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

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Aims

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 $CoCl_2$

Main methods

The ischemic injury induced in HK-2 cells by $CoCl_2$ was validated by reduced cell viability at different times and doses. Reverse transcription polymerase chain reaction for Nox4 and TGF-β1 was performed. Western blotting for Nox4 and Smad pathway were done. ROS production was detected using a DHE stain and Amplex red assay. HK-2 cells were transfected with siNox4 and pretreated with GKT137831(most specific Nox1/4 inhibitor). ELISA has been used to measure TGF-β1 levels. The effect of treatment with TGF-β1 type 1 tyrosine kinase inhibitor SB431542 on Nox4 expression was observed.

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 via Smad2/3 dependent cell signaling pathway. Silencing of Nox4 recued production of reactive oxygen species (ROS), down regulation of proinflammatory markers and reduced caspase 3/7 activity in hypoxic HK-2 cells. Pretreatment of GKT137831 replicated theses results.

Figure 1

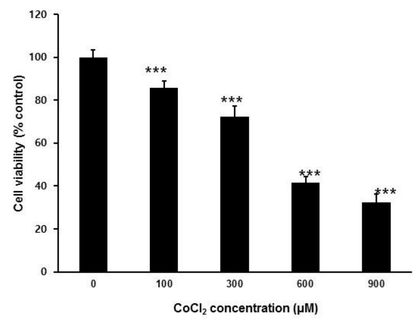


Figure 1. The effect of $CoCl_2$ on HK-2 cell viability. The cell toxicity of $CoCl_2$ was found to be dose dependent. We chose 300uM of $CoCl_2$ for hypoxic injury in this study., ****P*< 0.001 at each time point compared to control.

Figure 2

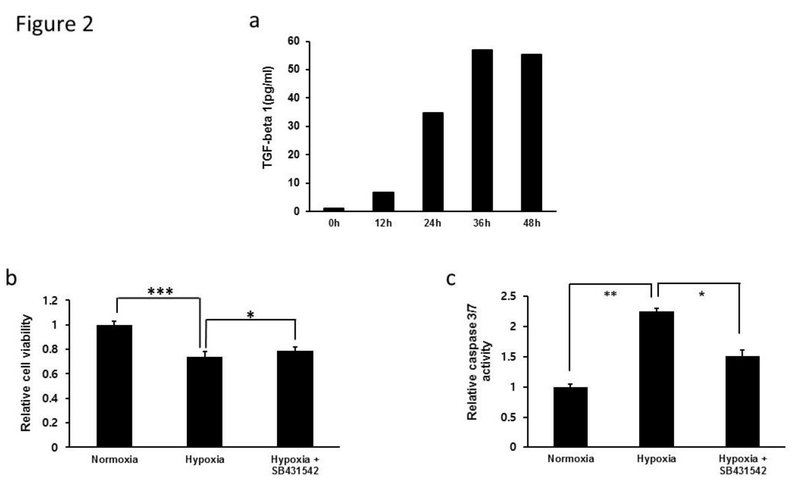


Figure 2. Role of TGF-β on apoptosis and cellular survival in hypoxia exposed HK-2 cells. (a) Concentration of TGF-β1 after hypoxic injury (ELISA) (b) Effect of TGF-β inhibition with SB431542 on cellular survival (MTT assay, 24 h later after 300 uM $CoCl_2$ exposure) (c) Effect of TGF-β inhibition with SB431542 on caspase 3/7 activation (24 h later after 300 uM $CoCl_2$ exposure) **P* < 0.10, ***P*<0.05, ****P*< 0.001 at each time point compared to control.

Figure 3

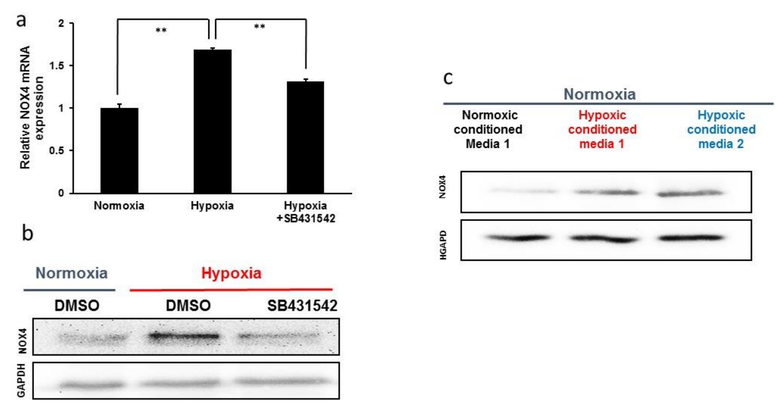


Figure 3. Hypoxia induced TGF-β associated Nox4 proliferation in HK-2 cells. (a) Effect of hypoxia on Nox4 expression with qPCR. (b) Western blot showing the effect of hypoxia and TGF-β on Nox4 protein expression. (c) Nox4 protein expression in normoxic conditioned media and in hypoxia conditioned media1(hypoxia conditioned media 2.5ml + new media 5ml) and hypoxia-conditioned media2 (hypoxia conditioned media 5ml + new media 5ml). **P* < 0.10, ***P*<0.05, ****P*< 0.001 at each time point compared to control.

Figure 4

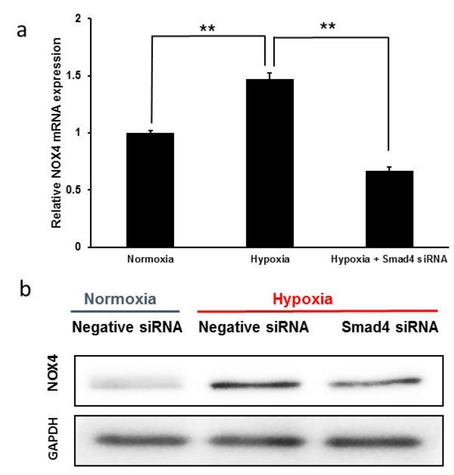


Figure 4. Nox4 is regulated by Smad pathway of TGF-β1 (a) Under hypoxia, Nox4 expression of HK-2 cells transfected with or without Smad4 siRNA with qPCR (b) Western blot showing the effect of Smad4 on Nox4 protein expression. **P* < 0.10, ***P*<0.05, ****P*< 0.001 at each time point compared to control.

Figure 5

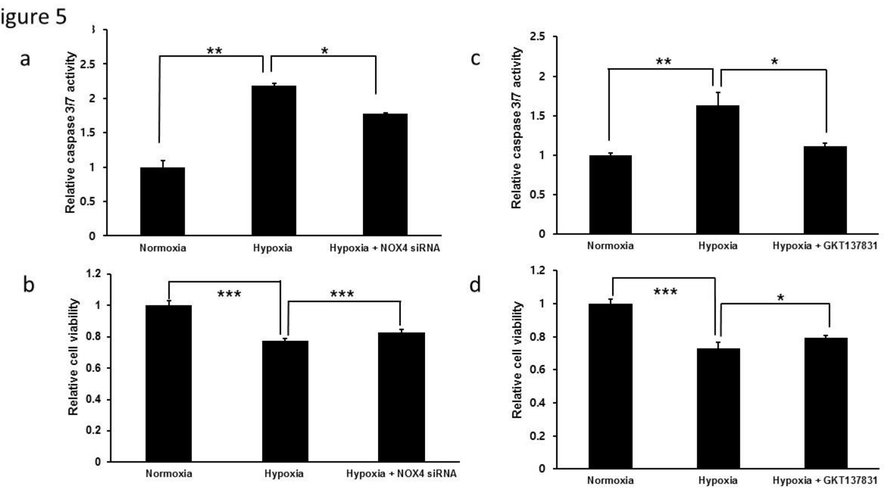


Figure 5. Role of Nox4 in hypoxia induced apoptosis in HK-2 cells. (a and b) Effect of knockdown of Nox4, (c and d) effect of pharmacologic inhibition of Nox4 with GKT137831. (a and c) caspase 3/7 activity, (b and d) MTT assay. **P* < 0.10, ***P*<0.05, ****P*< 0.001 at each time point compared to control.

Figure 6

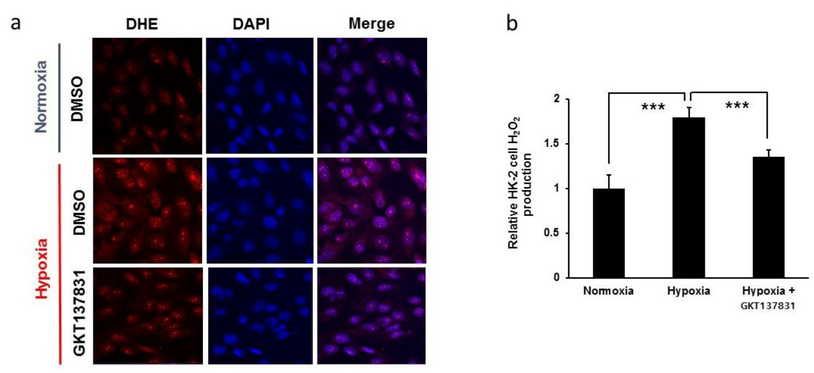
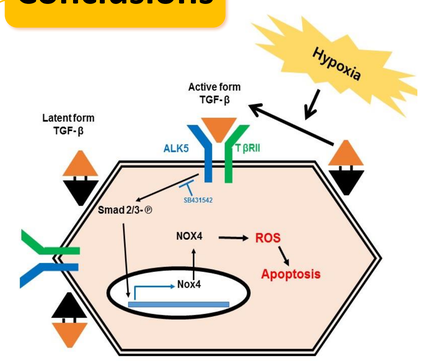


Figure 6. Effects of Nox4 on hypoxia induced ROS generation. Cells were exposed to CoCl2 l for 300uM with and without GKT137831. (**a**) Confocal microscopic images of cells subjected to dihydroethidium (DHE) staining. (**b**) H_2O_2, product of Nox4, was measured by Amplex red assay. **P* < 0.10, ***P*<0.05, ****P*< 0.001 at each time point compared to control.

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.

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