

Cell Proliferation and Activation of Survival Signaling Pathways by Hypoxia/reoxygenation in Renal Epithelial Cells

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Background : Numerous studies have shown that reperfusion of ischemic cells initiates reperfusion injury, a second phase of injury, which is attributed to generation of reactive oxygen species. On the other hand, during recovery from ischemia/reperfusion injury, surviving epithelial cells dedifferentiate and proliferate, eventually replacing the irreversibly injured tubular epithelial cells. However, molecular mechanism of proliferation occurring after ischemic injury has not been defined. We observed in renal epithelial cells that reoxygenation of hypoxic cells caused an increase in cell proliferation rather than induction of cell death. The present study was therefore undertaken to investigate the underlying mechanism of hypoxia/reoxygenation (H/R)-induced cell proliferation in renal epithelial cells using opossum kidney (OK) cell culture, an established renal proximal tubular cell line.

Methods : Cells were exposed to hypoxia (95% N₂/5% CO₂) and reoxygenation (95% air/5% CO₂) for various time periods. Cell proliferation was estimated by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, cell cycle, and bromodeoxyuridine (BrdU) binding assay. Activation of kinases and Wnt/b-catenin signal was evaluated by Western blot analyses.

Results : Reoxygenation after 4, 7, 10-hr hypoxia increased cell proliferation. H/R caused activation of extracellular signal-regulated kinase (ERK) and Akt signaling pathways. Hypoxia itself decreased cell growth in a time-dependent manner without any effect on activation of ERK and Akt. These changes were prevented by the antioxidant N-acetylcysteine and the inhibitor of growth factor receptor (suramin). The phosphatidylinositol 3-kinase (PI-3K) inhibitor LY294002 inhibited Akt phosphorylation but not ERK activation induced by H/R. MEK inhibitors (PD98059 and U0126) and LY294002 inhibited cell proliferation induced by H/R. Exposure of cells to H/R increased translocation of b-catenin into nucleus, indicating that H/R induces activation of Wnt/b-catenin signal. Transfection of constitutive active MEK1 and Akt stimulated cell proliferation in cells exposed to H/R. NF- κ B activation was not altered by H/R.

Conclusion : These results suggest that H/R causes activation of growth factor/MEK/ERK, PI-3-kinase/Akt, and Wnt/b-catenin signaling pathways to enhance cell proliferation and to maintain cell survival. Reactive oxygen species may initiate these signals.