242

Signalling Response Under Par: A PAR-1 mediated signalling pathway in podocytes is a consistent feature of 'circulating factor' initiated nephrotic syndrome.

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Background: Steroid resistant nephrotic syndrome (SRNS) often progresses to end-stage renal disease. Post-transplantation recurrence of SRNS is thought to be due to the presence of an unknown "circulating factor" which has been remained elusive so far. Our previous work suggests that an unknown circulating protease in recurrent SRNS plasma signals to podocytes through protease activated receptor-1 (PAR-1), a G protein-coupled-receptor and affects the actin cytoskeleton leading to increased motility via phosphorylation of vasodilator-stimulated phosphoprotein (VASP).

We also postulated that Th17 lymphocytes could be producing the circulating factor(s).

We have now further elaborated this signalling pathway in podocytes, and strengthened it using several models and in human disease biopsies

Methods: Conditionally immortalised human podocytes (ciPods) were exposed to 1) PAR-1 agonist; 2) Relapse and paired-remission plasma from SRNS patients, along with PAR-1 and TRPC6 inhibitors; and 3) Supernatant from Th17 cells

A transgenic mouse model was generated, expressing a podocyte-specific constitutively active PAR-1 construct. This mouse develops massive proteinuria and dies of renal failure by day 40.

Results: We found that PAR-1 agonist, patient relapse disease plasma, and Th17 lymphocyte supernatant, all induced phosphorylation of VASP, paxillin and JNK in human podocytes, and increased motility compared to relevant controls. This could be blocked by co-incubation of cells with TRPC6 or certain PAR-1 inhibitors. For the latter, out of 3 inhibitors only 1 was effective, suggesting a non-canonical agonism of PAR-1 by disease plasma.

In the mouse model we demonstrated the same signalling pathways upregulated in podocytes in vivo, and on human SRNS biopsy specimens we demonstrated increased VASP and JNK phosphorylation compared to minimal change disease or IgA nephropathy.

Conclusion: We reveal a signature signalling response to the 'circulating factor' that we have evidenced in vitro and in vivo: both in a murine disease model and in human disease. This signalling in SRNS that leads to podocyte effacement and proteinuria and suggests direct therapeutic targets.