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Super-resolution Imaging of the Kidney Glomerulus in Health and Disease Conditions

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Introduction & Aim: The kidney glomerulus is a capillary tuft where urine begins to form by ultrafiltration. This glomerular filter allows water and small solutes to pass freely, while restricting cells and proteins larger than 65 kD from entering the urinary space. The glomerular filtration barrier (GFB) is composed of three layers: the fenestrated endothelial cell layer, the glomerular basement membrane (GBM) layer and the podocytes with interdigitating foot processes. Slit diaphragms, small rod-like structures bridging the space between adjacent foot processes, are important for podocyte function[1]. Glomerular diseases lead to filtration barrier failure, causing protein leakage into the urine (proteinuria) that is often followed by eventual kidney failure. Proteinuria is usually accompanied by podocyte foot process effacement (FPE), a major change in podocyte shape characterized by simplification of the foot processes and direct adherence of the podocyte cell body to the capillary wall. This is a major shift in the podocyte actin cytoskeleton, and it occurs in almost all glomerular diseases regardless of the etiology. However, the exact causes of podocyte FPE and its relationship with proteinuria are unclear [2, 3].

Because the diffraction limit of conventional light microscopy restricts the resolution of structures smaller than 200 nm, such as podocyte foot processes and slit diaphragms, imaging the podocyte is challenging. Super-resolution imaging breaks the diffraction limit and allows for imaging structures on the nanoscale level. One super-resolution approach, Stochastic Optical Reconstruction Microscopy (STORM), relies on single molecule imaging to break the diffraction limit with a resolution of ~10-20 nm [4]. Previously, we developed a method that allowed us to use STORM to image the GFB's components in kidney tissue sections [5]. Now we have applied this method to study the molecular changes that accompaniy FPE and podocyte injury.

Methods: We developed a simplified method that supports multi-color super-resolution imaging using STORM and allows for easy and direct imaging of kidney sections. We used this method to study the molecular changes that accompany FPE in mouse models of glomerular disease as well as in human diseased kidney biopsy samples. For STORM imaging we used a wide array of antibodies that recognize key podocyte proteins in the foot processes and slit diaphragms. We used the STORM-imaged samples to generate deep-etch platinum replica electron micrograph images in order to combine the molecular and morphological information in the same kidney sample in what we call STORM-EM correlation [5].

In order to study the podocyte cytoskeleton *in vivo*, we developed a new method of cytoskeletal preservation that allowed us to directly image actin and actin-associated molecules in kidney glomeruli. A combination of 2D and 3D super resolution imaging enabled us to study the architecture of the podocyte in normal and diseased states.

Results: Using STORM on healthy kidney sections, we imaged slit diaphragm proteins nephrin, podocin and CD2AP. Using antibodies against the actin-bundling proteins synaptopodin and α-actinin-4, we could label individual podocyte foot processes. Using antibodies to myosin IIA, the major myosin II motor in the podocytes, we found that myosin IIA is present in the podocyte cell body and the major processes but not in the foot processes, both in mouse as well as in human podocytes (Fig. 1. A, A'; C, C' & C'').

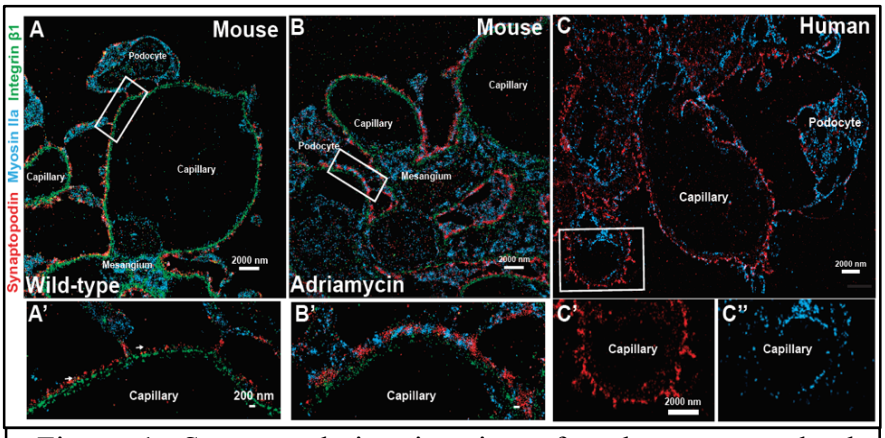

Figure 1. Super-resolution imaging of podocytes reveals the spatial distribution of myosin IIA in healthy and injured podocytes.

Using mouse podocyte injury models, we found apical translocation of slit diaphragm proteins after FPE. While the synaptopodin and α-actinin-4 clusters did not change upon injury, there was robust recruitment of myosin IIA to the basal aspect of the podocytes. Myosin IIA stained in alternating stripes with synaptopodin and α-actinin-4, suggesting the formation of thick contractile actin fibers after podocyte FPE (Fig. 1.A, A' ; B & B'). 3D super-resolution imaging of cytoskeleton-stabilized glomeruli confirmed that myosin IIA is part of the sarcomere-like structures adjacent to the GBM in the different injury models. This is in contrast to the normal podocytes, where myosin IIA was present only in the major processes.

Discussion & Conclusions: Using a new way of imaging the podocyte cytoskeleton in combination with novel super resolution microscopic methods, we visualized podocyte foot processes in both healthy and diseased conditions. Our data indicate that in the normal podocyte, contractile actin cables are present in major processes, while the actin filaments in the foot processes are non-contractile. After foot process effacement, contractile actin filaments form at the base of effaced podocytes and arrange in a sarcomere-like contractile network adjacent to the GBM.

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