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TGF-β1 induces Nox4 dependent hypoxia induced apoptosis in human kidney proximal tubular epithelial cells

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Objectives : Ischemia/reperfusion injury, resulting from hypoxic damage within a graft, is the leading cause of cell death and graft rejection. In this study, we investigated whether Nox4 have a great role in ischemic injury in a cellular model in which experimental hypoxia was induced using CoCl2

Methods : The ischemic injury induced in HK-2 cells by CoCl2 was validated by reduced cell viability at different times and doses. Reverse transcription polymerase chain reaction for Nox4 was performed. Western blotting for Nox4 and Smad pathway were done. HK-2 cells were pretreated with GKT137831 (most specific Nox1/4 inhibitor) and SB431542 (TGF-β1 type 1 tyrosine kinase inhibitor). The treatment effect of SB431542, GKT137831 on in HK-2 cell survival was observed. ELISA has been used to measure TGF-β1 levels. ROS production was detected using a DHE stain and amplex red assay.

Results : Expression of Nox4 in HK-2 cells significantly increased by hypoxic stimulation. TGF-β1 was secreted endogenously by hypoxic HK-2 cells. SB4315432 significantly inhibited Nox4 expression in HK-2 cells through the Smad2 / 3-dependent cell signaling pathway. Silencing of Nox4 reduced the production of reactive oxygen species (ROS) and attenuated apoptotic pathway. Finally Knocking down of Nox4 increased cellular survival in hypoxic HK-2 cells. Pretreatment of GKT137831 and SB4315432 replicated theses results.

Conclusions : Hypoxia induces HK-2 cell apoptosis through the signaling pathway involving Nox4 dependent ROS generation and TGF-β1 via Smad pathway. Therapies targeting Nox4 may be effective against ischemia induced kidney injury.

Keywords : Acute kidney injury, ischemia, Hk-2 cell, TGF-β1, GKT137831