Proteolytic processing in podocytes

Podocytes are crucial components of the glomerular filtration barrier in the kidney. Podocyte function at this barrier is dependent on proteases, such as cathepsins and matrix metalloproteases. New research has analysed protein cleavage events in podocytes using a novel proteome-wide 'degradomics' approach. "The degradomics method is very similar to mass spectrometry-based assessment of protein phosphorylation and relies on the purification of protein amino-termini that result from protease cleavage, thus reflecting protease activity," says Thomas Benzing. "By quantifying these termini in cells or tissues in different states of injury, we are able to obtain a snapshot of protease activity." The technique involves differential labelling of protein N-termini and negative selection from tryptic peptides generated for mass spectrometry.

First, the investigators tested the method in mouse glomeruli, and identified 3,815 N-termini — 2,448 termini were in unexpected positions (for example, not at known protease cleavage sites), and the

N-termini observed suggested that the majority of cleavage sites were outside predicted protein domains. Novel termini were identified in the slit diaphragm proteins podocin, nephrin and nephronectin, and in cytoskeletal proteins, such as the actin-binding protein α -actinin 4.

Next, the researchers analysed the effect of podocyte injury on protease activity, using puromycin aminonucleoside (PAN) treatment to induce damage in cultured human podocytes. In comparison to untreated podocytes, PAN treatment led to reduced cleavage of cytoskeletal proteins (classified by gene ontology), whereas cleavage of proteins associated with general cellular functions was increased. In particular, PAN treatment reduced cleavage of α -actinin 4 and the intermediate filament protein vimentin. PAN treatment also had distinct effects on different classes of proteases — arginine-specific proteases were inhibited, whereas caspase activity was induced.

Finally, the applicability of these *in vitro* results was investigated in PAN-treated rats and in a mouse model of focal segmental glomerulosclerosis. The reduced cleavage of α -actinin 4 observed *in vitro* was conserved in both *in vivo* models of nephropathy, whereas vimentin cleavage was only reduced in PAN-treated rats. "This proteome-wide atlas of protease cleavage activities in healthy and injured podocytes from living animals can be used to analyse the impact of proteolytic processes in glomerular diseases, which may enable the development of new treatment approaches," concludes Benzing.

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