CCAAT-Enhancer-Binding Protein Homologous Protein Promotes Liver Ischemia and Reperfusion Injury by Inhibiting Beclin-1-mediated Autophagy in Hepatocytes

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ABSTRACT

Background and Aims: Critical role of endoplasmic reticulum (ER) stress has been found in ischemia and reperfusion(IR) injury models. However, the role of CCAAT-Enhancer-Binding Protein Homologous Protein (CHOP) signaling in liver IR injury still remains unclear. The aim of this study is to determine the role and its underlying mechanism of CHOP signaling in liver IR injury.

Method: Wild-type (WT) and CHOP KO mice were subjected to a murine liver partial warm ischemia model. Liver injury and hepatocellular apoptosis was compared between groups. Autophagy and its regulatory signaling pathways were analyzed both in the liver tissues and the primary hepatocytes.

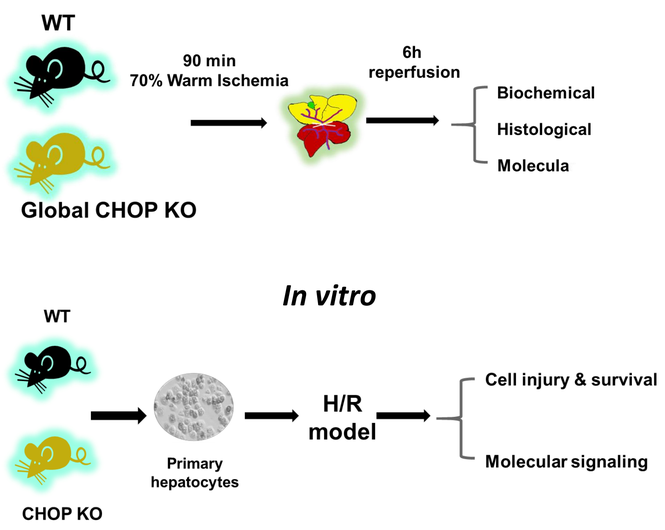
Results: CHOP KO significantly decreased liver IR injury, as evidenced by lower sALT levels and better preserved liver architectures. Liver IR induced marked hepatocellular cell apoptosis, as indicated by HE/TUNEL staining and western blot analysis of cleaved-Caspase-3, BCL-2 and BCL-XL. CHOP KO mice demonstrated much less hepatocellular apoptosis but enhanced autophagy. Primary hepatocytes were isolated and subjected to an in vitro hypoxia/reoxygenation (H/R) model. CHOP KO in hepatocytes resulted in reduced cell death, as measured by LDH and CCK8 assay. Autophagy was enhanced in CHOP KO hepatocytes as evaluated by LC3B staining and the electron microscope examination. Beclin-1 activation was significantly increased in CHOP KO hepatocytes post H/R. Functionally, Beclin-1 siRNA or autophagy specific inhibitor (3-MA) effectively blocked autophagy in hepatocytes and abrogated the protective role of CHOP KO in hepatocyte H/R models.

Conclusion: Our results indicated that CHOP activation promoted liver IR injury by inhibiting Beclin-1-mediated autophagy in hepatocytes. Strategies targeting CHOP or autophagy signaling may provide therapeutic effects against liver IR injury in patients.

OBJECTIVE

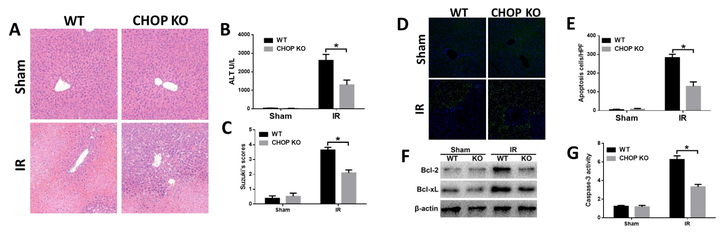
To determine the role of CHOP signaling in IR-stressed liver parenchymal cells.

METHODS



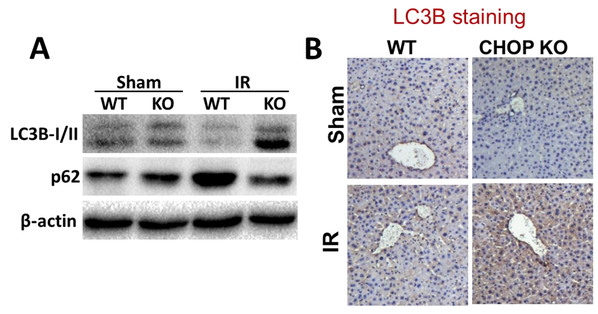
RESULTS

CHOP KO protects against liver IR injury



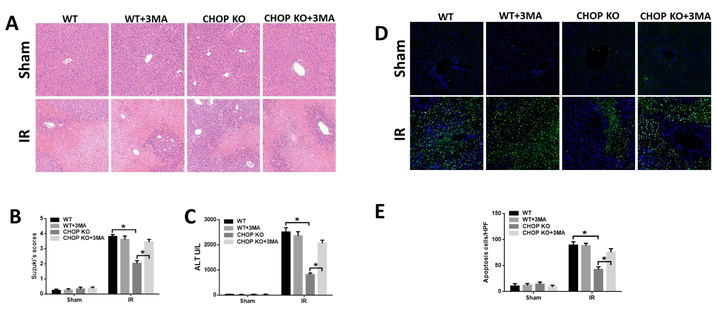
A: Liver IR injury was measured by HE staining, B,C: Serum ALT and Suzuki's score. n=6/group. D: Hepatocellular apoptosis was characterized by TUNEL staining, E: Quantification of apoptosis cells. F: Bcl-2, Bcl-XL and β-actin were determined by Western blot. G: Hepatocellular Caspase-3 activity n=6/group. *p <0.05.

CHOP KO promotes autophagy in livers post IR



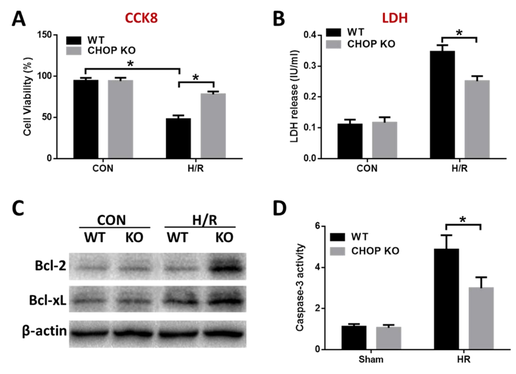
A: Western blot of LC3II/I and p62 from liver of mice in WT and CHOP KO livers. B: Representative immunohistochemical staining of LC3B in WT and CHOP KO liver. n=6/group.

Autophagy mediates the protection of liver IRI by CHOP KO



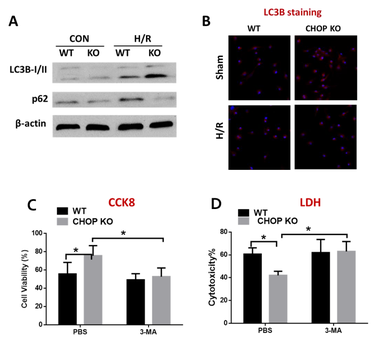
Liver injury after autophagy inhibition by 3-MA pretreatment i.p. 1h prior to ischemia. A: Liver IR injury was evaluated by HE staining, B,C: Serum ALT and Suzuki's score. D: Hepatocellular apoptosis was determined by TUNEL staining. E: Quantification of apoptosis cells. n=6/group. *p <0.05

CHOP KO inhibits primary hepatocyte apoptosis post H/R



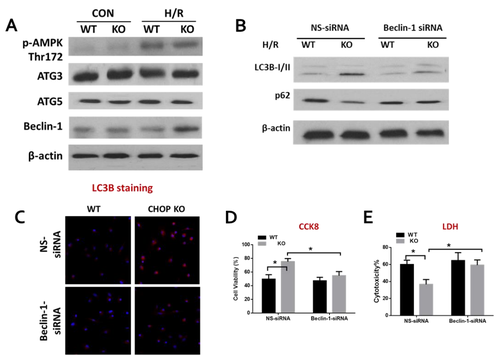
Primary hepatocytes were isolated and subjected to an in vitro hypoxia/reoxygenation (H/R) model. A: Cell viability was measured by CCK-8 assay. B: Cell injury level was determined by LDH release assay. C,D: Primary hepatocyte apoptosis was measured by Western blot of Bcl-2, Bcl-xL and Caspase-3 activity. n=6/group. *p <0.05

Autophagy mediates hepatocellular protection by CHOP KO



Primary hepatocytes were isolated and subjected to an in vitro hypoxia/reoxygenation (H/R) model. A: LC3-I/II and p62 were measured by Western blot . B: LC3B in primary hepatocyte was detected by immunofluorescence. Primary hepatocyte autophagy was inhibited by 3-MA treatment 1h prior to H/R. C: Cell viability was measured by CCK-8 assay. D: Cell injury level was determined by LDH release assay. n=6/group. *p <0.05

Beclin-1 mediates autophagy activation in CHOP KO hepatocyte



A: p-AMPK, ATG3, ATG5 and Beclin-1 were measured by Western blot. Primary hepatocyte was pretreated with Beclin-1 siRNA and NS-siRNA 1h prior to H/R. B: LC3-I/II and p62 were measured by Western blot. C: LC3B in primary hepatocyte was detected by immunofluorescence. D: Cell viability was measured by CCK-8 assay. E: Cell injury level was determined by LDH release assay. n=6/group. *p <0.05

SUMMARY

- CHOP KO significantly protected livers against IR injury.
- CHOP KO promoted autophagy in livers post IR
- Autophagy inhibition by 3-MA abrogated the protective role of CHOP KO in both liver IR model *in vivo* and hepatocyte H/R model *in vitro*.
- Beclin-1 was significantly activatdi in CHOP KO primary hepatocytes post H/R.
- Autophagy inhibition by Beclin-1 siRNA abrogated the protective role of CHOP KO in H/R induced cell injury.

CONCLUSIONS

CHOP KO alleviates liver IR injury and inhibits hepatocellular apoptosis via promoting Beclin-1 dependent autophagy activation in hepatocytes.

ACKNOWLEDGMENTS

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