IMAGING

Endocytosis in the proximal tubule

Reabsorption of protein and other filtered macromolecules via the processes of receptor-mediated and nonspecific fluid-phase endocytosis is a key function of the kidney proximal tubule. Now, Andrew Hall and colleagues report that the different segments of the proximal tubule have specialized endocytotic functions.

"Very little is known about the workings of the proximal tubule endolysosomal system in vivo," says Hall. "We had noticed subtle differences in endolysosomal system ultrastructure between the S1 and S2 segments of the proximal tubule using electron microscopy and wondered if these differences had functional significance."

To visualize proximal tubule endocytosis in real time in live mice, the researchers used multiphoton imaging of intravenously injected fluorescently labelled ligands. They also used a tissue clearing method to visualize solute uptake patterns in 3D in large sections of kidney cortex. These analyses showed that uptake of lysozyme and albumin by receptor-mediated endocytosis occurred almost exclusively in S1, whereas fluid-phase endocytosis of dextran occurred in both S1 and S2. However, solute uptake length and proximal tubule morphology were highly variable throughout the kidney. Quantitative PCR and immunostaining confirmed that with the exception of megalin, which binds low molecular weight proteins and albumin, the expression of endolysosomal proteins was much greater in S1 than in S2. Megalin expression was similar in both segments of the proximal tubule.

The researchers conclude that the S1 segment is highly specialized for receptor-mediated endocytosis. "Our findings add to the growing evidence from live imaging studies that S1 and S2 are functionally discrete entities — something that was previously not well appreciated because they appear to be so similar in routine histology," comments Hall. "They also help to explain topographical patterns of kidney injury in response to endocytosed toxins and suggest that patients with tubular proteinuria most likely have a defect in S1."

Ellen F. Carney

ORIGINAL ARTICLE Schuh, C. D. et al. Combined structural and functional imaging of the kidney reveals major axial differences in proximal tubule endocytosis. *J. Am. Soc. Nephrol.* https://doi.org/10.1681/ASN.2018050522 (2018)

PODOCYTE BIOLOGY

CXCL12 limits podocyte regeneration

The chemokine CXCL12 is secreted by podocytes and has been shown to regulate stem cell homing and activation. Now, Paola Romagnani and colleagues report that a CXCL12-mediated podocyte-progenitor feedback mechanism limits podocyte regeneration after glomerular injury.

Using lineage tracing of $PAX2^+$ parietal epithelial cells (PECs) in mice with adriamycin-induced nephropathy, the researchers show that CXCL12 blockade promotes de novo podocyte formation and attenuates glomerulosclerosis. Super-resolution microscopy imaging confirmed that the new podocytes were fully differentiated and integrated into the glomerular filtration barrier.

To quantify podocyte regeneration, the researchers used 3D tissue analysis. They report that CXCL12 blockade induced regeneration in up to 25% of glomeruli in the adriamycin model. "Podocyte regeneration was limited in juxtamedullary nephrons, whereas cortical nephrons frequently included new progenitor-derived podocytes, a difference that increased with CXCL12 inhibition," comments Romagnani.

The researchers also show that CXCL12 suppresses Notch signalling in renal progenitor cells but not in podocytes and that CXCL12 and its receptors are expressed in human podocytes and PECs. They suggest that podocyte-derived CXCL12 maintains the quiescence of podocyte progenitors by inhibiting Notch signalling. This mechanism maintains homeostasis in healthy kidneys but also limits podocyte regeneration after injury.

"Our results provide proof of concept that renal progenitors can be stimulated to regenerate podocytes and also explain the clinical observation that focal segmental glomerulosclerosis is most severe in juxtamedullary nephrons," says Romagnani. "The hope remains that CXCL12 inhibitors or other drugs that stimulate podocyte regeneration could improve long-term outcomes in chronic kidney disease."

Ellen F. Carney

ORIGINAL ARTICLE Romoli, S. et al. CXCL12 blockade preferentially regenerates lost podocytes in cortical nephrons by targeting an intrinsic podocyte-progenitor feedback mechanism. *Kidney Int.* https://doi.org/10.1016/j.kint.2018.08.013 (2018)

RENAL CELL CARCINOMA

A central anti-oncogenic pathway in ccRCC

The majority of clear cell renal cell carcinomas (ccRCC) harbour loss-of-function mutations in *VHL* that lead to increased levels of HIF2α, a pivotal oncogenic transcription factor. HIF2α also functionally interacts with secondary tumour suppressor genes such as *KDM5C* and *PBRM1*, which are often mutated in ccRCC. Now, Yaomin Xu, Qin Yan, Haifeng Yang and colleagues report that HIF2α and several chromatin regulators that are disabled in ccRCC enhance the expression of the transcription factor ISGF3, a regulator of the type I interferon response.

"We were intrigued by the mutations of multiple chromatin regulators such as *PBRM1*, *KDM5C*, *SETD2* and *BAP1* in ccRCC," explains Xu. The researchers show that in $VHL^{-/-}$ cell lines, loss of any of these chromatin regulators reduced the levels of the ISGF3 components IRF9 and STAT2, as well as the expression of ISGF3 target genes.

In a xenograft model, loss of any of the three ISGF3 components — IRF9, STAT1 or STAT2 — resulted in a significant increase in tumour size. Tumours in which IRF9 was knocked down were mainly composed of cancer cells, whereas immune cells were more abundant in control tumours. No differences were observed in cancer cell proliferation or apoptosis. These results suggest that ISGF3-mediated tumour suppression might be linked to immune modulation. Consistent with a protective role of ISGF3, loss of nuclear IRF9 staining in human ccRCC tissue samples was associated with reduced patient survival.

"Mutations in VHL activate tumour suppressive ISGF3 via HIF2α but mutations in any of the secondary tumour suppressors disable this negative feedback loop. The fact that the secondary tumour suppressors share the regulation of ISGF3 suggests that this is a central tumour suppressive pathway in ccRCC," concludes Yang. "Boosting ISGF3, either alone or in combination with other drugs, might be beneficial in patients with RCC," adds Yan.

Monica Wang

ORIGINAL ARTICLE Liao, L. et al. Multiple tumor suppressors regulate a HIF-dependent negative feedback loop via ISGF3 in human clear cell renal cancer. *eLife* https://doi.org/10.7554/eLife.37925 (2018)