Inflammation and Fibrosis

Results: Treatment of NRK-49F cells with Ang II increased the expression of c-Myc and integrin αv. Meanwhile, the activation of TGF-β signaling and the expression of fibronectin, collagen I and α-SMA were up-regulated. Knockdown on c-Myc expression or pharmacological blockade of c-Myc by 10058-F4 blocked the effects induced by Ang II. Overexpression of c-Myc significantly increased the level of integrin αv and activation of TGF-β signaling, and increased the synthesis of fibronectin, collagen I and α-SMA. Knockdown on integrin αv by siRNA abolished the effects induced by c-Myc overexpression. CHIP assay revealed that c-Myc was bound to the chromatin region of the integrin αv proximal promoter. UUO induced marked increases in fibronectin, collagen I and α-SMA expressions, and renal interstitial fibrosis in the obstructed kidneys. Those effects were significantly attenuated by the administration of 10058-F4.

Conclusion: C-Myc upregulation was involved in UUO-induced renal fibrosis by stimulating ECM expression in renal fibroblasts. Integrin αv mediated c-Myc-induced TGF-β signaling pathway activation and the related ECM overexpression.

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O120 Ablation of Prostaglandin E2 Receptor EP4 Gene Impairs Urinary Concentration in Mice

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Objective: The antidiuretic hormone arginine vasopressin (AVP) is a systemic effector in urine concentration. However, increasing evidence suggests that other locally produced factors may also play an important role in the regulation of water reabsorption in renal collecting ducts. Recently, prosta-glandin E2 (PGE2) receptor EP4 has emerged as a potential therapeutic target for the treatment of nephrogenic diabetes insipidus, but the underlying mechanism is unknown. The present study was designed to examine the role of EP4 in urinary concentration.

Methods: Mice with renal tubule-specific knockout of EP4 (Ksp-EP4-/-) and collecting duct-specific knockout of EP4 (AQP2-EP4-/-) were generated using the Cre/loxP system. Mouse primary inner medullary collecting duct (IMCD) cells and mouse IMCD cell line (IMCD3) stably transfected with the aquaporin-2 (AQP2) gene (AQP2-IMCD3) were used to study AQP2 expression and membrane targeting, respectively.

Results: Urine concentrating defect was observed in both Ksp-EP4-/- and AQP2-EP4-/- mice. Decreased AQP2 abundance and apical membrane targeting in renal collecting ducts were also evident. In vitro studies demonstrated that AQP2 mRNA and protein levels were significantly up-regulated in mouse primary IMCD cells after pharmacological activation or adenosine-mediated overexpression of EP4 in a CAMP-CREB dependent manner. In addition, EP4 activation or overexpression also increased AQP2 membrane accumulation in AQP2-IMCD3 cells, mainly through the CAMP/PKA and ERK pathways.

Conclusion: The EP4 receptor in renal collecting ducts plays an important role in regulating urinary concentration under physiological conditions. The ability of EP4 to promote AQP2 membrane targeting and increase AQP2 abundance makes it a potential therapeutic target for the treatment of clinical disorders including acquired and congenital diabetes insipidus.

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O124 Homocysteine Induces Collagen I Expression by Downregulating Histone Methyltransferase 9a

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Objective: Hyperhomocysteinemia (HHcy) leads to several clinical manifestations including renal fibrosis. This study aimed to demonstrate the epigenetic mechanism of HHcy-induced renal fibrosis.

Methods: Wild-type C57BL/6 mice, at the age of 6-9 weeks, were fed with either standard rodent Chow or high-methionine diet. After 2 weeks on the diet, mice were sacrificed. Kidney tissues were removed. HK-2 cells were cultured in DMEM containing 10% homocysteine. The expression of histone methyltransferase 9a and collagen I were examined by Western blot and qPCR. Renal pathological changes were assessed in tissue sections stained with collagen I and Masson staining. HK-2 cells were transfected with pcOL-GL3 reporter plasmid, pRL null together with siG9a or Flag-tagged G9a to examine the promoter activity of COL1A1. The level on G9a and H3K9me2 on the promoter of COL1A1 was assessed by CHIP assay.

Results: We demonstrated that elevated concentration of Hcy induced the expression of collagen type I in cultured HK-2 cells as well as in kidney tissue of HHcy mice. Meanwhile, Hcy inhibited the expression of G9a. Mechanistically, silencing endogenous G9a by siRNA enhanced the promoter activity of COL1A1 in HK-2 cells. Conversely, overexpressing G9a inhibited the promoter activity of COL1A1. CHIP assay demonstrated that G9a binds to the neuron-restrictive silencer element (NRSE) on the promoter of COL1A1. Hcy treatment decreased the binding of G9a on NRSE, which in turn decreased the level of H3K9me2 on the promoter of COL1A1, led to upregulation of COL1A1.

Conclusion: These results show that Hcy induces collagen I expression by downregulating histone methyltransferase 9a and provide a novel mechanism on explaining how HHcy promotes ECM production.

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O127 Retinoid X Receptor α (Rxrα) Forms a Complex with Nuclear Factor of Activated T-cells 5 (Nfat5) to Suppress Nfat5 Activity and to Contribute to Renal Osmoregulation and Osmoadaptation

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Objective: To investigate the potential roles of nuclear receptor Rxrα in osmoregulation and kidney function.

Methods: The protein-protein interaction between Rxrα and osmoregulatory transcriptional factor Nfat5 (also called toxicity response element binding protein/osmotic responsive element binding protein, TorEBP/OREBP) was analyzed by co-immunoprecipitation and bimolecular fluorescence complementation (BiFC) assays. Chromatin immunoprecipitation (ChIP) and luciferase reporter assays were performed to test the effects of Rxrα on Nfat5-mediated transcription. Additionally, in vivo renal Rxrα expression was achieved by in situ injection of lentiviruses expressing Rxrα.

Results: In cultured mouse inner medullary epithelial cells (mIMCD3), high-NaCl hypertonicity negatively regulated Rxrα expression in a time- and toxicity-dependent manner. Further, hypertonicity-induced down-regulation of Rxrα appeared to be also proteasome activity-dependent. Meanwhile, Rxrα overexpression repressed whereas Rxrα knockdown enhanced Nfat5 transcriptional activity as well as the mRNA and protein expression of heat-shock protein 70 (Hsp70) and aldose reductase (Ar), two Nfat5-regulated genes involved in renal osmoregulation and osmoadaptation. Co-immunoprecipitation and BiFC, on the other hand, revealed that the DNA binding domain (DBD) of Rxrα was capable of physically interacting with the Rel homology domain (RHD) of Nfat5. ChIP assays further indicated that probably through competing for RHD binding, overexpression of Rxrα prevented Nfat5 from binding to the osmotic response elements (ORE) of the promoters of Nfat5-regulated Hsp70 and Ar genes. In mouse renal medulla, Rxrα mRNA or protein expression was found to be negatively correlated with the renal toxicity and the mRNA or protein expression of Nfat5, Hsp70 and Ar. Finally, in vivo renal overexpression of Rxrα resulted in significant alterations in medulla morphology and suppressed expression of Hsp70 and Ar.
Conclusion: Rrxα is tonicity responsive and capable of interacting with Nfat5 to negatively regulate Nfat5 activity and to exert important regulatory roles in osmoregulation, osmoadaptation and renal function.

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0156
Lrp6 Mediated Wnt Signaling Acts Through Primary Cilia Leading to Renal Defects
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Objective: Congenital anomalies of the kidney and urinary tract (CAKUT) are one of the most common birth defects. Our previous results indicate that conventional deletion of the key Wnt receptor of Lrp6 leads to a spectrum of renal hypoplastic and cystic kidney disorders, which are major phenotypes for CAKUT. Primary cilia are cellular organelle existing in surface and serving as signalling centre for multiple pathways. We found cilia are defect in Lrp6-/- kidney, but the underlying mechanism is unclear. We aim to discover the roles of Lrp6 in cilia-mediated pathway which disrupts cellular proliferation, differentiation and organogenesis in renal defects.

Methods: Lrp6 beta-geo mice were raised and mouse embryonic fibroblasts (MEFs), murine inner medullary collecting duct (mIMCD3) was purchased from ATCC and cultured in UC Davis and Nanfang Hospital. Scanning electronic microscope (SEM) and immunofluorescence staining were performed according to standard protocol.

Results: (1) The embryonic kidney samples from E18.5 Lrp6-/- mice were examined under scanning electronic microscope (SEM). Primary cilia in mutant kidneys exhibit extra slim/long (up), ungrown, short or curved cilia (bottom) compared with littermate control. The picture in big frame in upper-left is the magnification of the picture in small frame (unpublished preliminary data). (2) The active state of phosphorylated Lrp6 (T1479) is co-localized with cilia markers of acetylated-tubulin and γ-tubulin at basal body in MEFs and mIMCD3 cells. Phosphorylated Lrp6 (green) is expressed in the basal body in control MEFs, but absent in Lrp6-/- MEFs. Phosphorylated Lrp6 (green) is co-localized with acetylated-tubulin (red) and basal body marker of γ-tubulin (red) in mIMCD3 cells (unpublished preliminary data).

Conclusion: Lrp6 mediated Wnt signaling may act through primary cilia with undiscovered mechanism which cause genetic renal defects and ciliopathy in CAKUT.

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0161
Reversal of Epithelial to Mesenchymal Transition Following Relief of Unilateral Ureteral Obstruction In the Rat
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Objective: Renal fibrosis begins with renal tubular epithelial mesenchymal transition (EMT); the progression thereafter depends upon a number of fibrotic factors. Unilateral ureteral obstruction (UUO) is a well-described model of EMT. We used an improved reversible unilateral ureteral obstruction (RUUO) model to investigate whether a progressive renal injury model of EMT could be reversed into the opposite direction, into mesenchymal-epithelial transition (MET) after relief of UUO in rats.

Methods: Rats were subjected to UUO or sham operation and the obstruction was removed five days later (or was left in place). Rats developed EMT after reversal of 2 or 4 weeks of ureteral obstruction as assessed by the expressions of fibrotic factors, EMT and MET markers in this post-obstructive model.

Results: We found a significant decrease in the kidney weight and renal cortical thickness in the RUUO group compared with the sham groups. This rise in the RUUO group was significantly reduced. The elevated level of TGF-β1, TGF-β receptors and core fucosylation in the UUO group was significantly reduced in the RUUO groups. The EMT markers staining showed results parallel to those of TGF-β1 expression levels. In addition, RUO rats exhibited pronounced inflammatory and intrinsin proliferative cellular responses, and ultimately fibrosis. By comparison, RUUO mice had more controlled and measured extrinsic and intrinsic responses to EMT with return to MET within several weeks after release of ureteral obstruction.

Conclusion: Our findings provide a model that allows investigation of the fibrotic factors during reversal of EMT that contribute to the development of fibrosis. EMT of the progressive renal injury could be actively reversed into MET and renal architecture is better maintained throughout injury and recovery from injury after relief of UUO in rats.

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0163
Effect of Podocalycin Expression Induced by Iopromide on Podocytes
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Objective: Contrast-induced renal tubular cells apoptosis represents a key mechanism of contrast-induced nephropathy (CIN). So most episodes of CIN are self-limiting and resolve within 1–2 weeks, but in some cases, reduced renal function progresses to chronic kidney disease. Albuminuria was observed after injection of contrast media in animal studies, which last until the function of renal tubular epithelial cell recover. Podocalcin, which has