Targeting Recipient-derived macrophage Xbp-1 protects early kidney allotransplant function by promoting renal cell regeneration through the downregulation of Klf4.

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Introduction

The IRE1α/XBP1 pathway is part of the unfolded protein response (UPR), a cellular stress response related to the endoplasmic reticulum (ER). The UPR is activated in response to an accumulation of unfolded or misfolded proteins in the ER. IRE1α/XBP1 pathway has been shown to play a role in developing various diseases, including kidney disease, and has been reported to regulate inflammatory responses in macrophages. However, the role of IRE1a/Xbp1 in macrophage in kidney transplantation has not been researched. This study aims to find the role of macrophage-derived IRE1a/Xbp-1 ER stress pathway in tubular epithelial cell regeneration after kidney transplantation.

Methods

The impact of transplant ischemia-reperfusion injury (IRI) on the ER stress response was examined. We used conditional Knockout mice lacking Xbp-1 in myeloid cells to investigate macrophage Xbp-1's role in modulating kidney graft epithelial cell regeneration in kidney transplantation and epithelial IRI models. A clinically relevant mice kidney transplantation model was used in this research, and a new epithelial cell cold ischemic-reperfusion injury model was established.

Results

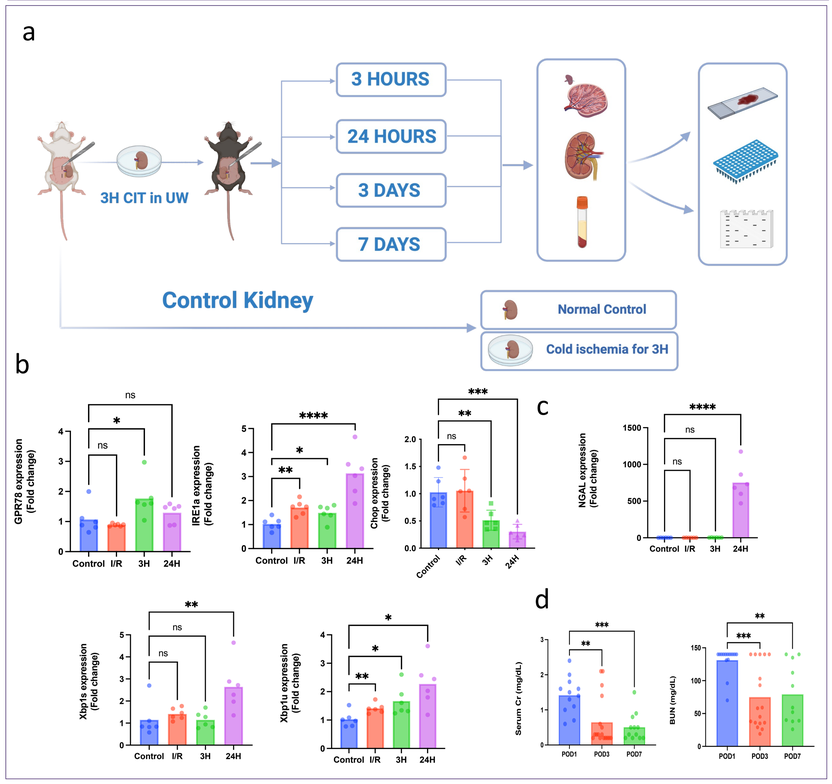

Figure 1| a, Flow chart of the experiment design. b, Activation of IRE1a-Xbp1 pathway kidney transplantation. c, Up-regulation of kidney injury biomarker after kidney transplantation. d, Graft function recovery after transplantation.

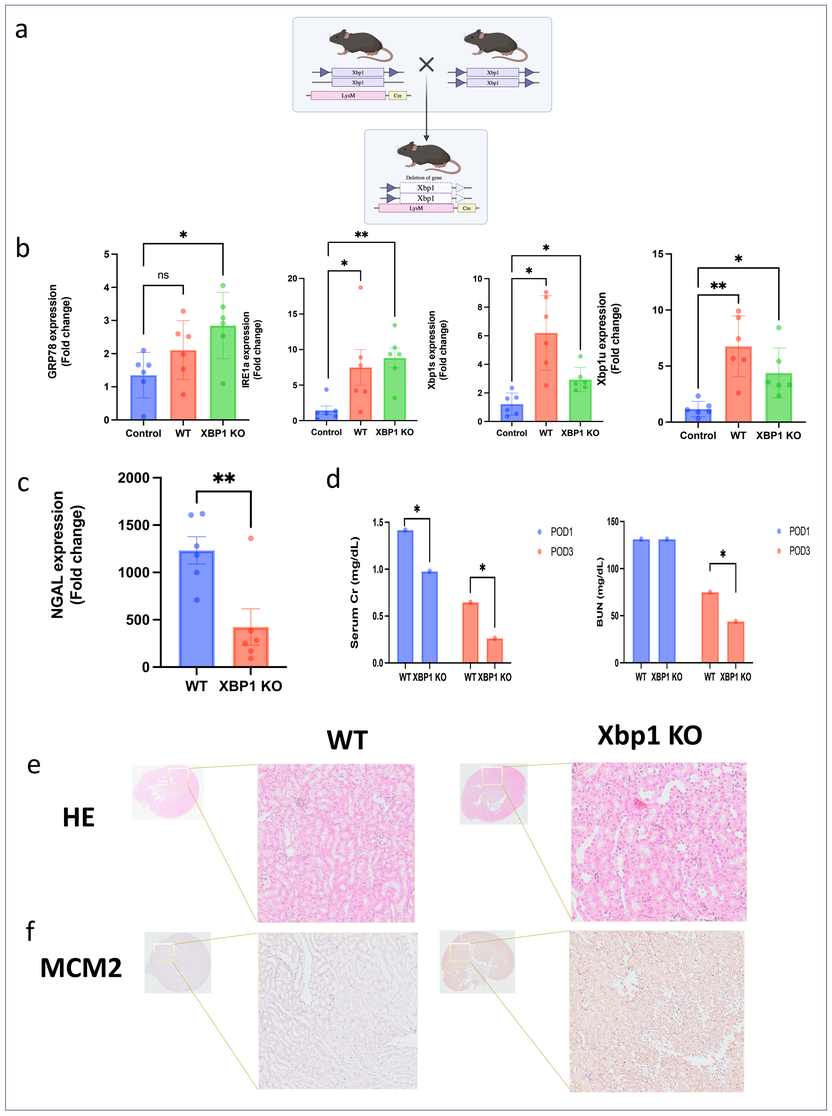

Figure 2| Conditional Knockout of Xbp1 in Myeloid cells helps recovery of graft function at the early stage after kidney transplantation. a, Generation of the conditional Knockout mice lacking Xbp-1. b, Upregulation of Xbp-1 upstream genes and Downregulation of both types of Xbp-1 after kidney transplantation in the Xbp1 KO group. c, Downregulation of kidney injury biomarkers in the Xbp1 KO group. d, Better graft function in the Xbp1 KO group 24 Hours and 3 Days post-transplantation. e, HE staining of WT and Xbp1 showing worse kidney injury in WT mice 24 hours post-transplantation. f, Kidney regeneration marker MCM2 staining.

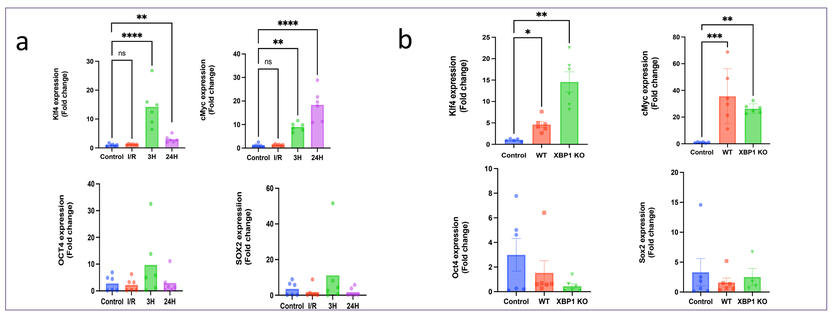

Figure 3| Kidney regeneration panel after transplantation. a, Kidney regeneration panel in WT at early stage after transplantation. b, Kidney regeneration panel in WT and Xbp1 KO mice 24 hours post transplantation.

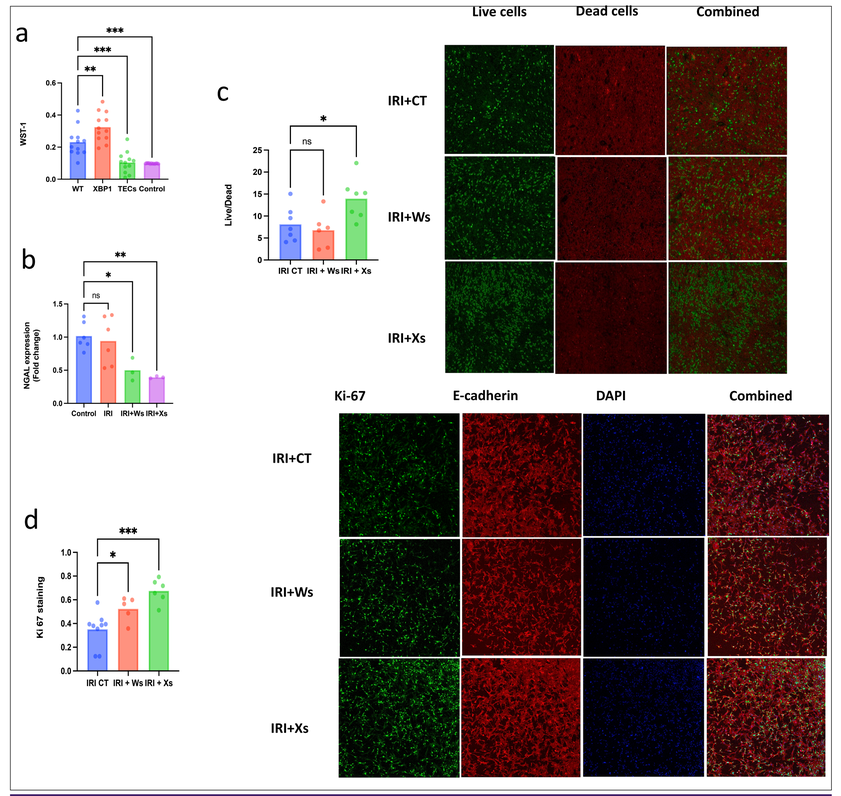

Figure 4| Crosstalk of macrophage and renal epithelial cells. a, Results of cell proliferation experiment WST-1 showing that macrophage supernatant has pro-proliferation ability, and the ability of Xbp-1 KO macrophage is stronger. b, The kidney injury biomarker NGAL expression was downregulated in epithelial cells with macrophage supernatant and better in Xbp1 macrophage supernatant. c, d Result of Live/Dead and of cell proliferation marker Ki67 staining showed better survival in epithelial with supernatant from Xbp1 KO macrophages.

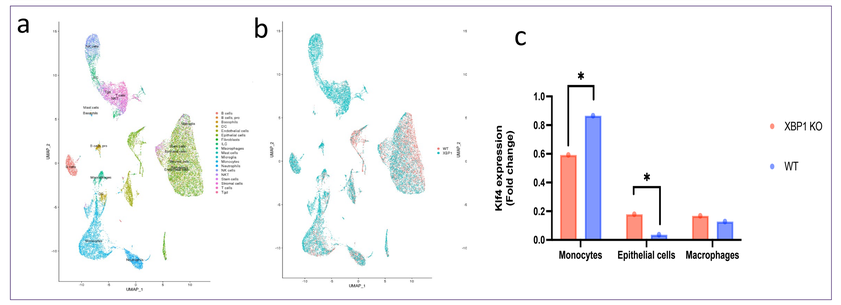

Figure 5| Single-cell analysis of WT and Xbp1 KO graft at 3 days post-transplantation. a, b, A composite Uniform Manifold Approximation and Projection (UMAP) from a graft of WT and Xbp1 KO recipient showing 19 various kidney and immune cell clusters(n=2 mice per group, four grafts total in aggregate). c, Klf4 expression in different cell clusters shows that Klf4 expression was downregulated in monocytes but upregulated in tubular epithelial cells.

Conclusion

Recipient-derived macrophage Xbp-1 protects early kidney allotransplant function by the downregulation of Klf4 to promote renal epithelial cell regeneration. Donor-derived macrophage IRE1α-XBP1 pathway can be explored as a potential future therapeutic target against IRI of graft.

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