3D-Structured Illumination Microscopy reveals increased Claudin-5 localization to the slit diaphragm in effaced podocyte foot processes

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Objective

The upregulation of tight junction proteins at the podocyte slit diaphragm has been demonstrated as a mechanism in nephrotic syndrome (Farquhar et al., 2009). In rat glomeruli, Claudin-5-expression in podocytes has been observed (Koda et al., 2011), but the expression in human and mice remains unknown. Using 3D-structured illumination microscopy (3D-SIM) we wanted to study Claudin-5 expression in Minimal Change Disease (MCD) and Focal Segmental Glomerulosclerosis (FSGS) and in the nephrotoxic serum nephritis mouse model (NTS).

Methods

For investigation of human Claudin-5 expression, biopsies of MCD and FSGS patients and excess kidney tissue of partial nephrectomies as controls were used. In mouse experiments we compared mice injected with NTS to PBS-injected control mice. After immunofluorescence staining with antibodies against Nephrin, Claudin-5 and CD31, all sections were imaged with Confocal Laser Scanning Microscopy (C-LSM) and 3D-SIM. We measured the Claudin-5- and Nephrin-positive area of the slit diaphragms in 3D-SIM images and calculated the Claudin-5/Nephrin-ratio. The filtration slit density (FSD) was measured in humans with the previously published Podocyte Effacement Measurement Procedure (PEMP) (Siegerist et al., 2017).

Results

In healthy human and mouse samples Claudin-5 was co-localized with Nephrin in the podocyte slit diaphragm in a granulated pattern and continuously in arterioles of the vascular pole. Claudin-5 expression in glomerular capillary endothelium could only be observed in human. In 3D-SIM images of MCD and FSGS samples we found an increase of continuous Claudin-5-positive lines in areas with broadened podocyte foot processes. This upregulation could also be seen in highly effaced foot processes of NTS-injected mice. Claudin-5/Nephrin-ratio in human and mouse glomerulopathies was significantly higher compared to controls. In human samples a highly significant negative correlation between Claudin-5/Nephrin-ratio and FSD could be observed.

Conclusion

3D-SIM and measurement of the Claudin-5/Nephrin-ratio reveal Claudin-5 localization to effaced parts of the slit diaphragm of MCD and FSGS patients as well as NTS mice. Taken together, herein we show that Claudin-5-localization to the slit diaphragm in proteinuric kidney diseases is tightly correlated with the degree of podocyte foot process effacement.