5.Two independent pathways, BNIP3 and sestrin2, mediate autophagy of renal tubular cells in acute kidney injury in vitro and in vivo

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Autophagy is one of the systems which protect life from many kinds of stresses. In the previous study we reported autophagy occurred in acute kidney injury (AKI) mice model. Autophagy is thought to play a protective role in renal tubules from oxidative stresses. However, little is known about signal transduction for autophagy in AKI. Bcl-2/adenovirus E1B 19kDa-interacting protein 3 (BNIP3) is one of the target proteins of hypoxia inducible factor -1a (HIF-1a). Sestrin2 is one of the target proteins of p53. Both BNIP3 and sestrin2 have relations to injured cell survival. The aim of this study is to reveal the roles of BNIP3 and sestrin2 in autophagic pathway in AKI.

We used rat ischemia/reperfusion (I/R) AKI model in vivo and cultured renal tubular cells as an in vitro AKI model. The expression of BNIP3 and sestrin2 are similarly up-regulated 12-24h after I/R in proximal tubules in immunostaining and immunoblotting. BNIP3 mRNA and protein expressions were up-regulated in the hypoxia condition via HIF-1a dependently in vivo experiment. On the other hand, sestrin2 mRNA and protein expressions were up-regulated in the oxidative stress condition (H2O2) via p53 dependently. Furthermore, to examine BNIP3 and sestrin2 regulate autophagy or not, we established NRK cells which stably transfected with a fusion protein between green fluorescent protein and light chain 3 (LC3-GFP) as a marker of autophagy. Overexpression of BNIP3 and sestrin2 both induced autophagy in NRK-LC3-GFP cells and induced LC3 protein expression. Interestingly, the autophagosome induced by BNIP3 localized mainly in mitochondria. Induction of autophagy by hypoxia was significantly reduced by sestrin2 siRNA. Meanwhile, induction of autophagy by H2O2 was reduced mainly by BNIP3 siRNA. These observations disclose that autophagy in renal tubules in AKI is induced by at least two independent pathways, p53-sestrin2 pathway and HIF-1a-BNIP3 pathway. These two pathways may work differently according to the types of stresses to protect renal tubules in AKI.