory feedback loop.

To further characterize the transcriptional effects of SMYD2, the researchers performed ChIP–sequencing analyses and identified 91 and 116 transcriptional targets of SMYD2 in wild-type and *Pkd1*-deficient cells, respectively. The ChIP assay also revealed that SMYD2 bound to the promoter of the tyrosine phosphatase PTPN13. The expression levels of this enzyme were reduced in the absence of *Pkd1* and were increased in *Smyd2*-depleted cells, which altered the phosphorylation of its transcriptional targets ERK, S6 and AKT. These proteins are also altered in other PKD-related pathways.

"This study, together with our previously published studies showing that histone deacetylases are regulators of cystogenesis, will establish a comprehensive mechanism of epigenetics in ADPKD," concludes Li. "We also found novel SMYD2 target genes, including several ciliopathy-related genes, which will broaden the role of SMYD2." In particular, Li and colleagues shwoed that SMYD2 regulates the transcription of *Pkhd1*; they now plan to investigate whether SMYD2 is involved in ADPKD and autosomal recessive PKD through similar or distinct mechanisms.

Andrea Aguilar

ORIGINAL ARTICLE Li, L. X. *et al.* Lysine methyltransferase SMYD2 promotes cyst growth in autosomal dominant polycystic kidney disease. *J. Clin. Invest.* http://dx.doi.org/10.1172/JCI90921 (2017)