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Inhibiting Core Fucosylation of Megalin and TGF-β receptor II Protects Against Proximal Tubular Epithelial Cell Injury Caused by Albumin

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Background: Albuminuria is a strong risk factor for renal interstitial injury that impairs proximal tubular epithelial
cells (PTECs) through both albumin endocytosis and non-endocytosis mechanisms in diabetic nephropathy. Megalin is essential for albumin endocytosis mechanism whereas transforming growth factor-β1 receptor II(TGFβRII) is responsible for albumin non-endocytosis mechanism. We try to find a common target to inhibit both endocytic and non-endocytic injury pathway.

Methods: Both megalin and TGFβRII are glycoproteins modified by core fucosylation. We investigated the role of core fucosylation in albumin induced-injury to HK-2 cells. RNAi was performed to suppress expression of megalin, TGFβRII and FUT8 genes. FACS and confocal microscopy were performed to observe effect of siRNAs on endocytosis of bovine serum albumin (BSA). Western blot, ELISA and FACS were performed to determine changes in levels of megalin, TGFβRII, p-Smad2/3, monocyte chemotactic protein 1 (MCP-1), nuclear factor-kB (NF-κB), reactive oxygen species (ROS), TGF-β1, Fibronectin, Collagen I and apoptosis after incubation with BSA for different time.

Results: After 4-h incubation with BSA, albumin endocytosis increased, followed by upregulation of ROS, MCP-1 and NF-κB. Inhibiting core fucosylation of megalin suppressed endocytosis of BSA, subsequently, it suppressed described endocytic injury above. In contrast, after 24-h incubation with BSA, expression of megalin decreased to 55%, while that of TGFβRII increased to 1.5-fold of its original level. At the 24-h time point, inflammation and oxidative stress were weaker than that at the 4-h time point, the expression of fibronectin and collagen I was significantly upregulated. Inhibiting core fucosylation of TGFβRII, suppressed activation of TGFβ/TGFβRII/smad2/3 signaling pathway after incubation for 24 h, followed by downregulation of fibronectin and collagen I.

Conclusion: Inhibiting core fucosylation of megalin and TGFβRII could inhibit albumin endocytosis and non-endocytosis injury to PTECs simultaneously, regulating core fucosylation is likely an effective strategy for preventing against albumin-induced injury to PTECs in diabetic nephropathy.

Keywords: Albuminuria, core fucosylation, megalin, PTEC, TGF-β receptor II