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REVIEW

Endoplasmic Reticulum Stress and Cancer

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The endoplasmic reticulum (ER) is the principal organelle responsible for multiple cellular functions including protein folding and maturation and the maintenance of cellular homeostasis. ER stress is activated by a variety of factors and triggers the unfolded protein response (UPR), which restores homeostasis or activates cell death. Multiple studies have clarified the link between ER stress and cancer, and particularly the involvement of the UPR. The UPR seems to adjust the paradoxical microenvironment of cancer and, as such, is one of resistance mechanisms against cancer therapy. This review describes the activity of different UPRs involved in tumorigenesis and resistance to cancer therapy.

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Key Words: Endoplasmic reticulum stress, Cancer, Unfolded protein response

INTRODUCTION

The endoplasmic reticulum (ER) is the principal intracellular organelle responsible for protein folding, translocation and post-translation modification. Disturbance in the ER environment by biochemical, physiological and pathologic stimuli causes nutrient deprivation, altered glycosylation, calcium depletion, oxidative stress, DNA damage and energy disturbance/ fluctuation, resulting in ER stress with subsequent accumulation of unfolded or misfolded proteins in the ER. These cells must overcome perturbations in ER function and ER stress to survive.

If unresolved ER stress can lead to apoptosis.[1] The imbalance between anti- and pro-apoptotic Bcl-2 proteins due to ER stress causes an increase in transcription of Bcl2-like11 (BIM), p53 unregulated modulator of apoptosis (PUMA), NADPH oxidase activator (NOXA), and BH3-only proteins. The interactions between PUMA and Bax are promoted by ER stress, leading to the release of cytochrome *c* and apoptosis through caspase-dependent cleavage of p53.[2]

In tumor cells, ER stress may restore homeostasis and make the adjacent environment hospitable for tumor survival and tumor expansion.[3] Various stressful conditions such as hypoxia, nutrient deprivation, pH changes or poor vascularization can be growth limiting for tumor cells, and thus activate the unfolded protein response (UPR). Both nutrient starvation[4,5] in tumor cells and nutrient excess under normal conditions produce ER stress.[6,7] The ER is the main site for the translation of excess nutrition into metabolic and inflammatory responses. During tumorigenesis, the high proliferation rates of cancer cells require increased activities of ER protein folding, assembly and transport, which are conditions that can induce physiological ER stress.[8] The ER stress response is considered cytoprotective and is involved in tumor growth and adaptation against harsh environments.[9,10]

Three ER stress signaling branches, inositol-requiring enzyme 1α (IRE1α), activating transcription factor 6 (ATF6) and pancreatic ER kinase-like ER kinase (PERK) localized in the ER, are involved

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in tumorigenesis. IRE1α and its down-signaling, X-box binding protein (XBP1) contribute to cancer progression.[11] XBP1 is increased in many human cancers such as breast cancer, hepato cellular carcinoma and pancreatic adenocarcinoma.[11] Similarly, another ER stress branch, PERK/eukaryotic initiation factor 2α (eIF2α)/ATF4, also contributes to cancer progression.[12] Separately, calreticulin, an ER resident chaperone, has been localized to the cell surface in tumor cells and is related to immunogenic cell death and the localization of calreticulin on the surfaces of tumor cells. This relationship may be associated with ER stress induction in tumor cells.[13,14]

ER stress is a potential target for developing drugs that interfere with specific signaling pathways to reduce adaptation to hypoxia, inflammation, and angiogenesis, thereby overcoming drug resistance.[15] Several anti-cancer agents have recently been studied in relation to ER stress, which may directly or indirectly affect tumors.[16] However, specific targets in cancer cells are not established. The effects of these drugs on nontumorigenic cells remain under investigation.[9] Even during treatment with ER stress-inducing anticancer agents, tumor cells might paradoxically be more resistant than normal cells.

Tumor cells grow continuously and require effective high-energy producing systems due to their high proliferation characteristic compared with nontumorigenic cells. Therefore, glycolysis is substantially greater in tumor cells than in nontumorigenic cells.[17-20] Hypoxia inducible factor 1α (HIF1α) plays an important role in tumor development and helps mediate angiogenesis, proliferation and invasiveness, as well as regulating the expression of glycolytic enzymes. Therefore, blocking the HIF1α signal might be a novel and promising therapeutic target for the treatment of hypoxic tumors.[21]

The regulation/inhibition of ER chaperones or one arm of the UPR components, such as ATF4, XBP1, and PERK, have been recently suggested as potential cancer therapies.[22,23] Glucose regulated protein 78 (Grp78), an ER chaperone, and UPR components are over-expressed in several tumor types such as breast, lung, hepatocellular, brain, colon, ovarian, glioblastoma, and pancreatic cancers. In a human tumor xenograft mouse model, ER stress exhibited pro-survival effects on tumor development and progression. Other ER resident proteins that participate in tumor survival include ATF4, which is increased in severe hypoxic conditions in human breast cancer tissues,[24,25] and spliced XBP1, which is increased in breast cancer, lymphoma and glioblastoma cells. PERK also supports beta cell proliferation and promotes angiogenesis in human tumor xenograft mice.[26]

However, the ER stress response is also directly involved in proapoptotic mechanisms in either UPR-dependent or -independent manners.[27] ER stress inducing agents are also potential anticancer therapies.[28,29] The cytosolic domain of IRE1α interacts with the Bax/Bak apoptotic pathway to induce IRE1α activation.[30] EI24/PIG8, a novel ER-localized Bcl2-binding protein, modulates Bcl-2 function and suppresses breast cancer invasiveness.[31] Bim also mediates breast cancer-derived MCF-7 cell death through the activation of ER stress-induced apoptosis.[32] ER stress causes spontaneous tumor cell apoptosis, which has been implicated in B cell chronic lymphocytic leukemia.[28] The activation of the CHOP-GADD34 axis is another potential anti-tumor strategy.[33,34] PERK is well-supported as a major factor in ER stress-induced cell death, as CHOP is the downstream target of PERK.[35] It has been reported that cells and live mice gain resistance to ER stress due to loss of CCAAT/enhancer binding protein homologous protein (CHOP), suggesting that CHOP stimulates the cell death program.[36] Similarly, CHOP induces cell death by promoting protein synthesis and oxidation in ER stress-exposed cells.[35,37]

UNFOLDED PROTEIN RESPONSE

The UPR is cytoprotective as well as being cytotoxic, depending on cell status. The purpose of the UPR is to balance the ER folding environment under ER stress. If ER stress is prolonged and the UPR fails to restore ER homeostasis, tumor cells will undergo cell death. The UPR can also protect tumor cells from apoptosis in conjunction with induced tumor dormancy and permitting regrowth of the tumor when favorable conditions have been restored.[38,39]

Through the UPR process, cells seek to maintain appropriate folding processes in the ER by the dissociation of Grp78/binding immunoglobulin protein (Bip), a main chaperone protein, from 3 membrane-bound ER stress sensors, including PERK, ATF6, and IRE1α.[40] After the dissociation of sensing proteins from Grp78/Bip (Fig. 1), activation of these sensors occurs sequentially with PERK which blocks general protein synthesis by phosphorylating eIF2α, being the first.[41-43] These processes also lead to inhibition of the transcription factor NF-κB during cellular stress. ATF6 is another transcription factor that is activated by translocation to the Golgi apparatus, where ATF6 is cleaved and the active form of the transcription factor is released to regulate gene expression.[44] After the activation of IRE-1 and its downstep, the splicing of XBP1, the spliced XBP1 protein translocates to the nucleus and activates the transcription of genes encoding chaperones or folding enzymes involved in protein folding, secretion or ER-associated protein degradation (ERAD).[45,46] During tumorige-

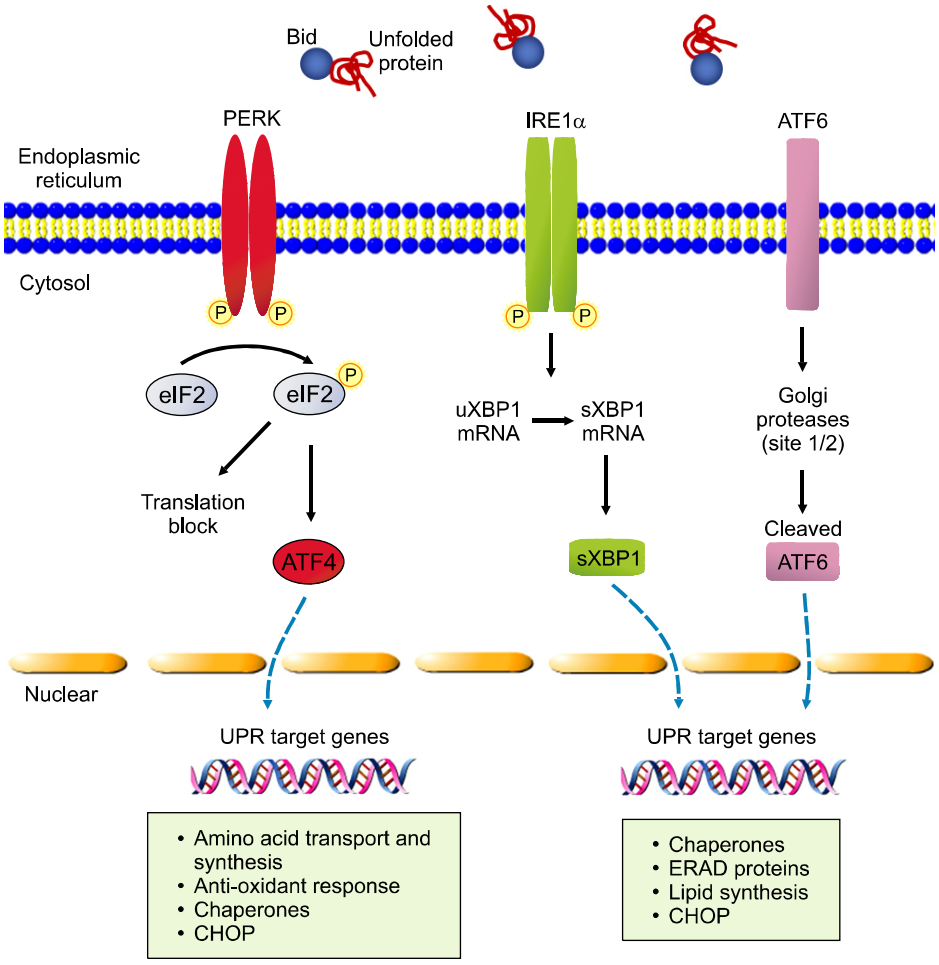

Figure 1. During endoplasmic reticulum (ER) stress, glucose regulated protein 78 binds to misfolded proteins, activating inositol-requiring enzyme 1α (IRE1α), activating transcription factor 6 (ATF6) and pancreatic ER kinase-like ER kinase (PERK). PERK is activated by dimerization and autophosphorylation and phosphorylates eukaryotic initiation factor 2α (elF2α). Phosphorylated elf2α inhibits protein synthesis and activates the transcription of ATF4, inducing the transcription of downstream genes. IRE1α produces a spliced form of XBP1 (XBP1s) due to its RNase activity. IRE1 assists protein folding and degradation. ATF6 translocates from the ER to the Golgi apparatus, where it is cleaved by protease activity, forming active nuclear ATF6 (N). CHOP, CCAAT/enhancer binding protein homologous protein, ERAD, ER-associated protein degradation.

nesis, there is rapid tumor growth and inadequate vascularization leading to microenvironmental stress (Table 1).[47]

CANCER

Cancer cells continuously divide and therefore tumor cells can be challenged by restricted supplies of nutrients and oxygen and decreased vascularization. Thus, ER resident proteins display altered expression patterns in cancer. ER stress has a dual impact on tumors. First, it has adaptive meaning, enhancing tumor growth. Second, it also has cytotoxic effects, inducing apoptosis. Cancer cells adapt to the surrounding microenvironment by the activation of UPR and macrophages create more favorable microenvironments for cancer cell development and invasiveness by secreting cytokines, growth factors and angiogenic factors.[48] Mahadevan et al.[49] described cross-talk between macrophages and cancer cells and documented such cross-talk between cancer cells. During ER stress, cancer cells induce cyclooxygenase-2 expression through NF-κB pathways, playing antiapoptotic roles. It also enhances pro-inflammatory NF-κB activation via CHOP and maintains production of IL-8 in human epithelial cells.[50,51] ER stress is one of multiple pathways through which apoptosisis induced. The caspase-12 family of proapoptotic cysteine proteases plays a major role in ER stress-induced apoptosis, associated with the ER membrane, but is not activated by other non-ER stimuli.[52] Grp78 expression is increased on the endothelial surface by vascular endothelial growth factor (VEGF) and enhances endothelial cell proliferation and angiogenesis. Knockdown of Grp78 suppresses endothelial cell proliferation through mitogen-activated protein kinase (MAPK) signaling.[53] Cells remain in a G0-like quiescent state through the action of P38MAPK.[54,55] In this quiescent state, the cells are resistant to drugs that damage DNA. PERK-elf2α also arrests the growth of cells at G0/G1 and inhibits tumorigenesis in subcutaneous xenograft models and a chicken embryo chorioallantoic membrane system (Fig. 2).[56]

Table 1. Endoplasmic reticulum stress markers that are increased in cancer

Cancer type	Sample type	ER stress marker expression	References
Breast	Human breast cancer tissues and breast carcinoma cell lines (MCF-7, MDA-MB-231, HS578T, and HCC1500 cells)	High levels of mRNA and protein Bip/Grp78	66, 143
	MCF7 cells	Increased ATF4 in severe hypoxia	24, 25
	Human breast cancer tissues	Higher levels of unspliced XBP1 mRNA favoring apoptosis of tumor cells and higher levels of spliced XBP1 mRNA increasing tumor survival	106
	Human breast cancer hormone-resistant cells, MCF-7/BUS-10	Hormone-resistant breast cancer cells promote Grp78 to the cell surface, which can be further elevated by ER stress	144
Prostate	Human prostate adenocarcinoma hormone-resistant cells, C4-2B	Hormone-resistant prostate cancer cells promote Grp78 to the cell surface, which can be further elevated by ER stress	144
Pancreatic	Human tumor xenograft mice	PERK supports beta-cell insulinoma proliferation and promotes angiogenesis	145
Liver	Human hepatocellular carcinoma tissues, human hepatocellular carcinoma cells SMMC7721	Grp78 promotes the invasion of hepatocellular carcinoma both in vitro and in vivo	73
Lymphoma	Patient	Splicing of XBP1 promotes tumor growth under hypoxic conditions	146
Brain, central nervous system	Human brain tumor specimens, glioma cell lines A172, U87, LNZ308, U251, LN-443, and LN-229	Grp78 is overexpressed	65
	U373 glioblastoma cells	XBP-1 depletion dramatically sensitized U373 cells to viral oncolysis	147
	Glioblastoma patient samples	Inhibiting IRE1α enhances oncolytic therapy	147
Colorectal	HT29 cells	Increases ATF4 in severe hypoxia	25
	Human colon carcinoma HT29, SW480, SW620, DLD1, and Lovo cell lines	Grp78 is found on CRC cell surfaces and promotes CRC cell migration and invasion	148
Ovarian	Patients	Grp78 is overexpressed	149

ER, endoplasmic reticulum; Bip, binding immunoglobulin protein; Grp78, glucose regulated protein 78; ATF4, activating transcription factor 4; XBP1, X-box binding protein; PERK, pancreatic ER kinase-like ER kinase; IRE1α, inositol-requiring enzyme 1; CRC, colorectal cancer.

1. Glucose regulated protein 78/binding immunoglobulin protein in cancer

The ER chaperone protein Grp78 is one of the most active components of cancer cells and is overexpressed in different kinds of cancers.[57,58] It has been interpreted as a chaperone protein that enhances cancer cell adaptation against hypoxic environments and as a resistance protein against anti-cancer therapy.[59,60] Grp78 regulates cell apoptosis, proliferation, invasion, inflammation and immunity, especially in cancer systems.[61] Recently, it has also been shown to be involved in tumorigenesis, metastasis, and angiogenesis.[8,62,63] The expression of Grp78 is correlated with both the rate of patient survival and the depth of tumor invasion. In human cancers, elevated Grp78 levels indicate higher pathologic grade, recurrence risk, and poor patient survival in breast, liver, prostate, colon, and gastric cancers, although lung cancer is an exception to these outcomes.[8] In the ER, Grp78 inhibits BIK-mediated apoptosis via physical and functional interactions with BIK, and confers resistance to estrogen starvation-induced apoptosis in human breast cancer cells.[64] It has been also shown that overexpression of Grp78 decreases the sensitivity of glioma cells to etoposide and cisplatin.[65] Indeed, some studies indicate that ER chaperones Grp78 and Grp94 are effective biomarkers that indicate aggressive behavior and poor prognosis in cancer.[66-69]

Grp78 expression is also positively correlated with increasing tumor thickness and with increasing dermal tumor mitotic index,[70] suggesting the potential to target Grp78 for cancer therapy. A Grp78-knockout model and Grp78 siRNA-transfected human prostate cancer cells showed that protein kinase B activation is reduced in phosphatase and tensin homolog-null prostate epithelium, reducing cancer development[71,72] Grp78 is suggested to be a novel approach to reducing tumorigenesis.[23] Overexpression of Grp78 leads to invasion activity in hepatocellular cancer.[73] Focal adhesion kinase (FAK) is involved in adhesion, invasion and migration activity, and overexpression of Grp78 increases the phosphorylation of FAK (PY397) and induces invasion by phosphorylating p190RhoGAP and inhibiting Rock kinase.[73] The

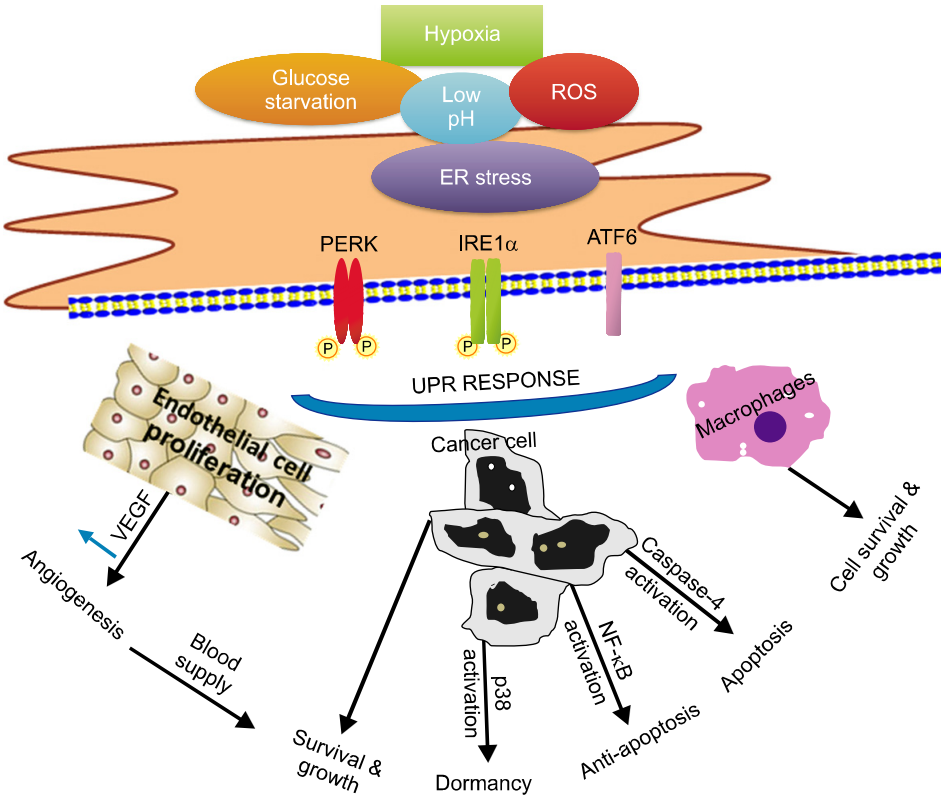

Figure 2. Cancer cells grow continuously, develop decreased nutrition supplies and increase reactive oxygen species (ROS) production, thereby inducing hypoxia and activating endoplasmic reticulum (ER) stress. ER stress activates the unfolded protein response (UPR). The UPR is both apoptotic and adaptive in tumor cells. The adaptive activity of UPR induces anti-apoptotic NF-κB, which inhibits p53 dependent apoptotic signals and induces angiogenic activity through increased vascular endothelial growth factor (VEGF) secretion. Mitogen-activated protein kinase (p38 MAPK) contributes to tumor cell dormancy during drug treatment through pancreatic ER kinase-like ER kinase (PERK)-eukaryotic initiation factor 2α, which arrests the growth of cells at G0/G1. Tumor-associated macrophages also secrete inflammatory cytokines that promote tumor growth, angiogenesis, invasion and metastasis during periods of ER stress. IRE1α, inositol-requiring enzyme 1α; ATF6, activating transcription factor 6.

phosphorylation of FAK facilitates invasion by activating the urokinase-type plasminogen activator system.[74] Grp78 translocates to the colorectal cancer cell surface, interacting with beta1-integrin and facilitating the cell-matrix adhesion process.[73] Insulin-like growth factor binding protein-3 stimulates the survival of breast cancer cells through interaction with Grp78.[75] Recently, the cytotoxic effects of Grp78 knockdown were confirmed in many cancer cell lines.[76,77] Specific Grp78 inhibitors have also been screened as anticancer agents,[78-80] suggesting that Grp78/Bip inhibition is a promising anti-cancer strategy. In addition, the possible translocation of Grp78 in cancer cells has been studied as a possible cancer treatment. Grp78 is primarily located inside the ER, but during ER stress, Grp78 may be translocated to the surface of tumor cells.[57,81] During ER stress, some fraction of Grp78 resides in the cytosol, nucleus and mitochondria in addition to the ER.[82,83] The inhibition of Grp78 translocation is another promising potential anticancer strategy.

2. Pancreatic endoplasmic reticulum kinase-like endoplasmic reticulum kinase in cancer

PERK/eIF2α plays regulatory roles in tumor initiation and survival, thereby facilitating adaptation in different situations such as hypoxia and oxidative stress.[3,9,84,85] Tumor cells grow rapidly, leading to the formation of new vasculature and finally linking to microenvironment and nutrient deprivation conditions. Increased demand for glucose and oxygen leads to cytotoxic conditions. As the production of ATP by glycolysis and NADPH in a reducing equivalent form is disturbed, reactive oxygen species (ROS) are generated. In the mitochondria, ROS accumulate and cause activation of ER stress.[86,87] The ER responds to alterations in nutrient deficiency with a cellular stress sensor that is associated with tumorigenesis. PERK is a trans-ER membrane serine/threonine protein kinase that contains an N-terminal ER luminal domain and a cytoplasmic C-terminal protein kinase domain.[88] Nrf2 transcription factor[89] and eIF2α are the 2 transcription factors that are phosphorylated by PERK. The phosphorylation of eIF2 inhibits the translation of most transcripts, simultaneously increasing the translation of selected mRNAs such as ATF4 transcription factor.[90,91] Nrf2 is phosphorylated by PERK and is released from an inhibitory E3 ligase complex containing Keap1 and cullin 3 and translocated into the nucleus, where it produces enzymes responsible for the elimination of intracellular ROS.[92-95] Thus, PERK is one of the key factors maintaining cellular redox homeostasis and reducing ROS-induced genotoxic stress. PERK has been considered to be a regulator of the growth of cancerous cells. A previous study examined whether the absence of PERK affected the ability of mammary carcinoma cells to form solid tumors in vivo.[96] Hypoxia is the most common feature of tumors,

downregulating protein synthesis by PERK inhibition and phosphorylation of elF2α at Ser51. When hypoxia occurs in tumors, the transcription regulator HIF1α is stabilized and fully activates the complete branch of UPR, i.e., PERK, leading to the phosphorylation of elf2α, ATF4, and GADD34. The phosphorylation of elf2α inhibits general protein synthesis, but ATF4, a transcription factor, is related to cancer cell proliferation and survival against nutrient deprivation through amino acid synthesis.[84]

3. Inositol-requiring enzyme 1α/X-box binding protein in cancer

IRE1α, an ER transmembrane sensor, plays a protective role against ER stress in cells and tissues.[1] During ER stress, IRE1α is activated by oligomerization and autophosphorylation, resulting in the activation of its endoribonuclease to cleave and initiate splicing of the XBP1 mRNA.[97] IRE1α-dependent decay of mRNAs (RIDD) helps to restore ER homeostasis by targeting mRNAs encoding secretory proteins and is distinct from XBP1 splicing. The activity of RIDD is regulated by IRE1α RNase activity.[98] RIDD has been the subject of very few studies, and further examinations of the mechanism of its pathway in apoptosis are necessary, due to the relatively new discovery of its role in ER stress. The IRE1α-XBP1 pathway has been also considered for a pro-survival role in the UPR.[97] However, under conditions of prolonged and uncompensated stress, the UPR leads to cellular apoptosis.[99] Another suggested pathway is IRE1α-TRAF2-ASK. IRE1α is activated by phosphorylation, binds to tumor-necrosis factor receptor associated factor 2 (TRAF2) and activates apoptosis signal-regulating kinase (ASK1), leading to the activation of JNK and p38 and ER-stressed induced cell death.[100,101] IRE1α and TRAF2 pathwaysare also involved in mitochondria-independent apoptotic response by directly activating procaspase-4.[102]

A number of recentstudies have suggested that IRE1α/XBP1 is essential for the maintenance of malignancy under oncogenic stress. XBP1-lacking cells displayan inability to grow in tumor xenograft mouse models.[103,104] Instead, XBP1-deficient cells exhibit increased apoptosis and decreased clonogenic survival under ER stress orhypoxia. Furthermore, expression of the dominant-negative form of IRE1α or inhibition of XBP1 gene expression reduce blood vessel formation during tumorigenesis.[105] However, the expression of spliced XBP1 restores angiogenesis in IRE1α dominant-negative expressing cells, suggesting that UPR signaling through IRE1α/XBP1 is crucial for angiogenesis in the early stages of tumor development. High expression levels of spliced XBP1 are associated with increased tumor survival, whereas high levels of the unspliced form of XBP1 increase the apoptosis of tumorcells.[106] IRE1α also regulates the expression of cyclin A1 and promotes cell proliferation by splicing XBP1 in prostate cancer, andis related to the cancer suppressor, p38MAPK. XBP1-deficient cells produce less catalase than normal cells, increasing ROS generation and p38 activation.[107] The IRE1α-XBP1 pathway has recently been suggested as an appealing target for cancer therapy.[97] However, the specific role of IRE1α in tumor characteristics such as growth and angiogenesis has not been clarified.[108]

THE THERAPEUTIC POTENTIAL OF TARGETING ENDOPLASMIC RETICULUM STRESS-ASSOCIATED MACHINERY

1. Targeting unfolded protein response

The importance of UPR in the maintenance of malignancy has inspired great interest in exploring the therapeutic potential of targeting UPR components. Tumor cells grow under oncogenic stress caused by hypoxia, nutrient deprivation, DNA damage, metabolic stress, and oxidative stress, leading to UPR as an adaptation strategy.[109] However, most normal cells are not subjected to stress and the UPR pathways remain inactive in these cells.

This difference between tumor and nontumorigenic cells might offer an advantage of targeting the UPR to achieve specificity in cancer therapy (Table 2).[110] If tumor cells are exposed to another form of ER stress, the intensity of the stress might be a threshold, thereby inducing specific cell death in tumor cells, with less effect on nontumorogenic cells. ER stress inducing mechanisms are also potential anti-cancer strategies through disturbing the adaptive response of UPR. A strategy of diminishing or removing UPR may also solve the problem of drug resistance against anti-cancer agents. Therefore, cancer therapeutic approaches might be divided into 2 categories: (1) increasing misfolded proteins in ER to overload protein folding requirements, therefore inducing more severe ER stress and cell death, and (2) inhibiting UPR adaptive and pro-survival pathways, leading to increased sensitivity to anticancer therapy.[110]

2. Targeting protein degradation machinery

Misfolded proteins in ER are identified by molecular chaperones and lectin-like proteins in the ERAD pathway and are subsequently degraded by ERAD as a part of the ER quality control mechanism. In cancer cells, there is continuous activation of

Table 2. Endoplasmic reticulum stress-/unfolded protein response-targeted drugs that inhibit cancer development

Therapeutic drugs	Therapeutic effect related to ER stress	Indication	References
Irestatin	Inhibits IRE1α activity	Malignant myeloma cells	110
Honokiol (HNK)	Binds to the unfolded ATPasedomain of GRP78 with consequent induction of ER stress	Melanoma, glioblastoma	134
Bortezomib A	Induces ER stress by inhibiting a 26S proteasome and thereby activating the ER-associated degradation pathway with misfolded proteins	Different types of cancer	150-152
Retaspimycin (IPI-504)	Inhibits HSP90 activities	Gastrointestinal stromal tumors, non-small cell lung, prostate	153
SNX-2112	Inhibits HSP-90 activities	Gastric cancer	154
MG-132	Inhibits 26S proteasome	Different types of cancer	151, 155, 156
Ritonavir	HIV protease inhibitor, activates certain UPR components such as CHOP and Grp78	Improves the antibody response and inhibits CD8+ T cell activity	9, 121
Epidermal growth factor (EGF)-SubA	GRP78 targeting cytotoxin	Prostate tumor	79
GSK2656157	Inhibits PERK and eIF2α phosphorylation, ATF4 translation and CHOP mRNA expression	Multiple myeloma, pancreatic cancer	139
Brefeldin A (BFA)	Inhibits protein transport from ER to Golgi complex	Cancer, leukemia	14, 150, 157
Delta(9)-tetrahydrocannabinol (THC)	Increases phosphorylation of eIF2α and activates ER stress response	Glioma cells	140
Resveratrol	Resveratrol induces GRP78 and CHOP, p-eIF2α and XBP1 splicing	Human leukemia K562 cell line	141
O(2)-[2,4-dinitro-5-(N-methyl-N-4-carboxyphenylamino)phenyl]1-(N,N-methylamino) diazen-1-ium-1,2-diolate (PABA/NO)	PDI inhibitor, leads to activation of PERK, eIF2α, XBP1 splicing, BiP, PDI, GRP94, and ERO1	Human leukemia (HL60), ovarian cancer cells (SKOV3).	142

ER, endoplasmic reticulum; IRE1α, inositol-requiring enzyme 1α; GRP78, glucose regulated protein 78; HSP90, heat shock protein 90; HIV, human immunodeficiency virus; UPR, unfolded protein response; CHOP, CCAAT/enhancer binding protein homologous protein; PERK, pancreatic ER kinase-like ER kinase; eIF2α, eukaryotic initiation factor 2α; XBP1, X-box binding protein; PDI, protein disulfide isomerase; BiP, binding immunoglobulin protein; ERO1, ER oxidoreductin-1.

ERAD, clearing misfolded proteins. Proteasomal activation is a main pathway for ERAD.[5] 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 as a single agent or in combination with chemotherapeutics against solid tumor malignancies.[111,112] In vitro studies have confirmed the cytotoxic effects of bortezomib on various kinds of cancer cells, including those of the prostate, lung, breast, and colon.[113-115] Although the mechanisms involved in its anticancer activity are still in the process of being elucidated, bortezomib was recently shown to cause accumulation of misfolded proteins in ER and apoptosis by inhibiting 26S proteasome activity and subsequent ERAD machinery[116,117]; moreover, bortezomib was shown to inhibit IRE1α endoribonuclease/kinase activity.[45,118] In the ERAD process, a cytosolic ATPase, p97, plays key roles in extracting misfolded proteins that are poly ubiquitinated and transporting them to the proteasome for degradation. Like bortezomib, Eeyarestatin I (EerI), achemical inhibitor that can block ERAD, induces an integrated stress response in the ER,leading to cell death. EerI activates CREB/ATF transcription factors ATF3 and ATF4, which form acomplex capable of activating BH3-only protein NOXA expression.[119] 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. Recently, an ERAD chemical inhibitor designed to block the ERAD pathway has also shown cytotoxic activity against cancer.[120] Ritonavir, used as a HIVprotease inhibitor, also interferes with ERAD machinery and activates UPR components such as CHOP and Grp78.[9,121]

3. Heat shock protein 90 inhibitor

The heat shock protein 90 (HSP90) complex is activated in cancer cells to regulate the folding and degradation of unfolded proteins.[122] Cancer development-associated proteins such as Akt, Flt3, Bcr-Abl, and Apaf and cyclin-dependent kinase are regulated by the HSP90 inhibitor. All 3 branches of UPR are activated by HSP90 inhibitors such as retaspimycin (IPI-504) and SNX-2112, activating a caspase-dependent cell death pathway.[123] The HSP90 inhibitor also leads to inactivation, destabilization and degradation of HSP90 client proteins. A number of drugs were discovered during the search for a HSP90 inhibitor (Table 2) such as HSP90 inhibitors and geldanamycin analogs like 17-allylamino-17-demethoxygeldanamycin. Recently HSP90 was found to regulate the UPR by stabilizing IRE1 and PERK.[124]

4. Brefeldin A

Brefeldin is an ADP ribosylation factor (ARF) inhibitor required for coatamer assembly on the Golgi membrane. Blocking ARF blocks the retrograde transport of protein from the ER to the Golgi and causes the accumulation of trapped secretory protein in the ER, subsequently activating the UPR. Activation of the UPR results in apoptosis in many cancer cell lines such as multiple myeloma (U266, NCI-H929), Jurkat, HeLa, leukemia (HL60, K562, and BJAB), colon (HT-29), and prostate and adenoid cystic sarcoma cells.[125-130] Brefeldin A may be an effective therapeutic drug. A related mechanism has been suggested to perturb intracellular protein trafficking and induce caspase activation and apoptosis through analysis of a chronic lymphocytic leukemia cell model. Brefeldin A was found to trigger Grp78 upregulation and ER dilation, markers of ER stress in follicular lymphoma cells.[131]

5. Glucose regulated protein 78/binding immunoglobulin protein inhibitor

Grp78 acts as a survival factor in solid tumor and cancer cells.[132] Its expression is correlated with metastasis or late stages of tumor progress. The expression of Grp78 may be related to resistance against anticancer therapy in which apoptosis signaling is involved.[8] In cancer cells, knockdown of BiP/Grp78 increases sensitivity against therapeutic drugs.[132] Epidermal growth factor-SubA (EGFSubA) is highly toxic to the growing of confluent epidermal growth factor-expressing cancer cells and Grp78, a causative protein for cancer, is rapidly cleaved following treatment with EGFSubA.[79] Epigallocatechin gallate, which binds to the ATP-binding domain of Grp78, blocks its UPR protective function and sensitizes glioma cells against chemotherapeutic agents such as temozolomide or etoposide. Glucose deprived tumor cells are more sensitive to versipelostatin because they exhibit inhibited UPR. Versipelostatin inhibits BiP/Grp78 transcriptional activation in combination with cisplatin, regulating tumor growth in a stomach cancer xenograft model.[133] Honokiol [2-(4-hydroxy-3-prop-2-enyl-phenyl)-4-prop-2-enyl-phenol], a cell-wall component of *M. grandiflora*, exhibits similar anti-tumor activity to EGCG and has been tested for the treatment of multiple melanoma and glioblastoma[134].

6. Inositol-requiring enzyme 1α inhibitor

IRE1α inhibitors inhibit IRE1α activity by binding at one of the 2 sites on the IRE1α: the catalytic core of the RNase domain or the ATP-binding pocket of the kinase domain. Salicylaldehydes (typified by 3methoxy-6bromosalicylaldehyde), 4μ8C, MKC3946, and STF083010 interact with the catalytic core of the RNAase domain and have high potential activity for IRE1α RNase activity.[97,135-137] Salicylaldehydes (typified by 3methoxy-6bromosalicylaldehyde) bind to IRE1α in an irreversible manner and inhibit XBP1 splicing and RIDD activity.[137] The 4μ8C forms a stable imine bond at the critical lysine 907 residue in the catalytic core of the RNase domain and blocks the cleavage of XBP1 mRNA and RIDD.[97,135,136] MKC-3946, in combination with a proteosome inhibitor, broteozomib, induces ER stress by inhibiting XBP1 mRNA splicing.[97] STF-083010 exerts an inhibitory effect on tumors in mice bearing human multiple myeloma xenografts.[138] Irestatin, an inhibitor of IRE1α, mediates the inhibition of XBP1s transcription activity and inhibitsthe UPR, disturbing the growth of malignant myeloma cells.[110]

7. Other inhibitors

A number of drugs are currently being screened to target different causes of cancer, with actions such as inhibiting ER signaling or activating the ER stress pathway. GSK2656157 inhibits PERK signaling and reduces cancer growth by impairing amino acid metabolism and angiogenesis.[139] Delta(9)-tetrahydrocannabinol, the main active component of marijuana, induces human glioma cell death through the stimulation of autophagy. This effect is associated with increasing phosphorylation of eIF2a and activation of an ER stress response that promotes autophagy.[140] Resveratrol, a natural plant polyphenol, has been reported to cause cell cycle arrest via induction of the UPR in human leukemia cell lines.[141] The polyphenol stimulates transcriptional induction of Grp78 and CHOP and phosphorylation of eIF2α and XBP1 splicing. Protein disulfide isomerase (PDI) is one of the most abundant ER proteins and maintains a sentinel function in the

organization of accurate protein folding. The PDI inhibitor, O(2)-[2,4-dinitro-5-(N-methyl-N-4-carboxyphenylamino)phenyl] 1-(N,N-methylamino) diazen-1-ium-1,2-diolate (PABA/NO), increases intracellular nitric oxide that causes S-glutathionylation of PDI. PABA/NO activates the UPR and leads to the activation of PERK, eIF2α, XBP1 splicing, BiP, PDI, GRP94, and ERO1 in human leukemia (HL60) and ovarian cancer cells (SKOV3).[142]

CONCLUSION

Accumulating evidence is helping to elucidate the role of the ER stress response in tumorigenesis and cancer resistance. These findings have raised the exciting possibility of targeting UPR components in cancer therapy and overcoming drug resistance, and may facilitate the discovery of distinct roles of UPR branches that produce survival or death signals in tumorigenesis.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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