

ER Stress Signaling and its Role in Cancer Cell Death

OPEN ACCESS

Volume: 8

Special Issue: 1

Month: February

Year: 2021

P-ISSN: 2321-788X

E-ISSN: 2582-0397

Impact Factor: 3.025

Citation:

Kavitha, CV, and SK Poornima. "ER Stress Signaling and Its Role in Cancer Cell Death." *Shanlax International Journal of Arts, Science and Humanities*, vol. 8, no. S1, 2021, pp. 81–86.

DOI:

<https://doi.org/10.34293/sijash.v8iS1-Feb.3937>

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Abstract

The endoplasmic-reticulum (ER) stress response represents a cellular process that is provoked by a number of circumstances that disturb folding of proteins in the ER. An evolutionarily conserved adaptive mechanism has been developed by Eukaryotic cells, termed the unfolded protein response (UPR) to clear the unfolded proteins and restore ER homeostasis. The cellular functions deteriorate, in the conditions when ER stress cannot be reversed and this sometime lead to cell death. The poor vascularization, low oxygen supply, nutrient deprivation, and acidic pH in the tumor microenvironment stimulate the ER stress. UPR has been shown to exert a significant cytoprotective part in speedily growing cancers as it helps folding of newly synthesized proteins required for the growth of the tumor. Accumulating evidence showed that ER stress-induced cellular dysfunction and cell death are the major contributors to many diseases and making the modulators of ER stress pathways potentially attractive targets for therapeutics discovery. Herein, we will briefly summarize gesticulating cascade activated upon ER stress and its role in cancer cell death.

Introduction

The endoplasmic reticulum (ER) is a dynamic intracellular organelle in the secretory pathway. Its major work is to promote translocation and folding of protein into their correct tertiary structures, and to instigate protein post-translational changes that further make room for the carrying of proteins to the Golgi apparatus. Disturbances in ER function, an action named "ER-stress", instigates the well-established signaling cascade, the unfolded protein response (UPR), to restore protein homeostasis. This complex mechanism is a significant way to reestablish cellular homeostasis and also to alleviate the inciting stress. In higher eukaryotes, UPR signaling begins by three ER transmembrane sensors: inositol-requiring enzyme 1 α (IRE1 α), pancreatic ER kinase-like ER kinase (PERK), and activating transcription factor 6 (ATF6). If there is no proper restoration of protein folding capacity in the ER is not done, the UPR upregulates genes such as CCAAT-enhancer-binding protein homologous protein (CHOP), which activates apoptotic pathways¹⁻³.

The luminal domains of PERK and ATF6 proteins are bound to the ER resident chaperone BiP (Binding immunoglobulin Protein) under physiological conditions. This keeps them inert. BiP is released from these complexes when unfolded proteins accumulate in the ER to help with the folding of proteins accumulated⁴. Comparing the UPR modulators with PERK and ATF6, which are modulated by association with BiP, IRE1 α appears to become activated when unfolded proteins bind directly to it. PERK, IRE1 α and ATF6 when activated, induce signal transduction events that erase the accumulation of misfolded proteins in the ER by enhancing expression of ER chaperones, avoiding the entry of protein into the ER by avoiding mRNA translation, and causing retro grade transport of misfolded proteins from the ER into the cytosol for ubiquitination and destruction by a process named ERAD (ER-assisted degradation). Thus ER stress is a major symptom of many common diseases in conditions where (a) the stress is so strong that it leads to the cells ultimately die or (b) the capacity to overcome ER stress is damaged due to pathological conditions.

This review focuses on the (a) UPR signaling and their connections to cell death mechanisms; (b) UPR-independent mechanisms of ER stress-induced cell death, and (c) role of ER stress in cancer cells.

UPR Signaling and their Connection to Cell Death Regulation

IRE1 α is a major molecule among the UPR signaling pathways and it functions as a rheostat that regulates the fate of a cell. IRE1 α binds directly to unfolded proteins, including structural studies of the conserved core of yeast IRE1 α ER luminal domain, analysis of synthetic peptide interactions with IRE1 α in vitro, and protein interaction studies performed using intact cells⁴. This is proved in several studies. It is shown that, BiP desensitizes IRE1 α to low levels of stress and acts as a timer to modulate the response time to the level of stress by assisting IRE1 α deactivation once ER homeostasis is reestablished. Several lines of evidence state that alternative outputs from IRE1 α -mediated downstream signaling dictate opposing cell fates (survival or death) during stress, which are greatly influenced by the intensity and longevity of ER stress⁵.

IRE1 α is a transmembrane protein that consists of an N-terminal luminal sensor domain, a single transmembrane domain and C-terminal cytosolic effector that is accountable for the activities of both protein kinase and endonuclease. Accumulation of unfolded proteins in the ER stimulates IRE1 α oligomerization in ER membranes and autophosphorylation of IRE1 α 's cytosolic domain⁶. Moreover, IRE1 α cleaves micro RNAs that control the levels of caspase family cell death proteases. The XBP-1 protein binds to promoters of several genes involved in UPR and ERAD (ER-assisted degradation) to restore protein homeostasis and promote cytoprotection. Along with performing its cytoprotective function, IRE1 α also stimulates activation of the Apoptotic-Signaling Kinase-1 (ASK1). This in turn causes activation downstream of stress kinases Jun-N-terminal kinase (JNK) and p38MAPK which promote apoptosis^{7,8}. Bcl-2 and Bim, are major apoptosis inducing substrates of JNK. These are both inhibited and activated, respectively, by JNK phosphorylation^{9,10}. In addition, p38 MAPK phosphorylates and activates the transcription factor CHOP, which causes changes in gene expression that favor apoptosis. This increases expression of Bim and DR5, and decreases expression of Bcl-2^{11,12}.

Upon ER stress ATF6 which is a transcriptional factor translocates to the Golgi compartment where it is cleaved by the action of two proteases. ATF 6 is cleaved in the luminal domain by the serine protease site-1 (S1P) and the metalloprotease site-2 protease (S2P) cleaves the N-terminal portion. Later the cleaved N-terminal cytosolic domain of ATF6 is translocated into the nucleus and binds to ATF/cAMP response elements (CRE) and ER stress-response elements (ERSE-1). The target genes such as BIP, GRP94 and CHOP are activated by this process. Astonishingly, it is shown in the functional experiments in a model of ischemia/reperfusion that ATF6 protected cardiomyocytes by inducing expression of the protein disulfide isomerase associated 6 (PDIA6)

gene, which encodes an ER enzyme that catalyzes protein disulfide bond formation and thus assists with protein folding in the lumen of the ER^{13,14}.

For attenuation of mRNA translation under ER stress, PERK is the major protein responsible as it prevents influx of newly synthesized proteins into the already stressed ER compartment. This translational attenuation is mediated by phosphorylation of eukaryotic translation initiation factor 2 (eIF2 α). The recycling of eIF2 α to its active GTP-bound form is inhibited by phosphorylation of eIF2 α . This is required for the initiation phase of polypeptide chain synthesis. The preferential translation of UPR-dependent genes such as the activating transcriptional factor 4 (ATF4) is allowed by Phosphorylation of eIF2 α . CHOP (transcriptional factor C/EBP homologous protein), GADD34 (growth arrest and DNA damage-inducible 34), and ATF3 are the important targets driven by ATF4. Along with eIF2 α , PERK can also phosphorylate nuclear erythroid 2p45-related factor 2 (NRF2). This contributes to dissociation of the NRF2-Keap1 complex and promotes expression of genes containing antioxidant response elements (ARE), preventing oxidative stress by induction of antioxidant genes such as hemeoxygenase 1 (HO-1)¹⁵.

Suppose the other UPR-induced mechanisms fail to alleviate ER stress, both the intrinsic and extrinsic pathways for apoptosis can become activated. Factors that lead to the cell death response comprises of (i) PERK/eIF2 α -dependent induction of the pro-apoptotic transcriptional factor CHOP; (ii) IRE1-mediated activation of TRAF2 (tumor necrosisfactor receptor associated factor 2), which stimulates the ASK1 (apoptosis signal-regulating kinase 1)/JNK (c-Junamino terminal kinase) kinase cascade, and (iii) Bax/Bcl2-regulated Ca²⁺ release from the ER. CHOP/GADD 153 (growth arrest/DNA damage) plays a convergent role in the UPR and this is regarded as one of the most important mediators of ER stress-induced apoptosis protein. Apoptosis relevant targets of the CHOP transcription factor include (i) GADD34;(ii) DR5 (TRAIL Receptor-2), a caspase-activating cell-surface death receptorof the Tumor Necrosis Factor Receptor family; and (iii) Ero1 α (endoplasmic reticulum oxidoreductase-1), which hyperoxidizes the ER and leads to cell death. The inositol triphosphate receptor (IP3R) can also be activated by Ero1 α stimulating excessive Ca²⁺ transport from the ER to the mitochondria, and thereby triggering cell death¹⁶. Release of the translational inhibition contributes to accumulation of unfolded proteinsin the ER compartment and, at the same time, permits translation of mRNAs encoding pro-apoptotic proteins. Another possible mechanism by which CHOP induces apoptosis is via direct inhibition of Bcl-2 transcription and induction of Bim expression. Additional ER stress-induced mechanisms contributing to cell death may include activation of the kinase function of IRE1 involving activation of ASK and p38 MAPK, where p38MAPK activates CHOP via phosphorylation of its transactivation domain¹⁷. Additionally, both p38MAPK and JNK are reported to promote phosphorylation and activation of pro-apoptotic protein Bax. Among the mechanisms of cell death induced by pro-apoptotic proteins Bax and Bak are their binding and pathological activation of IRE1 α .

UPR Independent ER Stress-Signaling and Cell Death Calcium

The major storage of intracellular Ca²⁺ and Ca²⁺- binding chaperones, the ER lumen mediate the proper folding of proteins in the lumen of the ER. It is proved beyond doubt that Ca²⁺ trafficking in and out of the ER regulates a variety of cellular responses and signaling transduction pathways relevant to stress response, modulation of transcriptional processes, and development. For instance, acute release of Ca²⁺ from the ER can trigger a number of signaling mechanisms that leads to death of cells mainly by Ca²⁺-mediated mitochondrial cell death¹⁸. Conversely, pulses of Ca²⁺ delivered via IP3Rs at contact sites of ER and mitochondria promote oxidative phosphorylation. This not only sustains ATP levels, but also leads to cell survival^{19,20} Bax and Bak are the other proteins involved

in ER Ca^{2+} -mediated apoptosis. Transient over-expression of Bax leads to the release of ER Ca^{2+} . This results in the increases of the mitochondrial Ca^{2+} which enhances cytochrome c release. As opposed to this, cells with deficiency of both Bax and Bak have lessened Ca^{2+} release from ER upon stimulation with IP₃ and other ER Ca^{2+} -mobilizing agents²². A key component for folding of newly synthesized proteins and for other quality control pathways of the ER is Calreticulin which is a major Ca^{2+} binding ER chaperon. The fluctuations of the levels of Ca^{2+} in the ER therefore can severely affect folding capacity and hasten cell death. To sum up, modifications in Ca^{2+} dynamics might play a major role both in the ER and some ER stress-associated mechanisms of cell death.

Cancer

Cancer cells that are confronted by microenvironments such as hypoxia and hypoglycemia lead to the induction of ER stress. Cellular dysfunction and cell death often occur when ER stress is chronically prolonged and the protein load on the ER greatly exceeds its fold capacity. IRE1 α and PERK are important for tumor cell survival and growth under hypoxic conditions amongst UPR branches. Tumors derived from PERK-deficient are changed with the lengthening of ER stress and the load of protein on the ER greatly exceeds its fold capacity mouse embryonic fibroblasts (MEFs) and it shows evidence of increased cell death rates and impaired ability to stimulate angiogenesis. A study with MEFs that expressed a non-phosphorylated form of eIF2 α that impaired cell survival under extreme hypoxia²³ has supported the significance of PERK/eIF2 α -mediated UPR signaling. The IRE1 α /XBP-1 pathway has been shown to be crucial for angiogenesis in the early stages of tumor development²⁴. Inhibition of the IRE1 α /XBP-1 pathway has been explored of late as a target for anticancer therapy. For example, small molecules that prohibit IRE1 α -mediated XBP-1 splicing majorly suppressed multiple myeloma cell growth in an animal model²⁵. Also, the transcriptional activity of XBP-1, that has been shown previously as a requirement for plasma cell differentiation³⁰, is repeatedly suppressed by proteasome inhibitors in myeloma cells²⁶. XBP1 inhibition was associated with high levels of apoptosis, indicating a role of this transcription factor in perpetuating the malignancy. Ultimately, elevated levels of the ER chaperone BiP in cancer cell lines and clinical tumor specimens are very often present in a wide variety of malignancies, with expression correlated with pro-survival mechanisms, chemo resistance to therapy, and poor prognosis²⁷. A number of reports indicated that many chemotherapeutic agents are known to induce ER stress and making them more efficient anticancer agents²⁸.

Conclusions and Future Perspectives

Cellular stress responses are the basic part of a normal cell physiology. As a matter of fact, the adaptation to the “adversity” has greatly formed the evolution of multi-cellular organisms and lead to the birth of a variety of cellular resilience mechanisms. The ER is a highly dynamic organelle that exerts an important role in coordinating signaling pathways that ensure cell adaptation, cellular resilience, and survival. Though a great number of evidences have indicated that a variety of diseases are caused by abnormal cellular responses to ER stress, yet, several questions need to be answered regarding the roles of ER stress in health and disease. What is the ideal level of UPR signaling is needed for enabling cellular adaptation and survival? To what extent are the adaptive versus destructive UPR and non-UPR responses to ER stress involved in the pathophysiology of diseases? How the ER stress responses in sick but not healthy cells can selectively be modulated? And finally, in what way the interconnection of ER with other cellular organelles regulates cell death? On these lines the research on ER stress and its signaling connections are ongoing and evidence for a role of ER stress-mediated cell death in a variety of diseases make this process an attractive target for therapy. Particularly, small molecule inhibitors of the kinase-components

of the UPR, PERK and IRE1 α , are potential druggable candidates for cancer. It is also true that targeting single branch of the ER stress pathway may not be sufficient to prevent cell death. Thus, a clear comprehension of the mechanisms that orchestrate the ER stress responses may help to devise future strategies of safely attuning this process for therapeutic benefit.

Acknowledgments

We thank the Government College for Women (Autonomous) Mandya for providing a chance to publish our review article.

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