PODO-GPCR2-A NOVEL PODOCYTE TARGET PLAYS A PATHOGENIC ROLE IN THE DEVELOPMENT OF DIABETIC NEPHROPATHY

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OBJECTIVES

Glomerular damage, mediated by TGF-B and EGF in podocyte cells, has an important role in the development of diabetic nephropathy (DN). In our study, we aim to identify novel podocyte-enriched receptors that could be targeted to manipulate pathogenic podocyte cell signaling in disease and therefore protect podocytes from diabetic damage.

METHODS

To identify molecular profile of diabetic glomeruli, RNA sequencing was performed in micro-dissected glomeruli isolated from 20 patients with DN and 20 healthy controls. Immunofluorescence, immunohistochemistry, real-time PCR and semi-quantitative immune-EM methods were conducted to validate key expression changes. Signaling pathways were analyzed in a human podocyte cell line by over/underexpressing key genes. Knockout mouse line was generated for podo-GPCR2 and streptozocin-induced diabetes model was generated to further unveil its in vivo biological function

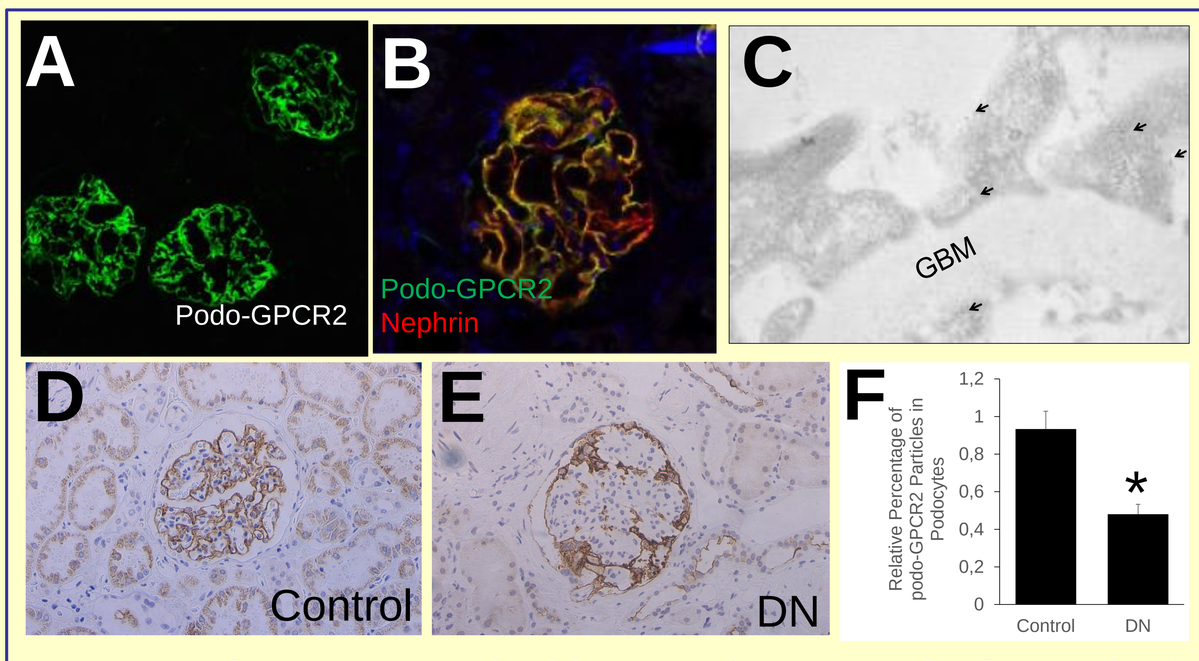

Fig 1. Podo-GPCR2 is highly specific to renal glomeruli (A) where it co-localizes with the podocyte marker, nephrin (B, C). Its expression is significantly downregulated in human DN as shown by semiquantitative immunohistochemistry (D, E, F).

RESULTS

1) Podo-GPCR2 was identified as one of the most highly expressed G-protein coupled receptors in human glomeruli via RNA sequencing and it was significantly down-regulated in DN.
2) Ectopic overexpression of it in in vitro cultured human podocytes showed that podo-GPCR2 inhibited EGF induced EGFR activation and Smad2/3 phosphorylation. Concomitantly, TGFB1 and its downstream extracellular matrix expression were also remarkably blunted, suggesting the potential role of podo-GPCR2 in glomerulus fibrosis.
3) Inactivation of podo-GPCR2 in mouse resulted in thickening of glomerular basement membrane and activation of mesangial cells, which are hallmarks signs of diabetic glomerular damage in humans.
4) Transcription of EGFR and TGFB1 in knockout mouse showed up-regulation in isolated glomeruli.
5) Moreover, in streptozocin-induced diabetes model, podo-GPCR2 knockout mice showed more albuminuria, podocyte foot process effacement and glomerular fibrosis.

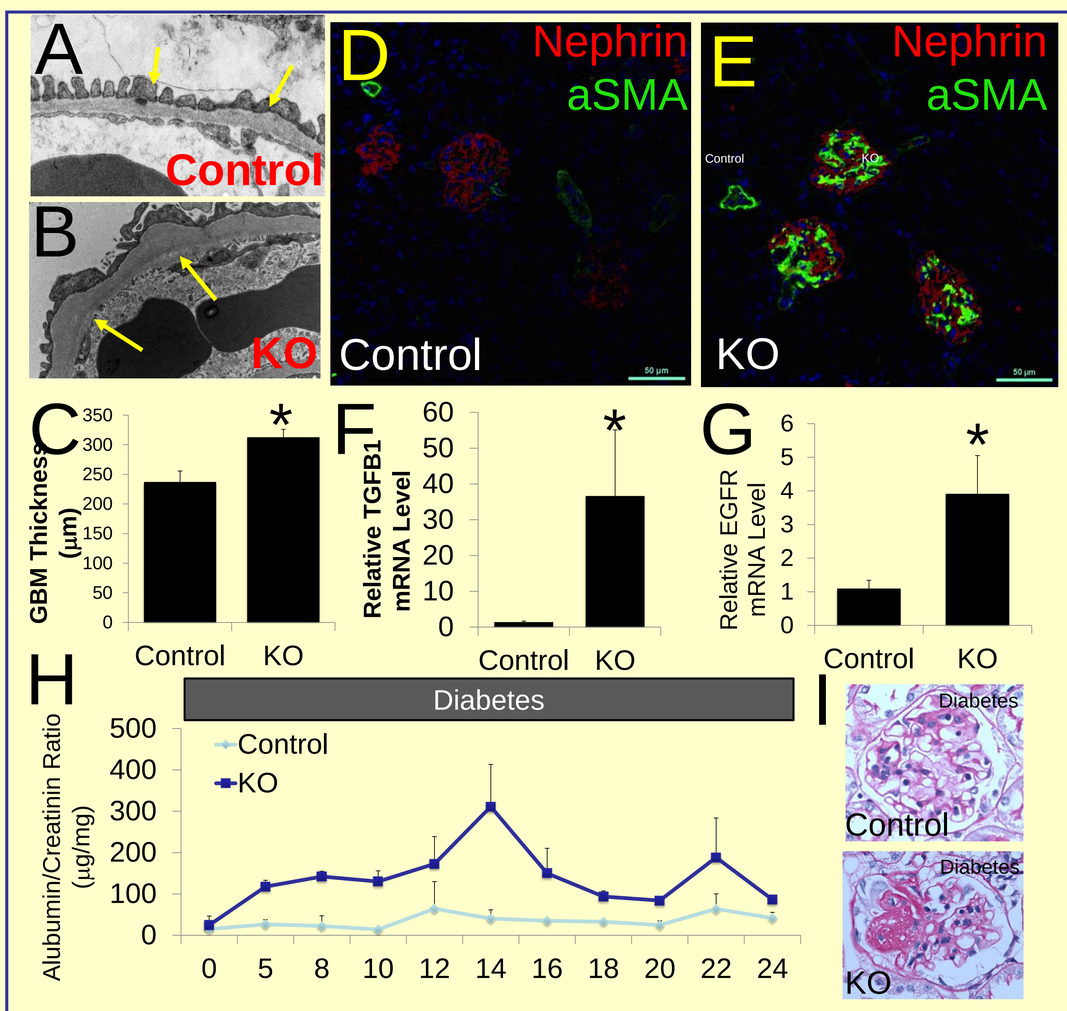

Fig 3. Podo-GPCR2 KO mice exhibit thickening of GBM (A-C) and mesangial activation (D, E), coming along with higher TGFB1 and EGFR expression in glomeruli (F,G). In a mouse of diabetes, podo-GPCR2 deficiency aggravates glomerular damage (I) and albuminuria (H).

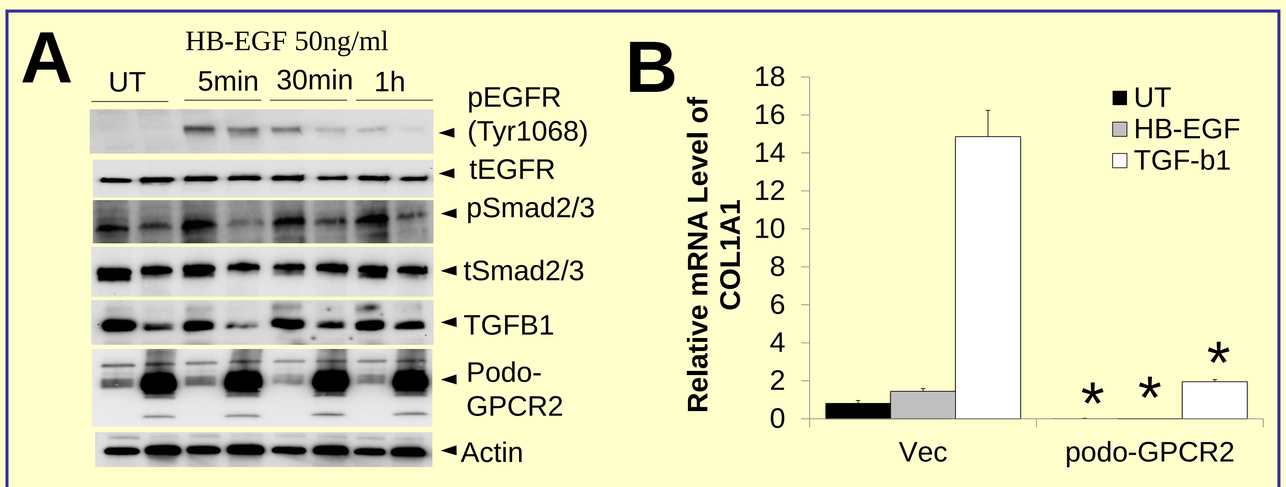

Fig 2. Podo-GPCR2 inhibits EGFR and TGF-β signaling (A), as well as their downstream fibrotic pathway (B).

CONCLUSIONS

Podo-GPCR2 and its downstream signaling pathways play a protective role in maintaining podocyte and glomerular health. We propose that manipulation of podocyte signaling via podo-GPCR2 may represent a novel, targeted therapeutic option for DN. We have iniated screening of small molecular libraries in order to deorphanize the receptor.

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