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

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ABSTRACT
Unfolded protein response (UPRs) directs adaption or apoptosis depending on the severity of endoplasmic-reticulum (ER) stress. We found that apoptotic signaling by inositol requiring enzyme 1α (IRE1α), 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.

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 1α (IRE1α) (also known gene name EIF2AK3, ATF6, ERN1, respectively). 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. Uprogulation 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. Importantly, although robust ER stress is observed in most tumor cells, they escape cell fate toward apoptosis triggered by apoptotic switch of UPR signaling.

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. 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 IRE1α, one of the three UPR sensor proteins, as a novel substrate for MITOL. IRE1α is a unique protein integrated in the ER membrane with dual catalytic activity, kinase and RNase. Under mild ER stress, IRE1α undergoes dimerization to induce cell adaptation by X-box-binding protein-1 (XBP1) mRNA splicing. In contrast, under severe or prolonged ER stress, IRE1α 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 IRE1α at ER-mitochondria membrane contact sites. MITOL inhibits excessive oligomerization of IRE1α, thus, prevents IRE1α-dependent decay of mRNA/miRNAs by adding K63-linked polyubiquitin chain, which regulates the activity of substrate but not relates to proteasome degradation, to IRE1α. In MITOL deficient cells under ER stress, a drastic cell death was triggered by activation of IRE1α branch of the UPR signaling. Furthermore, overexpression of the IRE1α mutant K481R, unrelated to the ubiquitination by MITOL, phenocopied the enhanced alternative IRE1α signaling observed in MITOL deficient cells, such as excessive IRE1α oligomerization and remarkably decay of anti-apoptotic miRNA. Thus, our findings indicate that the ubiquitination of IRE1α K481 is one of the key regulatory mechanisms directing IRE1α signaling to apoptosis (Figure 1).

Discussion
Among the three UPR branches, the IRE1α-XBP1 pathway is strongly associated with tumor development. Cells inside solid
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\(\alpha\)-dependent apoptosis is scarcely induced in the tumor cells in spite of its obvious activation of the IRE1\(\alpha\)-XBP1 pathway. In a recent study, we found that a key mitochondrial regulator MITOL inhibits the apoptotic switch of ER-sensor IRE1\(\alpha\) by K63-linked polyubiquitin chain at ER-mitochondria contact sites.

Previous reports have indicated that MITOL is highly expressed in breast cancers, which caused efficient and excessive tumor proliferation. Therefore, there may be cases that hyperactivation of MITOL suppresses cell death by inhibiting excessive oligomerization of IRE1\(\alpha\) 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. The dualistic role of MITOL in tumor development may be resulted from the multi-functional aspects of MITOL. Therefore, there may be cases that hyperactivation of MITOL suppresses cell death by inhibiting excessive oligomerization of IRE1\(\alpha\) 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. The dualistic role of MITOL in tumor development may be resulted from the multi-functional aspects of MITOL.

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

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.
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