

Mitochondrial retrograde signaling to the endoplasmic-reticulum regulates unfolded protein responses

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ABSTRACT

Unfolded protein response (UPRs) directs adaptation or apoptosis depending on the severity of endoplasmic-reticulum (ER) stress. We found that apoptotic signaling by inositol requiring enzyme 1 α (IRE1 α), a transducer of UPRs, is suppressed by mitochondrial ubiquitin ligase MITOL/MARCH5 on ER-mitochondria contacts, suggesting that mitochondria regulate cell fate under ER stress.

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Introduction

The endoplasmic reticulum (ER) is the largest cellular organelle involved in the synthesis and folding of membrane/secretory proteins, lipid metabolism, and calcium storage. Therefore, adaptive ER response is a pivotal intracellular signaling for cell adaptation to intra – and extracellular environmental changes. Various physiological and pathological environmental changes, such as hypoxia, low-nutrition, oxidative stress, and increase in protein synthesis, promote adaptive ER reactions through the activation of ER stress responses, also known as the unfolded protein response (UPR) signaling. UPR signaling is initiated by three ER-sensor proteins, protein kinase R-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol requiring enzyme 1 α (IRE1 α) (also known gene name *EIF2AK3*, *ATF6*, *ERN1*, respectively).¹ When the ER perceives the imbalance in ER homeostasis, the UPR signaling mediates cell survival and adaptation to eliminate ER stress. However, when ER stress becomes severe and irreversible, UPR signaling triggers cell death.

The molecular understanding of the balance between cell adaptation and cell death in response to environmental changes may contribute to clarify the pathogenesis of various diseases caused by micro-environmental changes. Upregulation of ER functions has also been reported to allow tumor cells to adapt to cell-intrinsic and cell-extrinsic stresses. Several studies have indicated that tumor cells maintain their high proliferative capacity even in the severe stress conditions, such as hypoxia and acidic extracellular pH, depending on the adaptive UPR signaling.² Importantly, although robust ER stress is observed in most tumor cells, they escape cell fate toward apoptosis triggered by apoptotic switch of UPR signaling.²

We have previously identified mitochondrial ubiquitin ligase (MITOL, also known gene name *MARCH5*), which is integrated into the mitochondrial outer membrane, and demonstrated that MITOL plays critical roles in mitochondrial homeostasis and signaling.^{3,4} We noticed that MITOL is abundantly localized in the proximal junction between the ER

and mitochondria, suggesting a possibility that MITOL may regulate cellular signalings provoked not only from mitochondria but also from the ER.

Results

Recently, we have identified IRE1 α , one of the three UPR sensor proteins, as a novel substrate for MITOL. IRE1 α is a unique protein integrated in the ER membrane with dual catalytic activity, kinase and RNase.⁵ Under mild ER stress, IRE1 α undergoes dimerization to induce cell adaptation by X-box-binding protein-1 (XBP1) mRNA splicing. In contrast, under severe or prolonged ER stress, IRE1 α forms self-oligomers and cleaves various mRNA/miRNAs, including pro-survival mRNA and anti-apoptotic miRNA, thereby leading to cell death. We have demonstrated that MITOL directly ubiquitinates K481 of IRE1 α at ER-mitochondria membrane contact sites. MITOL inhibits excessive oligomerization of IRE1 α , thus, prevents IRE1 α -dependent decay of mRNA/miRNAs by adding K63-linked polyubiquitin chain, which regulates the activity of substrate but not relates to proteasome degradation, to IRE1 α . In MITOL deficient cells under ER stress, a drastic cell death was triggered by activation of IRE1 α branch of the UPR signaling. Furthermore, overexpression of the IRE1 α mutant K481R, unrelated to the ubiquitination by MITOL, phenocopied the enhanced alternative IRE1 α signaling observed in MITOL deficient cells, such as excessive IRE1 α oligomerization and remarkably decay of anti-apoptotic miRNA. Thus, our findings indicate that the ubiquitination of IRE1 α K481 is one of the key regulatory mechanisms directing IRE1 α signaling to apoptosis (Figure 1).

Discussion

Among the three UPR branches, the IRE1 α -XBP1 pathway is strongly associated with tumor development.^{6,7} Cells inside solid

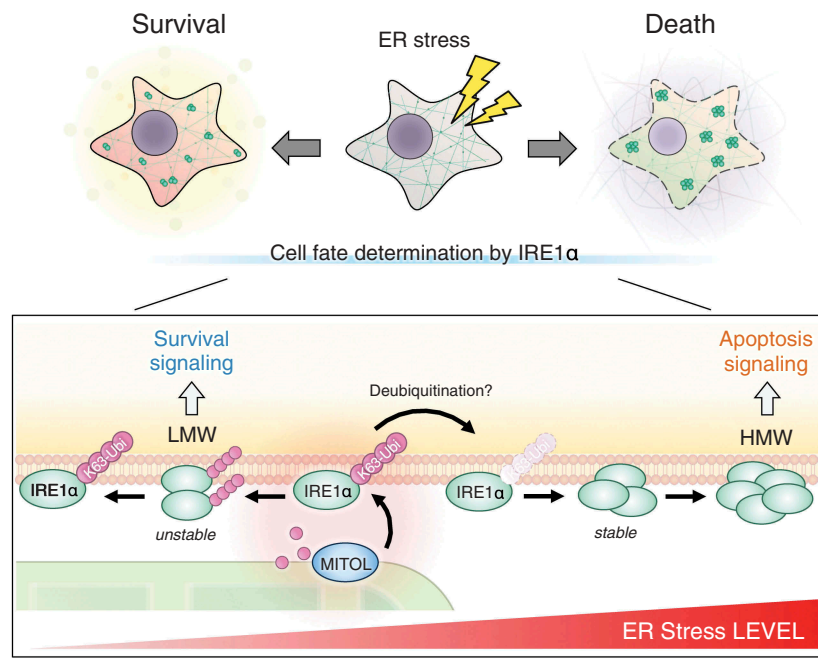


Figure 1. Mitochondrial ubiquitin ligase (MITOL)-dependent mitochondrial retrograde signaling in inositol requiring enzyme 1 α (IRE1 α) regulation. Under basal conditions or low level of endoplasmic-reticulum (ER) stress, MITOL interacts with and ubiquitinates IRE1 α by the K63-linked polyubiquitin chain (K63-Ubi), preventing excessive oligomerization and continuous activation of IRE1 α . This regulatory machinery mediated by MITOL contributes to cell survival under the permissible range of ER stress. Conversely, under irremediable ER stress, the IRE1 α ubiquitination by MITOL is decreased by unclear mechanisms, thereby triggering IRE1 α hyper-activation with mRNA/miRNA decay and apoptosis. LMW: low molecular weight, HMW: high molecular weight.

tumors were exposed by stress conditions including hypoxia, glucose starvation, and an increase of protein synthesis for high proliferation. These stresses have reported to activate UPR signaling; thus, intrinsic/extrinsic environmental changes in tumor cells are considered to result in the activation of UPR signaling. Several studies have showed that XBP1 activation is pivotal for environmental adaptation and cell survival in tumor cells. In contrast, the IRE1 α -dependent apoptosis is scarcely induced in the tumor cells in spite of its obvious activation of the IRE1 α -XBP1 pathway. In a recent study, we found that a key mitochondrial regulator MITOL inhibits the apoptotic switch of ER-sensor IRE1 α by K63-linked polyubiquitin chain at ER-mitochondria contact sites.⁵ Previous reports have indicated that MITOL is highly expressed in breast cancers, which caused efficient and excessive tumor proliferation.⁸ Therefore, there may be cases that hyperactivation of MITOL suppresses cell death by inhibiting excessive oligomerization of IRE1 α in tumor cells. On the other hand, mutations of the *MITOL/MARCH5* gene, which is identified in somatic endometrial cancers, lead to loss of catalytic-activity of MITOL.⁹ The dualistic role of MITOL in tumor development may be resulted from the multi-functional aspects of MITOL. Therefore, in some cases such as endometrial cancers, MITOL dysfunction and subsequent decrease in mitochondrial homeostasis may cause metabolic changes within the cancer and induce the Warburg effect, contributing to cancer development. Other proteins, such as BIM (also known gene name *BCL2L11*), have also been reported to regulate excessive IRE1 α oligomerization by direct interaction, which is important for apoptotic switch of IRE1 α signaling. Thus, in cases including endometrial cancers, other factors may inhibit apoptotic switch of IRE1 α signaling independently of

MITOL. Further understating of apoptotic switch of IRE1 α signaling is required to develop a novel therapy targeting UPR signal switch.

Recently, ER stress has also reported to regulate anti-tumor immunity. The tumor microenvironmental conditions, such as secreted factors and acidosis, triggers ER stress in tumor-infiltrating immune cells. IRE1 α contributes to secretion of inflammatory cytokines as well as proteostasis in the ER via XBP1 up-regulation, although excessive and sustained activation of IRE1 α impairs cellular functions of the infiltrating immune cells, thereby leading to tumor immune evasion. These facts suggest that optimal ER stress response in tumor-infiltrating immune cells is required for anti-tumor immunity. Interestingly, we found that the K63-linked ubiquitination of IRE1 α by MITOL is decreased following prolonged ER stress (Figure 1). Thus, under chronic ER stress, UPR signaling by IRE1 α may shift to the alternative UPR signaling causing cell death by reduction of its inhibitory ubiquitination. Dissociation of K63-linked polyubiquitin chain from IRE1 α is a faster than IRE1 α protein turnover, suggesting that ubiquitin-specific proteases (USPs) or deubiquitinase-enzymes (DUBs) also determines the apoptotic switch of IRE1 α via releasing of the polyubiquitin chain. Thus, the identification of unknown USP/DUBs involving in the IRE1 α regulation may lead to more efficient drug development to control the apoptotic switch of IRE1 α signaling, and the drug(s) may also be available for cancer treatment and immunotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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