C/EBP HOMOLOGOUS PROTEIN (CHOP) DEFICIENCY ATTENUATES ISCHEMIA REPERFUSION-INDUCED ACUTE KIDNEY INJURY: ROLES OF REACTIVE OXYGEN SPECIES

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Introduction

Renal ischemia-reperfusion (IR) is a major cause of acute renal failure. The mechanism of IR injury included endoplasmic reticulum (ER) stress, inflammatory responses, hypoxia, and reactive oxygen species (ROS). CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP) is involved in ER stress signal pathway. CHOP is a transcription factor and a major mediator in ER stress-induced cell apoptosis. The role of CHOP in renal IR injury is still unclear.

Materials & Methods

Experimental animal - B6.129S-*Ddit3*^{tm1Dron}/J mice (CHOP-knockout mice) & C57BL/6j mice (wild-type mice)

IR protocol - Bilateral arteries clamped by non-traumatic clamps for 15 mins, followed by reperfusion.

Genotyping process - All the mice's RNA was extracted and changed to complementary DNA and amplified by polymerase chain reaction (PCR).

Histological Examinations - The 15 fields from every sample were chosen to quantifier the degree of renal injury induced by IR. Tubular injury include renal tubule dilation, tubular epithelial injury and cast formation were graded with a score from 0 to 4.

Apoptotic cells were detected by Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining. Apo-ONE® homogeneous caspase-3/7 assay kit (Promega) was use to detect caspase 3/7 activity. Tissue oxidative stress induced by IR was detected by Malondialdehyde (MDA) assay.

Results & Figures

CHOP deficiency recovered the loss of renal function after IR in mice. The renal proximal tubule damage and collagen deposition were induced by IR in mice, but the degrees of alterations were significantly slighter in CHOP knockout mice. CHOP deficiency could also decrease the IR-induced renal cell apoptosis and caspase-3 activation. Lipid peroxidation was increased after 24 h of IR in mice, which could be reversed by CHOP deficiency. In an *in vitro* model to mimic the renal cell injury induced by ROS produced from IR, siRNA targeting CHOP significantly decreased H_2O_2-induced renal tubular cell apoptosis and caspase-3 activation, but mitochondria-related apoptosis signals Bax and Bcl-2 were not changed. The siRNA targeting CHOP could also reverse the H_2O_2-induced NFκB activation and COX-2 protein expression in renal tubular cells.

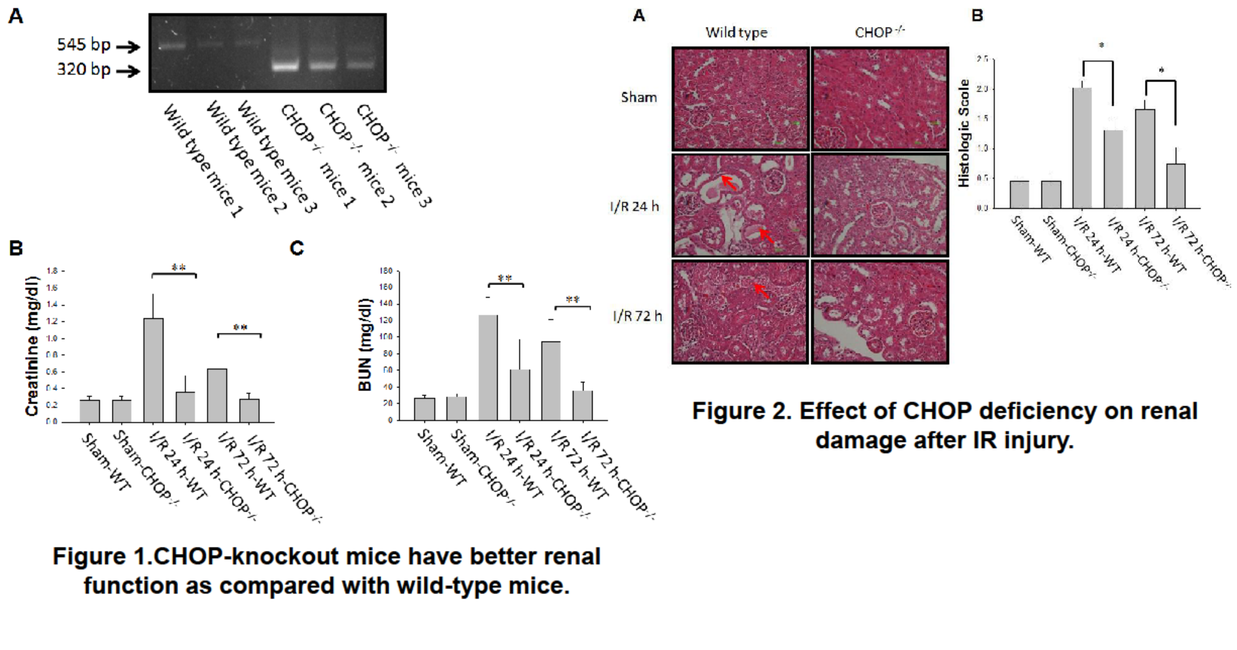

Figure 1.CHOP-knockout mice have better renal function as compared with wild-type mice.

Figure 2. Effect of CHOP deficiency on renal damage after IR injury.

Results and Figures

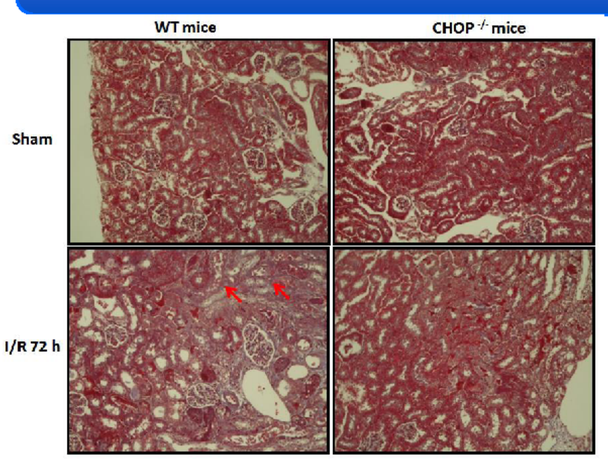

Figure 3. CHOP deficiency attenutates collagen deposition after IR 72 h.

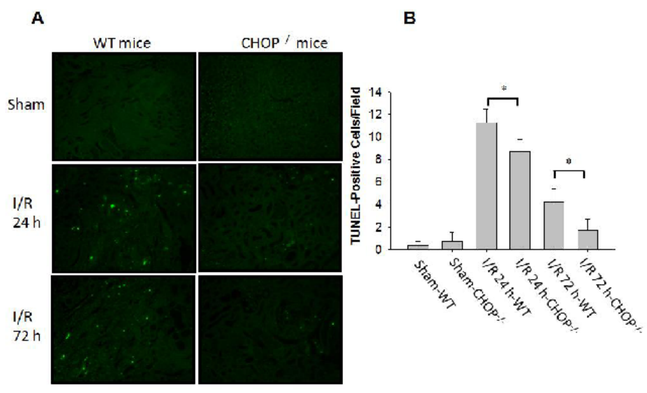

Figure 4. Effect of CHOP deficiency on apoptosis subjected to IR.

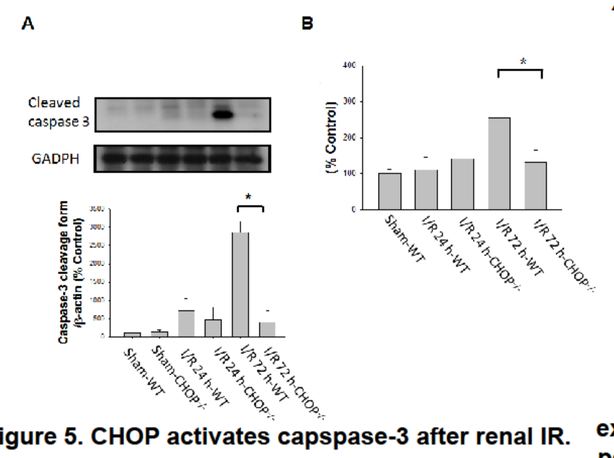

Figure 5. CHOP activates capspase-3 after renal IR.

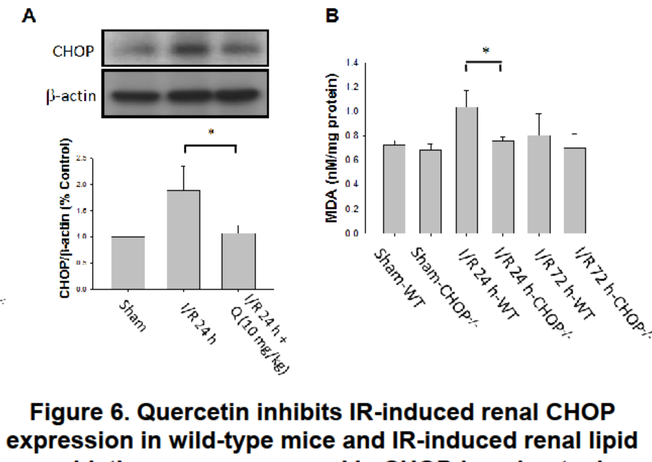

Figure 6. Quercetin inhibits IR-induced renal CHOP expression in wild-type mice and IR-induced renal lipid peroxidation was suppressed in CHOP-knockout mice.

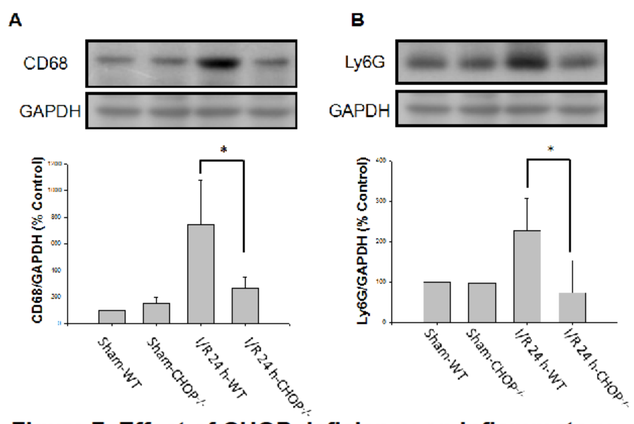

Figure 7. Effect of CHOP deficiency on inflammatory cell infiltration after IR injury.

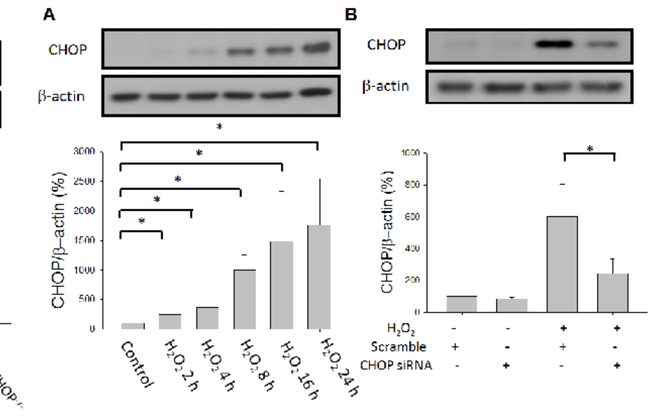

Figure 8. CHOP expression in NRK-52E cells after H_2O_2 treatment followed a time dependent manner.

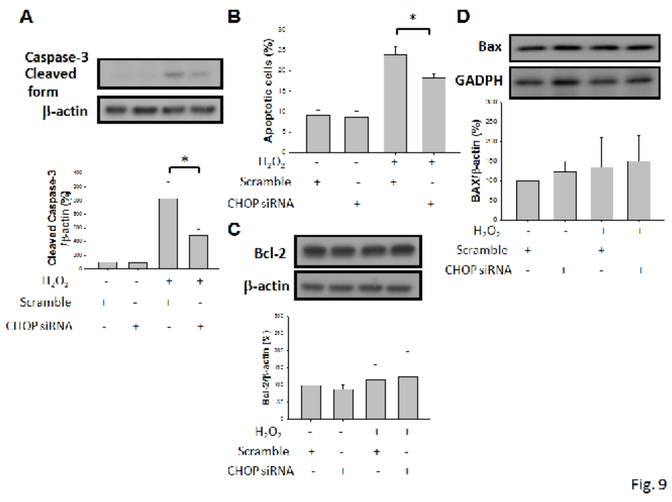

Fig. 9

Figure 9. CHOP knockdown by siRNA decreases H_2O_2-induced cell apoptosis.

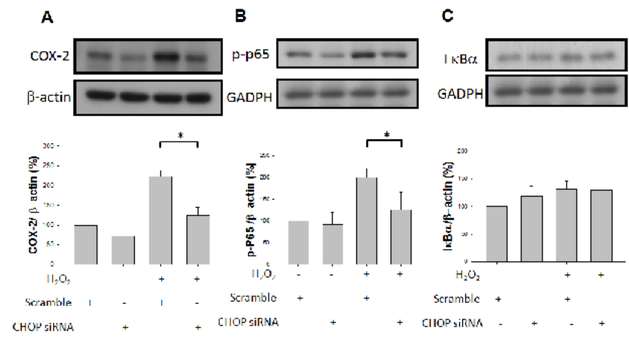

Figure 10. CHOP knockdown by siRNA decreases H_2O_2-induced inflammatory signals.

Summary

In conclusion, this study demonstrates for the first time that CHOP deficiency attenuates oxidative stress and renal IR injury. IR-induced renal injury may occur through the CHOP-regulated cell apoptosis, oxidative stress, and inflammatory responses. These findings suggest that CHOP may play an important role in pathophysiology of IR-induced renal injury.

References

1. Chiang CK et al. *Mol. Med.* 2011; **17:**1295-1305.
2. Inagi R et al. *Curr. Opin. Pharmacol.* 2010; **10:**156-165.
3. Chen CM et al. *PLoS ONE* 2012; **7:**e40801.