0084

Blockade of Wnt/β-catenin Signaling Exhibits Superior Therapeutic Efficacy Compared to RAS Inhibitors in CKD

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Background: Chronic kidney disease (CKD) has become a public health problem worldwide. At present, treatment options for CKD are limited and often ineffective, underscoring enormous unmet medical need. The mainstay of clinical therapy for CKD is inhibition of renin-angiotensin system (RAS), using angiotensin-converting enzyme inhibitor (ACEI) or angiotensin II type 1 receptor (AT1) blocker (ARB). However, current therapy with RAS inhibition is insufficient, partially because of compensatory upregulation of renin expression. Thus, it is paramount to develop new therapeutic strategy with better outcomes.
Methods: Using two mouse models of CKD induced by adriamycin (ADR) or unilateral ischemic/reperfusion injury (UIRI), we directly compared the therapeutic efficacy of small-molecule Wnt/β-catenin inhibitor ICG-001 with trandolapril (ACEI) alone, or the combination of trandolapril and losartan (ARB). The effect of renin on fibroblast activation was also assessed in vitro.
Results: Compared to ACEI, or ACEI plus ARB, ICG-001 displayed superior therapeutic efficacy in both models. ICG-001 almost completely abolished proteinuria, ameliorated glomerular injury and fibrotic lesions and reduced serum creatinine in ADR nephropathy, whereas trandolapril, or trandolapril plus losartan only displayed as 50% efficacy as ICG-001. Similar results were obtained in UIRI model. We found that ICG-001 completely abolished renal expression of all RAS components including angiotensinogen, renin, ACE and AT1 in both models. However, trandolapril or trandolapril plus losartan actually induced angiotensinogen and renin expression in the kidneys. In vitro, incubation of kidney interstitial fibroblasts (NRK-49F) with renin protein induced fibronectin expression, and this effect was dependent on ERK-1/2 activation. Losartan did not block renin-induced fibronectin expression, suggesting that renin elicited its effect by an angiotensin II-independent mechanism.
Conclusion: Our studies demonstrate that blockade of Wnt/β-catenin, the master upstream regulator of all RAS genes, has superior therapeutic efficacy for the treatment of CKD than RAS inhibitors.

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0095

Yi Qi Qing Re Gao Formula Ameliorates Puromycin Aminonucleoside-induced Nephrosis by Suppressing Inflammation and Apoptosis

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Objective: Yi Qi Qing Re Gao (YQQRG) formula is a traditional Chinese herbal medicine used to treat chronic nephritis. This study was designed to evaluate the underlying mechanism in the use of YQQRG formulato treat nephrosis induced by puromycinaminonucleoside (PAN).
Methods: Thirty-six male Wistar rats were randomly divided into three groups of 12 rats: a sham group, a vehicle-treated PAN model group (PAN) and a group treated with YQQRG (PAN + YQQRG). The PAN model was established by a single intravenous injection of PAN at a dose of 40 mg/kg body weight; the rats in the sham group received the same volume of saline. Twenty-four hour urinary protein was measured 0, 3, 5, 10, and 15 days after the injection. The rats were sacrificed at day 10 and day 15 and the serum lipid profile examined. The renal cortex of each rat was stained with periodic acid-Schiff reagents and the pathological alterations and ultrastructural changes were examined by transmission electron microscopy. In situ cell apoptosis was detected by a terminal deoxynucleotidyltransferase-mediated uridine 5—triphosphate-biotin nick end-labeling (TUNEL) assay. Transcriptive levels of inflammatory markers and molecules associated with apoptosis were detected by a real-time polymerase chain reaction and the expression of proteins was examined by either immunohistochemistry or Western blot.
Results: YQQRG remarkably decreased the levels of urinary protein and lowered serum lipid levels. YQQRG also attenuated histological lesions in the rat kidneys. The activation of inflammatory markers was largely restored by the administration of YQQRG. The TUNEL assay showed that YQQRG decreased the number of apoptotic cells. Both the mRNA and protein levels of caspase-3 were significantly reduced in the group treated with YQQRG, whereas the Bcl-2 protein increased in the YQQRG group.
Conclusion: YQQRG alleviated kidney injuries of PAN-treated rats, possibly through anti-inflammatory anti-apoptosis effects.

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0107

High Glucose-induced Fibronectin Upregulation in Cultured Mesangial Cells Involves Caveolin-1-dependent RhoA-GTP Activation via Src Kinase

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Background: Increasing evidence indicates that diabetic-mediated renal interstitial fibrosis through extracellular matrix protein (ECM) accumulation is a key event in the development of diabetic kidney disease (DKD). High glucose (HG) promotes excessive accumulation of ECM proteins and expression of fibrotic factors in mesangial cells (MCs), which leads to subsequent diabetic renal dysfunction. The activation of RhoA and its downstream mediator Rho-kinase act as crucial mediators of strain-induced the matrix protein fibronectin (FN) secretion in MCs, which depend on intact caveolae. However, the involvement of caveolae/caveolin-1 in HG-induced dysfunction of MCs has not been assessed.
Methods: Primary MCs were obtained from Sprague-Dawley rat glomeruli by differential sieving and cultured in DMEM supplemented with supplemented with 20% fetal calf serum, streptomycin, and penicillin. Experiments were carried out using cells between passages 6 and 15. We then examined the influence of HG on caveolin-1/RhoA signaling and FN secretion in mouse MCs.
Results: We showed that high levels of glucose time and dose dependently increased matrix protein FN production in primary rat MC. Rho pathway inhibition blocked HG-induced FN upregulation. HG-induced RhoA activation was prevented by disrupting caveolae with filipin III or caveolin-1 siRNA and rescued by exogenous caveolin-1. HG also increased caveolin-1/Src association and activated Src kinase, and Src inhibitor blocked RhoA activation and FN upregulation. Src mediated phosphorylation of caveolin-1 on Y14 has also been implicated in signaling responses. Overexpression of nonphosphorylatable caveolin-1 Y14A mutant prevented HG-induced RhoA activation and FN upregulation.
Conclusion: HG-induced FN upregulation require caveolae and caveolin-1 interaction with RhoA and Src kinases. Interference with Src/caveolin-1/RhoA signaling may provide new avenues for the treatment of DKD.

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0119

C-Myc is Involved in Unilateral Ureteral Obstruction-induced Renal Fibrosis via Upregulating Integrin αv

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Objective: To explore the effect of c-Myc, a pleiotropic transcriptional factor, in the progression of kidney fibrosis and investigate its related downstream pathway.
Methods: Cultured renal fibroblast NRK-49F and unilateral ureteral obstruction (UUO) mice were used in the experiment. Small interfering RNA (siRNA) was used to downregulate the expression of c-Myc or integrin αv while an adenoviral vector harbouring c-Myc gene was used to overexpress c-Myc. HE and Masson's trichrome staining were used to observe the histological changes in kidney. Western blotting or real-time PCR was used to measure the protein or mRNA level of c-Myc, integrin αv, fibronectin, collagen I, α-smooth muscle actin (α-SMA) and transforming growth factor-β (TGF-β). ChIP assay was used to identify the binding of c-Myc to the integrin αv proximal promoter.

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 signalling pathway activation and the related ECM overexpression.

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0120
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 urinary 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, prostaglandin 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 adenovirus-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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0124
Homocysteine Induces Collagen I Expression by Downregulating Histone Methyltransferase G9a

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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 DL-homocysteine (Hcy). The expression of histone methyltransferase G9a 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 G9a and provide a novel mechanism on explaining how HHcy promotes ECM production.

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0127
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 tonicity response element binding protein/osmotic responsive element binding protein, TonEBP/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 tonicity-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-regualted 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 tonicity 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*.