HOMEOSTASIS

Pax proteins regulate urine concentration

Pax proteins have key roles in kidney development. Now, Gregory Dressler and colleagues report that Pax2 and Pax8 also function as regulators of salt and water homeostasis in adult kidneys.

"We previously proposed that Pax proteins recruit histone methyltransferases via the adaptor protein PAX-interacting protein 1 (PTIP) to imprint a renal epithelial cell fate," explains Dressler. "Once this fate is established in the adult kidney, are Pax proteins still needed to maintain or regulate this fate? This is the essential question that our study was investigating."

The researchers report that in adult mice, both Pax2 and Pax8 are expressed in the nuclei of the collecting ducts and the medulla, but only Pax8 is highly expressed in the proximal tubules. Adult mice with induced deletion of either *Pax2* or *Pax8* seemed to be healthy but those in which both genes were deleted showed severe polyuria and dehydration. Consistent with this phenotype, gene expression analysis and immunostaining of kidney sections indicated that solute carriers, including urea transporter 1 (UT1), UT2, aquaporin 2 (AQP2), AQP3 and AQP4, were significantly downregulated in the double-knockout mice.

Culture of mouse inner medullary collecting duct cells in high-salt conditions led to upregulation of Pax2 and Pax8 within 4–8 hours and of *Uta1* and *Uta3* mRNAs within 16 hours. Further studies showed that Pax8, but not Pax2, directly binds to the promoter region of *Slc14a2*, which encodes UT1 and UT2, and recruits the histone methyltransferase complex via PTIP, leading to upregulation of these transporters in response to high-salt conditions.

"I think our study has clinical relevance beyond the mechanistic aspects of urine concentration," says Dressler. "Pax proteins are essential developmental regulators, yet we know little about their functions in adult tissues. Our results could apply to other Pax family members expressed in the nervous system, eyes, muscle or skeleton."

Ellen F. Carney

ORIGINAL ARTICLE Laszczyk, A. M. et al. Pax2 and Pax8 proteins regulate urea transporters and aquaporins to control urine concentration in the adult kidney. *J. Am. Soc. Nephrol.* https://doi.org/10.1681/ASN.2019090962 (2020)

ACUTE KIDNEY INJURY

Targeting inflammation in AKI by P2Y14 receptor inhibition in mice

Acute kidney injury (AKI) is common among hospitalized patients and is frequently associated with immune cell infiltration into the kidney. Now, Sylvie Breton and colleagues show that inhibition of the P2Y14 receptor reduces inflammation and attenuates AKI following ischaemia–reperfusion injury (IRI). They also report a correlation between urinary levels of the P2Y14 receptor ligand, uridine diphosphate-glucose (UDP-Glc) and the development of AKI among patients in the intensive care unit (ICU).

The mechanism by which trauma to a distant organ or the kidney itself can lead to AKI is a topic of much debate. In previous work, Breton and colleagues implicated UDP-Glc as a damage-signalling molecule involved in this process. "When released from damaged or stressed tissues, UDP-Glc activates P2Y14 within the kidney, triggering an inflammatory cascade," says Breton. "The cascade then creates a self-reinforcing cycle that causes further kidney inflammation, ultimately leading to AKI." The researchers now show that the pro-inflammatory response of intercalated cells to IRI and subsequent recruitment of macrophages and monocytes is attenuated by pharmacological or genetic inhibition of P2Y14 in mice. Moreover, inhibition of P2Y14 reduced the extent of proximal tubule damage and indices of kidney dysfunction.

To assess the relevance of this pathway, the researchers investigated urinary UDP-Glc levels and outcomes of patients admitted to the ICU. "Our longitudinal pilot study showed a striking correlation between urinary levels of UDP-Glc and a subsequent AKI diagnosis in ICU patients, particularly among patients undergoing cardiac surgery," explains Breton. "Not only do our findings suggest that urinary UDP-Glc may serve as a biomarker of AKI, they also provide mechanistic insights into the inflammatory storm that can lead to AKI".

Susan J. Allison

ORIGINAL ARTICLE Battistone, M. A. et al. Pro-inflammatory P2Y14 receptor inhibition protects against ischemic acute kidney injury in mice. *J. Clin. Invest.* https://doi.org/10.1172/JCI134791 (2020)

ACUTE KIDNEY INJURY

CDKL5 controls RTEC fate during AKI

Cyclin-dependent kinase-like 5 (CDKL5) is a key regulator of renal tubular epithelial cell (RTEC) death, according to a new study by Navjot Pabla and colleagues. The researchers suggest that targeted CDKL5 inhibition is a potential strategy to protect against acute kidney injury (AKI).

"Although the US FDA has approved >50 small-molecule protein kinase inhibitors, the role of protein kinases in the pathogenesis of non-oncological diseases is underexplored," says Pabla. "We utilized an unbiased kinome-wide small-interfering RNA screening approach to identify protein kinases that contribute to RTEC death in order to identify therapeutic targets for AKI."

This screening approach and in vivo validation experiments identified CDKL5 as a key regulator of RTEC death. "CDKL5 is highly expressed in the brain and *CDKL5* loss-of-function mutations are associated with neurodevelopmental disorders," comments Pabla. "Whether CDKL5 controls any biological processes in non-neuronal tissues, such as the kidneys, is unknown."

In mouse and porcine models, CDKL5 activity in RTECs increased during the early stages of AKI. Furthermore, conditional knockout of *Cdkl5* in RTECs or treatment with a small-molecule CDKL5 inhibitor, AST-487, protected mice from ischaemia-associated or cisplatin-mediated AKI, suggesting a pathogenic role of CDKL5. "We found that CDKL5 phosphorylates the pro-survival transcription factor SOX9, resulting in reduced SOX9 stability and functional inactivation during AKI," says Pabla.

The researchers caution that future studies are required before their findings can be clinically translated. "First, it will be critical to explore the potential toxicities associated with CDKL5 inhibition," says Pabla. "Second, it will be crucial to examine the effects of CDKL5 inhibition-dependent SOX9 stabilization on regeneration, fibrosis and AKI-to-chronic kidney disease transition. Finally, we are interested in identifying CDKL5 substrates that might be involved in the pathogenesis of AKI."

Ellen F. Carney

ORIGINAL ARTICLE Kim, J. Y. et al. A kinome-wide screen identifies a CDKL5–SOX9 regulatory axis in epithelial cell death and kidney injury. *Nat. Commun.* **11**, 1924 (2020)