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Endoplasmic reticulum quality control in cancer: friend or foe

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Abstract

Quality control systems in the endoplasmic reticulum (ER) mediated by unfolded protein response (UPR) and endoplasmic reticulum associated degradation (ERAD) ensure cellular function and organismal survival. Recent studies have suggested that ER quality-control systems in cancer cells may serve as a double-edged sword that aids progression as well as prevention of tumor growth in a context-dependent manner. Here we review recent advances in our understanding of the complex relationship between ER proteostasis and cancer pathology, with a focus on the two most conserved ER quality-control mechanisms – the IRE1 α -XBP1 pathway of the UPR and SEL1L-HRD1 complex of the ERAD.

Keywords

Protein folding; ER homeostasis; UPR; IRE1 α ; XBP1; ERAD; SEL1L-HRD1; tumorigenesis

1. Introduction

In eukaryotic cells, approximately one third of the total proteome is folded to maturity in the endoplasmic reticulum (ER) prior to transportation to various subcellular or extracellular compartments. A myriad of chaperones, folding enzymes and nascent proteins crowd the molecular environment of the ER lumen all the while maintaining a delicate homeostasis in its protein folding machinery. Various perturbations to this equilibrium, including both physiological and pathological stimuli, can lead to an accumulation of misfolded proteins inside the ER, subjecting the cell to a condition called “ER stress” and activating a series of adaptive mechanisms to alleviate the stress and restore ER homeostasis. These mechanisms consist of two major ER quality control machineries, including unfolded protein response (UPR) and ER-associated degradation (ERAD) [1–3].

Originally discovered as a response to nutrient depletion, autophagy is a cellular process involved in the lysosomal degradation of cellular components and in the maintenance of

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energy homeostasis through recycling of amino acids and nutrients [4]. Several studies suggest that autophagy is activated as an adaptive mechanism in cells experiencing ER stress and may play a role in the maintenance of ER homeostasis in cancer [5, 6]. However, as the role of autophagy goes beyond the ER [7], whether the effect of autophagy in cancer is related to its function in the ER remains to be established. Hence, as the role of autophagy in cancer has recently been extensively reviewed [8–10], it will not be the focus here.

Owing to a high proliferation rate, cancer cells often experience impaired ATP generation, hypoxia, hypoglycemia and specific mutations which may perturb ER homeostasis and trigger the activation of UPR [2]. Persistent ER stress often activates pathways that lead to cell death, effectively eliminating cells with a potential to go rogue. On the other hand, tumor cells may hijack the ER quality control machineries to provide survival signals required for neoplasm growth and eventually avoid cell death [11]. Researchers have considered targeting various components of UPR and ERAD as potent therapeutic means to specifically modulate the survival of cancer cells [12]. In this review, we will discuss the involvement of two most highly conserved branches of the ER quality control systems – the IRE1 α signaling pathway of the UPR and the SEL1L-HRD1 complex of the ERAD – in cancer pathogenesis.

2. The IRE1 α signaling pathway

IRE1 is a type-1 ER-resident membrane protein with bifunctional cytosolic kinase and endoribonuclease (RNase) domains [13, 14]. In mammals, IRE1 exists in two isoforms, IRE1 α [15] and IRE1 β [16]. IRE1 α is ubiquitously expressed and global knockout of the gene results in early embryonic lethality [17, 18]. In contrast, IRE1 β expression is limited to the gastrointestinal epithelial cells [19] and has no RNase activity towards the classical IRE1 α substrate X-box binding protein 1 (*Xbp1*) mRNA [20]. While IRE1 β knockout mice are viable, they are hypersensitive to experimental colitis [19], which may be in part due to reduced mucin biosynthesis [20].

Upon ER stress, IRE1 α undergoes dimerization and/or oligomerization and trans-autophosphorylation, which triggers conformational change and activation of its RNase domain. Activated IRE1 α splices 26 nucleotides from *Xbp1* mRNA, leading to translational frameshift and the generation of an active transcription factor XBP1s. Subsequently, XBP1s enters the nucleus, where it transactivates various target genes, including those involved in protein folding, ERAD, protein trafficking, and lipid biosynthesis (Figure 1) [21]. Additionally, IRE1 α has been shown to degrade a subset of mRNAs via a process called Regulated IRE1-Dependent Decay (RIDD) (Figure 1) [22–25]. Moreover, IRE1 α cleaves some premature microRNAs as a means of regulating apoptosis [26] as well as its own mRNA level [27, 28]. The physiological significance of these extra-*Xbp1* activities of IRE1 α in vivo remains poorly characterized.

Similar to IRE1 α -deficient mice, global deletion of XBP1 leads to embryonically lethal in mice [17, 18, 29]. Using cell type-specific knockout mouse models, studies have demonstrated a critical role of IRE1 α -XBP1 pathway in secretory cells, most notably B cell-derived plasma cells and pancreatic β cells. Mice with B cell-specific *Xbp1* deficiency show

a profound defect in plasma cell production, along with decreased levels of antigen-specific immunoglobulin [30–32]. Intriguingly, IRE1 α deficiency in B cells affects not only plasma cell differentiation, but also early stage of B cell development [17]. While VDJ rearrangement occurs normally in XBP1^{-/-} B cells [30], this event is severely defective in the pro-B cell stage of IRE1 α ^{-/-} B cells [17]. The authors propose that the cytoplasmic domain of IRE1 α may directly regulate transcriptional activation of genes involved in VDJ recombination such as *Rag1* (recombination-activating gene 1), *Rag2* (recombination-activating gene 2), and *TdT* (terminal deoxynucleotidyl transferase).

In vitro, IRE1 α can be activated by glucose in a concentration-dependent manner [33] and hyperactivation of IRE1 α by high glucose may lead to insulin mRNA degradation in pancreatic β cells [34]. Intriguingly, β cell-specific deletion of *Xbp1* in mice results in islet atrophy and hyperglycemia associated with impaired β cell proliferation, insulin maturation and secretion at basal level [35]. Moreover, deficiency of XBP1 caused constitutive hyperactivation of IRE1 α , leading to attenuation of *insulin* mRNA via RIDD. On the other hand, while IRE1 α deficiency in β cells causes disruption in glucose homeostasis and impairs β cell proliferation under metabolic stress, it did not affect pancreatic structure or islet area [36]. These differential phenotypes observed in β cell specific IRE1 α - and XBP1-null mice suggest that each component of this pathway may have its own unique function in cellular physiology. Alternatively, it points to a possible role of the unspliced form of XBP1u, whose physiological role awaits further investigation. Taken together these studies highlight the indispensable role of the IRE1 α -XBP1 pathway in ER expansion and survival of highly secretory cell types.

3. The role of IRE1 α -XBP1s signaling pathway in cancer

Figure 2 depicts various possible molecular mechanisms underlying the role of IRE1 α in cancer. The role of IRE1 α in cancer is best illustrated and characterized in multiple myeloma (MM). MM is a malignant proliferation of plasma cells in the bone marrow and share phenotypical characteristics with long-lived plasma cells. Due to abundant synthesis of secretory proteins in the ER, MM cells are hypersensitive to the activation of UPR that aggravates as the disease advances [37]. Thus, these cells require a large capacity of folding and disposal in the ER and are particularly sensitive to compounds targeting proteostasis. IRE1 α activation can contribute to cancer progression in several pathways mediated by its substrate XBP1s, which is highly expressed in MM [38]. Blocking of IRE1 α RNase activity by IRE1 inhibitors such as STF-083010 or 4 μ 8C or similarly reducing XBP1 expression by proteasome inhibitor or toyocamycin, an XBP1 inhibitor, attenuates the growth of MM cells, via apoptosis [39–42]. Conversely, forced expression of XBP1s in B cells promotes multiple characteristics of myeloma pathogenesis with lytic bone lesions, plasmacytosis and increased monoclonal antibodies [43]. More than 1,000 genes are upregulated in XBP1s-transgenic myeloma cells compared to non-transgenic B cells, including Cyclin D1, Cyclin D2, MAF and MAFB, many of which are known to be involved in human MM pathogenesis. In clinical studies, human MM patients with high ratio of *Xbp1s* mRNA to *Xbp1u* mRNA have a significantly lower survival rate [38]. Collectively, these studies suggest a potential causative role of XBP1s in diseases pathogenesis in some MM patients and implicate IRE1 α -XBP1 axis as a potential therapeutic target in MM.

In addition to MM, the IRE1 α -XBP1 signaling pathway has been implicated in colon and breast cancers. As substantial evidence is lacking in most cases, we will discuss these studies in brief. XBP1 has been implicated in colon carcinogenesis in a 2007 clinical study, where higher levels of total *Xbp1* mRNA and protein assessed via RT-PCR and immunohistochemistry, respectively, were found in colorectal polyps, colon carcinomas and colon cancer cell lines as compared to normal and stromal tissue [44]. It should be pointed out that this study was limited by a small dataset of only 11 patients. In mice, loss of *Xbp1* in the intestinal epithelium leads to an increase in intestinal stem cell numbers in an IRE1 α -dependent manner and Stat3-dependent hyper-proliferation of intestinal epithelial cells [45]. Consequently, these *Xbp1* null mice are more susceptible to colitis-associated cancer as well as genetic-induced colorectal cancer associated with the mutation of adenomatous polyposis coli (*Apc-min*).

In addition to its role in ER maintenance, the IRE1 α -XBP1 signaling pathway may aid in the regulation of hypoxia in highly aggressive triple-negative breast cancer (TNBC) [46]. In breast cancer cell lines and xenograft models, loss of *Xbp1* reduces tumor growth and metastasis due to impaired angiogenesis, independently of cell proliferation or apoptosis. ChIP-seq analysis coupled with co-IP experiments reveals that XBP1s and HIF1 α may function within the same transcriptional complex to regulate the expression of genes involved in survival and angiogenesis such as *VEGFA* (Vascular endothelial growth factor A), *PDK1* (Phosphoinositide-dependent kinase 1), *GLUT1* (Glucose transporter 1), *DDIT4* (DNA-damage-inducible transcript 4) via the recruitment of RNA polymerase II [46]. In line with this notion, inhibition of IRE1 α in gliomas reduces the expression of pro-angiogenic genes such as *VEGF-A*, *IL-6*, *IL-8* and *IL-1 β* , while having an opposite effect on anti-angiogenic factors and matrix proteins such as *thrombospondin-1*, *decorin* and *osteonectin* (also known as *secreted protein acidic and rich in cysteine* or *SPARC*) [47, 48]. Indeed, in tumors expressing a dominant negative IRE1 α , where IRE1 α transmembrane and luminal domains (aa 1–555) is fused upstream to full length Nck-1 (non-catalytic region of tyrosine kinase adaptor protein 1), there is a marked decrease in angiogenesis, tumor vascular density and growth [47, 49]. Taken together, these studies point to a critical role of IRE1 α in mediating hypoxia and angiogenesis in tumor growth and identify the IRE1 α -XBP1s signaling pathway as a candidate for therapeutic intervention in targeting the angiogenic switch in tumor development.

IRE1 α may be involved in cancer pathogenesis via *Xbp1*-independent pathways as well (Figure 2). Various RIDD targets have recently been implicated in pathways promoting tumor growth and metastasis. Among them lies SPARC, a matrix-associated protein that retards cell-cycle progression, triggers changes in cell shape and induces synthesis of extracellular matrix, thereby promoting tumor cell invasiveness. [22, 50]. IRE1 α , via its RNase activity, leads to a downregulation of *Sparc* mRNA levels as shown in a rat glioma model. Expression of the same aforementioned dominant negative IRE1 α transgene leads to an increase in tumor cell attachment and migration along with upregulation of SPARC and activation of its mediator RhoA, a cytoskeleton regulator protein [49, 51].

Glypican-3 (GPC3) is an RIDD substrate as its mRNA is cleaved at the 3' UTR by IRE1 α in an ER stress-independent manner [53]. GPC3 is a glycosylphosphatidylinositol (GPI)-

anchored membrane protein that has been associated in diseases such as Wilms tumors and Simpson-Golabi-Behmel syndrome, and is highly overexpressed in hepatoblastoma and hepatocellular carcinoma (HCC) [54]. GPC3 stimulates canonical Wnt signaling by forming complexes with Wnts, thereby promoting aggressiveness, tumor growth and poor prognosis [55]. In the GPC3^{high} HCC subgroups, the oncogenic microRNA, miR-1291, is particularly up-regulated [53]. Intriguingly, miR-1291 downregulates IRE1 α via destabilization of its mRNA by targeting a site in its 5' UTR region [53]. This regulatory circuit whereby the oncomiR-1291 downregulates IRE1 α leading to high levels of GPC3 may promote tumor growth and invasiveness via stimulation of canonical Wnt signaling in liver cancer cells.

Finally, IRE1 α has also been implicated in the pathogenesis of several cancers via mechanisms yet unknown. Studies conducted using glioblastoma, serous ovarian cancer and lung adenocarcinoma models have demonstrated that mutations often accrue in IRE1 α genomic loci [56]. We recently analyzed the activities of some cancer-associated IRE1 α mutants (P830L, S769F, L474R, and R635W) and found that a highly conserved proline residue at position 830 (Pro830) is crucial for maintaining IRE1 α structural integrity [57]. This residue seems to act as a structural linker with adjacent tyrosine (Tyr945) and tryptophan (Trp833) residues to link the kinase and RNase domains of IRE1 α . The P830L mutation destabilizes IRE1 α and renders both kinase and RNase domains of IRE1 α inactive. Similarly, the Ser769Phe mutation abolishes IRE1 α activation and signaling, although the mechanism remains unclear [57]. It is possible that cancer cells may accrue these loss-of-function IRE1 α mutations to attenuate the pro-apoptotic function of IRE1 α as recently proposed [58]. However, as whether or not IRE1 α signaling exerts pro- and/or anti-apoptotic effects remains controversial [58, 59], further investigations are required to delineate physiological significance of IRE1 α mutations in tumorigenesis.

4. ERAD

ERAD is responsible for the recognition, retrotranslocation and ubiquitination of misfolded proteins in the ER for proteasomal degradation in the cytosol [60]. The ERAD system revolves around transmembrane ubiquitin E3 ligase proteins that connect together the substrate recognition machinery in the ER lumen and the ubiquitin-proteasome system in the cytosol. Failure to remove misfolded ER proteins may result in their accumulation and aggregation, which may account for the pathogenesis of various diseases such as cystic fibrosis, α 1-antitrypsin deficiency and type-1 diabetes [61, 62]

There are two principle E3 ERAD complexes in yeast (Hrd1p and Doa10p), and at least half a dozen in metazoans, each of which recognizes a subset of misfolded proteins in the ER [60]. Hrd1p forms a complex with Hrd3p in yeast [63, 64] and with Suppressor/Enhancer of Lin-12-like (Sel1L) in mammals [65, 66]. As shown in Figure 1, Sel1L nucleates the Hrd1 ERAD complex by interacting with multiple ERAD components such as Hrd1, Derlin1/2, p97, OS9, and E2 enzyme UBC6e [65–67]. A recent proteomic analysis has implicated both Sel1L-dependent and -independent Hrd1-mediated degradation, which may be dictated by substrate topology or accessibility of specific E3 ligases [67].

Physiological importance of SEL1L and HRD1 in vivo is recently emerging. Global deletion of *Sel1L* causes embryonic lethality in mice [68, 69]. In the absence of *Sel1L*, the development of embryonic pancreatic epithelial cell was blocked [70]. Using inducible and adipocyte-specific *Sel1L*-deficient mouse and cell models, we recently demonstrated that *Sel1L* plays a critical role in the stabilization of HRD1 protein in mammals [69, 71] and that the Sel1L–Hrd1 complex plays a critical role in mammalian ERAD, ER homeostasis and survival in vivo [69]. Acute loss of *Sel1L* in adult mice causes premature lethality and severe pathologies of secretory tissues with striking abnormalities of the ER structure integrity, suggesting a crucial role of *Sel1L* in secretory cell types in particular. On the other hand, loss of *Sel1L* in adipocytes leads to resistance to diet-induced obesity and postprandial hypertriglyceridemia due to the ER retention of lipoprotein lipase [71]. In addition, variants in the *SEL1L* gene have also been identified in humans with Alzheimer's diseases [72] and SEL1L mutations have been linked to early-onset cerebellar ataxia in canines [73], pointing to a possible role of SEL1L in maintaining homeostasis in neuronal/glial cells.

Global deletion of Hrd1, also known as Synoviolin (encoded by the gene *Syvn1*), also causes embryonic lethality in mice [74]. Loss of Hrd1 in the liver upregulates the expression of Nrf2 (Nuclear factor (erythroid-derived 2)-like 2) protein and its target genes *Nqo1* (NAD(P)H quinone oxidoreductase 1) and *Gclm* (glutamate-cysteine ligase, modifier subunit). This study demonstrated that Nrf2 is a substrate for Hrd1-mediated proteasomal degradation in the pathogenesis of liver cirrhosis. In this context, ER and oxidative stress response signaling pathways converge as ubiquitination of Nrf2 by HRD1 results in downregulation of the Nrf2-mediated antioxidant response pathway [75]. In dendritic cells (DC), Hrd1 seems to regulate the expression of MHC (major histocompatibility complex) class II via regulating the protein turnover of a key transcriptional repressor BLIMP1 (B lymphocyte induced maturation protein 1) [76]. Loss of *Hrd1* causes the accumulation of BLIMP1, which represses gene transcription of MHC class II. Dendritic cell (DC)-specific *Hrd1* knockout mice exhibit splenomegaly with increased B cell numbers and defects in CD4+ T cell priming in the autoimmune inflammatory response [76]. How Hrd1 mediates the degradation of nuclear transcription factor and whether this function of Hrd1 is Sel1L dependent remain to be demonstrated.

5. The role of SEL1L-HRD1 ERAD in cancer

While the role of ERAD in cancer remains largely unknown, SEL1L has been implicated in cancer pathogenesis. Ectopic SEL1L induction in pancreatic cancer cells leads to G1 phase cell cycle arrest via the induction of PTEN – a phosphoinositide-3-phosphatase and well-known tumor suppressor that normally inhibits cell proliferation, growth and motility. High levels of SEL1L in these cells also leads to reduction in invasiveness possibly via negative modulation of genes encoding matrix metalloproteinase inhibitors [77]. Furthermore, a 2012 study discovered that the SEL1L SNP (rs12435998) shares a close association with the age at diagnosis of pancreatic ductal adenocarcinoma and the patient survival time with or without pancreaticoduodenectomy by analysis of DNA obtained from Caucasian (non-smoker) patients [78].

In the context of colorectal cancer, while basal SEL1L expression level in normal mucosa of the epithelial lining is low, it is elevated in adenoma and adenocarcinoma cells [79]. Nonetheless, SEL1L expression pattern has so far been found to lack correlation with patient survival and the grade of colon cancer. In an investigation involving glioma stem cell lines and valproic acid (VPA), a histone deacetylase inhibitor, the same group reported that SEL1L downregulation in glioma stem cell lines leads to an impairment of neurosphere size and proliferative rate, while inducing differentiation towards a neuronal fate via Notch1 signaling [80]. VPA, a promising therapeutic agent owing to its anti-cancer and minimally toxic properties, was found to upregulate the expression of SEL1L and other UPR genes in glioma stem cells. siRNA-mediated SEL1L knockdown in these cells negatively affects their self-renewal potential and exacerbates the cytotoxic effects of VPA. These data suggest that SEL1L may protect against VPA-mediated cytotoxicity via the maintenance of cancer stem cell properties [81]. In addition, correlations between low SEL1L protein levels (detected by monoclonal antibody staining) and poor prognosis have been reported in breast carcinoma patients [82]. In the context of esophageal cancers, while absent in normal cells, SEL1L is expressed in early neoplastic events and persists in later stages of esophageal cancer [83].

It should be noted that, as most of these studies implicating SEL1L in cancer pathogenesis are based on association studies, interpretation of their findings should proceed with caution. Many outstanding questions remain, for example, what is the mechanism by which SEL1L is involved in tumorigenesis? How do changes in SEL1L level in tumor cells affect ER homeostasis or specific ERAD substrates? How ER homeostasis affects tumorigenesis? Nonetheless, these studies are important because they have opened avenues for more definitive future investigations using animal models.

Another well-studied ERAD component is the lectin protein osteosarcoma amplified-9 (OS9) involved in the recognition and recruitment of misfolded glycoprotein or nonglycoproteins to the ERAD complex [84, 85]. Under hypoxic conditions, OS-9-mediated ubiquitination and subsequent degradation of HIF-1 α , aided by an E3 ligase and tumor suppressor von Hippel–Lindau (VHL), is instrumental in downregulating genes that promote cell survival, proliferation, invasion, angiogenesis and metastasis [86, 87]. A recently identified gene CIM (Cancer Invasion or Metastasis-related) also known as ERLEC1 (Endoplasmic Reticulum Lectin-1) has been found to sequester OS-9 away from the HIF-1 α complex in lung cancer cells, causing HIF-1 α stabilization and accumulation thereby aiding tumor growth and metastasis [88]. This study proposes OS9 to be an important link between hypoxia regulation and cancer progression.

Intriguingly, we have recently shown that in the absence of Sel1L, OS9 accumulates and is stabilized [69, 71], suggesting that OS9 is a substrate of the Sel1L-Hrd1 ERAD complex. Thus, the Sel1L-Hrd1 ERAD complex, along with other ERAD components (such as VHL) may exert its role in tumorigenesis via the regulation of either ER homeostasis in general or specific substrates such as OS9.

6. Therapeutics

Maintaining ER proteostasis assumes high importance in cancer cells due to the increased pressure on protein folding owing to their enhanced metabolic needs, as is evident from the high basal level of expression of UPR markers in these cells. Consequently, developing interventions that aim to sensitize tumor cells to various anti-cancer agents by selectively inhibiting UPR has become a popular therapeutic strategy of late.

A recent endeavor that tested the efficacy of several IRE1 α inhibitors (STF-083010, 3-Ethoxy-5,6-dibromosalicylaldehyde, 2-Hydroxy-1-naphthaldehyde, toyocamycin etc.) in a dosage and time-dependent manner on 14 pancreatic cancer cell lines observed growth retardation due to either cell cycle arrest or induced apoptosis as well as reduction in invasiveness demonstrated by soft agar assays and xenograft experiments [89]. Irestatin is another IRE1 α endonuclease activity inhibitor that has been seen to impair proliferation survival under starvation conditions of malignant myeloma cells [90]. Interestingly, while there is considerable variability in cellular responses to these IRE1 α inhibitors, synergistic effects have been observed when using various drug treatments in combination [90].

The use of oncolytic virus therapy (OVT) – viral induction of tumor cell lysis and recruitment of the immune system to the infected tissue – is quickly rising in popularity owing to its selectivity in targeting malignancies based on the inherent abnormalities of cancer cells. A major drawback of this approach lies in the great variation in the response rates of these viruses on patients. In this context, a genome-wide RNAi screen recently identified various ER stress pathway components including IRE1 α whose inhibition results in preconditioning of cancer cells to undergo apoptosis when challenged with rhabdoviral oncolysis. This sensitization to caspase-2-dependent cell death occurs via pro-apoptotic factors such as MCL1 (myeloid cell leukemia 1), RAIDD (RIP-Associated ICH1/CED3-Homologous Protein With Death Domain) and PIDD (p53-induced death domain) [91]. This “one-two punch” tactic was validated using primary patient samples and can be useful in combating cancers that are otherwise individually resistant to UPR inhibition and oncolytic viruses.

Other means of sensitizing tumor cells towards proteotoxicity involve drugs that block proteasomal activity. Popular among these are the following – Bortezomib (BTZ) which leads to abrogation of NF- κ B function and increases sensitivity to TNF α -related and caspase-mediated apoptosis; Nelfinavir which blocks cellular proteasomal activity by virtue of its own protease property and elicits pro-apoptotic effects marked by amassing of polyubiquitinated proteins [92]; Eeyarestatin I (EerI) which is an agent that targets p97 (ATPase functioning in the transportation of ubiquitinated proteins) as a means to blocking ERAD [93]. Most of these therapeutic agents and their modes of action in combating cancer have been summarized in Table 2.

A 2003 study by Lee *et al.* [41] using MM cell line demonstrated XBP1 to be an important therapeutic target in cancer as proteasome inhibition in MM cells suppresses IRE1 α RNase activity and XBP1s generation, resulting in an increased apoptotic cell death [41]. However, despite recent advances in therapy with proteasome inhibitors (e.g. bortezomib,

carfilzomib), MM remains incurable due to the resistance to most drugs [94]. A recent study by Leung-Hagesteijn and colleagues demonstrated that silencing of IRE1 α or XBP1 in MM cell lines confers resistance to proteasome inhibitors [95]. The loss of XBP1 in MM results in attenuation of Ig production and a decline in ER stress and ERAD function, which reduces ER stress hypersensitivity in MM cells and accounts for the resistance to proteasomal inhibitors. Moreover, a subset of Xbp1s-negative MM cells lacks plasma cell features [95]. These findings may explain the inability of proteasomeal inhibitors in treating MM patients, while underscoring the importance of targeting both the committed plasma cells and the progenitors in therapeutic treatment to overcome drug resistance.

7. Conclusions

When normal cells experience stress due to the accumulation of misfolded proteins inside the ER, a series of adaptive mechanisms are initiated, namely, UPR and ERAD. These pathways lead to global translational attenuation, the induction of specific signaling cascades and clearance of misfolded proteins in the ER aimed at restoring ER homeostasis in the cell. However, in the context of cancer cells, insults from hypoxia, nutrient deprivation, genetic mutations, and enhanced metabolic needs, can often lead to a gross accumulation of faulty proteins in the ER. Therefore, the survival of tumor cells may become especially dependent, relative to normal cells, on the adaptation to stressful conditions via UPR and ERAD. This can potentially serve as the Achilles heel of cancer cells and allow us to view UPR and ERAD components as lucrative therapeutic targets for cancer treatment. On the other hand, it is the same UPR activation that is wielded by the tumor cells in order to mount resistance to various anti-cancer drugs, thus becoming a double edged sword in tumor cell survival. Linking ER homeostasis to cancer progression may shed further light on the cell-intrinsic processes in tumor cells and allow us to potentially target them specifically over surrounding normal cells. However, most of the studies implicating various UPR and ERAD components in cancer pathogenesis are limited by their model system of study (cell culture based experiments) and small sample sizes (sampling of patient tissue). Hence our understanding of the mechanisms by which the ER quality control systems contribute to the function and survival of cancer cells still warrants further thoughtful investigations.

8. Perspectives

Although ER stress is thought to occur in many physiological and pathological conditions, what is lacking in most studies to date is the direct and accurate measurement of stress levels in the ER. Since cancer cells probably have a elevated protein turnover rate and are likely to accost this enhanced metabolic need by adaption via UPR and ERAD, it is quite possible that the basal levels of various components of ER chaperones are higher in tumor cells. Therefore, the induction or inhibition of these so-called “UPR markers” such as GRP78 and CHOP may not serve as a reliable indicator of ER stress. Thus, although many studies have suggested various possible roles of ER stress and IRE1 α signaling pathways in tumor progression, we still lack a deep and accurate understanding of exactly what the status of ER stress and the contribution of UPR pathways in cancer pathogenesis are. Hence we would like to emphasize the urgent need to directly quantitate ER stress at the level of UPR sensors

such as IRE1 α and PERK activation. Using the phos-tag-based approach, one can directly measure and quantitate the extent of IRE1 α phosphorylation (Fig. 3), which we have shown to correlate with the stress level in the ER. As this method is very sensitive and can detect mild ER stress under physiological and pathological conditions [57, 69, 71, 96–98], it promises to provide insights into several outstanding questions, including when and to what extent UPR and IRE1 α are activated during tumorigenesis, how small molecules affect IRE1 α signaling and ER homeostasis in tumors, and how perturbation of ER homeostasis affects the survival and death of cancer cells in humans.

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Fig. 1. Schematic diagrams depicting the roles of IRE1α in UPR and SEL1L-HRD1 in ERAD
 Upon sensing ER stress, IRE1α undergoes dimerization or oligomerization, and trans-autophosphorylation, activating its cytosolic endonuclease activity. Subsequently, IRE1α alternatively splices *Xbp1* mRNA to generate Xbp1s which translocates into the nucleus and regulates different genes. Furthermore, activated IRE1α can selectively degrade particular mRNAs by a process called regulated IRE1-dependent decay (RIDD). Unlike IRE1α-XBP1 pathway, physiological significance of other IRE1α pathways are not well established. (B) Misfolded proteins in the ER lumen are recognized, ubiquitinated and retrotranslocated by the HRD1-SEL1L ERAD complex to the cytosol for proteasomal degradation. BiP and OS9 may be involved in the recognition of misfolded substrates.

Fig. 2. The role of IRE1 α -mediated signaling pathways in cancer pathogenesis

IRE1 α can exert XBP1s-dependent and -independent functions in cancer cells. In XBP1s-dependent pathways, IRE1 α activation can trigger plasma cell maturation and multiple myeloma cell survival; protection from colon cancer via maintenance of intestinal homeostasis; induction of transcription of angiogenic factors and other tumor promoting components in complex formation with HIF1 α , as discovered in gliomas and breast cancer. Via RIDD, IRE1 α downregulates SPARC mRNA thereby preventing RhoA-mediated increase in glioma invasiveness. In liver cancer, the oncogenic miR-1291 downregulates IRE1 α , thereby allowing its otherwise RIDD substrate Glypican-3 to promote tumor growth and aggressiveness via canonical Wnt signaling.

Figure 3. Methods for quantitation of IRE1α activation and stress levels in the ER
 (A and C) Immunoblots of IRE1α and PERK in HEK293T cells transfected with the indicated plasmids for 24 h. NHK, the unfolded form of α1-antitrypsin; p97-QQ, dominant negative form of p97-WT. ER-dsRed and GFP, negative control plasmids. HSP90, a position and loading control. (B and D) Quantitation of percent of phosphorylated IRE1α in total IRE1α protein in Phos-tag gels shown in A, C. Values are mean ± SEM *, $P < 0.05$ using unpaired two-tailed Student's t-test. This data is taken from [98].

Table 1

The role of IRE1 α in various types of cancer.

Type of cancer	System	Mechanism	Reference
Multiple myeloma	B cell specific <i>Xbp1s</i> transgenic mice and patient samples	Aiding in secretory maturation of plasma cells and resistance to anti-cancer drugs	[38, 43, 94, 95]
Triple negative breast cancer	Breast cancer lines and mice with mammary glands injected with tumor cells	High Xbp1s levels aid in sustaining hypoxia (co-localizing with hypoxia markers), promoting angiogenesis and invasion in collaboration with HIF1 α via transcriptional regulation of various genes including <i>VEGFA</i> , <i>PDK1</i> , <i>GLUT1</i> , <i>DDIT4</i>	[46]
Colon cancer	Eleven patient primary tumor samples	High levels of Xbp1 expression in colorectal carcinoma and adenoma	[44]
Glioma	Human U87 glioma cell line; mouse orthotopic brain model; chick chorioallantoic membrane	Supports tumor vascularity, blood vessel cooption and invasiveness by promoting pro-angiogenic VEGFA, IL-6, IL-8, IL-1 β , and by inhibiting anti-angiogenic thrombospondin1, decorin.	[48]
Several cancers	Cell lines	IRE1 α mutations identified in tumor cells are defective in signaling	[57, 58]
Liver cancer	Hepatoma HuH7 cell line	Downregulates GPC3 (mediator of tumor growth via canonical Wnt signaling) by RIDD; itself silenced in cancer cells by oncomiR miR-1291	[53]
Colon cancer	In vivo Xbp1 intestine-specific-null mouse model	Protects against colitis-associated-cancer and Apc-mediated colorectal cancer; depletion of Xbp1 predisposes intestinal epithelium to inflammatory diseases and tumorigenesis	[45]
Glioma	Human U87 glioma cell line	Suppresses attachment and migration properties by downregulating RhoA and SPARC (a matrix protein that retards cell cycle, induces cell shape change and promotes invasiveness)	[51]

Abbreviations – Apc: Adenomatous polyposis coli; DDIT4: DNA-damage-inducible transcript 4; GLUT1: Glucose transporter 1; GPC3: Glypican 3; HIF1 α : Hypoxia inducible factor 1 alpha; IL-6, -8, -1 β : Interleukin 6, 8, 1beta; IRE1 α : Inositol requiring enzyme 1 alpha; miR: microRNA; PDK1: Phosphoinositide-dependent kinase 1; RIDD: Regulated IRE1-dependent decay; RhoA: Ras homolog gene family member A; SPARC: Secreted protein acidic and rich in cysteine; VEGFA: Vascular endothelial growth factor A; XBP1s: Xbp1 spliced.

Table 2Therapeutic interventions in cancer targeting IRE1 α and ERAD.

Agent	Mechanism	Effect	Functional partners	Reference
STF-083010, 3-Ethoxy-5,6-dibromosalicylaldehyde, 2-Hydroxy-1-naphthaldehyde, toyocamycin	IRE1 α inhibition	Growth retardation owing to cell cycle arrest or induced apoptosis; reduction in invasiveness	Synergistic effects seen when used in combination or with gemcitabine/bortezomib	[89]
Rhabdovirus	Oncolytic virus therapy plus IRE1 α inhibition	IRE1 α inhibition preconditions cancer cells specifically and sensitizes them to apoptosis following rhabdovirus infection – called “one-two punch”	shRNA or small molecule inhibitors targeting IRE1 α	[91]
Bortezomib	Blocks proteasome activity	Leads to abrogation of NF- κ B function, increased sensitivity to apoptosis, thereby pushing tumor cells towards proteotoxicity	TNF α and caspase (mediating apoptosis)	[90]
Nelfinavir	Blocks proteasome activity	Exercises protease property in blocking cellular proteasome activity and inducing pro-apoptotic effects via amassing polyubiquitinated proteins	BiP/GRP78, CHOP and caspase activation induced	
Eeyarestatin I	Targeting p97 ATPase	Blocks ERAD by preventing de-ubiquitination of ERAD substrates and preferentially pushing cancer cells towards cytotoxicity		
Irestatin	IRE1 α RNase inhibitor	Blocks UPR by abrogating XBP1s transcription, thereby impairing proliferation or inhibiting tumor cell survival in oxygen-starvation conditions		

Abbreviations – BiP: Binding immunoglobulin protein; CHOP: C/EBP homologous protein; ERAD: Endoplasmic reticulum associated degradation; IRE1 α : Inositol requiring enzyme 1 alpha; GRP78: 78 kDa glucose-regulated protein; NF- κ B: Nuclear factor kappa-light-chain-enhancer of activated B cells; shRNA: short hairpin ribonucleic acid; TNF α : Tumor necrosis factor alpha; UPR: Unfolded protein response; XBP1s: Xbp binding protein 1 spliced.