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XBP1U overcomes the XBP1S-mediated upregulation of the iNOS gene expression in ERS

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Introduction: The human inducible nitric oxide synthase (hiNOS) can be induced to expressed under inflammatory, pathogens invision, endoplasmic reticulum stress (ERS) and many pathological conditions. Upon these conditions, the unfolded protein response (UPR) will be activated. The IRE1-XBP1 pathway, as the most conserved branch of UPR, participates in UPR signal pathway regulation. There are two types of X-box binding protein1 (XBP1), XBP1 unspliced (XBP1U) and XBP1 spliced (XBP1S). Here we demonstrate that the transcriptional regulation mechanism of the human iNOS gene by XBP1 in ERS. We found that (1) XBP1S activates iNOS expression via AABS(A-activator-binding site) element. (2) There exists protein interaction between XBP1U and XBP1S.(3) XBP1U expression will be reduced and inhibit XBP1S expression in ERS. (4) XBP1U can affect XBP1S-mediated regulation of iNOS in ERS. Take together, There is the dynamic interplay between XBP1U and XBP1S, XBP1U is a negative regulator of XBP1S and regulate on iNOS gene expression via XBP1S to adapt to physiological changes during ERS.

Methods: Electrophoretic Mobility Shift Assay (EMSA); Reporter Gene Assay; Chromatin Immunoprecipitation (ChIP);Co-immunoprecipitation (CoIP) Assay; Immunofluorescent Cell Staining; siRNA assay.

Results and Discussion: XBP1S binds to the AABS in iNOS promoter in vitro and in vivo — The EMSA assay were performed to determine whether XBP1S binds to iNOS promoter in vitro, and the result showed that XBP1S is able to bind AABS domain in iNOS promoter (Fig.1A-a). The ChIP assay were performed to determine whether XBP1S binds to iNOS promoter in vivo, As shown in Fig. 1A-b,c, these results demonstrate that endogenous XBP1S binds to the native AABS in the iNOS gene in transfected living cells.

XBP1S upregulates iNOS-specific reporter construct activities —The core sequence of iNOS promoter is found from-286 to +96bp, And it is indicated that the transactivation of iNOS gene expression depends on both AABS and NF-ĸB sites located in iNOS promoter with Reporter Gene Assay(Fig.1B,C).

XBP1U and XBP1S form a heterodimer in vivo in ERS — In HepG2 cells with ERS, Immunoprecipitation of the cell lysates with antibodies to either one of the two proteins, but not control IgG, immunoprecipitated all two proteins, It demonstrated that endogenous XBP1U specifically binds to XBP1S in vivo in ERS(Fig.1D).

Knockdown of XBP1U enhances the XBP1S-mediated upregulation of the iNOS gene expression in ERS — Immunofluorescent cell staining with human HepG2 cell transfected with pSUPER-XBP1U or pSUPER vector demonstrated that expression of the specific siRNAs efficiently reduced the levels of the corresponding proteins (Fig.1E). Next,XBP1U expression was reduced in ERS after transfected with pSUPER XBP1U(Fig.1F-a).XBP1S and iNOS expression were increased after treated with different dosage of si-XBP1U in ERS(Fig.1F-b,c).

In conclusion, this study provides evidence showing that XBP1U associates with XBP1S and inhibits XBP1S-mediated upregulation of iNOS gene. On basis of these findings,we propose a model for explaining the transcriptional control of iNOS gene expression in ERS(Fig.2).During ERS, XBP1U will be spliced and produced XBP1S, more XBP1U will inhibit XBP1S expression and overcomes the XBP1S-mediated upregulation of iNOS expression.

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Fig.1

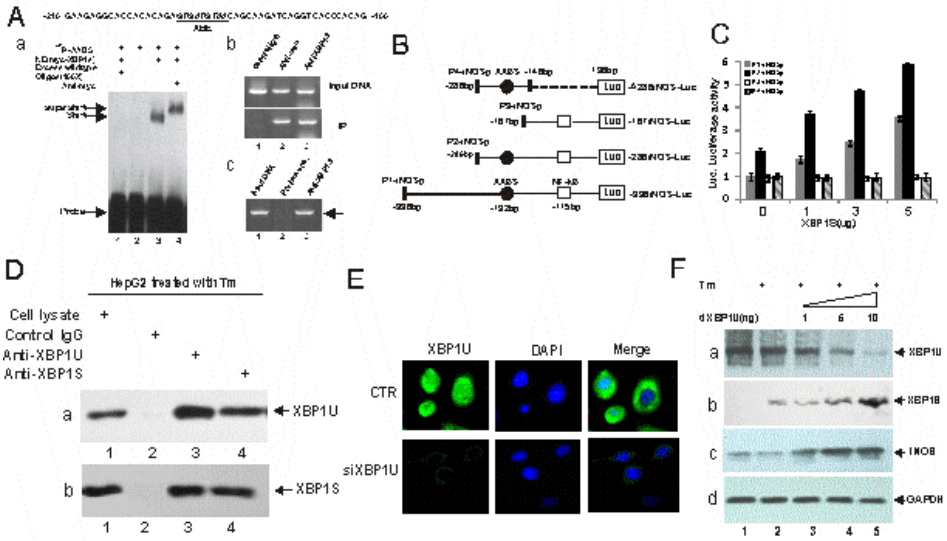

Fig.1.(A) XBP1S binds to the AABS of the iNOS promoter in vitro(EMSA) and in vivo(CHIP). (B) schematic structures of four iNOS-specific reporter constructs.(C) Reporter gene assay.XBP1S-mediated iNOS transactivation is dose-dependent. (D)XBP1U and XBP1S formed a complex in HepG2 cells in ERS (Co-IP).(E) siRNAs against XBP1U efficiently suppress the expression of their target molecules, assayed by immunofluorescence cell staining. (F)Western blotting assay of XBP1U(a), XBP1S(b) and iNOS(c) expression in HepG2 cell lines with different dosage pSUPER-XBP1U siRNA in ERS.

Fig.2

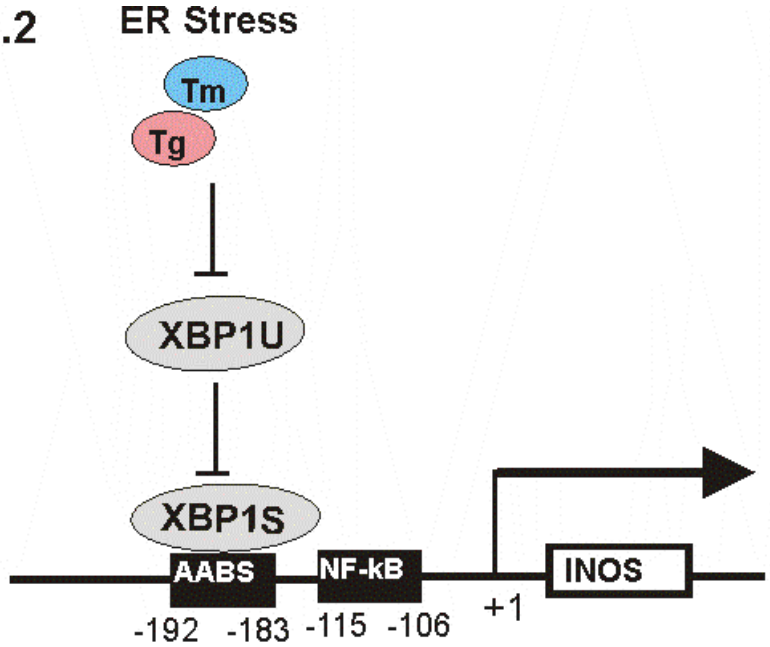

Fig.2.A proposed model for explaining the transcriptional control of iNOS gene expression in ERS. Numbers indicate distances in nucleotides from the first nucleotide of intron 1. "→" and "⊣" indicate activation and repression, respectively.

Keywords: Endoplasmic Reticulum Stress, Unfolded Protein Response, X-box Binding Protein 1, Inducible Nitric Oxide Synthase