The role of siRNA knockdown of X-box-binding protein 1 (XBP1) in mitigating renal ischemic reperfusion injury

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Introduction

Renal ischemic reperfusion injury (IRI), a large increase in cell death and poor function due to cold preservation and subsequent implantation, is the major cause of acute kidney injury (AKI) in the transplantation setting. Tubular epithelial cells (TECs) are the primary target of IRI and a source of the resulting cellular stress and inflammatory response. Targeting the unfolded protein response (UPR) has been implicated in the possible mitigation of this injury. Our previous scRNA-seq analysis showed that XBP1-mediated UPR pathway was robustly activated. We thus aimed to determine the role of XBP1, the vital transcription factor in the UPR pathway, in TECs using a novel in vitro model of TEC IRI.

Methods

Kidneys from wild-type (WT) B6 and Balb/C mice were harvested and TECs were isolated and cultured. These TECs, after reaching confluency, underwent XBP1-siRNA transfection or treatment as usual (control). To mimic IRI in vitro we developed an in vitro model which subjected TECs to hypoxia for 6hr in cold UW solution (4°C, CIT) followed by 24h of reperfusion with fresh media (37°C, IRI). This created 4 separate experimental groups: control, XBP1-siRNA, 24h IRI, and 24h IRI + XBP1-siRNA. We then used qPCR to quantify gene expression differences of injury markers or inflammatory cytokines, as well as live/dead staining to detect the cell death between groups.

Results

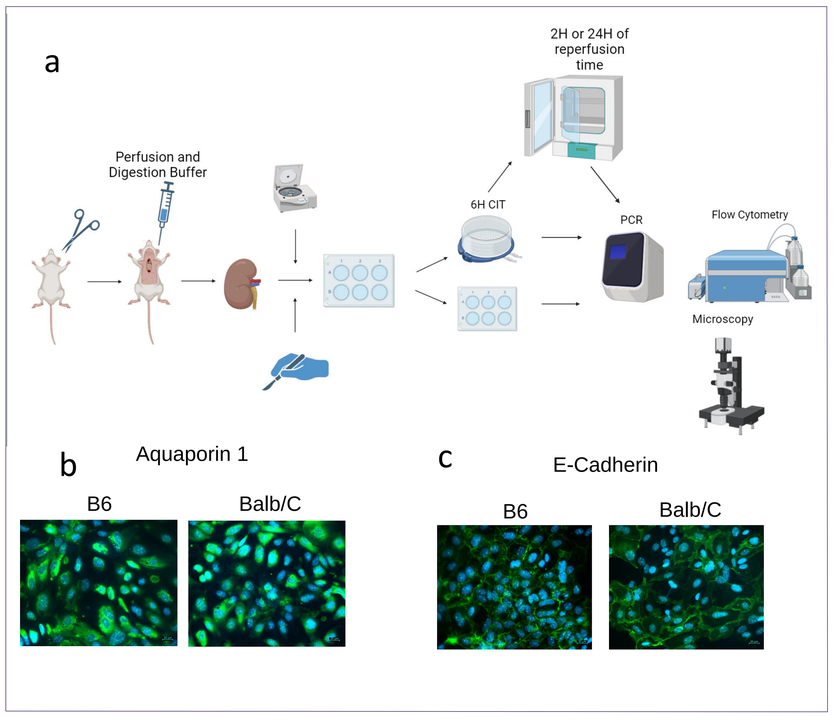

Figure 1| Experiment design and Characterization of TECs. a, Flow chart of the experiment design. b, c, Staining of Aquaporin 1 and E-Cadherin confirming the characterization of isolated TECs (Balb/C).

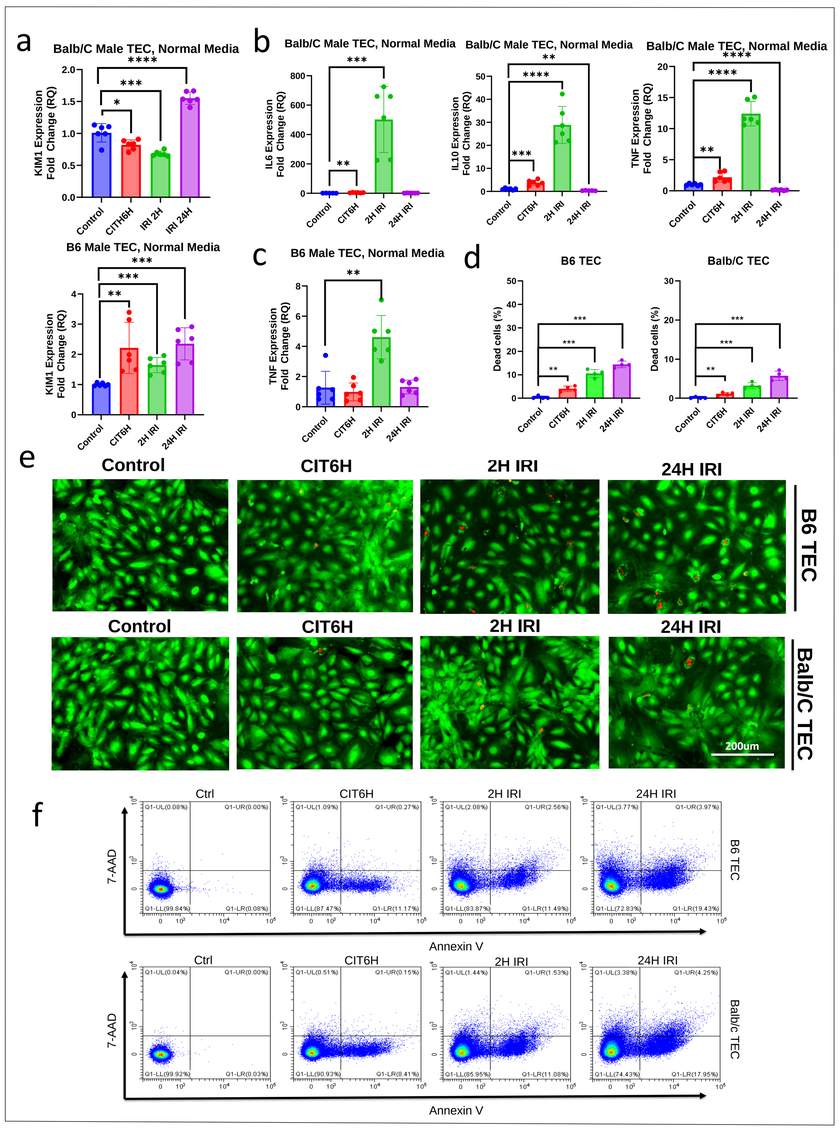

Figure 2| Confirmation of TEC injury. a, Upregulation of kidney injury biomarkers after CIT and IRI. b, Up-regulation of inflammatory biomarker after CIT and IRI (Balb/C). c, Up-regulation of inflammatory biomarker after CIT and IRI (B6). d-e, Live/Dead staining (Green = Live, Red = Apoptotic) of TECs with no injury, CIT6H, 2H IRI, 24H IRI. Quantification of Live/Dead staining. f, flow cytometry of Annexin V/7-AAD apoptosis assay.

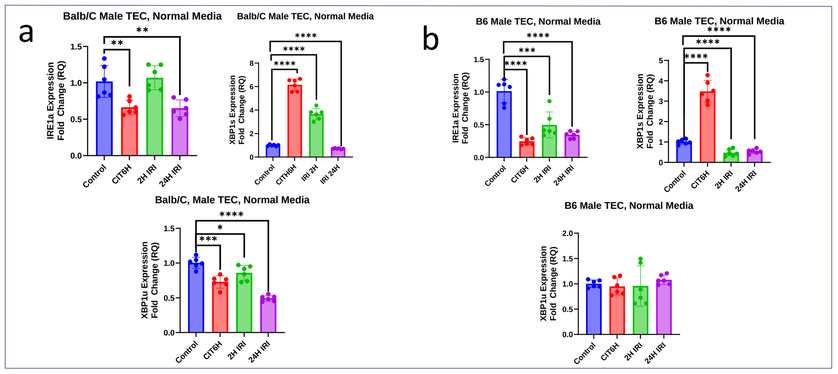

Figure 3| | Activation of the ER stress pathway has been implicated in the possible mitigation of IRI. XBP1 specifically has been seen to be upregulated during injury. a, Significant upregulation of XBP1s after injury (Balb/C). b, Significant upregulation of XBP1s after injury (B6).

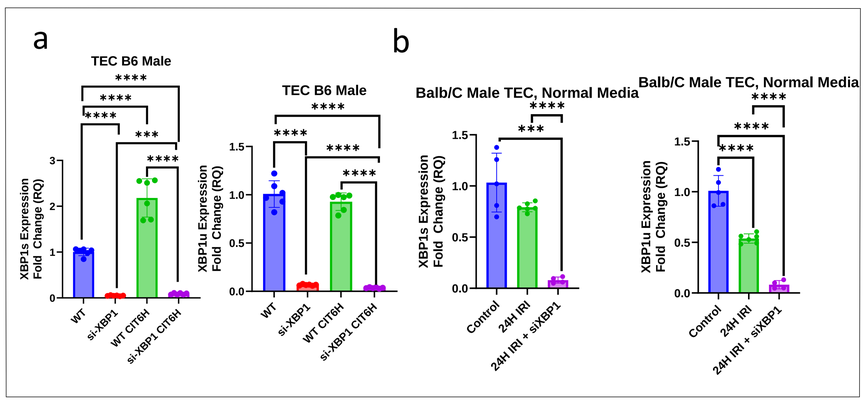

Figure 4| XBP1 siRNA KD efficiency. a, XBP1s and XBP1u expression for B6. siRNA KD was very effective. B, XBP1s and XBP1u expression for Balb/C, siRNA KD was very effective.

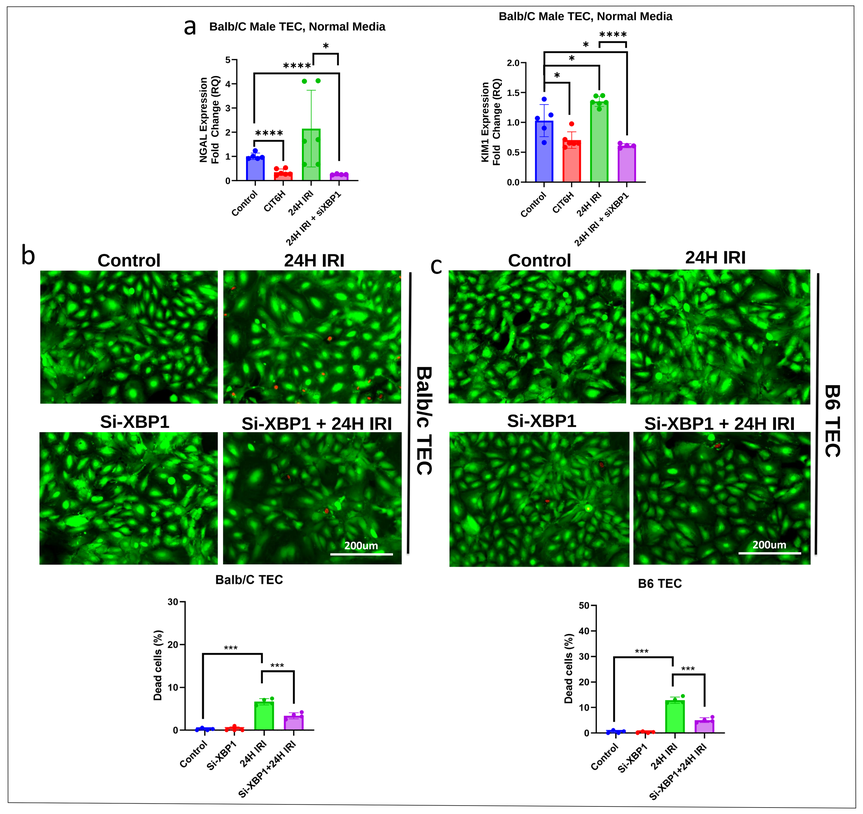

Figure 5| Alleviation of TEC injury via si-XBP1 treatment. a, Treatment with si-XBP1 greatly reduces kidney injury markers in the 24H timepoint that would otherwise have upregulated expression of kidney injury biomarkers (Balb/C). b, Live/Dead staining of Balb/C TECs, showing reduced cell death in 24H IRI + si-XBP1 condition compared to 24H IRI. c, Live/Dead staining of B6 TECs, showing reduced cell death in 24H IRI + si-XBP1 condition compared to 24H IRI.

Conclusion

Our in vitro IRI model exhibited a classic injury phenotype of TECs, which partially mimicked the IRI process in vivo. In addition, TECs displayed the upregulation of XBP1s expression resulting from IRI. Knockdown of XBP1 protected the TECs from IRI-mediated cell death and inflammatory response, which implicates XBP1 as a promising target for therapeutics to alleviate renal IRI and improving kidney transplant survival.

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