FR-PO706

mTOR-Dependent Autophagy Regulates Slit Diaphragm Density in Podocyte-Like Drosophila Nephrocytes

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Background: Both mTor signaling and autophagy are important modulators of podocyte homeostasis, regeneration and aging, and have been implicated in glomerular diseases. However, the mechanistic role of these pathways for the glomerular filtration barrier remains poorly understood.

Methods: Drosophila presents a well-established model to study mTor signaling and autophagy with versatile genetic tools like tissue-specific RNAi-mediated knockdown or overexpression of wild type or functionally modified proteins. We used Drosophila nephrocytes as a podocyte model to investigate the connection of mTor signaling and autophagy and the homeostasis and maintenance of the nephrocyte ultrastructure and function as a storage kidney.

Results: We found that in the podocyte-like nephrocytes, mTor signaling positively controls cell size, survival and the extent of the subcortical actin network. Surprisingly, the inhibition of mTor signaling resulted in increased slit diaphragm spacing, whereas gain-of-function of mTor signaling did not affect slit diaphragm spacing, suggesting that additional cues limit the maximal density. Interestingly, both activation and inhibition of mTor signaling led to decreased nephrocyte function indicating that a fine balance of signaling activity is needed for proper function. We showed that basal autophagy in nephrocytes is required for survival and limits expression of nephrin (neph), but does not directly affect slit diaphragm formation or endocytic activity. However, using a genetic rescue approach, we demonstrated that excessive autophagy associated with loss of mTor function is primarily responsible for slit diaphragm misspacing.

Conclusions: Utilizing the Drosophila nephrocyte model to study the mechanistic role of mTor signaling and autophagy for the glomerular filtration barrier, we discovered a direct regulatory impact on the slit diaphragm architecture.

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Podocytes Respond to Mechanical Forces to Spatially Orient Their Processes on Glomerular Capillaries

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Background: It has recently been proposed by our group that the main role of podocyte foot processes is to counteract forces resulting from filtration pressure in order to compress the basement membrane, thereby optimizing sieving properties. We here expand on these models by studying the role of spatial orientation of foot processes on glomerular capillaries.

Methods: We apply novel imaging protocols which allow for confocal in-situ 3D imaging of intact glomerular capillaries at a resolution sufficient to resolve foot processes. This allows for analyzing several thousands of podocyte processes and quantitatively determine their spatial orientation with regards to the capillary orientation.

Results: We report the novel finding that podocyte processes display a non-random distribution on glomerular capillaries, which is lost in different types of kidney disease. This finding suggests that the orientation of foot processes is important for the function of the filtration barrier. We further observe a more prominent orientation preference in elongated and more cylindrical capillary segments, where the difference between circumferential and longitudinal wall stress is highest. This strongly indicates that podocytes possess a machinery to regulate and maintain the spatial orientation of their processes based on the forces acting on them.

Conclusions: We consider the various forces that foot processes are exposed to and conclude that the observed orientation of foot processes in parallel with the orientation axis of capillaries is likely to ensure that slit diaphragm molecules (e.g. nephrin, nephi) are preferably aligned in parallel with the axis of highest wall stress. This adds further evidence to the theory that foot processes and the slit diaphragm act to mechanically counteract lateral wall stress, but also possesses a mechanosensory machinery for maintaining orientation on capillaries.

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The Redundant and Unique Interactors of YAP and TAZ in Podocyte Homeostasis and Disease

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Background: The two effector proteins of the Hippo signaling pathway, YAP and TAZ, play a pivotal role in the cellular homeostasis of podocytes and the pathogenesis of focal segmental glomerulosclerosis (FSGS). The two proteins share 46% amino acid identity and are often regarded as homolog proteins. However, the podocyte-specific knockout of TAZ results in milder proteinuria and FSGS than the podocyte-specific YAP knockout. We aim to unravel the unique and redundant functions of YAP and TAZ in podocytes by identifying podocyte-specific interactors in health and disease.

Methods: We used immortalized podocytes (hMPs) and co-immunoprecipitation YAP or TAZ with specific antibodies. To overcome drawbacks resulting from these two proteins’ homology, we generated hsMPs expressing FLAG-tagged YAP or TAZ using TALEN-based genome editing. For in vivo purposes, we generated transgenic mice expressing 3xFLAG.YAP and TAZ.3xFLAG using CRISPR/Cas9. YAP or TAZ were pulled down in vitro from podocytes and in vivo from isolated glomeruli, followed by mass spectrometry analysis. Further, we generated YAP or TAZ podocyte-specific knockout mice as well as double knockouts, to shed light on common, distinct, and possible compensatory roles of YAP and TAZ in podocyte disease.

Results: Within the interactome analyses of the hsMPs, we identified shared and non-shared interacting proteins between YAP and TAZ. Of all interactors, 60% overlapped for both, while 40% were unique. These results comprise known and novel interactors, including Fat1, Actn4, or Nephi. Interactome analysis of the nuclear fraction identified specifically nuclear interactors of YAP and TAZ, including known transcription factors (e.g. TEADs) and also ~30% of new nuclear interacting proteins. Currently, we are investigating the mechanistic role of novel candidates in FSGS while we are working on the in vivo models.

Conclusions: YAP and TAZ are critical proteins in the podocyte’s homeostasis with divergent functions and interactors. Overlapping and distinct candidates identified in interactome analyses conducted both in vitro and in vivo suggest both shared and unique podocyte-specific functions. These novel unique and shared interactors of YAP and TAZ in podocytes will help to understand the specific impact of YAP and TAZ in the development of FSGS and recovery from podocyte injury.

FR-PO709

Mutation in Transient Receptor Potential Cation Channel Subfamily C Member 6 (TRPC6) Regulates Yes-Associated Protein 1 (YAP1) Phosphorylation

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Background: Transient receptor potential cation channel subfamily C member 6 (TRPC6), is a cation channel associated with hereditary focal segmental glomerulosclerosis. Angiotensin II (Ang II) activates TRPC6 and its downstream signalling. We investigated signalling regulated by TRPC6 in podocytes to understand the disease pathogenesis.

Methods: We developed conditionally immortalized wild-type and TRPC6 knockout (T6K) podocytes. Lentiviral transduction was used to express GFP-tagged TRPC6 in T6K cells. Phosphoproteomic analysis was used to identify changes in signalling pathways mediated by knocking out TRPC6. Immunoblotting and quantitative reverse transcription PCR (RT-qPCR) were applied for validation.

Results: T6K podocytes showed increased phosphorylation of Yes-associated protein (YAP) compared with the control. Ang II treatment increased YAP phosphorylation in control podocytes but not in T6K. RT-qPCR showed decreased expression of connective tissue growth factor (CTGF) and cellular communication Network Factor 1 (CCN1). YAP

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral; PO - Poster; PUB - Publication Only Underline represents presenting author.