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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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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.