Neuronal proteins are novel components of podocyte major processes and their expression in glomerular crescents supports their role in crescent formation

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The podocyte has a central role in the glomerular filtration barrier typified by a sophisticated morphology of highly organized primary (major) and secondary (foot) processes. The molecular makeup of foot processes is well characterized, but that of major processes is poorly known. Previously, we profiled the glomerular transcriptome through large-scale sequencing and microarray profiling. Unexpectedly, the survey found expression of three neuronal proteins (Huntingtin interacting protein 1 (Hip1), neurofascin (Nfasc), and olfactomedin-like 2a (Olfml2a)), all enriched in the glomerulus. These proteins were expressed exclusively by podocytes, wherein they localized to major processes as verified by RT–PCR, western blotting, immunofluorescence, and immunoelectron microscopy. During podocyte development, these proteins colocalized with vimentin, confirming their association with major processes. Using immunohistochemistry, we found coexpression of Hip1 and Olfml2a along with the recognized podocyte markers synaptopodin and Pdlim2 in glomerular crescents of human kidneys, indicating the presence of podocytes in these lesions. Thus, three neuronal proteins are highly expressed in podocyte major process. Using these new markers we found that podocytes contribute to the formation of glomerular crescents.

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The glomerulus comprises a tangled network of capillaries surrounded by a urinary space and Bowman's capsule. The filtration of primary urine occurs through the capillary wall of the glomerulus. This filtration barrier is essentially composed of three layers: (1) the glomerular basement membrane, which serves as a foundation and support; (2) fenestrated endothelial cells, located on the inside; and (3) visceral epithelial cells (podocytes), located on the outside of the glomerular capillary.[1]

The podocyte is a highly specialized epithelial cell with a remarkably sophisticated architecture.[2] It has a prominent cell body with large cytoplasmic projections named major processes, which further extend into smaller processes known as foot processes. The foot processes interdigitate as they wrap around and envelope the capillaries. The adjacent foot processes interact and are connected by a specialized cell–cell junction termed the slit diaphragm.[2–4] Mutations in slit diaphragm proteins cause inherited forms of proteinuria,[1] which underlines the importance of these structures in the filtration barrier.

Podocytes are dynamic cells with a prominent cytoskeleton that can effectively respond to changes in their extracellular environment.[5] This cytoskeletal machinery is vital for a sustained kidney filtration, as defects in cytoskeleton-regulating proteins of podocyte foot processes result in proteinuria.[5] Bundles of microtubules and intermediate filaments support major processes, whereas the cytoskeleton of foot processes is composed mainly of actin filaments.[2,5,6] This feature confirms an obvious morphological difference between these two structures, thus contributing to their molecular and functional divergence. The molecular composition of foot process is rather well characterized.[4] This includes basal, slit diaphragm, and apical plasma membrane protein complexes connected by a number of linker proteins and actin cytoskeleton.[4,5] In contrast to this, the molecular nature of major processes is still poorly understood. Besides microtubules and the intermediate filament protein

vimentin,[2,6] the components that form these cellular projections are largely unknown.

We have previously identified several highly glomerulus-enriched transcripts using large-scale sequencing and microarray profiling.[7] In this study, we are characterizing further three of these glomerular transcripts. All three proteins in the kidney are expressed only by podocytes, wherein they localize to major processes. The specific expression in podocyte major processes suggests that they have a dedicated role in the formation of these peculiar cellular projections. Furthermore, we use these novel podocyte markers to demonstrate that podocytes are present in glomerular crescents, an issue that has been a matter of controversy.

RESULTS

Previously, we have profiled the glomerular transcriptome through large-scale sequencing and microarray profiling.[7] In this approach, we chose three transcripts, Huntingtin interacting protein 1 (Hip1), neurofascin (Nfasc), and olfactomedin-like 2a (Olfml2a), which were enriched in the glomerulus, for a more detailed analysis. These genes were chosen for further studies because their biology was poorly understood, and their role and expression in the kidney were completely uncharacterized.

Expression of Hip1 in mature and developing kidney

First, we analyzed the expression of Hip1 in the kidney through reverse transcriptase–PCR (RT–PCR). The PCR product for Hip1 was amplified in glomeruli, whereas no expression was detected in the rest of the kidney lacking glomeruli (Figure 1). The control experiment with glyceraldehyde 3–phosphate dehydrogenase showed strong signal in both lanes, whereas podocin, a known glomerulus-specific transcript, gave significantly stronger signal in the glomerular fraction. Next, we generated a polyclonal antibody against the human Hip1 protein. By using western blotting of human kidney lysates, our anti-Hip1 antibody detected a band of expected size (~115–kDa) in the glomerulus as opposed to the rest of the kidney, which appeared completely negative (Figure 1). A similar expression pattern was observed for nephrin, a known podocyte-specific protein. The control experiment with β-actin antibody showed clear signal in both lysates.

In immunofluorescence, Hip1 antibody showed intense staining in the glomeruli, and no signal was detected in the extraglomerular areas (Figures 2a and 3a). Double staining with foot process marker nephrin showed that Hip1 was located at the side of the urinary space of nephrin (Figure 3b and c). This indicated expression in podocytes. Occasionally, some overlapping reactivity with nephrin was observed (arrow, Figure 3c). Double labeling with vimentin showed that Hip1 colocalized with this major process marker (Figure 3d). Immunoelectron microscopy was used to confirm the subcellular localization of Hip1. Gold label for Hip1 was observed mostly in major processes (Figure 4a). Quantitatively, we detected a total of 176 gold labels in glomeruli, of which 96 (55%) were located in major processes. In addition, few labels were occasionally detected in foot processes (Figure 4a). No significant signal for Hip1 was found outside podocytes.

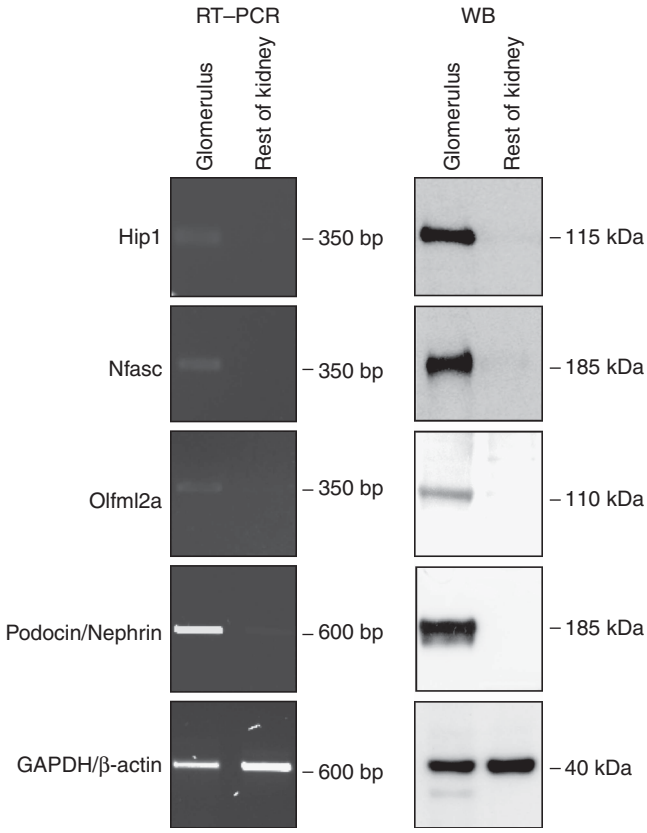

Figure 1 | Expression of Huntingtin interacting protein 1 (Hip1), neurofascin (Nfasc), and olfactomedin-like 2a (Olfml2a) in the kidney as analyzed with reverse transcriptase–PCR (RT–PCR) and western blotting (WB). We compared the expression in the glomerular fraction with the rest of the kidney depleted of glomeruli. The transcripts are all amplified in the glomeruli, whereas no (or very weak) expression is detected in the rest of the kidney. The control reaction with podocin, a known glomerulus-specific transcript, is detected in the glomerular fraction, whereas glyceraldehyde 3–phosphate dehydrogenase (GAPDH) is detected in both the glomerular fractions and the rest of the kidney fractions. In WB, the antibodies directed against Hip1, Nfasc, and Olfml2a show immunoreactivity in the glomerular fraction, whereas no (or very weak) band is visible in the rest of the kidney lysate. The sizes of proteins detected are as follows: Hip1 ~115 kDa, Nfasc ~185 kDa, and Olfml2a ~110 kDa. The control reaction with nephrin antibody recognizes the protein with the expected size (~185 kDa) in the glomerular fraction. β-Actin antibody, used as a loading control, recognizes a protein ~40 kDa in both lanes.

To confirm the association of Hip1 with major processes, we investigated the expression of Hip1 during podocyte development. The two earliest stages of development, vesicle and S-shaped stages, showed no Hip1 expression (data not shown). In the capillary stage glomerulus, a stage wherein the formation of major processes begins, immunoreactivity for Hip1 was first detected (Figure 5a and b). At this stage,