SIRT1 deficiency, especially in the podocyte, is responsible for the change. In contrast, diabetic endothelium SIRT1 deletion did not have significant albuminuria and developed diabetic kidney damage without blood pressure increase. Blood pressure was determined by tail-cuff method. Endothelium selective SIRT1 knockout was generated by crossing the floxed SIRT1 mice with SCLC-reER, and recombination was induced by tamoxifen. Podocyte selective SIRT1 knockouts were generated by crossing the floxed SIRT1 mice with Podocin-Cre.

Results: In the diabetic kidney, SIRT1 expression was significantly reduced. In mice with one allele of SIRT1 deletion, urinary albumin excretion was markedly increased by 4 fold compared with SIRT1 intact mice. Renal histology showed mesangial expansion in the diabetic SIRT1 heterozygotes; Sirius red staining revealed more fibrosis, and qPCR indicated higher expression of PAI-1, aSMa, Col 1β1, Lox12 gene in diabetic SIRT1 heterozygotes compared with wild type control. Meanwhile, losing one allele of SIRT1 did not alter blood pressure in the diabetic mice. To further examine the mechanism, endothelium and podocyte SIRT1 was selectively deleted respectively. Following STZ challenge, the podocyte SIRT1 deficient mice showed higher albuminuria and developed diabetic kidney damage without blood pressure change. In contrast, diabetic endothelium SIRT1 deletion did not have significant change in albuminuria and kidney damage compared with wild type.

Conclusion: SIRT1 deficiency, especially in the podocyte, is responsible for the development of albuminuria and renal fibrosis. The mechanism by which podocyte SIRT1 protects the kidney from diabetic kidney damage remains to be explored.

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Resveratrol Inhibits Renal Interstitial Fibrosis in Diabetic Nephropathy by Regulating AMPK/NOX4/ROS Signaling

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Objective: Renal interstitial fibrosis is a major pathologic feature of diabetic nephropathy, while the pathogenesis and therapeutic interventions of diabetic renal interstitial fibrosis are not well established. This study investigated the renoprotective effects of resveratrol and its underlying mechanisms in diabetic renal fibrosis.

Methods: Renal fibroblast (NRK-49F) cells were pre-incubated with resveratrol before exposure to high glucose or normal glucose for 48 hours. We measured cell proliferation and differentiation, reactive oxygen species (ROS) production, nicotinamide adenine dinucleotide phosphate oxidase (NOX) and AMP-activated protein kinase (AMPK) activity in renal fibroblast cells. We further examined whether resveratrol-treated type 2 diabetic db/db mice were resistant to the development of renal fibrosis. Phospho-AMPK and NOX expression in the kidney were also assessed.

Results: High glucose is capable of inducing renal fibroblast cell proliferation and differentiation to myofibroblasts, manifested by increased expressions of α-SMA and fibronectin. The proliferation and myofibroblastic activation of renal fibroblast cells induced by high glucose is accompanied by an increase in the intracellular levels of ROS derived from the up-regulation of NOX4. Resveratrol, like the NOX inhibitor diphenyleneiodonium, can markedly inhibit high glucose-induced fibroblast proliferation and activation by reducing NOX4-derived ROS production. It is then revealed that the increase in the expression of NOX4 after high glucose treatment is due to the inactivation of AMPK, which can be reversed by resveratrol. Further in vivo investigation shows that resveratrol treatment significantly attenuates renal fibrosis in type 2 diabetic mice, accompanied by an up-regulation of phospho-AMPK and a down-regulation of NOX4.

Conclusion: Our results suggest that high glucose can directly promote renal fibroblast proliferation and activation in a ROS-dependent manner, and resveratrol is a potential therapeutic agent against diabetic renal fibrosis via regulation of AMPK/NOX/ROS signaling.

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0149
Calcitriol Protects Against Renal Tubular Cell Apoptosis by Promoting M2 Macrophages Polarization in Diabetic Nephropathy Rats

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Objective: Renal tubular apoptosis is a key event in initiating kidney damage in DN. Heterogeneity of macrophage phenotype and function ultimately determines the outcome of diabetic nephropathy (DN). Recently studies suggested that promoting anti-inflammatory M2 macrophage polarization inhibits renal tubular cell apoptosis in renal ischemia reperfusion injury. Therefore, we sought to investigate whether calcitriol, known as an important renal-protective drug, is sufficient to protect against tubular cell apoptosis by promoting M2 macrophage in DN rats.

Methods: DN model rats were established by intraperitoneal injection with streptozocin (STZ). The rats were subsequently receiving either calcitriol (0.1 μg/kg/d) or vehicle by gavage. Rats were sacrificed at 18w for histological and molecular analysis. In addition, we performed in vitro study using Raw264.7 cells cultured with either high glucose or high glucose followed by 1,25-dihydroxyvitamin D3 medium to assess macrophage phenotype.

Results: Calcitriol significantly improved renal function and ameliorated renal histology in DN rats. The increased tubular cell apoptosis in DN rats was alleviated by calcitriol. Calcitriol up-regulated the expression of anti-apoptotic protein Bcl-2, and down-regulated the expression of pro-apoptotic protein such as Bax and caspase-3. Interestingly, calcitriol significantly enhanced M2 macrophage polarization in interstitium with elevated expressions of M2 markers, including CD163, Arg-1 and MR. Moreover, the ratio of CD163/CD68 considered as the proportion of M2 macrophage was about 2.9 fold highly after calcitriol treatment.