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Conclusion: MMT contributes to the chronic progression of renal allograft rejection. MMT is derived from bone marrow macrophages and is regulated via a Smad3-dependent mechanism.

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0043
Long Noncoding RNA-7949 Regulates Macrophage Activation in Renal Inflammation via the TLR4/NF-KB Pathway

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Objective: Increasing evidence shows that long noncoding RNAs (LncRNAs) play a role in renal inflammation. By using advanced RNA-sequencing technique, we previously identified lncRNA_7949 is markedly upregulated in unilateral ureteral obstruction (UUO) mouse model. In this study, we aimed to investigate the regulatory mechanisms and functional role of lncRNA_7949 in renal inflammation.
Methods: LncRNA_7949 in UUO kidney was quantitatively analyzed by real-time PCR and its full sequence was obtained by a rapid amplification of cDNA ends (RACE). Expression patterns and regulatory mechanisms of lncRNA_7949 were investigated in mesangial cells (MC), tubular epithelial cells (TEC), and macrophages by using inhibitors to TLR4 and NF-kB and by chromatin immunoprecipitation (ChIP) assay. The functional role of lncRNA_7949 in renal inflammation was determined in macrophages with knockdown of lncRNA_7949.
Results: LncRNA_7949 was dramatically upregulated in UUO kidney with prominent macrophage accumulation and fibrosis. Interestingly, LncRNA_7949 was expressed by mouse macrophage cell line (RAW264.7) and bone marrow-derived macrophages but not by TEC and MC. LncRNA_7949 was selectively induced in macrophages by LPS, but not by TGF-β1, IL-1β, or TNF-α. Moreover, lncRNA_7949 was positively regulated by the TLR4-NF-kB pathway since LPS-induced lncRNA_7949 was blocked by a TLR4 inhibitor (CLI-095) and by a NF-kB inhibitor (Bay11-7985). ChIP assay identified the interaction between NF-kB p65 and lncRNA_7949 promoter, indicating that lncRNA_7949 may be a transcriptional target of NF-kB p65. Importantly, knockdown of lncRNAs_7949 with siRNA blocked LPS-induced MCP-1 but not IL-6 and TNF-α expression.
Conclusion: LncRNA_7949 is a macrophage-specific lncRNA and is positively regulated by the TLR4/NF-kB signaling pathway. lncRNA_7949 may regulate macrophage-dependent renal inflammation by inducing MCP-1 transcription. Thus, targeting lncRNA-7949 may represent a novel and specific therapy for kidney inflammatory disease.

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0049
ADH-induced Stimulation of Na-activated K Channels is Responsible for Maintaining Basolateral K Conductance of the Thick Ascending Limb (TAL) in EAST/Sesame Syndrome

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Objective: The aim of the present study is to test whether antidiuretic hormone (ADH)-induced stimulation of the Na-activated 80—150 pS K channel is responsible for compensating the lost function of Kcnj10 in the thick ascending limb (TAL) of subjects with EAST syndrome.
Methods: Patch-clamp technique, immunostaining, Western blot.
Results: (1) Immunostaining and Western blot show that the AQP2 expression in the knockout mice was higher than those of WT and Kcnj10$^{+/-}$ mice (Western blot: 250 ± 50% of the WT control, n = 3). (2) The treatment of the TAL in Kcnj10$^{-/-}$ mice with 100 nM AVP significantly caused a hyperpolarization of K reversal potential (an index of the membrane potential from 62 ± 5 mV to 73 ± 6 mV, n = 4). (3) The application of AVP increased the 80—150 K channel activity from 1.05 ± 0.15 to 1.70 ± 0.18 (n = 5); the inhibition of PKA with H89 and AVP in sequence, NP_o: H-89, 1.05 ± 0.15; H89+AVP, 1.03 ± 0.16 (n = 5); a V2 receptor (V2R) antagonist (tolvaptan, 0.92 ± 0.16, tolvaptan+AVP, 0.96 ± 0.15, n = 5). (4) The water restriction for 24 hours significantly increased the probability of finding the 80—150 pS K channel in the TAL from 4% to 14.4%, and the channel activity (NP_o: control 0.59 ± 0.20, water restriction, 0.92 ± 0.18). (5) The results showed that AVP activated the 10 pS Cl channel in the TAL, and adding Ba^{2+} to inhibit the basolateral K channels in a cell-attached mode also reduced Cl channel activity and decreased NP_o from 1.65 ± 0.19 to 0.2 ± 0.10 (n = 5).
Conclusion: **(1)** ADH stimulates the 80—150 pS K channel in the TAL was mediated by V2R and PKA-dependent pathway. **(2)** The ADH-induced stimulation of K channels is responsible for compensating lost function of Kcnj10 thereby rescuing the basolateral K conductance which is essential for the transport function in the TAL.

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0053
Megalin/cubulin-lysosome-mediated Albumin Reabsorption is Involved in Tubular Cell Activation of NLRP3 Inflammasome and Tubulointerstitial Inflammation

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Objective: 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 (6 h, 12 h, 24 h, 48 h) 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-κB 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.
Conclusion: Taken together, our findings suggest that megalin/cubilin and lysosome rupture are involved in albumin-triggered 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.

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0054
Elevated Serum Platelet Microparticles Contribute to Aorta Endothelial Injury: A Potential New Mechanism for Atherosclerosis in Diabetes

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Objective: An early complication in diabetes is the development of endothelial dysfunction, characterized by altered endothelial cell function, impaired nitric oxide (NO) bioavailability and accelerated thrombosis. Platelet microparticles