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The impact of Sirtuin 3 in renal tubular cell apoptosis under diabetic conditions

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Objectives : Reactive oxygen species (ROS) play important roles in various metabolic and kidney diseases, including diabetic nephropathy, via mediating renal tubular epithelial cell injury. Central to tubular injury is mitochondrial dysregulation which is manifested in a reduction in cell respiration and ATP production, resulting in ROS overproduction. In this context, recently, the role of mitochondrial sirtuins (SIRTs) has been reported to be implicated in numerous ROS-mediated diseases. Since SIRT3 is mainly localized in the mitochondria and regulates mitochondrial function via deacetylation of mitochondrial proteins, SIRT3 has also been suggested to be involved in the pathogenesis of various kidney diseases, including acute kidney injury. However, the impact of SIRT3 on tubular cell apoptosis under diabetic conditions has never been elucidated.

Methods : In vitro, rat proximal tubular epithelial cells (NRK-52Es) were cultured in DMEM media containing 5.6 mM glucose (normal glucose, NG) or NG + TGF-β1 (10 ng/ml) with or without plasmid SIRT3 transfection. After 48 hours, cells were harvested and mitochondrial fraction was isolated. In vivo, 12 C57BL/6 mice were intraperitoneally injected with saline (Control, C) (N=6) or STZ (50 mg/kg/d) for 5 consecutive days (Diabetes, DM) (N=6), and were sacrificed after 6 weeks. The protein expression of SIRT3, MnSOD, and apoptosis-related proteins (Bax, Bcl-2, cleaved-caspase 3, cytochrome C, and p53) were determined by western blot analysis. Immunofluorescent staining for SIRT3 and Mitotracker staining were also performed with cultured cells, TUNEL assay was conducted with mice renal tissues.

Results : Compared to NG cells, the protein expression of mitochondrial SIRT3 was significantly decreased in TGF-β1-stimulated renal tubular cells, while SIRT3 protein expression in the cytoplasm was comparable between the two

groups. Bax, cleaved-caspase 3, and p53 protein expression were significantly increased, whereas the protein expression of Bcl2, MnSOD, and cytochrome C were significantly decreased in tubular cells exposed to TGF-β1. In contrast, transfection with plasmid SIRT3 significantly abrogated the changes in apoptosis-related protein expression in TGF-β1-stimulated cells. A significant decrease in SIRT3 expression was also demonstrated in the kidney of DM mice compared to the C kidney along with a significant increase in TUNEL-positive tubular epithelial cells in the DM kidney.

Conclusions : These results suggest that SIRT3 plays a protective role in tubular injury under diabetic conditions and that SIRT3 can be a promising therapeutic target in patients with diabetic nephropathy.

Keywords : SIRT3, diabetic nephropathy, tubular injury