Conclusion: miR-214 expression in children with CKD increased significantly, and miR-214 expression was positively correlated with the severity of proteinuria. It suggested that miR-214 in kidney is associated with tubular injury during proteinuria.

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0329
Reactive Oxygen Species-initiated Autophagy Opposes Aldosterone-induced Podocyte Injury

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Objective: To investigate the role of autophagy in Aldo-induced podocyte damage and the underlying mechanism.
Methods: Mouse podocytes were treated with Aldo or H_2O_2 in the presence or absence of 3-methyladenine (3-MA) and NAC. Cell apoptosis was investigated by detecting Annexin V conjugates, apoptotic bodies, caspase-3 activity, and the alteration of podocyte protein nephrin. Autophagy was evaluated by measuring the expressions of LC3, p62, beclin-1 and Atg5 and evaluating the extent of autophagic flux using ad-mRFP-GFP staining.
Results: Aldo (10^{-7} mol/L) induced podocyte apoptosis, autophagy and downregulation of nephrin protein in a time-dependent manner. After 24 hours of Aldo treatment, the apoptosis rate was increased significantly ($P < 0.05$) as compared with control group. After 48 hours of stimulation, the apoptosis rate was further increased by 41.8%. Nephrin protein expression was significantly decreased ($P < 0.05$) with Aldo for 24 hours. Typical autophagosomes appeared in podocytes 24 hours after aldosterone treatment. Transmission electron microscopy and Ad-mRFP-GFP-LC3 showed that Aldo induced the formation of autophagosome bodies. Moreover, western blot analysis demonstrated that the autophagy marker protein LC3-I (18 kDa) was converted to LC3-II (16 kDa), p62 was decreased and Atg5 was increased in a time-dependent manner after cells were treated with Aldo. Aldo-induced apoptosis was further promoted by the inhibition of autophagy via 3-MA and Atg5 siRNA pretreatment. Moreover, Aldo time-dependently increased ROS generation, and H_2O_2 (10^{-4} mol/L) application remarkably elevated podocyte autophagy. After treatment with NAC, the autophagy induced by Aldo or H_2O_2 was markedly attenuated, suggesting a key role of ROS in mediating the autophagy formation in podocytes. Inhibition of ROS also could lessen Aldo-induced podocyte injury.
Conclusion: ROS-triggered autophagy played a protective role against Aldo-induced podocyte injury, and targeting autophagy in podocytes may represent a new therapeutic strategy for the treatment of podocytopathy.

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0331
Peroxisome Proliferator-activated Receptor-gamma Agonists Inhibit TGF-β1-induced Epithelial-Mesenchymal Transition in Renal Proximal Tubular Epithelial Cells

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Background: Emerging evidence suggested epithelial-mesenchymal transition (EMT) plays an essential role in the pathogenesis of renal tubulointerstitial fibrosis (TIF) which is the common pathway of end-stage renal failure (ESRF). TGF-β1-induced EMT is a key contributor to fibrotic scar in diabetic nephropathy by statistical analysis. Therefore, inhibition of EMT can be an important therapeutic strategy to inhibit kidney fibrosis. Studies suggest that peroxisome proliferator-activated receptor γ (PPAR-γ) agonists protect kidney frbrosis in vivo and vitro which promote us to explore the effect on TGF-β1-induced EMT.
Methods: Treatment with 10 ng/ml TGF-β1 for 3 days induced EMT. PPAR-γ agonists (Refine Gundam Zeta and 15-deoxyprostaglandin J2) or inhibitor T007 were used to treat culture cells before the treatment of TGF-β1. Changes in morphology were observed, expression of E-cadherin, snail and α-smooth muscle actin (α-SMA) were analyzed by western blot and real-time PCR. We also used PPAR-γ plasmid to transfect HK-2 cells.
Results: We firstly found that TGF-β1 lessened PPAR-γ expression and activity in dose and time dependently manner, and activation of PPAR-γ by RGZ and 15d-PGJ2 prevents TGF-β-induced loss of E-cadherin expression and inhibits the induction of α-SMA and snail1, two typical marks involved in EMT. Moreover, pretreated with antioxidant *N*-acetyl-L-cysteine, mitochondrial respiratory chain complex I inhibitor rotenone (Rot) and NADPH oxidase inhibitor apocynin suggested that ROS mediated the inhibition of TGF-β1 on PPAR-γ expression and activity, and PPAR-γ agonists blocked TGF-β1-induced ROS production.
Conclusion: Based on these findings, we confirmed that activation of PPAR-γ by RGZ and 15d-PGJ2 or overexpression of PPAR-γ protected TGF-β-induced EMT via inhibition of ROS production suggesting that PPAR-γ agonist might be a new therapeutic target against kidney fibrosis.

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0343
Presence of Vitamin D Receptor and Change of the Main Signaling Pathway in 1,25-Dihydroxyvitamin D_3 Cultured Human Glomerular Mesangial Cells

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Objective: To investigate the presence of vitamin D receptor (VDR) and the change of the main signaling pathway in 1,25-dihydroxyvitamin D_3 (1,25(OH) $2D_3$) cultured human glomerular mesangial cells (HMC).
Methods: HMC cultured in vitro were randomly divided into two groups: normal control group; 1,25(OH)$2D_3$ (10—8 mol/L) group. VDR expression was detected by real-time fluorescence quota PCR analysis, western-blot and three parallel sets of monochromatic fluorescent cRNA micro-array. Besides, the micro-array different genes chose by the 1.5-fold change standard, were analyzed by GO function classification, KEGG analysis and pathway enrichment analysis.
Results: The expression of VDR in each group was not seen in the cellular level. A small amount of VDR genes were detected in the cRNA micro-array results. Micro-array data analysis indicated and confirmed that PI3K/AKT/mTOR signaling pathway was inhibited and RAS pathway was activated.
Conclusion: Firstly, there has VDR in the HMC. The different results of the cellular level may be caused by such two reasons: the amount of the VDR in the HMC is very low or the RAS pathway inhibited the expression of the VDR. Secondly, when 1,25(OH)$2D_3$ cultured HMC, PI3K/AKT/mTOR signaling pathway was inhibited and the RAS signaling pathway was activated.

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0344
Targeted Deletion of Numb from Proximal Tubules Attenuates Interstitial Fibrosis by Mitigating G2/M Arrest

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Objective: Progressive tubulointerstitial fibrosis (TIF) is the final common pathway leading to end stage renal disease. Tubular epithelial cells (TECs) have a crucial role in the pathogenesis of TIF. The goal of the present study was to examine the physiologic and pathologic role of Numb in kidney.
Methods: We examined the expression and distribution of Numb in normal adult mouse kidney as well as in mouse model of renal fibrosis induced by unilateral ureteral obstruction (UUO). To explore Numb's role in renal fibrosis, we generated a conditional knockout mouse model in which Numb is selectively ablated from proximal tubules (PEPCK-Numb-KO). To confirm the role of Numb in regulating cell cycle, Numb was overexpressed in NRK52E cells by infecting with a Numb adenovirus (Ad-Numb) and endogenous Numb was knocked down by siRNA in HK-2 cells before aristolochic acid (AA) treatment. To examine the role of p53 in Numb-induced G2/M arrest, Ad-Numb infected HK-2 cells were incubated with pifithrin-α, a p53 inhibitor.