MEGALIN/CUBILIN-LYSOSOME-MEDIATED ALBUMIN REABSORPTION IS INVOLVED IN THE TUBULAR CELL ACTIVATION OF NLRP3 INFLAMMASOME AND TUBULOINTERSTITIAL INFLAMMATION

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Introduction and Aims: Albuminuria contributes to the development and progression of chronic kidney disease (CKD) by inducing tubulointerstitial inflammation (TI) and fibrosis. However, the exact mechanisms of TI in response to albuminuria are unresolved. We previously demonstrated that NLRP3 and inflammasomes mediate albumin-induced lesions in tubular cells. Here, we further investigated the role of endocytic receptors and lysosome rupture in NLRP3 inflammasome activation.

Methods: We established an albumin-overload induced rat nephropathy model. The adult male Wistar rats that were uninephrectomized or sham operated under anesthesia 5 days before starting BSA injection. In vitro, tubular epithelial cell line (HK-2) was cultured with or without megalin/cubilin gene siRNA transfection and then stimulated with BSA for different time durations (6h, 12h, 24h, 48h) and concentrations (5, 10, 20, 40 mg/ml). Cell lysates and supernatants were collected and determined by western blotting and ELISA. Cathepsin B and Cathepsin D with or without their inhibitors were detected by western blotting and immunofluorescence staining.

Results: The priming and activation signals for inflammasome complex formation were evoked simultaneously by albumin excess in tubular epithelial cells. The former signal was dependent on albumin-triggered NF-kappa B pathway activation. This process is mediated by the endocytic receptor, megalin and cubilin. However, the silencing of megalin or cubilin inhibited the albumin-induced NLRP3 signal. Notably, subsequent lysosome rupture and the corresponding release of lysosomal hydrolases, especially Cathepsin B, were observed in TECs exposed to albumin. Cathepsin B release and distribution is essential for NLRP3 signal activation, and inhibitors of Cathepsin B suppressed the NLRP3 signal in TECs.

Conclusions: Taken together, our findings suggest that megalin/cubilin and lysosome rupture are involved in albumin-induced tubular injury and TI. This study provides novel insights into albuminuria-induced TI and implicates the active control of albuminuria as a critical strategy to halt the progression of CKD.