ENHANCED EPITHELIAL-TO-MESENCHYMAL TRANSITION ASSOCIATED WITH LYSOSOME DYSFUNCTION IN PODOCYTES: ROLE OF P62/SEQUESTOSOME 1 AS A SIGNALING HUB

Guangbi Li, Cai-Xia Li, Min Xia, Joseph K. Ritter, Todd W. B. Gehr, Krishna Boini, Pin-Lan Li
Department of Pharmacology, School of Medicine, Virginia Commonwealth University, Richmond, VA 23298

BACKGROUND AND AIMS

> One of the major hypotheses for the mechanism leading to podocyte injury proposes that injured podocytes undergo epithelial-to-mesenchymal transition (EMT), which may lead to disruption of its delicate architecture, impairing glomerular filtration membrane function and triggering glomerular injury and sclerosis.

> Previous studies have demonstrated that podocyte differentiation and maturation are highly dependent upon normal autophagy.

> We have recently reported that the regulation of lysosome function importantly contributes to autophagic flux or autophagy maturation in mouse podocytes and that lysosome dysfunction or injury due to derangement of its regulatory mechanisms resulted in deficiency of autophagic flux and consequent EMT.

So far, it remains poorly understood how podocyte EMT is activated and regulated in response to different pathological stimuli. The present study tested a hypothesis that lysosome dysfunction may induce podocyte EMT due to the accumulation of autophagosomes, p62 aggregation, and activation of associated signaling pathways.

METHODS

Cell culture

Conditionally immortalized mouse podocytes were cultured on collagen I-coated flasks in RPMI 1640 medium supplemented with recombinant mouse IFN-γ at 33°C. After differentiated at 37°C for 10-14 days without IFN-γ, podocytes were used for proposed experiments.

Western blot analysis

Homogenates from cultured podocytes were prepared using sucrose buffer containing protease inhibitors and phosphatase inhibitors. Then proteins were boiled, subjected to SDS-PAGE, transferred onto a PVDF membrane, and blocked by TBS with dry milk or BSA. After the membrane was probed with antibodies, the immunoreactive bands were detected by chemiluminescence methods and visualized on films.

Immunofluorescence microscopy

Cultured podocytes were used to detect the expressions of podocin, ZO-1, P-cadherin, α-SMA, and FSP-1 in podocytes where Alex-488-conjugated second antibody was used to label podocin, and Alex-555 was used to label ZO-1, P-cadherin, α-SMA, and FSP-1.

siRNA transfection

Podocytes were serum starved for 12 h and then transfected with CDK1 siRNA or scrambled siRNA using Lipofectamine 2000. After 24 h of incubation at 37°C, the medium was changed, and Baf (5 nM) was added into the medium for indicated time span in different protocols.

CONCLUSIONS

> Lysosome dysfunction induces the accumulation of autophagosomes and p62, leading to EMT in podocytes.

> Inhibition of the autophagosome formation attenuates EMT induced by lysosome dysfunction in podocytes.

> Enhanced EMT by lysosome dysfunction is attributed to reduction of CDK1-dependent phosphorylation of p62 vs total p62.

Phosphorylated vs. Total p62 was Reduced during Accumulation of Autophagosomes by Lysosome Dysfunction

Figure 5. To test the mechanism by which podocyte EMT is enhanced by autophagosome accumulation, we tested the effects of RO-3306 (RO), a CDK1 inhibitor and its siRNA, given that CDK1-mediated phosphorylation of p62 is required for participation in cell differentiation and homogenization. It was found that RO-3306 produced similar effects to similar bafilomycin, namely, decreased P-cad with increased α-SMA in podocytes. In the presence of RO-3306, bafilomycin had not further effects (A, B). CDK1 siRNA led to a significant reduction on podocyte EMT (C, D).

Podocyte EMT Enhanced by Lysosome Dysfunction was due to Accumulation of Autophagosomes

Figure 6. We also found that RO-3306 markedly decreased phosphorylated p62 vs total p62 level in podocytes no matter whether cells were stimulated by bafilomycin, although it had no effect on the level of phosphorylated p62. Similarly, CDK1 siRNA was found to reduce the ratio of phosphorylated p62 vs total p62. It appears that the relative reduction of phosphorylated p62 vs. total p62 is critically involved in the control of EMT.