Xbp1 Knockdown Produces a Protective Effect Against IRI in Mice and Human TECs

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Introduction

Renal ischemia-reperfusion injury (IRI) is the major cause of acute kidney injury (AKI) in the transplant setting. Tubular epithelial cells (TECs) are the primary target of IRI and the source of the resulting stress response. Activation of the endoplasm reticulum (ER) stress has been implicated in the possible aggravation of this injury. We thus sought to determine how Xbp1, a vital transcription factor in the ER stress pathway, affected kidney IRI using both TEC culture system and kidney transplantation in mice.

Methods

We developed an in vitro IRI model of TEC culture, in which primary mouse TECs (mTECs) or human TECs (hTECs) were subjected to cold ischemia time (CIT, 4℃) for 6hr followed by replacement with fresh media (37℃) for 2 or 24hr. mRNA-sequencing was performed using the normal mTECs and 2hr IRI mTECs to explore the predominantly regulated pathways. The in vitro IRI model was used in combination with Xbp1-siRNA transfection or 4μ8C, an IRE1α/Xbp1 inhibitor, to investigate the effects or co-cultured with bone marrow-derived macrophages (BMDMs). In the kidney transplant model, donor kidneys were kept in cold storage for 3hr, then transplanted into allogeneic recipients and analyzed at 3 or 24hr post-transplant.

Results

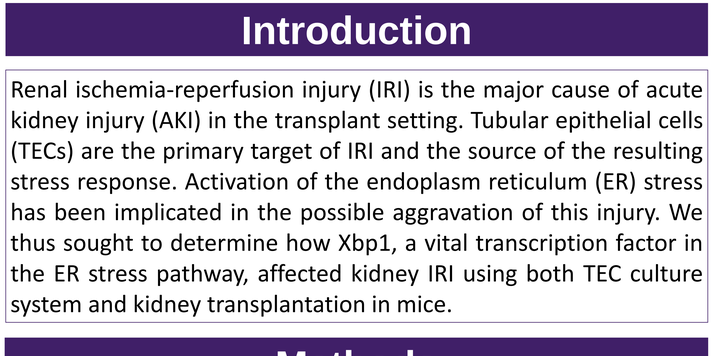

Figure 1| Experiment design and effect. (a) Flow chart of the experiment design. (b) LIVE/DEAD fluorescence staining of mTECs displaying proof of IRI as a result of the experiment. Cell death significantly increases over time as IRI progresses.

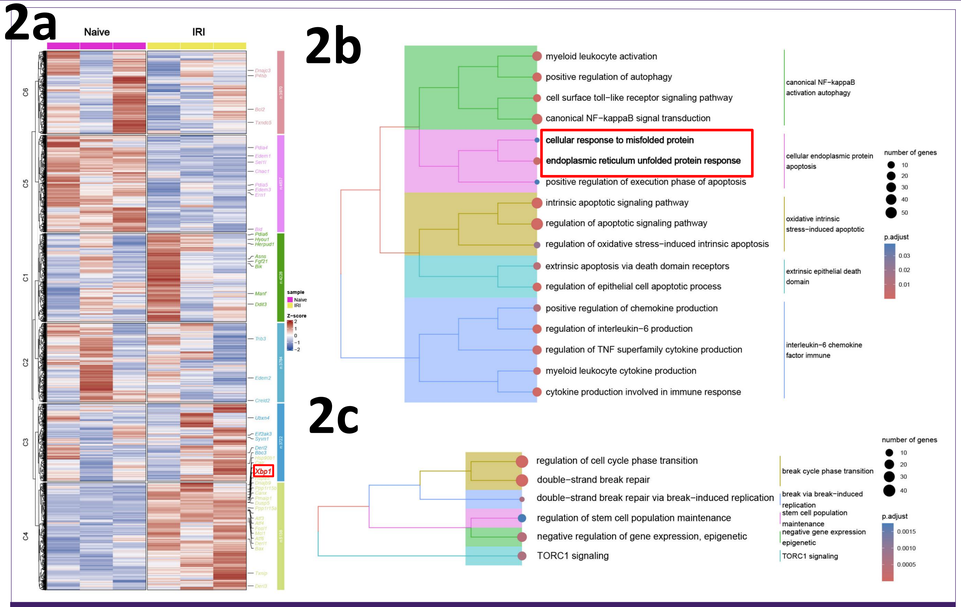

Figure 2 | mRNA-seq analysis between the control TECs and 2H IRI TECs. (a) Heatmap showed the differences between the two groups. Specially, Xbp1, the key transcriptional factor in ER stress, was remarkably up-regulated. (b-c) The DEGs were further determined using Gene Ontology (GO) analysis. Consistently, endoplasmic reticulum unfolded protein response was hyperactivated upon IRI treatment.

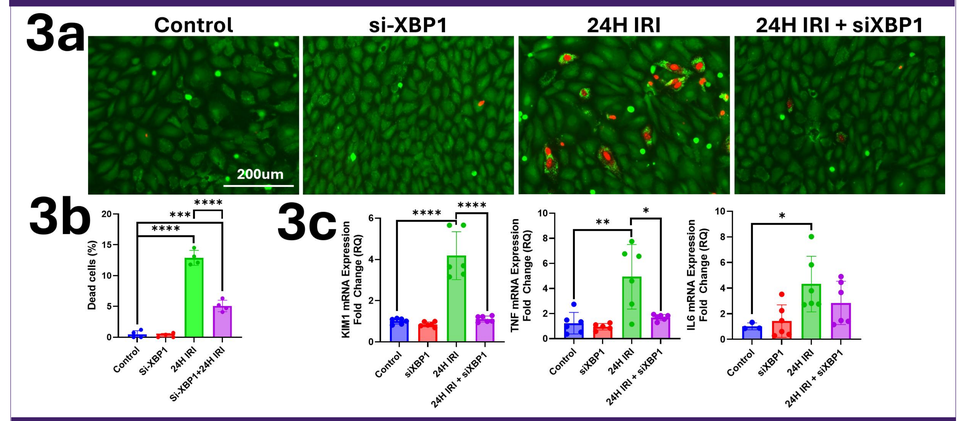

Figure 3 | The effects of knocking down XBP1 on injury of TECs via cold ischemia and IRI. (a) LIVE/DEAD fluorescence staining of mTECs. siRNA transfected TECs at 24H IRI exhibit significantly less cell death than untreated TECs that have undergone 24H IRI. (b) Quantification of LIVE/DEAD staining showing percentage of dead TECs. (c) KIM1, TNF, and IL6 mRNA expression for siRNA and

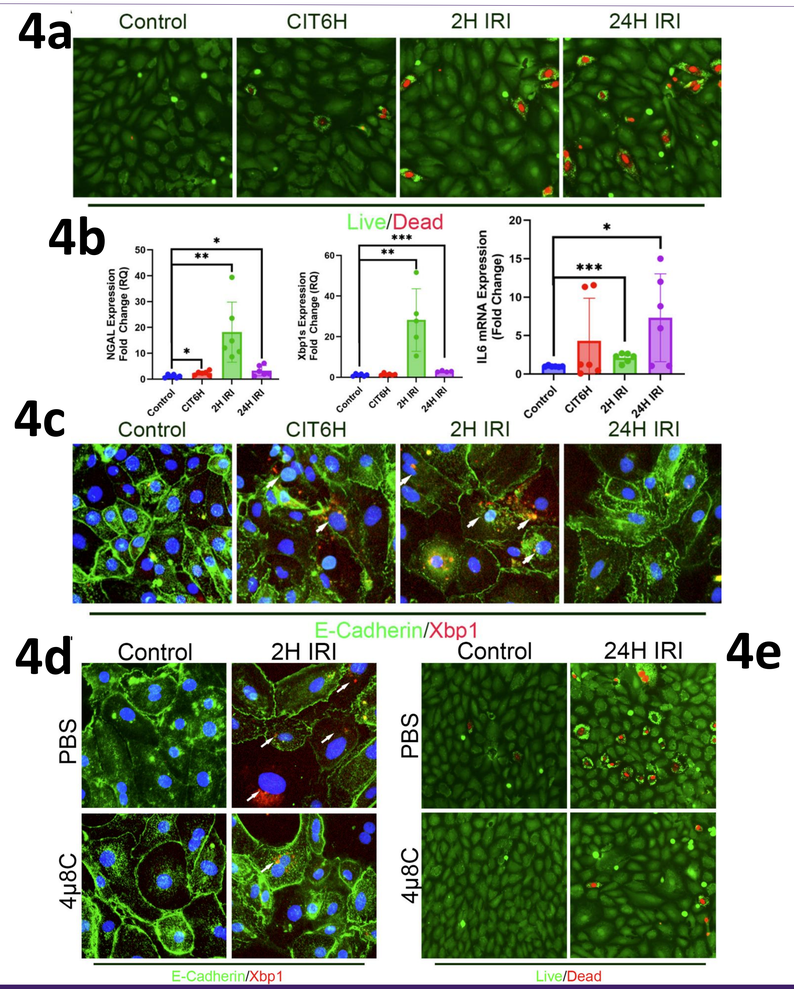

Figure 4 | IRI and 4μ8C treatment of hTECs. (a) LIVE/DEAD fluorescence staining of hTECs displaying an increase in cell death over time during post-hypoxia reperfusion. (b) NGAL, Xbp1s, and IL6 mRNA expression for CIT6H, 2H IRI, and 24H IRI. (c) DAPI, E-cadherin, and Xbp1 fluorescence staining of hTECs. (d) DAPI, E-cadherin, and Xbp1 fluorescence staining of hTECs for 4μ8C treated and PBS treated groups at the control and 2H IRI timepoints. (h) LIVE/DEAD fluorescence staining of hTECs at the control and 24H IRI timepoints with and without 4μ8C treatment.

Conclusion

These findings indicate that IRI-induced activation of TEC intrinsic ER stress pathways drives pro-inflammatory response and cell death. The inhibition of the Xbp1-dependent ER stress pathway presents as a promising approach to mitigating IRI.

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