Establishment of a glomerular endothelial glycocalyx EXT1/HS deficiency model and its impact on normal kidney physiology

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Background:
The endothelial glycocalyx is a key determinant of vascular function, and studies have pointed to the glycosaminoglycan heparan sulphate (HS) as having a particularly important role in diabetic kidney disease. HS loss has been associated with kidney damage in human pilot studies. HS chains are assembled in the endoplasmic reticulum by the actions of a series of enzymes and exostosin (EXT) 1 is an essential and rate-limiting enzyme for HS chain polymerisation.

Aim
To examine in detail the role of glomerular endothelial HS in normal kidney physiology.

Methods
Eps Homology Domain (Ehd3) is highly and selectively expressed in glomerular endothelial cells in the kidney in comparison to other organs, tissues and cells, making Ehd3 a key tool in specifically targeting the glomerular endothelium. An inducible Ehd3 knock-in mouse model, expressing Cre-recombinase (Ehd3-eGFPCreERT_2) under the Ehd3 promoter, was generated to excise floxed genes specifically in glomerular endothelial cells. The experimental and littermate control mice were injected intraperitoneally with 75 mg tamoxifen/kg body weight for five consecutive days. Glomerular albumin permeability, FACS, lectin and immunofluorescence staining were used to fully characterise the mouse model.

Results
GFP staining, which denotes Cre recombinase expression, colocalises with CD31 staining, an endothelial marker, but not with nephrin, a podocyte marker. The colocalisation was confirmed by Pearson correlation coefficient analysis, establishing Cre recombinase in glomerular endothelial cells in the kidney. EXT1 gene expression was significantly reduced in FACS-glomerular endothelial cells. No significant change in EXT1 expression was observed in non-endothelial renal cells, pointing to the selective knockdown of EXT1 in glomerular endothelial cells. Lectin LEL staining, known to bind to N-acetylglucosamine ([GlcNAc]1-3) found on HS chains, shows reduced glomerular endothelial glycocalyx coverage but no change in other microvasculature, e.g. cardiac endothelial glycocalyx coverage, assessed by our novel confocal peak-to-peak analysis, confirming specificity to glomerular endothelial glycocalyx. Importantly, EXT1 knockdown leads to an increase in glomerular capillary albumin permeability, assessed by our sensitive ex vivo glomerular albumin permeability assay.

Conclusions
We have successfully established an in vivo animal model that allows glomerular endothelial cells to be specifically targeted in the kidney. Moreover, our study shows for the first time that genetic deletion of EXT1 selectively in glomerular endothelial cells reduced endothelial glycocalyx HS coverage and increased leakage of albumin in the glomerular capillaries. Strategies targeted at restoring glomerular endothelial HS could be of potential therapeutic value in diabetic kidney disease.