Introduction

Cellular homeostasis is supported by a symphonic balancing act involving protein synthesis, folding, modification, trafficking, and degradation. In eukaryotic cells, the endoplasmic reticulum (ER) is a specialized organelle that is the central site of synthesis and folding of secretory, membrane, and a number of organelle-targeted proteins. Correct folding and post-translational modifications of these proteins are enabled by the presence of ATP, Ca²⁺, molecular chaperones, as well as an oxidizing redox environment. The imbalance between the load and capacity of protein folding results in a cellular condition known as ER stress.

Conclusion

The imbalance between the load and capacity of protein folding results in a cellular condition known as ER stress. Accumulating evidence demonstrates the importance of ER stress signaling pathways in maintaining function and differentiation of secretory cells such as B lymphocytes, pancreatic beta-cells, osteoblasts, and liver cells (reviewed elsewhere, [5]). However, these same pathways are linked to the etiology and progression of a number of human diseases, including Alzheimers, Parkinsons, diabetes mellitus, atherosclerosis, ischemia, liver and heart disease, as well as in cancer progression and the activation of inflammatory processes in and around the tumor microenvironment [6] (Table 1).

ER stress is accompanied by the activation of a number of highly conserved integrated transcriptional and translational signaling cascades aimed at restoring ER homeostasis. Collectively, these signal-transduction pathways are termed the unfolded protein response (UPR).

The UPR initially counters ER stress by transiently arresting protein synthesis, Figure 1. This is followed by the activation of ERAD pathways and increased transcription of chaperone genes, such as the glucose-regulated protein GRP78 (BiP), calreticulin, calnexin, and protein disulfide isomerases (PDI), each with a role in promoting protein folding and alleviating protein aggregation. Failure of these pathways to restore ER homeostasis results...
Protein Disulfide Isomerase Superfamily in Disease and the Regulation of Apoptosis

Table 1. The role of ER stress pathways in pathology.

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Figure 1. Signal transduction pathways in ER stress-induced apoptosis. The aggregation of misfolded/unfolded proteins in the ER results in GRP78 dissociation and the sequential activation of the three ER stress receptors: pancreatic ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1). Activated PERK phosphorylates eukaryotic initiation factor 2α (eIF2α), which suppresses global mRNA translation and protein synthesis and activates ATF4 translation, which induces the transcription of genes aimed at restoring ER homeostasis. PERK phosphorylation of nuclear factor-E2-related factor 2 (Nrf2) regulates the expression of a number of genes involved in antioxidant defense pathways. Under chronic ER stress, activation of PERK induces the expression pro-apoptotic pathways involving ATF4-dependent CHOP expression. ATF6 is activated by proteolysis mediated by proteases after its translocation from the ER to the Golgi apparatus. Following proteolysis, active ATF6 translocates to the nucleus and regulates the expression of ER chaperones to facilitate protein folding, secretion, and degradation in the ER. Under severe ER stress ATF6 induces CHOP expression and mediates caspase activity. Activated IRE1 induces the expression of genes involved in ER-associated mRNA protein degradation and mediates the splicing of XBP1 mRNA. Spliced XBP1 translocates to the nucleus and controls the transcription induction of ER chaperones. If ER load is overwhelming IRE1 activates proapoptotic pathways through c-Jun NH2-terminal kinase (JNK) and nuclear factor-kappa-B (NF-κB). Apoptosis is additionally activated through IRE1 recruitment of TNF-receptor-associated factor 2 (TRAF2), and caspase activation. ATF-4/6, activating transcription factor 4/6; CHOP, CCAAT/enhancer-binding protein (C/EBP) homologous protein; ER, endoplasmic reticulum; eIF2α, eukaryotic initiation factors 2; GRP78, glucose regulated protein 78; IRE1, inositol-requiring enzyme 1; JNK, c-Jun NH2-terminal kinase; NF-κB, nuclear factor-kappa-B; Nrf2, nuclear factor-E2-related factor 2; PERK, pancreatic ER kinase; TRAF2, TNF-receptor-associated factor 2 UPR, unfolded protein response; Xbp1, X-box binding protein 1
in the activation of apoptotic pathways. The complexities
underlying the molecular mechanisms that define this switch
from pro-survival to pro-death response are evidenced by recent
studies that reveal the roles of specific chaperone proteins as
integration points in signaling pathways that determine cell fate
[7-12]. The following review discusses the dual role of PDI in cell
death and survival during ER stress.

The UPR: where do we go from here?

In mammalian cells three main ER trans-membrane receptors
modulate ER stress and signal the downstream pathways that
define the UPR; PERK (pancreatic ER kinase), IRE1 (inositol
requiring enzyme 1) and ATF6 (activating transcription factor
6). Under homeostasis all three ER stress receptors are
negatively regulated through direct protein-protein interactions
with the ER chaperone GRP78. Accumulation of unfolded or
misfolded proteins in the ER lumen induces the dissociation of
GRP78 from the three sensors, allowing for activation of
their signaling functions. Under ER stress, PERK is activated
by auto-phosphorylation and dimerization. UPR mediated
PERK phosphorylation alters two integrated signaling
pathways, serving to restore ER homeostasis and combat
oxidative stress [13,14]. PERK-mediated phosphorylation of
eukaryotic initiation factor 2 (eIF2a) results in attenuation of
transient global mRNA translation in addition to the selective
translation of the transcription factors and ER stress genes
that carry certain regulatory sequences in their 5’ untranslated
regions. These include ATF4 and ER stress genes such as
amino acid transporters and cellular redox control genes [13].
Simultaneously, PERK phosphorylation of nuclear factor-E2-
related factor 2 (Nrf2) regulates the expression of a number of
genes involved in antioxidant defense pathways. Similar to
PERK, trans-phosphorylation and homodimerization activate
IRE1. The unique endoribonuclease activity of IRE1 catalyzes
mRNA splicing of the bZIP transcription factor, X-box binding
protein 1 (XBP-1). This forms a stable frameshift splice variant
XBP-1s (spliced) that induces a number of genes involved in
ER quality control and ERAD components, ER/Golgi biogenesis
and activates pathways involved in redox homeostasis and
oxidative stress responses [15]. XBP-1s also induces the
expression of the HSP40 family member p58™, which binds
to and inhibits PERK, resulting in a negative feedback loop and
translational recovery [16]. p58™ induction may represent UPR
success and the restoration to normal cellular functioning or
alternatively allow for the synthesis of pro-apoptotic proteins.
Additional roles for IRE1 in translational attenuation and in
mediating ER stress responses have been reported [17,18].
Following GRP78 dissociation, ATF6 activation involves redox-
mediated regulated intra-membrane proteolysis (RIP) in the
Golgi [19]. Proteolytic processing of ATF6 enables transcription
factor binding and the regulation of a number of ER stress
response genes, including the expression of ER chaperones,
ERA components, and gene products with roles in lipid
biogenesis and ER expansion [20,21].

ER-stress mediated apoptosis

The UPR pathways outlined above define the early pro-survival
response signals that are aimed at restoring ER homeostasis.
When the folding capacity is not restored all three UPR pathways
contribute to cell apoptosis (Figure 1). Recent studies suggest
that the degree of ER stress and the differential stability of pro-
survival and pro-death mRNAs/proteins may partially regulate
 cell fate [22]. PERK, IRE1 and ATF6 mediated apoptosis have
been linked to the downstream transcriptional activation of the
C/EBP homologous protein (CHOP/growth-arrest-and DNA-
 damage-inducible gene 153 (GADD153), PERK regulated ATF4
expression directly mediates CHOP expression and apoptotic
cell death. ER stress induced ATF6 expression may play a role
in the determination of cell fate and the transition from anti-
to pro-apoptotic pathways, depending on the level of expression.
Morishima et al found that ATF6 regulated increased expression
of WW domain binding protein 1 (WBPI) resulted in myoblast
apoptosis [23]. Although ATF6 can induce CHOP mRNA, reports
that directly link ATF6-mediated CHOP expression to ER stress-
induced apoptosis are lacking. CHOP sensitizes cells to ER stress
through the down-regulation of the anti-apoptotic protein Bcl-2
and the activation of growth-arrest and DNA-damage-inducible
gene 34 (GADD34) and the redox-altering ER oxidase, ERO1alpha.
CHOP expression also leads to mitochondrial initiated apoptotic
pathways via Bax mitochondrial translocation and caspase
activation. Murine caspase-12 and human caspase-4 pathways
have been directly implicated in ER stress-induced cell death.

Apoptosis is additionally activated through IRE1 recruitment
of TNF-receptor-associated factor 2 (TRAF2) and apoptosis-
signal-regulating kinase 1 (ASK1), and through the activation of
the mitogen-activated protein kinase (MAPK) signaling pathways
of p38 and c-Jun NH²-terminal kinase (JNK). The importance of
ASK1 in ER stress mediated apoptotic pathways is exemplified by
either over-expression or knockout studies. In lung epithelial cells
and mouse embryonic fibroblasts (MEFs), ASK1 over-expression
induced apoptosis through mitochondria-dependent caspase-3
and -9 activation; while the neuronal cells of ASK1-/- mice showed
resistance to prolonged ER stress [24,25]. IRE1 mediated TRAF2
recruitment also permits TRAF2 dissociation from pro-caspase-12
(pCP12), allowing for the activation of pCP12 and downstream
caspases. JNK mediated phosphorylation of Bcl-2 and Bim induces
apoptosis both by suppressing the anti-apoptotic activity of Bcl-2
and enhancing additional pro-apoptotic pathways [26]. Regulation
through IRE1 association/dissociation with Jun activation domain
binding protein 1 (JAB1) may play a role in determining the choice
between the induction of cell survival or death responses [27].
The complete in vivo signaling pathways involved in ER stress-
associated apoptosis have not been entirely elucidated and
are further confounded by research that suggests that cellular
responses may be tissue, insult, and/or redox dependent [28-30].

Protein Disulfide Isomerase

The ER lumen is a highly organized cellular compartment that
contains numerous chaperones and oxidoreductases that assist
in protein folding. Protein disulfide isomerases (PDI) compose a superfamily of more than 20 members reported to date that have diverse sequences and multiple functions including, acting as molecular chaperones, protein-binding partners, and hormone reservoirs [31,32]. The highly abundant redox-active enzyme PDI (P4HB, PDIA1) was one of the initial ER chaperones to be reported and plays a crucial role in catalyzing disulfide bond formation, reduction, and isomerization. PDI may also act as an intracellular anti-inflammatory molecule through negative regulation of nuclear factor-kappa-B (NF-κB), inhibiting lipopolysaccharide (LPS)-induced pro-inflammatory cytokine production in macrophages [33].

The majority of PDI family members are characterized by at least one thioredoxin (Trx)-like Cys-Xaa-Xaa-Cys catalytic domain responsible for regulating PDI redox activity and a KDEL-like ER retention signal sequence. While PDI is primarily an ER protein, PDI expression has been documented on the cell surface as well as in the mitochondria, nucleus, and cytosol [34-37]. The ability to bypass KDEL-mediated ER anchoring has also been reported in the chaperone proteins GRP78 and GRP94, which can similarly display cell surface expression and extracellular secretion [38,39]. Furthermore, ER stress can induce leakage of PDI into the cytoplasm [40]. The functional consequence of KDEL-containing PDI in non-ER compartments is poorly understood.

Oxidative Protein Folding and the UPR

The oxidative environment of the ER is primed for the formation of disulfide bonds where glutathione (GSH) is the main redox buffer. PDI mediated folding of nascent proteins in the ER lumen involves repeated cycles of thiol oxidation and reduction; processes which become up-regulated during ER stress. During disulfide bond formation, PDI is reduced following the acceptance of electrons from cysteine residues (Figure 2). During this process PDI interacts with flavin adenine dinucleotide (FAD) -dependent ER oxidoreductin (Ero1) enabling the passage of electrons to molecular oxygen and the restoration of oxidized PDI [41]. Inhibition of Ero1 activity results in the accumulation of PDI and the cessation of disulfide bond formation [42]. PDI also has molecular chaperone activity that can include non-Cys containing proteins [43]. In vivo, it is possible that this chaperone activity prevents nonspecific interactions. In an elegant study using the yeast UPR model system, Delic et al introduce the importance of inter-compartmental redox homeostasis between the ER and cytosol during the UPR and a role for cytosolic glutathione peroxidase in the oxidative protein folding pathway [44]. Under conditions of ER stress and activation of the UPR, the redox potential within ER environment becomes increasingly reduced [45]. PDI may provide a redox-dependent switch by acting as a chaperone rather than a disulfide isomerase in its reduced state. In vitro studies show that reduced PDI binds and

Figure 2. The formation of disulphide bonds in the ER is driven by PDI and ERO1. During disulfide bond formation, ERO1 operates in association with the flavin, FAD. ERO1 uses a FAD-dependent reaction to transfer electrons from PDI to molecular oxygen (O₂), which results in the production of reactive oxygen species (ROS) in the form of H₂O₂ and the oxidation of PDI. PDI is reduced following the acceptance of electrons from protein-folding substrates, thereby oxidizing thiol (SH) groups in the target protein’s cysteine residues and forming disulphide bonds. Reduced GSH assists in disulphide-bond reduction, resulting in the production of oxidized glutathione (GSSG). Under conditions of ER stress and the UPR, GSH is consumed and the ER environment becomes increasingly reduced. Inhibition of Ero1 activity results in the accumulation of PDI and the cessation of disulfide bond formation. ER, endoplasmic reticulum; ER, endoplasmic reticulum; ERO1, ER oxidoreductin; FAD, flavin adenine dinucleotide; GHS, glutathione, PDI, protein disulfide isomerase.
unfolds cholera toxin in the ER lumen whereas PDI oxidation results in toxin release and cytosolic translocation [46,47]. In the context of enhanced UPR-mediated protein folding, a more reduced ER environment may enable PDI-mediated disulfide reshuffling of mis-oxidized proteins, a prerequisite for ERAD [48]. Oxidative stress pathways have been linked with the pathology and etiology of diseases. For example, Nardai et al. found a selective reductive shift in the redox status of PDI and Erp57 in the liver of diabetic rats when compared to healthy animals [49]. This shift was accompanied by an increase in levels of oxidized Erp1 and a significant decrease in PDI enzymatic activity. The redox state of other proteins, including calnexin, Grp78 and Grp94 and the inactive PDI-homolog Erp28, remained unchanged. Systemic ER redox stress in diabetic animals may influence hepatic PDI redox state and enzymatic activity, ultimately disrupting hepatic protein secretion.

It is broadly accepted that redox regulation through the reversible oxidation of susceptible cysteine residues influences the activity of proteins such as enzymes, transcription factors or transporters [50-52]. Given that PDI's active site cysteines are targets for redox defined post-translational modifications, such as S-glutathionylation and S-nitrosylation, and that these modifications regulate the chaperone and isomerase activities of PDI, it is likely that an interplay between cytoplasmic and ER redox environment govern the role of PDI in ER stress pathways [8,53-55]. The importance of PDI post-translational modifications and their role in ER-stress mediated apoptosis will be discussed in more detail in following sections.

ER stress, PDI and disease

Aging, genetic mutations, and environmental stressors may collectively and/or independently lead to pathologically chronic ER stress and the accumulation of misfolded proteins. Diseases that are a direct result of this protein aggregation are collectively known as ‘conformational’ or ‘folding diseases’ [56]. These include atherosclerosis, ischemia, liver and heart disease, diabetes, inflammation and neurodegenerative disorders, including Alzheimer’s disease, Parkinson’s disease and bipolar disorders. ER stress signaling is also associated with the pathology in cancer (Table 1).

**Neurodegenerative diseases**

While neurodegenerative diseases are linked with aging and/or have an underlying genetic cause, disease pathogenesis and cell cytotoxicity have been directly associated with an accumulation misfolded, aggregated proteins that form plaques [57,58]. In Alzheimer’s disease patients, the formation of senile plaques, composed of fibrillar β-amyloid (Aβ), is associated with disease progression. In *vivo*, the addition of high concentrations of Aβ to mature neurons results in dendritic and axonal retraction followed by neuronal death [59]. In Parkinson’s disease patients, there is an accumulation of α-synuclein in plaques referred to as Levy Bodies. In *vivo* models showed that α-synuclein accumulation was toxic to cultured dopamine-dependent human dopaminergic neurons, but exhibited neuroprotective activity in non-dopaminergic cortical neurons [60]. In diseases that involve aberrant protein conformational changes, ER stress responses frequently result in chaperone up-regulation, particularly PDI, to protect against misfolded protein aggregation. Using cell-free in *vivo* assays, Cheng et al. showed that PDI prevents α-synuclein aggregation [61]. PDI expression is upregulated in the cerebrospinal fluid of amyotrophic lateral sclerosis patients manifesting intracellular aggregates of mutant superoxide dismutase (mtSOD1), as well as in the dopaminergic neurons and Lewy bodies of Parkinson’s disease patients. PDI also co-localizes with ubiquitin-positive neuronal inclusions of torsin-A in a transgenic mouse model of dystonia as well as with neurofibrillar tangles in Alzheimer’s patients and is up-regulated in glial cells in response to brain ischemia [62-66]. In cellular prion disease models, the up-regulation of the PDI family member Erp57 (PDIA3; GRP58) correlates with the accumulation of misfolded prion protein [57]. Wang et al. have recently reported a role for PDI as a pleiotropic regulator in the cellular management of misfolded prion protein [7].

**PDI: neuroprotective or proapoptotic?**

ER stress-mediated PDI up-regulation in neurodegenerative diseases attenuates misfolded protein accumulation and prevents neurotoxicity. In neuronal cell models of Parkinson’s disease and amyotrophic lateral sclerosis, PDI overexpression decreases synphilin-1 protein and mtSOD1, respectively [53,68]. The protective role for PDI in glial cells in response to brain ischemia has similarly been attributed to roles in decreasing protein misfolding [68]. It is worth noting however, that PDI does not decrease the number of inclusions formed by the variant of α1-antitrypsin linked with liver disease [69]. This suggests that PDI-mediated cellular protection may be disease and protein-specific.

Discovery of S-nitrosylated PDI in brains manifesting sporadic Alzheimer’s, Parkinson’s, and amyotrophic lateral sclerosis suggest that redox-dependent posttranslational modifications may impact PDI chaperone and isomerase activity and contribute to neurodegenerative conditions. Uerara et al. reported nitric oxide (NO) -induced S-nitrosylation of the active-site thiol of PDI (PDI-SNO) and showed that this modification abrogates activity, thus triggering activation of the UPR and ultimately inducing neuronal cell death [53]. Interestingly, in transgenic mouse models of amyotrophic lateral sclerosis, treatment with reticulon-4A (Nogo-A) induced cellular redistribution of PDI. Deletion of Nogo expression exacerbated disease progression and prevented PDI translocation, thus emphasizing the importance of PDI subcellular localization in these pathways [70].

PDIs can have similar anti-apoptotic/neuroprotective roles in transmissible neurodegenerative prion disorders. PDI overexpression protects against prion-induced neurotoxicity. Furthermore, infected animals exhibit S-nitrosylation of PDI, while blocking cellular NOS inhibits PDI-SNO formation and associated cell cytotoxicity [7]. Prion disease-induced ER stress...
responses are also associated with Erp57 up-regulation. Erp57 overexpression in a neuroblastoma cell line model protected against caspase-12 activation and misfolded prion protein aggregation induced neuronal apoptosis [67]. Given that many neurodegenerative diseases, including prion diseases, are characterized by excessive generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), it is likely that PDI-SNO or redox-dependent post-translational modifications of other chaperone molecules may influence the pathophysiology of such disorders.

Interestingly, in a rat brain cell-based model for Huntington’s disease, expression of mutant Huntington exon 1 resulted in PDI concentration at ER-mitochondrial junctions and the subsequent induction of apoptotic pathways that lead to mitochondrial outer-membrane permeabilization (MOMP). Selective small-molecule PDI inhibitors or shRNAs that inhibited the enzymatic activity of PDI suppressed the toxicity of the misfolded Huntingtonin and of Aβ peptides processed from the amyloid precursor protein. PDI inhibition did not protect against other apoptotic stimuli, suggesting specificity for misfolded protein-mediated response [9]. GRP78 knockdown did not influence PDI-mediated MOMP induction nor anti-apoptotic pathways resulting from PDI knockdown. These data imply a role for non-UPR mediated pathways. The authors suggest that cellular death pathways are initiated when the accumulation of PDI in response to misfolded proteins reaches a threshold, similar to p53 expression, where cell apoptosis is induced at extreme thresholds of DNA damage.

The concept of PDI as a pleiotropic apoptotic regulator is further supported by a recent study examining apoptotic pathways in a prion disease [7]. ER-targeted PDI overexpression in hamster brain cells expressing prion protein caused attenuation, but did not reverse caspase-12 mediated cellular apoptosis by reversing increases in Bax/Bcl-2 ratios. These events may represent early-stage infection. However, knockdown of endogenous PDI preferentially amended cytoxicity associated with a mutant prion protein with an extra octa-repeat insertion [7]. Wang et al., suggest that in late stages of prion infection, disruptions in oxidative homeostasis induce formation of PDI-SNO and facilitate apoptotic pathways. Both the cellular location and the mutant type of prion influenced PDI-mediated cell death.

Collectively these studies suggest that PDI may participate in multiple phases of ER stress, where initial responses are protective and defined by attempts to process accumulated levels of unfolded proteins, while subsequent effects are pro-apoptotic, when protein repair has been deemed ineffective. It may be speculated that subcellular localization of PDI and/or protein aggregates, redox environment and post-translational modification of susceptible thiols, may contribute to defining and regulating the protective or apoptotic roles of PDI (Figure 3).

Furthermore, important considerations of PDI binding partners in the neuroprotective roles of PDI are emphasized in studies that identify ubiquilin as an endoplasmic reticulum-associated

Figure 3. The protective or apoptotic roles of PDI are regulated by the subcellular localization of PDI, redox environment and degree of ER stress. When unfolded/misfolded proteins accumulate in the ER lumen, ER stress triggers the UPR. Transient UPR induces cell survival pathways while a prolonged UPR elicits apoptotic pathways. PDI mediates proper protein folding. Under oxidative or nitrosative stress (as seen in neuronal disorders), the S-glutathionylation or S-nitrosylation of PDI inhibits normal protein folding and induces the activation of ER stress. Prolonged UPR contributes to aberrant protein accumulation and cell apoptosis. ER luminal chaperones localized outside of the ER lumen may have proapoptotic roles. However, in some cell lines, overexpression of PDI constructs lacking the KDEL ER retention sequence increased cell survival following the induction of apoptotic stimuli. Elevated expression of cell surface PDI is associated with high-generation invasive tumors, suggesting that cell-surface PDI may have function in mediating apoptotic pathways. See text for additional discussion of these pathways. ER, endoplasmic reticulum; PDI, protein disulfide isomerase; UPR, unfolded protein response.
protein in PDI-mediated acquisition of tolerance against ischemic stress in glial cells [71]. Indeed, additional investigation into how PDI-family members interact with each other and with other ER and non-ER resident proteins will further elucidate the apoptotic roles of PDI.

**PDI-mediated apoptotic signaling in other disorders**

**Diabetes**

ER stress pathways may have a role in the predisposition and onset of diabetes mellitus. Due to perpetual, fluctuating demands on insulin and glucagon synthesis and folding, β-cells are highly affected by deregulation of the UPR. PERK and eIF2α targeted knockout mice individually manifest pathologies associated with Type 1 diabetes mellitus, while CHOP knockout animals exhibit delayed onset [72-74]. Similar to PERK−/− mice, P58ΔF deficient mice showed increased apoptosis of pancreatic islet cells and develop diabetes [75]. Enhanced expression of both ATF4 and CHOP following P58ΔF gene silencing suggest the induction of apoptotic pathways most likely resulting from prolonged PERK activity, and the translational attenuation of pro-survival proteins. The up-regulation of chaperone proteins, including GRP78 and GRP94, in the insulin-secreting β-cells of diabetic mouse models further implies a role for additional ER stress-mediated pathways. In Type 2 diabetes models, ectopic overexpression of the molecular chaperones oxygen-regulated protein 150 (ORP-150) and GRP78 in β-cells improves insulin resistance, most likely by improving ER folding capabilities and protecting cells from ER stress-induced apoptosis [76,77]. However, PDI overexpression induced the opposite effect, resulting in elevated levels of misfolded pro-insulin and a reduction in glucose-stimulated insulin secretion and additional ER stress induction. Such data suggest that the regulation of PDI isomerase and associated chaperone activities may be essential in maintaining β-cell function and viability, whereas PDI overexpression may be lethal.

**Cardiovascular Disease**

A number of recent studies indicate that ER stress and the UPR are chronically activated in atherosclerotic cells [78]. Atherosclerosis associated ER stress has been linked to the accumulation of homocysteine, an intermediate amino acid in the metabolism of methionine and cysteine. Homocysteine accrual results in the up-regulation of ER stress pathways, including those involving BIP, GRP94, and CHOP-mediated apoptosis of macrophages [79,80].

Additional reports indicate a role for oxidized low-density lipoproteins (oxLDLs) in sustained ER stress, UPR pathways and the apoptosis of vascular endothelial cells and macrophages. A series of siRNA studies confirmed apoptosis mediation through IRE1α/JNK mediated XBP-1s and CHOP expression [81]. The pro-apoptotic and pro-atherogenic role of prolonged ER stress is exemplified in studies with CHOP−/− mice where knockout animals exhibit a reduction in atherosclerotic plaque necrosis and apoptosis [82]. PDI overexpression counteracts ER stress and oxLDL-induced apoptosis. A recent study found that electrophilic aldehydes present in oxLDLs disrupt PDI redox state and induce formation of carbonyl-PDI. PDI modification resulted in enzymatic inactivation and cellular apoptosis, through the activation of CHOP and XBP-1s. Furthermore, carbonylated-PDI was detected in lipid rich areas of advanced human atherosclerotic lesions, suggesting that PDI modification directly contributes to ER-stress-induced apoptotic events in vivo [83].

PDI also has a protective role in ischemic cardiomyopathy and in the inhibition of apoptosis during post-infarction remodeling. PDI expression is upregulated in hypoxia-treated myocardial capillary endothelial cells in viable peri-infarct and infarct regions of mouse hearts. Increased PDI expression was correlated with protection against endothelial cell apoptosis as well as in cellular migration, adhesion, and tubular formation. siRNA-mediated PDI inhibition negates these effects while induced PDI expression confers additional protection against hypoxia-induced apoptosis [84,85]. While this study did not evaluate subcellular localization of PDI, cell-surface PDI may act as a reductase on the disulfide bond of cell surface proteins or interact with integrins to mediate endothelial cell adhesion and migration [86]. Additional discussion of cell-surface PDI in cancers is included in the following section. The PDI-mediated signaling pathways involved in endothelial cell survival and in angiogenesis have yet to be clearly defined, but may involve the enzymatic rather than chaperone functions of PDI, since point-mutations in PDIs active sites blocked apoptotic protection. Severino et al, reported the increased expression of Ero1 and IRE1 in cardiomyocytes following hypoxic stress as well as PDI-mediated cellular protection from superoxide through increased SOD1 activity [87]. There are indications that systemic disease conditions may impact the redox state of PDI and its importance in protecting cardiomyocytes from apoptosis [87]. In a mouse model of diabetes, diabetic mice exhibited increases in the reduced form of PDI and decreased post-infarction remodeling as compared to healthy animals. The authors suggest that while diabetic animals showed elevated PDI expression, these increased levels of PDI were confounded by redox-mediated inactivation in diabetic hearts.

The type of cell stress or injury impacts which PDI family member might participate in the regulation of apoptosis. While PDI up-regulation has a protective role in ischemic cardiomyopathy, hyperoxia-induced down-regulation of ERP57 in neonatal rat lungs and in cultured endothelial cells conferred similar cellular protection. Furthermore, siRNA-mediated ERP57 knockdown inhibited caspase-3 activation and further protected against hyperoxia-induced apoptosis, while ERP57 overexpression exacerbated hyperoxia- or tunicamycin-induced apoptosis and augmented caspase-3 activation, while reducing GRP78 induction [88].

**Cancer**

Many cancer-related pathologies are associated with deregulation of ER homeostasis and the induction of ER stress and UPR pathways. ER stress may be the result of
high protein folding demands associated with increased rates of cancer cell proliferation or may be a consequence of tumor microenvironment. Hypoxia, pH, nutrient limitation, or therapeutic intervention strategies can all lead to perturbation of ER function and ER stress in cancer cells. A number of ER-resident proteins are upregulated in cancer and have diverse functions. Elevated ER stress chaperone protein expression has been correlated with tumor initiation, malignancy and metastasis. Chaperone proteins GRP78 and GRP94 are involved in anti-apoptotic pathways and tumor progression, and may serve as prognostic as well as diagnostic biomarkers [89,90]. ORP-150 influences tumor angiogenesis through the mediation of extracellular vascular endothelial growth factor-A (VEGF-A) release [91]. PDI upregulation has been documented in a number of human cancer cells including ovarian, prostate, lung, lymphoma, glioma, acute myeloid leukemia, and melanoma [92-97]. Increases in GRP94 and PDI in multiple myeloma cell lines have been correlated with upregulated secretory demands. Targeting these cells with mitochondrial inhibitors overwhelmed existing ER stress responses, exacerbated Ca\(^{2+}\) leakage, and induced CHOP associated apoptosis [98].

Cell surface PDI is associated with high-generation invasive glial tumors. Targeting PDI with PDI monoclonal antibodies and the PDI-inhibitor, bacitracin, inhibited tumor cell migration and invasion, implying that cell-surface PDI may be involved in mediating apoptotic pathways [97]. Several PDI family members are associated with non-ER locations and have been assigned a broad array of functions [83]. For example, ER luminal chaperones localized outside of the ER lumen may have pro-apoptotic roles. Cell surface expression of the ER chaperone calreticulin is involved in antigen processing as a recognition ligand in phagocytic processes and is involved in determining cancer cell immunogenicity during apoptosis [99,100]. ERP57-calreticulin interactions are necessary for calreticulin membrane translocation. Furthermore, ER-stress in prostate cancer cells induces GRP78 translocation to the cell surface where it serves to initiate FADD/caspase8/caspase3 apoptotic signaling via interaction with extracellular prostate apoptosis response 4 (Par-4) [101]. Bcl-2 proteins may have a role in increasing ER permeability and facilitating luminal protein translocation during ER-stress induced apoptosis [102]. Interestingly, cytosolic PDI acts as a substrate for caspase-3 and 7, and overexpression of PDI constructs lacking the KDEL ER retention sequence increased cell survival following the induction of apoptotic stimuli in premyeloid HL-60 cells [103]. PDI may also modulate apoptosis through the reversal of iron(III) mediated caspase-3 inhibition through the formation of iron-sulfur complexes at active site thiols [104].

Cell-surface redox environment defines thiol content, disulfide bond formation and chaperone function. PDI-mediated reduction of \(\beta\)-integrin disulfide bonds mediates cell migration and adhesion, while its redox-sensitive interaction with other surface proteins serves to facilitate viral entry [37,86,105,106]. Furthermore, S-glutathionylation of cell surface proteins by oxidized glutathione impacts intracellular and extracellular redox homeostasis [107]. The cell-surface redox environment may have a pleiotropic impact on PDI-mediated apoptotic pathways, acting directly at the cell membrane as well as intracellularly. Indeed, human cancer cells treated with redox-modulating agents that modify cell surface thiols show increases in the S-glutathionylation of PDI (PDI-SSG) which leads to inhibition of PDI activity and the induction of UPR-mediated cell death [9,107]. Recently, S-glutathionylation of the tumor necrosis factor (TNF) receptor family member, Fas (CD95, Apo-1), was found to be defined by subcellular localization and interactions with ERP57 and glutathione S-transferase pi (GSTP), influencing pathways that determine the strength of apoptosis. Inhibition of ERP57 or GSTP decreased Fas-ligand dependent Fas S-glutathionylation (Fas-SSG) and enhanced cell survival, where increased expression of ERP57 and GSTP resulted in translocation of Fas-SSG from the ER and eventually caspase-mediated cell death [10]. This study exemplifies the importance of the subcellular redox environment and protein-protein interactions in the complex pathways that determine cell survival.

PDI as Therapeutic Target

As outlined above, dysregulation of PDI plays a role in a number of human pathologies and as such presents a plausible target for therapeutic intervention. In particular, small molecules that mimic chaperone function or induce endogenous chaperone function may be viable targets in diseases characterized by protein aggregation, defective protein secretion or ischemia. Alternatively, agents that specifically target chaperone function would benefit in treating cancers that are defined by constitutive UPR activation and/or chemotherapeutic resistance, where chaperone inhibition sensitizes cancer cells to therapy-induced apoptosis.

Therapeutic application of PDI inhibitors in the treatment of cancers that are closely associated with poor prognosis and therapy resistance have shown promise in a number of studies, including those using melanoma, glioblastoma, breast cancer and ovarian cell lines/xenograft models [92,94,97,108]. PDI knockdown in melanoma and ovarian cell models inhibits xenograft formation and tumor growth and induces cellular apoptosis, in response to ER inducing agents and as a solo treatment, respectively [92,94]. However, therapeutic application of PDI inhibition may be confounded by studies that suggests that the UPR of different tumor cell lines differentially respond to various UPR stimuli and by differential regulation of ER chaperones. PDI induction was induced following tunicamycin but not ritonavir treatment in sarcoma cell lines [109]. Furthermore, in a single study, siRNA-mediated PDI knockdown induced varying degrees of cytotoxicity in human breast cancer, neuroblastoma, and cervical cancer cell lines [108]. The therapeutic success of PDI-targeted therapies may depend on understanding how ER stress response pathways are integrated in individual cell types and in individual pathologies.

Drug resistant tumors and cancer cells have elevated levels of GSTP. The development of nitric oxide (NO) releasing pro-
drugs that are metabolized by GSTPs have recently shown preclinical therapeutic potential in treating malignancy through tumor-specific activation [110,111]. Treatment of premalignant HL60 and ovarian cancer cells with Oβ(2,4-dinitro-5-(N-methyl-N-4-carboxyphenylamino)phenyl)1-(N,N-methylamino)diazon-1-um-1,2-diolate (PABA/NO) resulted in elevated intracellular nitric oxide and the activation of UPR-mediated cytotoxic pathways [5,55,112]. PABA/NO-induced cytotoxicity is linked to the S-glutathionylation of cysteines within PDI’s active site, which blunts activity and leads to activation of the UPR and JNK activated apoptosis [55]. Recent studies suggest that nitrosative/oxidative induced S-glutathionylation of PDI also impacts the chaperone activity. Specifically it was shown that PDI-SSG mediates UPR activation and cell death through abrogation of estrogen receptor α (ERα) stability and signaling in ERα-positive breast cancer cells [54]. In a model of PABA/NO acquired drug-resistance, HL60 cells were shown to have a constitutively activated UPR, including elevated levels of PDI, blunted cellular differentiation and decreased levels of S-glutathionylation caused by adaptive GSTP down-regulation [112]. Collectively, these data indicate the involvement of multiple extrinsic and intrinsic ER stress-mediated apoptotic cascades that may be harnessed individually or in concert to overcome drug resistant cancer pathologies.

Balancing the protein folding capacity within the cell is critical and the PDI superfamily plays a key role in both opposing pro-survival and death pathways. The emerging role of PDI in human pathologies remains poorly understood yet underscores the keystone role in normal physiology.

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