Mini reviews

A B S T R A C T

Perturbation of endoplasmic reticulum (ER) homeostasis induces a stress condition described as “ER stress”, which in turn leads to a well-regulated program termed as unfolded protein response (UPR). The principal purpose of UPR is to reestablish the ER homeostasis. Some of the physiological and pathological situations that disrupt the homeostasis include hypoxia, glucose limitations, nutrient deprivation, low pH, genomic instability, and some cytotoxic compounds are frequently observed during the core formation and progression of tumors. These stressful microenvironments around the tumors affect the innate and adaptive immune responses. The ER stress is usually induced to activate the UPR and to handle the stress. Although the UPR mechanism is primarily a pro-survival process, preserved and/or prolonged stress may induce cell death. In tumors, ER stress may modify apoptotic and autophagic cell death and, thereby provokes drug resistance of cancerous cells to current therapies. In this mini-review, at first, we highlight the role of UPR and its mediators in cancerous cells fate and then discuss their potential opportunities in cancer therapy.

Keywords: ER stress, unfolded protein response, cancer therapy
INTRODUCTION:

ER stress responses

Numerous physiological or pathological conditions (e.g. hypoxia, low glucose, oxidative stress, inflammation, and mutations in specific proteins) can cause an increase in the unfolded proteins in the endoplasmic reticulum (ER), which leads to activating unfolded protein response (UPR)\(^1\),\(^2\). The responsibility of UPR is maintaining homeostasis by augmenting the protein folding capacity in the ER or upregulates ERAD (ER-associated degradation) pathways to eliminate unfolded/ misfolded proteins. It also suppresses the general protein translation. Although the primary aim of UPR is the maintenance of the cell survival, the persistence of the stress activates cell death signaling pathways\(^3\).

The initiation of UPR function is induced by Grp78/Bip activation as ER chaperone. In normal cells, Grp78 interacts with three integral ER membrane proteins and makes them inactivated. These are IRE-1 (inositol-requiring protein 1), PERK (PKR-like ER kinase), and ATF6 (activating transcription factor 6). During stress stimulation, the Grp78 is released and these three transmembrane proteins are activated\(^4\). IRE1 has two functional enzymatic domains, a Ser/Thr kinase domain and an endoribonuclease domain as shown in figure 1. The exact substrates for kinase activity have not been clearly known yet\(^5\). However, it can active RNase domain that cleaves the intron of XBP1 (X-box–binding protein 1) pre mRNA and makes the XBP1s form, a transcription factor that can induce some genes involved in UPR and ERAD cascade. IRE1 pathway has also an ability to cleave some other mRNAs to reduce the more loading of proteins in ER to reestablish the homeostasis. The process, which is known as IRE1 dependent decay (RIDD), is upregulated during hyperactivation of IRE1\(^6\). RIDD also decreases the expression of some microRNAs (miRNAs), including miR-17, miR-34a, miR-96 and miR-125b\(^3\). In some conditions, IRE1 branch of the UPR is activated earlier than the other ones and also is decreased rapidly\(^4\).

PERK as another kinase transmembrane protein in the ER is autophosphorylated in Ser/Thr kinase domain upon activation and then can phosphorylate eIF2\(\alpha\) (eukaryotic initiating factor 2 subunit \(\alpha\)) factor to suppress the whole protein translation. However, some other mRNAs such as ATF4 are translated as a transcription factor to promote the expression of some other ER chaperones, including the genes involved in glutathione synthesis, amino acid metabolism, and resistance to oxidative stress\(^7\). In addition, ATF4 activates CHOP (C/EBP homologous protein) to induce cell death. CHOP is known as growth arrest and DNA damage-inducible protein GADD34 which is activated in response to DNA damage\(^8\). The dephosphorylation of eIF2\(\alpha\) is done by GADD34 that is a protein phosphatase 1 (PP1)-interacting protein. GADD34 can affect PPI to dephosphorylate eIF2 and eradicate the translational inhibition. Originally, the expression of GADD34 leads to apoptosis using an unknown mechanism\(^9\). During ER stress, all three arms of UPR can trigger CHOP. However, CHOP up-regulation is merely seen in PERK–eIF2–ATF4 branch of UPR. In fact, in the presence of stress, both PERK and IRE1 possibly affect each other to induce CHOP. The role of CHOP is to suppress BCL2 gene expression, which enhances the pro-apoptotic Bcl2 family proteins\(^10,\)\(^11\). ATF6 as the other arm of UPR is activated by RIP (regulated intramembrane proteolysis). Upon ER stress, ATF6 is transported to the Golgi and then cleaved by SP2 proteases. Its cytosolic domain is translocated into...
the nucleus to induce the expression of CHOP, ER chaperones, and ERAD components. AT-F6α can also suppress lipid biosynthesis during glucose deprivation.

**Role of UPR in cancer**

Cancerous cells survive during exposure to both intrinsic and extrinsic tension factors such as hypoxia, nutrient deprivation, and low pH. In addition, cancer cells generate reactive metabolic by-products that avidly modify ER-resident proteins and chaperones. Notably, the induction of various UPR-related factors has been commonly reported in patients with various cancer types and their overexpression usually correlates with poor prognosis and resistance to therapy.

Interestingly, treatment of tumor-bearing mice with the ER stress inducer, thapsigargin, increased the tu-

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**Figure 1.** ER stress initiation pathway. Following ER stress, the UPR main modulators, PERK and IRE1 are activated by dimerization and phosphorylation, ATF6 is cleaved in the Golgi apparatus, leading to transcription of key genes involved in resolving ER stress. Under long-term ER stress, the adaptive UPR pathway fails to rescue the cells, and the apoptotic UPR pathway, namely the PERK–eIF-2α–ATF4 or IRE1–TRAF2–ASK1–JNK pathway, is induced.

Abbreviations: ASK1, Apoptosis Signal-regulating Kinase 1; ATF, Activating Transcription Factor; eIF-2α, Eukaryotic Translation Initiation Factor 2α; ER, Endoplasmic Reticulum; IRE1, Inositol-Requiring Protein 1; JNK, C-Jun N-Terminal Kinase; PERK, Pancreatic eIF-2α; TRAF2, TNF receptor-associated factor 2; UPR, Unfolded Protein Response; XBP1, X-box Binding Protein 1; XBP1s, spliced form of XBP1.
mor growth, whereas global UPR inhibition using chemical chaperones, such as 4-Phenybutyric acid (4-PBA) or Tauroursodeoxycholic acid (TUDCA), delayed tumor progression and metastasis. Thus, the UPR as a potential therapeutic target is introduced for cancer treatment.

**UPR and tumor cell survival**

During cell stress e.g. hypoxia and oxidative stress, UPR supports the cell to survive. In other words, UPR initially helps the cells to survive and cope with the stresses. IRE1 promotes both adaptive and death pathways by its RNase activity. The XBP1s is a cytoprotective factor, while the RIDD induces both adaptation and death signals. Thus, both RNase functions of IRE1 may be good targets for cancer therapy. PERK as another arm of UPR also helps tumor development during hypoxia tolerance. It has been shown that some tumor cells, which are PERK-/- under the hypoxic condition, have lower viability with reduced ability to form new blood vessels. In fact, PERK induces ATF4 activation, which promotes some stress response genes. In addition, PERK can phosphorylate Nrf2, as a transcription factor, inducing ARE (antioxidant response elements) expression. Nrf2 activation inhibits CHOP expression and reduces cell death. Primary tumors under hypoxia condition may have the ability to survive and show metastasis. Therefore, UPR inhibition may not block cancer cells survival but may slow down their survival during metastatic process.

**UPR and tumor dormancy**

The UPR is involved in cancer cells survival in dormancy time. Dormancy time in tumors is a long-lasting period in which no sign of tumors is seen. Detection and treatment of tumors during dormancy are a challenging process. Tumor dormancy has also shown poor angiogenesis. Dormant tumors have ability to be activated in an appropriate time and grow up rapidly. The cause of tumor dormancy is quiescence of tumor cells in some cases. For example, in squamous cell carcinoma study, the T-HEp-3 and its dormant derivative D-HEp3 have shown high PERK-eIF2a signaling to maintain cell survival and also promote G2/G1 arrest. ATF6 activation is also important for the survival of the long-standing dormant tumor and may be a good potent target for eradicating cancer cells. Moreover, the dormant tumor is formed due to the inability of cancer cell growth as a result of apoptosis or poor vascularization. Since the tumor gets bigger, the oxygen and nutrients are limited and hypoxia and ischemia are locally formed. Following adaptation of the cell to hypoxia, HIF (hypoxia-inducible factor) expression promotes the cell to survival position. Hypoxia itself is a potent UPR induction. The UPR then through its three arms, IRE1, PERK, and ATF6, promotes VEGF (vascular endothelial growth factor) to maintain the survival. Targeting UPR in this stage helps to suppress cancer cell survival.

**UPR and tumor stem cell differentiation**

During neuronal differentiation of mouse embryonic stem cells, UPR is activated to promote neuronal differentiation. The involvement of UPR in differentiation of cancer stem cells is also confirmed. It has been shown that the colon cancer stem cells are resistant to conventional therapy than differentiated ones. But through UPR induction, the colon cancer stem cells differentiate and are sensitive to therapy in both in vitro and in vivo study.

**UPR and tumor cell death**

The UPR is responsible for reducing accumula-
tion of unfolded proteins. When the aggregation of proteins is prolonged, pro-apoptotic signaling is initiated. The most important mediators of UPR are PERK, ATF6 and IRE1, which can initiate pro-apoptotic signaling indirectly through activation of downstream molecules such as CHOP, and Bcl2 family proteins that are discussed as follows19.

PERK activity in its first step involves maintenance of the survival of the cell during mild and even moderate stress. However, it can switch the survival position to pro-death signaling through CHOP induction20. Following ATF6 activation, it moves into the nucleus to induce the genes with an ER stress response element (ERSE) in their promoter. Although CHOP is one of the most important genes, there is no report of apoptosis induction for ATF6. Thus, it seems that the role of ATF6 in UPR is pro-survival but not cell death17.

IRE1 has both pro- and anti-apoptotic activity. It has been shown that the importance of IRE1 is in the initiation of the pro-apoptotic mechanism. Upon UPR initiation, PERK and then ATF6 are activated to resolve the stress before IRE1 function. In prolonged cellular stress, IRE1 is activated to splice XBP1 and induce P58IPK to terminate more protein translation. If the cell returns to the normal situation or even the stress continues, IRE1 induces apoptosis signals by recruiting ASK1 and JNK27.

RIDD has both anti-and pro-oncogenic roles in different cells. For example, in glioblastoma, RIDD increases the cell migration and activates pro-inflammatory mechanisms. The research on RIDD is limited and the effect of most inhibitors on RIDD activity has not been investigated yet17.

ER stress and autophagy

Although ER stress and autophagy operate independently, they share some common duties such as protecting cell from stress and inducing cell death under severe stress28. Although making changes to one system may influence the other, the relationship between these pathways is not fully understood30. When there is a deficiency of foodstuff, autophagy aids the cell to survive by providing nutrient supply through cell’s components breakdown. On the other hand, if autophagy keeps going uncontrollable, cell death will occur31-33. The exact mechanism of this decision is not yet fully understood. Many studies have shown the activation of autophagy pathway by ER stress and also promotion of ER stress-induced cell death by autophagy inhibition. Indeed, accumulation of misfolded proteins affects both ER stress and autophagy31. For example, unwanted proteins might be removed by autophagic pathways or ERAD pathway, which transfer these proteins to proteasomes32. If autophagy is suppressed, the removal of all misfolded proteins is done by ERAD pathway that promotes more ER stress responses to switch the cell survival into death1. Accordingly, ER stress induces autophagy as a compensatory mechanism through PERK and IRE1 arms to sustain survival or even cell death31, 34.

ER-stressed and anti-tumor immunity

Although the role of the UPR in the survival/death of tumor cells has been much considered, its function in anti-tumor immunity needs to be addressed more13. Tolerogenic activity is observed in tumor-infiltrating myeloid cells, showing the important role of tumor microenvironment in the control of myeloid cell function35. The previous studies have reported the induction of ER stress in dendritic cells (DCs), macrophages, and myeloid-derived suppressor cells (MDSCs) with their decreased ability to induce T cell responses36. Also, more research has shown that cancer cells with ER stress activity release soluble factors which can affect the immune system.
In fact, microenvironment surrounding the tumors and some subsets of immune regulatory myeloid populations are obstacles to effective innate and adaptive immune responses and immunotherapy. There are some different chemotherapeutic agents such as anthracycline family which can trigger UPR in cancer cells and induce immunogenic cell death (ICD)\textsuperscript{37}. However, the mechanism of ICD induction by ER stress still is not fully understood but some evidence shows that it is mediated by elevation of ROS levels and activation of the NLRP3 inflammasome\textsuperscript{37}. In fact, preserved ER stress responses in transformed cells can promote immune-suppression, while the over-activation of the UPR following acute chemo- or radiotherapy may promote immune-stimulatory responses. The level of UPR mediators’ expression in tumor cells also is associated with different stages of the tumor, aggressiveness, and different malignancies. The UPR activity affects the survival of tumors through IRE1α-XBP1 and CHOP by regulation of myeloid cell activity\textsuperscript{13}.

**The therapeutic potential of targeting endoplasmic reticulum stress-associated machinery**

While tumor cells can grow under ER stress conditions such as hypoxia, limited nutrients, DNA damage and oxidative stress, the UPR is inactive in most normal cells. Therefore, targeting UPR mediators in cancer cells may be a potent strategy in cancer treatment. It is important to consider the different roles of UPR arms that may contribute to cell survival or cell death in response to chemotherapy. This informative data will be crucial for drug designing in the management of cancer therapy\textsuperscript{38}. In the following section, the ER stress mediators that are used to target most cancers will be discussed further (Table 1). It should be noted that both inhibition and induction (up to a death threshold) of these targets may propose for cancer therapy, depending on the condition.

**1- Glucose-regulated protein 78/binding immunoglobulin protein (Grp78/Bip)**

Grp78/Bip acts as a survival factor in tumor cells. The expression of Grp7 is associated with metastasis and drug resistance. It has been shown the knockdown of BiP/Grp78 in cancer cells, enhances sensitivity to chemotherapy\textsuperscript{39}. There are some Grp78 inhibitors to suppress the tumor cell growth. For example, Epidermal Growth Factor-SubA (EGFSubA) is highly toxic to cancer cells growth and could cleave Grp78 to inhibit the growth. Another example is epigallocatechin gallate, which binds to the ATP-binding domain of Grp78 in glioma cells. In this situation, the glioma cells sensitize to temozolomide or etoposide\textsuperscript{40}.

**2- Inositol-requiring enzyme 1α (IRE1)**

IRE1 promotes both adaptive and death pathways by its RNase activity. The XBP1s is a cytoprotective factor, while the RIDD induces both adaptation and death signals. Thus, all RNase functions may be good targets in cancer therapy\textsuperscript{17}. IRE1 kinase inhibitor type I could target ATP binding site and suppress the phosphorylation. These molecules such as APY29 and sunitinib stabilize the splicing of XBP1 mRNA. Whereas type II IRE1 kinase inhibitors inhibit XBP1 splicing\textsuperscript{41}.

**3- PRKR-like ER kinase (PERK)**

As earlier discussed, PERK promotes pro-death signals. Targeting PERK/eIF2 signaling for eIF2 phosphorylation inhibition or its prolonged phosphorylation is now considered. There are two PERK inhibitors, including GSK2606414 and GSK2656157, with an ATP-competitive activity which could interact with eIF2. Therefore, the phosphorylation of eIF2 is inhibited and the load of more proteins is de-
creased. The fate of the cell is apoptosis due to the reduction of ER stress adaptation. There is another PERK inhibitor with the prolonged phosphorylation of eIF2. Thus, it could induce apoptosis through TNF-related apoptosis-inducing ligand (TRAIL)38.

4- ATF6 signaling targeting

ATF6 function depends on the redox mechanism with PDI involvement and dissociation from Grp78. PDI family A member 5 (PDIA5) controls ATF6 activation through disulfide bond arrangement. Indeed, PDI blocks ATF6 translocation from ER to the Golgi, as a result of which ATF6 is inactivated. There are some PDI inhibitors, which inhibit the disulfide exchange capacity and effect on tumor growth. Although there is still no specific ATF6 inhibitors, studies are being done to find potent inhibitor molecules for presenting therapeutic approaches38.

5- Targeting the UPR as an adjuvant therapy

Adjuvant therapy is known as additional therapy that is given besides initial treatment to enhance the effectiveness of the drugs and keep cancer from returning. There are a lot of drugs for cancer therapy which act both as UPR inducer and anticancer specific drugs. These two agents push the cells to apoptosis and also decrease the drug resistance15. For instance, in hepatoma both salubrinal as ER

Table 1: The therapeutic potential of targeting the UPR mediators and endoplasmic reticulum stress-associated machinery in cancer cells

<table>
<thead>
<tr>
<th>UPR components</th>
<th>Mechanism of action</th>
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<tbody>
<tr>
<td>Grp78</td>
<td>Survival factor, involved in metastasis and drug resistant</td>
</tr>
<tr>
<td>IRE1</td>
<td>Pro death and pro survival</td>
</tr>
<tr>
<td></td>
<td>XBP1 mRNA splicing is cyto protective factor</td>
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<td></td>
<td>RIDD induces both adaptation and death signals</td>
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<tr>
<td>PERK</td>
<td>Tumor development in mild stress</td>
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<td></td>
<td>Apoptosis inducer in prolong stress</td>
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<tr>
<td>ATF6</td>
<td>Survival in moderate stress</td>
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<tr>
<td></td>
<td>Apoptosis inducer in long lasting stress</td>
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<tr>
<td>proteasome</td>
<td>Cell death</td>
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<tr>
<td>HSP</td>
<td>Stabilizing IRE1 and PERK</td>
</tr>
<tr>
<td>ARF</td>
<td>Cytoprotective factor</td>
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<tr>
<td>SERCA</td>
<td>Cytoprotective agent</td>
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<tr>
<td>Histone deacetylase</td>
<td>Epigenetic control of gene transcription and cell growth</td>
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<tr>
<td>Autophagy pathway</td>
<td>Cell survival and cell death</td>
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</tbody>
</table>

stress inducer and bortezomib as anticancer drug could increase cancer cell death. Another example is toyocamycin as ER stress inducer and bortezomib which decreases drug resistance and increases the apoptosis in multiple myeloma.

6- ER Associated Protein Degradation machinery (ERAD)
Misfolded proteins in ER are eradicated by ERAD mechanism through proteasomal activity. The ubiquitin-proteasome system has recently become the main target for drug development in cancer therapy. Bortezomib as a common proteasome inhibitor has been used in multiple myeloma with cytotoxicity effect. It has been shown that Eeyarestatin I (EerI), a chemical inhibitor of ERAD, has antitumor activities similar to bortezomib. EerI could induce apoptosis through up-regulation of the Bcl-2 homology3 (BH3)-only pro-apoptotic protein NOXA.

7- Heat shock protein 90 inhibitor
All three UPR branches in ER are activated by HSP90 inhibitors such as retaspimycin (IPI-504) and SNX-2112 to induce cell death. HSP90 complex is responsible for regulating protein folding and degrading unfolded proteins in tumor cells. There are some cancer development-associated proteins such as Akt, Flt3, Bcr-Abl, and Apaf and cyclin-dependent kinase that are regulated by HSP90 inhibitors. Indeed, HSP90 was found to regulate the UPR by stabilizing IRE1 and PERK suggesting a good target in drug development.

8- ARF (ADP-ribosylation factor)
ARF is required for coatamer assembly on the Golgi membrane. ARF inhibitors such as Brefeldin block the protein transfer from ER to the Golgi. Thus, the protein accumulation in ER is increased and subsequently, UPR is activated to induce cell death in many cancer cell lines such as Jurkat, Hela and some leukemia cell lines. Brefeldin A is a potent drug in cancer therapy in order to induce apoptosis through caspase activation.

9- Sarcoplasmic/Endoplasmic Reticulum Calcium ATPase (SERCA)
SERCA is a transmembrane protein in ER that pumps calcium ions into the ER. SERCA inhibitors such as Thapsigargin (Tg) are model agents for ER stress inducer. Although Tg is a good potent drug for anticancer therapy in vitro, it has cytotoxicity effect in systemic chemotherapy. Another SERCA inhibitor is celecoxib, which could induce ER stress both in vitro and in vivo in animal models. Celecoxib exerts anti-tumor activity by decreasing the ER calcium storage.

10- Histone deacetylase (HDAC)
There are some Histone deacetylase (HDAC) inhibitors (HDIs) for epigenetic control of gene transcription, cell growth arrest and apoptosis. Recently, studies have shown the link between ER stress and HDI. HDAC6 enzyme could interact with misfolded proteins and transfer them to aggresome. Aggresomes as cytoprotective response are involved in the removal of unused proteins through autophagic pathway. HDAC6 inhibitor induces more unwanted proteins loading and severe ER stress and subsequent apoptosis. Albeit, more study is needed concerning this topic.

11- Autophagy inhibitors
There are some autophagy inhibitors to induce ER stress through aggresome control. Autophagy is involved removing unwanted proteins through autophagolysosomes and hence, inhibition of this pathway effects on loading more misfolded proteins in ER and induction of severe stress. Chloroquine is an autophagy inhibitor, which is used widely in some experimental systems for chemosensitivity of tumor
cells. The effect of Chloroquine is increased when used as adjuvant drug with temozolomide in glioblastoma in clinical trial.  

**CONCLUSION:**
ER stress has a dual function in cancer either by repressing or supporting cancer initiation and progression. However, UPR may help cancer cells to survive in the face with a stressful situation, promote angiogenesis and induce drug-resistant for cancerous cells. Accordingly, due to personalized medicine progress, therapeutic strategies, instead of modulating general UPR pathways, is better to design for selectively targeting a specific mediator in the UPR pathway. Concerning drug designing for the UPR and its components, both activator or inhibitor agents can be proposed depending on the condition, but more research is required to reveal their therapeutic importance for UPR targeting in cancer.

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