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Review Article

Endoplasmic reticulum stress response in cancer: molecular mechanism and therapeutic potential

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Abstract: In eukaryotic cells, the endoplasmic reticulum (ER) is an organelle that is responsible for protein folding and assembly, lipid and sterol biosynthesis, and free calcium storage. In the past decade, intensive research effort has been focused on intracellular stress signaling pathways from the ER that lead to transcriptional and translational reprogramming of stressed cells. These signaling pathways, which are collectively termed Unfolded Protein Response (UPR), are critical for the cell to make survival or death decision under ER stress conditions. In recent years, research in the cancer field has revealed that ER stress and the UPR are highly induced in various tumors and are closely associated with cancer cell survival and resistance to anti-cancer treatments. Identifying the UPR components that are activated or suppressed in malignancy and exploring cancer therapeutic potentials by targeting the UPR are hot research spots. In this review, we summarize the recent progress in understating UPR signaling in cancer and its related therapeutic potential.

Keywords: Endoplasmic reticulum, ER stress, unfolded protein response, cancer, malignancy, cancer therapy

Introduction

Inside a eukaryotic cell, there are numerous organelles that play essential roles in cell survival and functions. Besides the nucleus, the largest organelle is endoplasmic reticulum (ER), an extensive membranous labyrinth network of tubules, vesicles and sac that surrounds the nucleus and expands to the cytosol [1]. The ER has been primarily recognized as a compartment for protein folding and assembly, a pool of free calcium, and a site for lipid and sterol biosynthesis [2, 3]. Approximately one-third of newly synthesized proteins are translocated into the ER where they fold and assemble with the help of a series of molecular chaperones and folding catalysts. Inside the ER, co- and post-translational modifications, including disulfide bond formation and N-linked glycosylation, play important roles in the folding and oligomeric assembly of proteins. The ER provides a high-fidelity quality control system to ensure that only correctly folded proteins can be transported out of the ER while unfolded or misfolded proteins are retained in the ER and eventually degraded [2]. As a protein-folding compartment, the ER is exquisitely sensitive to alterations in homeostasis. A number of biochemical, physiologic to pathologic stimuli, such as those that cause ER calcium depletion, altered glycosylation, nutrient deprivation, oxidative stress, DNA damage, or energy perturbation/ fluctuations, can interrupt the protein folding process and subsequently cause accumulation of unfolded or misfolded proteins in the ER - a condition referred to "ER stress" [3-8]. To ensure the fidelity of protein folding and to handle the accumulation of unfolded or misfolded proteins, the ER has evolved a group of signal transduction pathways, the unfolded protein response (UPR) signaling pathways, to alter transcriptional and translational programs [3, 7]. The basic UPR pathways in mammalian cells consist of three main signaling cascades initiated by three primary ER-localized protein stress sensors: IRE1α (inositol-requiring 1 alpha), PERK (double-strand RNA-activated protein kinase-like ER kinase), and ATF6 (activating transcription factor 6).

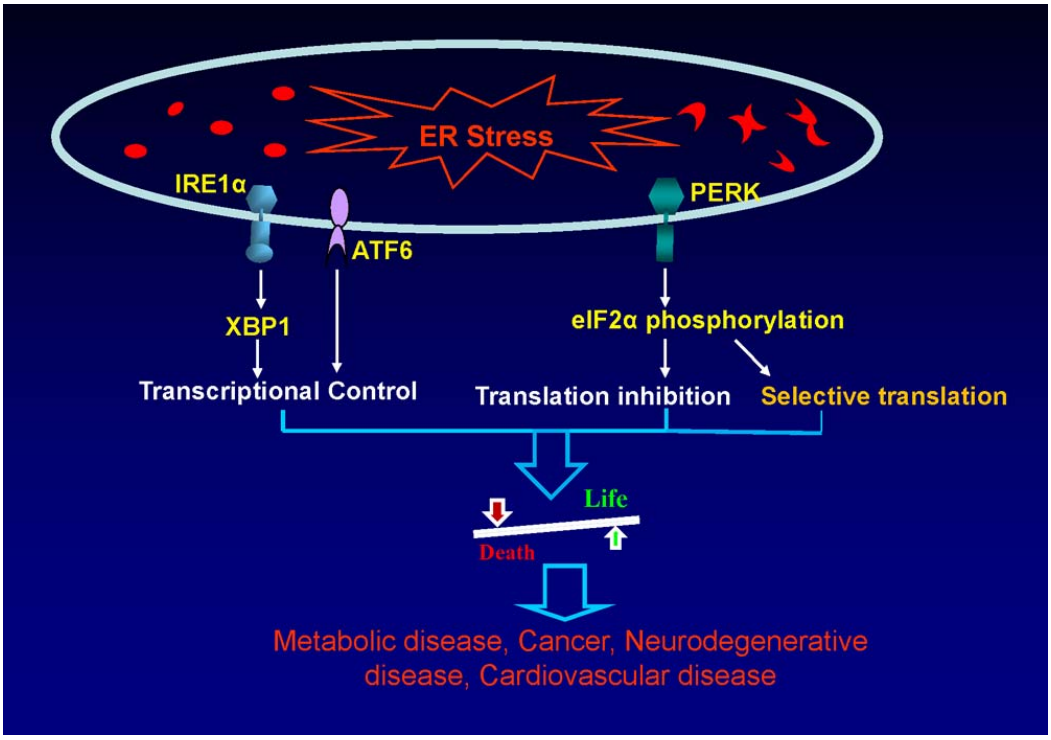

Figure 1. Role of UPR signaling in health and disease. Under ER stress, three ER stress sensors IRE1α, PERK and ATF6, are activated to alter transcriptional and translational programs to protect the cell from stress caused by the accumulation of unfolded or misfolded proteins. The UPR is critical for the cell to make survival or death decisions under ER stress conditions by altering translational and transcriptional programs. Regulation through UPR signaling is crucial for the development of a variety of diseases, including metabolic disease, cancer, neurodegenerative disease, and cardiovascular disease.

IRE1α is a protein kinase and endoribonuclease [9, 10], PERK is a protein kinase that is known to phosphorylate alpha-subunit of eukaryotic translation initiation factor (eIF2α) [11, 12], and ATF6 is a basic leucine zipper (bZIP) transcription factor of CREB/ATF family [13]. The primary role of the UPR is to prevent the cell from ER stress by reducing the amount of proteins translocated into the ER lumen, increasing retrotranslocation and degradation of ER-localized proteins, and augmenting the protein-folding capacity of the ER (**Figure 1**). However, if the attempt to recover from ER stress fails, the UPR will induce cell death programs to eliminate the stressed cells [14].

During tumorgenesis, the high proliferation rate of cancer cells requires increased activities of ER protein folding, assembly, and transport, a condition that can induce physiological ER stress [15]. Moreover, as the tumor grows, cancer cells experience increasing nutrient starvation and hypoxia, which are strong inducers for the accumulation of unfolded or misfolded proteins in the ER and the activation of the UPR pathways [15, 16]. Indeed, accumulating evidence has demonstrated that the UPR is an important mechanism required for cancer cells to maintain malignancy and therapy resistance. Additionally, the possibility of targeting the UPR signaling as a novel therapeutic strategy has greatly inspired the cancer research community and pharmaceutical industry.

The UPR pathways

When cells encounter ER stress, an immediate response will be the activation of ER stress sensor PERK through its homo-dimerization and auto-phosphorylation [17]. Activated PERK phosphorylates translation initiation factor eIF2α, leading to protein translational attenuation in general. PERK-mediated translation attenuation provides a survival signal, as this can reduce the ER workload by preventing newly-synthesized proteins from entering into the ER which is saturated by unfolded or misfolded proteins. This is evidenced by the fact that the

inactivation of PERK-mediated UPR pathway reduces cells' ability to survive ER stress [18, 19]. However, while general protein translation is inhibited, PERK-mediated eIF2α phosphorylation can lead to preferential translation of specific mRNAs that contain multiple upstream open reading frames in their 5'-untranslated regions (ORFs). These upstream ORFs are bypassed only when eIF2α is phosphorylated, thus allowing translation of the mRNA [20]. One of those mRNAs is known to encode the transcription factor 4 (ATF4). Under ER stress, phosphorylated eIF2α selectively initiates translation of *atf4* mRNA [21]. ATF4 subsequently activates expression of genes involved in cell metabolism, anti-oxidative response, and ER stress-associated apoptosis [18, 22].

Along with PERK-mediated translational repression, IRE1α- and ATF6-mediated UPR pathways are also activated to increase protein folding capacity and ER-associated protein degradation. Under ER stress, IRE1α is activated through its homo-dimerization and auto-phosphorylation. Activated IRE1α can function as an endoribonuclease to initiate removal of a 26 nucleotide intron from the mRNA encoding X -box binding protein 1 (XBP1) [17]. This unconventional mRNA splicing generates a translation frameshift that enables the spliced *Xbp1* mRNA to encode a functional potent bZIP transcription factor. The spliced XBP1 can activate expression of a group of ER chaperones and enzymes to help protein folding, maturation, secretion, as well as degradation of misfolded proteins [23]. In addition to its endoribonuclease activity, phosphorylated IRE1α can also serve as a scaffold protein that recruits tumour-necrosis factor (TNF)-receptor-associated factor 2 (TRAF2), leading to activation of JUN N-terminal kinase (JNK)-mediated signaling pathways [24]. Notably, the pro-apoptotic B-cell lymphoma 2 (BCL-2) family members BAX and BAK can directly bind to the cytosolic domain of IRE1α and augment its kinase and endoribonuclease activities [25, 26]. The interaction of BCL-2 family members with IRE1α may provide a molecular link between ER stress and apoptosis pathways. On activation of the UPR, ATF6 is also released from the ER membrane, and transits to the Golgi compartment where it is processed by proteases to produce an activated bZIP transcription factor that activates expression of UPR target genes [13]. Similar to spliced XBP1, cleaved ATF6 also activates expression of a group of genes involved in protein folding, secretion, and degradation in the ER [23, 27]. However, recent evidence suggests that ATF6, but not XBP1, is dispensable for the differentiation, function, or survival of specialized cell types where the UPR signaling is required [28, 29].

If the stressed cells fail to adapt to and recover from ER stress through the UPR-mediated survival programs, the UPR will initiate apoptotic pathways to remove the stressed cells. The well-defined pathway involved in the transition from ER stress to apoptosis is mediated by a transcription factor called GADD153/CHOP that is downstream of the PERK/eIF2α UPR pathway [14, 30-32]. Under prolonged ER stress, activated PERK phosphorylates eIF2α, which can selectively induce translation of the mRNA encoding ATF4. ATF4 induces a pro-apoptotic factor GADD153/CHOP to mediate ER stress-induced apoptosis. This is probably a case in some viral infections in which the organism utilizes ER stress-induced apoptosis to eliminate the infected, stressed cells in order to limit viral replication [33, 34]. Additionally, as part of the UPR program, ER-associated Protein Degradation (ERAD) is responsible for the degradation of aberrant or misfolded proteins in the ER and, in addition to this "quality control" function, also accounts for the degradation of several metabolically regulated, active ER proteins [35, 36]. During the process of ERAD, molecular chaperones and associated factors recognize and target substrates for retrotranslocation to the cytoplasm, where they are polyubiquitinated and degraded by 26S proteasome. ERAD is essential for maintaining ER homeostasis, and disruption of ERAD is closely associated with ER stress-induced apoptosis [37].

The UPR in malignancy

Cancer cells possess rapid glucose metabolism and fast growth rate, which leads to poor vascularisation of tumor mass, low oxygen supply, nutrient deprivation, and pH changes [16, 38]. On the other hand, cancer cells can express mutant proteins that cannot be correctly folded, and experience insufficient ER energy supply, alteration of the redox environment, and viral infection [39]. All of these can cause ER stress and activation of the UPR. Increasing evidence suggests that the UPR provides survival signaling pathways required for tumor growth. Indeed, increased expression of the UPR components, including the UPR *trans*-activators XBP1 and

ATF6, ER stress-associated pro-apoptotic factor CHOP, as well as ER chaperones GRP78/BIP, GRP94, and GRP170, have been detected in breast cancer, hepatocellular carcinomas, gastric tumors, and esophageal adenocarcinomas [40]. Cancer cells may adapt to ER stress and evade stress-induced apoptotic pathways by differentially activating the UPR branches [41-43]. Here, we discuss recent advances in understating the roles of different UPR components in malignancy.

ER chaperone GRP78/BiP

GRP78/BiP (glucose-regulated protein of 78 kDa) is an abundant ER chaperone that uses ATP/ADP cycling to regulate the protein folding process [44, 45]. It has been proposed that the initial activation of three ER stress sensors, including IRE1α, PERK and ATF6, depends on the dissociation of GRP78 in response to ER stress [7]. Recent studies suggested that GRP78 plays critical cytoprotective roles in oncogenesis [38, 44]. Increased expression of GRP78 has been observed in a variety of cancers [46-48]. GRP78 over-expression was shown to provide important survival signals for cancer cells during oncogenesis and confers drug resistance in both proliferating and dormant cancer cells [15].

The evidence that GRP78 is required for cancer cell survival came from the observation that suppression of GRP78 in fibrosarcoma cells inhibited their ability to form tumors *in vivo* [49]. GRP78 has also been implicated in promoting tumor cell proliferation. Over-expression of GRP78 correlated with increased proliferation rates of a range of glioma cells, while the knockdown of GRP78 resulted in decreased proliferation rates of glioma cells [41]. Dong et al showed that *Grp78* heterozygosity prolonged the latency period and significantly impeded tumor growth in a genetic mouse model of breast cancer where GRP78 expression level was reduced by half [50]. Their results suggested that GRP78 regulates cancer progression through three mechanisms, including enhancement of tumor cell proliferation, protection against apoptosis, and promotion of tumor angiogenesis. Recently, in a large series of breast cancer cases, expression of GRP78 and XBP-1 was observed in 76% and 90% of the breast cancers [47]. The results suggested that the UPR is activated in the majority of breast cancers and probably confers resistance to chemotherapy.

Additionally, a link between the high GRP78 expression level and poor clinical outcome of cancer therapy has been observed. For example, high levels of GRP78 expression correlate with increasing tumor grade in hepatocellular carcinoma, poor clinical outcome in breast cancer, high rates of recurrence and mortality in prostate cancer, and high rates of nodal metastasis and reduced survival in gastric cancer[48, 51-53].

UPR signaling through IRE1α/XBP1

The UPR signaling through ER stress sensor IRE1α and *trans*-activator XBP1 controls the upregulation of a broad spectrum of UPR-related genes involved in protein folding, transport, and ERAD [23]. In addition to classical UPR -related genes, the IRE1α/XBP1 arm of the UPR also regulates expression of the genes involved in cell differentiation, inflammation, lipogenesis, and apoptotic pathways [54]. A number of recent studies suggested that the IRE1/XBP1 arm of the UPR is essential for malignancy maintenance under oncogenic stress. Transformed mouse embryonic fibroblasts or human fibrosarcoma tumor cells (HT1080) that lack XBP1 displayed the inability to grow as tumor xenografts in SCID mice [55, 56]. Instead, XBP1-deficient cells showed increased apoptosis and decreased clonogenic survival under ER stress or hypoxia condition. Furthermore, expression of the dominant-negative form of IRE1α or inhibition of XBP1 by RNAi reduced blood vessel formation during tumorgenesis in an intradermal angiogenesis model or a human tumor xenograft model [57]. However, expression of spliced XBP-1 restored angiogenesis in IRE1α dominant -negative expressing cells, suggesting that the UPR signaling through IRE1α/XBP1 is crucial for angiogenesis in the early stage of tumor development. Interestingly, the un-spliced form of *Xbp1* mRNA was shown to encode a rapid-turnover protein that can function as a dominant negative factor to inhibit spliced XBP1 activities [58, 59]. While high expression levels of spliced XBP1 were associated with increased tumor survival, high levels of the unspliced form of XBP1 caused increased apoptosis of tumor cells [60].

UPR signaling through PERK/eIF2α

During tumorgenesis, cancer cells need to tolerate a subset of oncogenesis-associated cellular stresses including DNA damage, hypoxia, pro-

teotoxic, mitotic, and oxidative stress [16]. In order to adapt to and overcome the stress, tumor cells remodel the transcriptional and translational programs by activating pro-survival signaling pathways. The UPR signaling through PERK/eIF2α has been demonstrated to confer a survival advantage for tumor cells under hypoxic stress [61]. Hypoxic stress can activate PERK, leading to phosphorylation of eIF2α in tumor cells [61, 62]. Transformed mouse embryonic fibroblasts from the PERK-deficient animals and HT29 colorectal carcinoma cells expressing dominant-negative PERK exhibited lower survival rates under hypoxic conditions, compared to wild type cells [62]. These cells formed smaller tumors and displayed higher levels of apoptotic activity in hypoxic areas than the wild-type control cells [63]. Additionally, tumors derived from PERK-deficient mouse embryonic fibroblasts exhibited limited ability to stimulate angiogenesis [64]. Furthermore, the critical role of PERK/eIF2α-mediated UPR signaling in hypoxia survival is supported by a study with mouse embryonic fibroblasts expressing a "knock-in" mutant of eIF2α (S51A) that cannot be phosphorylated by PERK [63]. These cells displayed an increased susceptibility to hypoxia with virtually no survival under prolonged hypoxia conditions.

The therapeutic potential of targeting the UPR components

The importance of the UPR in malignancy maintenance has inspired great interest in exploring therapeutic potentials by targeting the UPR components. Tumor cells grow under oncogenic stress caused by hypoxia, nutrient deprivation, DNA damage, metabolic and oxidative stress and therefore rely on an activated UPR for survival [15, 47]. However, most normal cells are not subjected to stress, and the UPR pathways remain inactive state in these cells. This discrepancy between tumor cells and normal cells may offer an advantage for the agents that target the UPR to achieve the specificity in cancer therapy. In the following, we provide some representative evidence for cancer therapeutic applications by targeting UPR components.

GRP78/BiP as a cancer therapeutic target and biomarker

Expression of GRP78 protein correlated with both the rate of patient survival and the depth of tumor invasion. In human cancers, elevated GRP78 level generally indicates the higher pathologic grade, recurrence, and poor patient survival in breast, liver, prostate, colon, and gastric cancers, although lung cancer is an exception [15]. Additionally, GRP78 expression was positively correlated with increasing tumor thickness and with increasing dermal tumor mitotic index [65]. These observations have inspired the idea of targeting GRP78 for cancer therapy. Indeed, recent studies supported that knockdown of GRP78 can suppress cancer cell growth and improve the sensitivity of cancer cells to the treatments. Knockdown of GRP78 by siRNA could slow down the growth of glioma cells and increase their sensitivity to chemotherapeutic agents, including temozolomide, 5-fluorouracil and CPT-11 [41]. The cytotoxic effect of GRP78 knockdown has been confirmed in many cancer cell lines [66, 67], although one study suggested that the pro-survival role of GRP78 in tumorgenesis is possibly cell-line specific [68]. Researchers have been actively screening for a specific GRP78 inhibitor as an anticancer agent [69-71].

GRP78 is an abundant molecular chaperone that localizes to the ER lumen. However, recent evidence suggested that a sub-fraction of GRP78 localized to the surface of specific cell types, including malignant cells [72]. Preferential expression of GRP78 on the surface of tumor cells but not in normal organs suggests that surface GRP78 can serve as a biomarker for cancer-specific therapy. Indeed, some of recent studies supported that ER chaperones GRP78 and GRP94 are effective biomarkers for indicating aggressive behavior and poor prognosis in cancer [51, 53, 73, 74], although there is evidence that GRP78 as a cancer biomarker might be tumor-type specific [15, 52, 75].

Proteasome inhibitors

The ubiquitin-proteasome pathway is one of central players in the regulation of several diverse cellular processes. Proteasome inhibitors can block the action of proteasomes, inhibit the degradation of proteins critically involved in regulation of cell proliferation and survival, and eventually lead to growth inhibition and apoptosis. Proteasome inhibitors have been intensively studied in the treatment of cancers. Bortezomib (Velcade; PS-341) is a highly selective and reversible proteasome inhibitor that has been approved for clinical use against multiple myeloma and is in clinical trials as a single

agent or in combination with chemotherapeutics against other solid tumor malignancies [76, 77]. The *in vitro* studies have confirmed the cytotoxic effects of bortezomib on a broad range of cancer cell types, including prostate, lung, breast, colon, and non-Hodgkin's lymphoma [78-81]. It can induce additive or synergistic cytotoxic activities against cancer cells when combined with other antineoplastic agents [81-83]. Although the mechanisms involved in its anticancer activity are still being elucidated, bortezomib was recently shown to cause the accumulation of misfolded proteins in the ER and ER stress-associated apoptosis by inhibiting 26S proteasome activity and subsequent ERAD machinery [58, 84-86]. Moreover, bortezomib was shown to suppress the IRE1α/XBP1 arm of the UPR by inhibiting IRE1α endoribonuclease/kinase activity and by stabilizing the unspliced form of XBP1, a dominant negative for the functional XBP1 protein [58, 59]. In addition to bortezomib, therapeutic potentials of other proteasome inhibitors were also investigated. For example, BU-32 (NSC D750499-S), a highly selective proteasome inhibitor, was effective in suppressing *in vitro* and *in vivo* breast cancer cells, on which bortezomib has limited effect [87].

ERAD inhibitor

Under ER stress, ERAD removes aberrant or misfolded proteins from the ER through protein retrotranslocation and ubiquitin-proteasome degradation systems [36, 88]. Defects in ERAD cause the accumulation of misfolded proteins in the ER and thus trigger ER stress-induced apoptosis [37]. In the process of ERAD, a cytosolic ATPase named p97 plays key roles in extracting misfolded proteins that are polyubiquitinated and transporting them to the proteasome for degradation. Recently, Eeyarestatin I (EerI), a chemical inhibitor that can block ERAD, has been shown to have preferential cytotoxic activity against cancer cells [89, 90]. EerI can target p97 complex to inhibit deubiquitination of p97-associated ERAD substrates, which is required for the degradation process [90]. Like bortezomib, EerI induces an integrated stress response in the ER as well as apoptosis via the Bcl-2 homology3 (BH3)-only pro-apoptotic protein NOXA. EerI activates the CREB/ATF transcription factors ATF3 and ATF4, which form a complex capable of binding to the NOXA promoter and activate NOXA expression [89]. Interestingly, EerI was found to be able to block ubiquitination of histone H2A to relieve its inhibition on NOXA transcription [89]. These studies suggested that the ERAD inhibitor EerI may represent a novel class of anticancer drugs that integrate ER stress response with epigenetic mechanisms to induce cell death.

Other therapeutic potential associated with ER stress

Several other distinct agents have been reported to have anti-cancer potentials by modulating ER stress response. Versipelostatin, a novel macrocyclic compound, showed highly selective cytotoxicity to glucose-deprived tumor cells and *in vivo* tumors by inhibiting GRP78 induction and expression of the UPR transactivators XBP1 and ATF4 [69, 91]. An engineered fusion protein, epidermal growth factor-SubA (EGF-SubA), was reported to be highly toxic to growing and confluent epidermal growth factor receptor-expressing cancer cells, and its cytotoxicity is thought to be mediated by rapid cleavage of GRP78 [70]. Systemic delivery of EGF-SubA resulted in a significant inhibition of human breast and prostate tumor xenografts in mouse models. Additionally, Salazar et al reported that δ-tetrahydrocannabinol, the main active component of marijuana, induces human glioma cancer cell death through stimulation of ER stress-associated autophagy [92]. δ-tetrahydrocannabinol can induce ceramide accumulation and the ER stress response that triggers autophagy through inhibition of the Akt/mammalian target of rapamycin complex 1 axis. The δ-tetrahydrocannabinol-induced autophagic death of human and mouse cancer cells suggested that cannabinoid administration may be an effective strategy for cancer therapy.

Concluding remarks

Significant progress has been made in elucidating the mechanism and role of the ER stress response in oncogenesis and cancer therapy resistance. The related findings have raised an exciting possibility of targeting the UPR components as an effective strategy for cancer therapy and overcoming drug resistance. For future research, it is important to delineate the distinct roles of the UPR branches that may provide survival or death signal in tumorgenesis or cancer therapy. The related information will be essential for pharmaceutical design toward controlling cancer through modulating UPR signaling. Research in this topic will significantly advance our understanding of cancer biology and be infor-

mative to its therapeutic application against cancer.

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