

FORUM REVIEW ARTICLE

ERO1-PDI Redox Signaling in Health and Disease

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Abstract

Significance: Protein disulfide isomerase (PDI) and endoplasmic reticulum oxidoreductase 1 (ERO1) are crucial for oxidative protein folding in the endoplasmic reticulum (ER). These enzymes are frequently over-expressed and secreted, and they contribute to the pathology of neurodegenerative, cardiovascular, and metabolic diseases.

Recent Advances: Tissue-specific knockout mouse models and pharmacologic inhibitors have been developed to advance our understanding of the cell-specific functions of PDI and ERO1. In addition to their roles in protecting cells from the unfolded protein response and oxidative stress, recent studies have revealed that PDI and ERO1 also function outside of the cells.

Critical Issues: Despite the well-known contributions of PDI and ERO1 to specific disease pathology, the detailed molecular and cellular mechanisms underlying these activities remain to be elucidated. Further, although PDI and ERO1 inhibitors have been identified, the results from previous studies require careful evaluation, as many of these agents are not selective and may have significant cytotoxicity.

Future Directions: The functions of PDI and ERO1 in the ER have been extensively studied. Additional studies will be required to define their functions outside the ER. *Antioxid. Redox Signal.* 35, 1093–1115.

Keywords: PDI, ERO1, oxidative ER stress, unfolded protein response, disease, and inhibitors

Introduction

OXIDATION AND ISOMERIZATION of intramolecular disulfide bonds are essential for appropriate protein folding and maturation in the endoplasmic reticulum (ER). Dysregulation of this process often results in cell and tissue pathology and may lead to neurodegenerative diseases and diabetes. Thiol isomerases, such as the prototypic enzyme, protein disulfide isomerase (PDI), are catalysts that have critical roles in facilitating disulfide bond modification. After PDI has catalyzed the oxidation of one or more disulfide bonds in a protein substrate, the reduced protein is enzymat-

ically reoxidized by ER oxidoreductase 1 (ERO1). This interaction between ERO1 and PDI represents a catalytic redox cycle that is critical for oxidative protein folding in the ER.

Studies with conditional knockout (CKO) mice and pharmacological inhibitors have advanced our understanding of the physiological roles played by both PDI and ERO1. Both PDI and ERO1 are upregulated in various cell types in response to pathologic conditions, and small amounts of each enzyme are secreted extracellularly. These aspects highlight the rationale for developing novel therapies that target PDI and ERO1 and suggest that these enzymes might be developed as biomarkers to predict disease severity. In this review,

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we focus on the structure, function, and pathophysiological roles of PDI and ERO1 and the identification and development of their inhibitors as novel therapeutics.

Structure of PDI

PDI includes four major domains (a, b, b', and a'). The a and a' domains contain a TrpCysGlyHisCysLys active site. The b and b' domains are catalytically inactive but can interact with substrates primarily *via* a hydrophobic region in the b' domain (29). The b' and a' domains of PDI are linked with a flexible linker peptide containing 19 amino acids. This linker region regulates substrate binding by capping and uncapping a hydrophobic site on the b' domain (119, 180). Many acidic residues in the C-terminal region maintain the functional conformation of PDI and prevent self-aggregation (170). PDI has an ER retrieval sequence (LysAspGluLeu) at its C-terminus that promotes binding a receptor in the cis-Golgi and facilitates its return to the ER (24).

Despite the ER retention signal, PDI can be released from cells and has been detected on the plasma membrane where it is bound to molecules on the cell surface (51, 62, 97). Previous studies suggested several molecular mechanisms by which ER-resident molecules can be exported and released; these include (1) masking of the LysAspGluLeu sequence by glycosylation (2, 210) saturation or inactivation of LysAspGluLeu receptors (3, 202) membrane translocation of LysAspGluLeu receptor 1 (10) and (4) alteration of cytosolic calcium levels (20, 173).

The crystal structures of yeast and human PDI were resolved at resolutions of 2.4 and 2.5 Å, respectively (169, 181). Human and yeast PDI share 31% amino acid sequence identity. The four domains (a, b, b', and a') are arranged in a "U" shape with the a and a' active sites facing each other. In the reduced form of human PDI, the a, b, and b' domains are in a linear configuration, with the a' domain angled at ~45° from the plane; in this configuration, the distance between the sulfur atoms of Cys53 and Cys397 is maintained at 27.6 Å (181). In contrast, the four domains are organized differently in the oxidized form of human PDI, with a 40.3 Å distance separating the two active sites in the same plane.

Based on the relative positions of the substrate-binding pocket and catalytic sites, reduced and oxidized forms of PDI are considered to be in a closed and open conformation, respectively. Although most proteins are capable of interacting with oxidized PDI, it is critical to note that some substrates can bind preferentially to the reduced form. For example, we showed that the β -switch region of the platelet-specific receptor, glycoprotein Ib α (GPIb α), interacts with the b' domain of reduced PDI on the platelet surface (97). Further, we found that the Cys4–Cys17 disulfide bond in the β -finger region of GPIb α is closer to PDI Cys53 when complexed with the reduced rather than the oxidized form of the enzyme (16 Å vs. 28 Å, respectively). These results suggest that GPIb α may bind more favorably to the reduced form of PDI.

The existence of different conformations of the b' domains (181) implies the possibility of substrate interactions that favor either the oxidized or the reduced forms of PDI. In addition, it was recently reported that Fam20C, a Golgi-associated secretory pathway kinase, phosphorylates Ser357 in the x-linker region of PDI in response to ER stress. Phosphorylation at this site induces an open confor-

mation with a 70 Å distance between the a and a' domains and results in a functional switch from an oxidoreductase to a molecular chaperone (203). Future studies are required to explore the impact of Ser357 phosphorylation on the interaction between PDI and ERO1 α and ERO1 α -mediated PDI oxidation.

Function of PDI

PDI was first identified in 1963 as a microsomal enzyme of rat liver and pigeon/chicken pancreas that was capable of oxidizing reduced bovine pancreatic ribonuclease (1, 47). Twenty-four years later, PDI was identified as the β -subunit of prolyl 4-hydroxylase subunit β (P4HB) (132). Since prolyl 4-hydroxylase forms an $\alpha 2\beta 2$ tetramer and catalyzes the formation of 4-hydroxyproline in collagen, the PDI subunit could contribute to oxidoreductase and/or molecular chaperone activity as a monomer and as a part of the prolyl 4-hydroxylase tetramer together with the α -subunit.

A previous study revealed that PDI forms disulfide-independent dimers within cells and that dimerization impairs both the catalytic and chaperone function by blocking substrate-binding sites in the b and b' domains (11). However, because the results of this study were based on PDI overexpression, it is not clear whether endogenous PDI can undergo dimerization under pathophysiological conditions or the nature of its functional role in this conformation.

PDI catalyzes disulfide bond oxidation and isomerization in unfolded substrates in the ER and thus plays an indispensable role in the process of protein folding (152). In a highly oxidizing environment, reduced Cys thiols in substrates form transient disulfide bonds with thiols with active site residues in PDI (CysGlyHisCys), thereby resulting in an oxidized, folded protein. Misfolded proteins are reduced and isomerized by PDI and converted to their appropriate native conformation. Reduced PDI is subsequently reoxidized by oxidases such as ERO1, a topic that will be discussed later in this review. To facilitate this process, the active site of PDI is maintained in a reduced state, and glutathione (GSH) and nicotinamide adenine dinucleotide phosphate (NADPH) promote cleavage of the disulfide bonds (15, 111).

In addition to oxidoreductase activity, PDI-mediated chaperone activity prevents the accumulation of misfolded proteins that are formed in response to oxidative ER stress and facilitates the maturation and transport of secretory proteins. As described earlier, phosphorylation of Ser357 in PDI results in a significant decrease in its isomerase activity and a significant increase in its chaperone activity (203). This result suggests that the post-translational modification in PDI prevents aggregation of misfolded proteins under conditions of ER stress.

The expression and function of PDI in the ER are indispensable for survival in yeast, with no apparent compensation provided by any of the four additional members of the PDI enzyme family (53, 90). Consistently, our unpublished data suggest that deletion of the gene encoding PDI results in an embryonic lethal phenotype in mice, suggesting that none of the 21 PDI oxidoreductase family members can provide adequate compensation (16). Importantly, dysregulation of PDI function in the ER has been associated with numerous disease processes, including neurodegenerative disorders, diabetes, and cardiovascular diseases (CVDs). Specifically, these

disorders have been characterized by accumulations of misfolded and immature proteins or by impaired function of cell surface molecules, which will be discussed later in this review.

Structure of ERO1

The yeast gene, *Ero1*, encoding Ero1p was discovered in 1998 (42, 134). Two human genes, *ERO1-L* and *ERO1-L β* , encoding ERO1 α and ERO1 β , respectively, were identified thereafter (23, 126). Although ERO1 α is expressed in most cell types (126), ERO1 β is mainly expressed in intestinal and pancreatic β cells (7, 36). ERO1 α and ERO1 β share 65% amino acid sequence identity. Inaba *et al.* reported the crystal structure of hyperactive and inactive human ERO1 α at resolutions of 2.35 and 3.07 Å, respectively (58). Interestingly, one recent study showed that there are no substantial differences between the conformations of active and inactive forms of human ERO1 α (209).

Consistent with findings from mass spectrometry (4), a crystallographic analysis revealed that hyperactive and inactive ERO1 α form a single globular domain enriched in α -helices with five intramolecular disulfide bonds (Cys35–Cys48, Cys37–Cys46, Cys85–Cys391, Cys208–Cys241, and Cys394–Cys397) (58). Although the results of one earlier study suggested that the intramolecular Cys85–Cys391 disulfide bond regulates ERO1 activity (9), subsequent studies revealed a structural role for this linkage (6, 121, 208). Further, two essential Cys triads (Cys85–Cys94–Cys99 and Cys391–Cys394–Cys397) are highly conserved in yeast and human ERO1 and serve to control enzymatic activity (17).

The results from mutational studies revealed that ERO1 α is activated by the formation of a Cys94–Cys99 disulfide bond, whereas it is inactivated on disulfide bond formation between the Cys94–Cys131 and Cys99–Cys104 (4, 6). A protruding β -hairpin that includes Trp272 interacts with the substrate-

binding site of the b' domain of reduced PDI, and the Cys94–Cys99 pair then transfers electrons from PDI to the Cys394–Cys397 disulfide bond, resulting in PDI reoxidation (104).

As a flavin adenine dinucleotide (FAD)-binding enzyme, ERO1 oxidizes reduced PDI during oxidative protein folding. Our docking study confirmed the FAD-binding site in human ERO1 α and identified additional molecular interactions between FAD and ERO1 α as compared with a previous report (58). Among these results, we found that FAD interacts with ERO1 α *via* multiple hydrogen bonds with Arg187, Thr189, Trp197, Trp200, Ser252, Asn259, Arg287, and Arg300 and two π - π interactions with His255 and Tyr191 (Fig. 1A, B). One study reported that the carbon 4a in the isoalloxazine ring of bound FAD is only 3.3 Å away from Cys397 of ERO1 α , implicating the possibility of an interaction between FAD and Cys397 that takes place after cleavage of the Cys394–Cys397 disulfide bond (58).

Function of ERO1

Deletion of *Ero1* has a marked effect on oxidative protein folding and cell viability in yeast (42). In contrast, mice with loss-of-function mutations in *Ero1-l* and *Ero1-l β* are viable and exhibit minimal defects (214). The *Ero1-l* and *Ero1-l β* mutant mice are hypersensitive to β -adrenergic blockade, whereas an *Ero1-l* gene deletion alters calcium homeostasis in cardiomyocytes and protects against the progression of heart failure (27). Consistent with its abundant expression in pancreatic β -cells, deletion of *Ero1-l β* results in compromised oxidative folding of proinsulin and promotes glucose intolerance (214). The different outcomes resulting from disruption of ERO1 function are largely due to the presence of compensatory mechanisms for ERO1-mediated thiol oxidation in mice but not in yeast.

The expression of ERO1 α is upregulated *via* hypoxia-inducible factor-1 α (HIF-1 α) under hypoxia (105), whereas

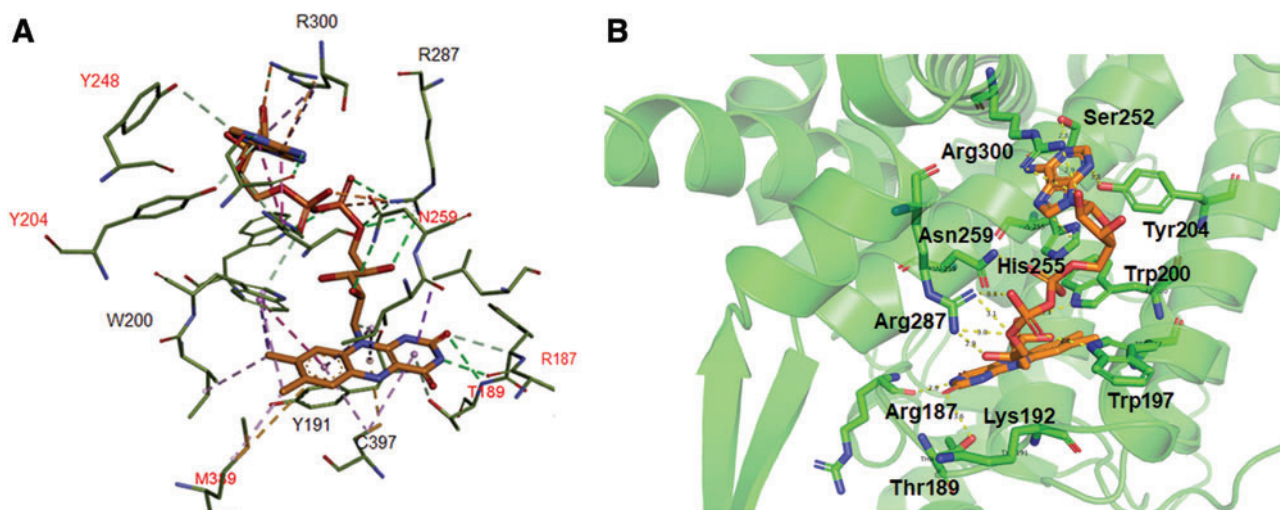


FIG. 1. The binding site of FAD in ERO1 α . (A, B) Glide docking was performed to verify the binding of FAD to ERO1 α . Our docked model confirmed molecular interactions between FAD and ERO1 α (in black) that were similar to those described in a previous report (3AHQ) (58) and identified new molecular interactions as shown in red. The abbreviation HIE255 represents His with hydrogen on nitrogen in the epsilon position. ER, endoplasmic reticulum; ERO1, ER oxidoreductase 1; FAD, flavin adenine dinucleotide. Color images are available online.

the expression of ERO1 β is enhanced by the unfolded protein response (UPR) (126). ERO1 α serves as the primary oxidase that restores reduced PDI and other PDI family thiol isomerases, which together constitute an electron transfer network of ER oxidoreductases. However, given the known dissociation constants for each oxidoreductase, ERO1 α is likely to bind preferentially to PDI ($K_d = 1.7 \mu M$), compared with ERp44 ($K_d = 21 \mu M$), ERp5 ($K_d = 70 \mu M$), ERp57 ($K_d = 180 \mu M$), ERp72 ($K_d = 160 \mu M$), or ERp46 ($K_d = 280 \mu M$) (5).

One study revealed that peroxiredoxin 4 preferentially recognizes and oxidizes ERp5 and ERp46 and that peroxiredoxin 4-catalyzed oxidation of ERp5 and ERp46 is accelerated in combination with PDI (150). Interestingly, ERO1 α -catalyzed PDI oxidation was impaired in the presence of a hyperactive mutant of ERp44 (104). Collectively, these results suggest that ER oxidases target specific thiol isomerases during protein folding and that thiol isomerases cooperate or compete with one another to promote protein reoxidation.

A previous study using full-length and mutant PDI revealed that the b'-x-a' domains of PDI provide the structural basis for the preferential binding and oxidation of ERO1 α

(183). Figure 2 summarizes ERO1 α -PDI redox activity and oxidative protein folding in the ER. A study using docking simulations and mutations suggested that Trp272 in ERO1 α interacts with the hydrophobic residues (Phe240, Phe249, and Phe304) of the b' domain of reduced PDI (104).

Preferential oxidation of the PDI a' domain is catalyzed by nucleophilic attack initiated by the C-terminal active site in PDI targeting the Cys94–Cys99 shuttle disulfide bond in ERO1 α . This results in a mixed-disulfide bond that forms a transient link between the C-terminal active site of PDI and Cys94 of ERO1 α . Oxidized PDI shuttles a disulfide bond to an unfolded substrate bound to its hydrophobic pocket. ERO1 uses O₂ as an electron acceptor in this reaction and produces H₂O₂ as a byproduct, inducing ER stress (183). Therefore, the ERO1-PDI redox cycle is tightly regulated under homeostatic conditions.

The ER-resident peroxidases, GSH peroxidase 7 (GPx7), and GPx8 utilize ERO1 α -generated H₂O₂ to oxidize PDI, facilitating oxidative protein folding (118, 184). One recent study suggested that GPx7 reacts more efficiently with H₂O₂ and promotes higher levels of PDI oxidation than GPx8. The differential activity observed was attributed to the presence

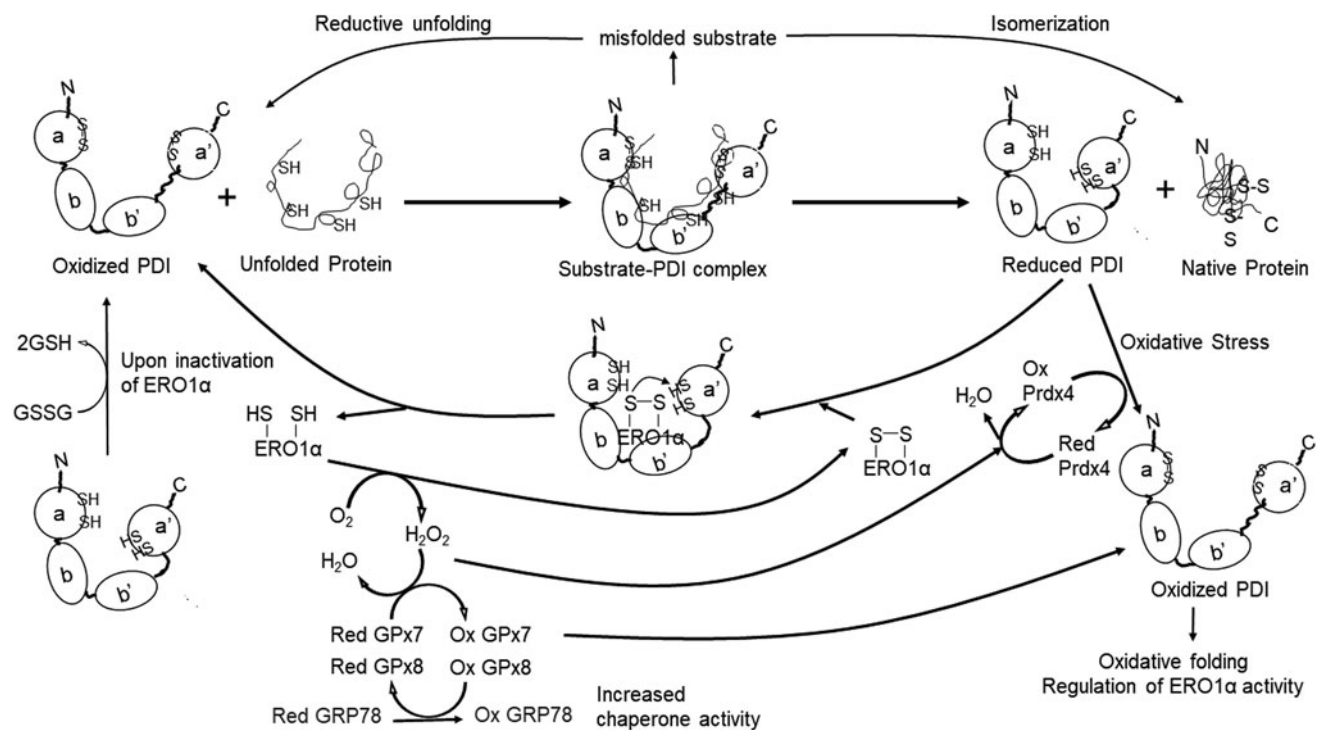


FIG. 2. ERO1 α -PDI redox cycling in the ER. In the highly oxidizing environment of the ER, unfolded proteins interact with the b' domain of oxidized PDI and undergo oxidative protein folding. Misfolded substrate proteins can be reduced and refolded or isomerized to the appropriate native protein conformation. Reduced PDI is then reoxidized by ERO1 α via direct hydrophobic interactions with its b' domain and electron transfer from the a' domain via a flexible Cys94 residue, resulting in the production of H₂O₂. ERO1 β is expected to participate in similar redox cycling. ERO1 α expression is upregulated by hypoxia, whereas ERO1 β is expressed in response to the UPR. Mammals express several peroxidases, including peroxiredoxins (e.g., Prdx4) and GSH peroxidases (GPx7 and GPx8), to reduce H₂O₂-induced ER stress and can scavenge excess H₂O₂, thereby tightly regulating its concentration. Further, Prdx4 can utilize H₂O₂ to oxidize reduced PDI and restore the homeostatic redox state of ER. GPx7 and GPx8 also convert H₂O₂ to H₂O. Oxidized GPx7 and GPx8 interact with and oxidize GRP78 (also known as BiP), thereby augmenting its chaperone activity. The increased levels of oxidized PDI promote the formation of the inhibitory disulfide bonds in ERO1 α (Cys94–Cys131 and Cys99–Cys104) via which GSSG can induce oxidation of PDI. GPx7, GSH peroxidase 7; GRP78, glucose-regulated protein 78; GSH, glutathione; PDI, protein disulfide isomerase; UPR, unfolded protein response.

of Gln92 in the GPx7 catalytic tetrad; this residue stabilizes sulfenylated Cys57 that forms a hydrogen bond with ERO1 α -generated H₂O₂ (70).

Mouse embryonic fibroblasts deficient in GPx7 exhibit increased production of reactive oxygen species (ROS) and, thus, oxidative stress (188). This study also showed that oxidized GPx7 generates disulfide bonds and activates the chaperone protein, glucose-regulated protein 78 (GRP78). However, a very high concentration of H₂O₂ (20 mM) was used in this study, which raises questions regarding physiological relevance. Deletion of GPx8 results in leakage of ERO1 α -produced H₂O₂ from the ER to the cytosol and induces ER stress and cell death (139). Further, knockdown of peroxiredoxin 4 results in impaired cellular tolerance to ER stress (165). Taken together, these results suggest that cells are protected from H₂O₂-induced ER stress by the actions of GPx7, GPx8, and peroxiredoxin 4.

Because the reduced form of PDI is necessary for isomerization of thiol-disulfide bonds, H₂O₂-induced ER stress must be tightly controlled. Molteni *et al.* demonstrated the antagonistic roles of cytosolic GSH and ERO1 α in regulating the ER redox state (111). GSH depletion accelerates the formation of protein disulfides most likely *via* enhanced ERO1 oxidase activity and the formation of high-molecular protein aggregates. The rate of protein oxidation is consistently higher in cells that overexpress ERO1 α . These results suggest that pathological conditions that alter the ratio of oxidized to reduced glutathione (*i.e.*, GSSG/GSH) would have an indirect effect on disulfide bond formation *via* its impact on the activity of ERO1 and/or thiol isomerases.

A study performed in yeast revealed that oxidative protein folding alters intracellular levels of GSSG but not GSH and thus influences the GSSG/GSH ratio (35). Increased expression of Pdi1p and Ero1p together reduces the cytosol redox state, whereas Pdi1p expression alone supports oxidation. Thus, maintaining the appropriate redox state in intracellular organelles can be a complex issue. Overall, oxidative protein folding and ER stress have an impact on the cytosolic redox balance and may be a key factor in the pathogenesis of protein folding-related diseases.

Pathological Roles of ERO1 α and PDI

Neurodegenerative diseases

The accumulation of misfolded proteins triggers the UPR and induces ER stress (130). Of particular note, the accumulation and ultimately the aggregation of amyloid β , tau, and α -synuclein together with oxidative stress induced by ROS have been associated with the development of several neurodegenerative diseases (67, 151). Among the most common of these neurodegenerative diseases is Alzheimer's disease (AD), which can have a profound impact on memory and cognitive judgment (151). Similarly, Parkinson's disease (PD) impairs mobility and mental ability (67) and is a major cause of morbidity and mortality in the elderly. Figure 3 illustrates the roles of PDI and ERO1 α in the pathogenesis of neurodegenerative diseases.

PDI has been found in S-nitrosylated form in the brains of patients with sporadic AD or PD (175). S-nitrosylation of PDI impairs both its isomerase and chaperone activities and thus induces protein misfolding and ER stress. The binding of PDI to tau protein prevents tau misfolding and fibrillization (198).

One recent report documented that the chaperone activity of PDI inhibits the phosphorylation and abnormal aggregation of tau and thus protects cells from mitochondrial damage and tau-mediated cytotoxicity (182). PDI is recruited to tissues by tau protein in liquid droplets, inhibiting phase separation and stress granule formation at these sites. By contrast, PDI that is S-nitrosylated at Cys312 in the b' domain is unable to recognize and undergo recruitment in response to tau protein (182). Likewise, intra-hippocampal injection of fibrillar amyloid β in rats increases the production of nitric oxide (NO) and decreases PDI activity; these responses result in the accumulation of misfolded protein in the ER lumen and ER stress (75).

Amyotrophic lateral sclerosis (ALS) is a disease in which degeneration of motor neurons in the brain and spinal cord leads to muscle weakness and paralysis (143). Many gene mutations have been associated with the familial form of ALS, including mutations in *SOD1* (cytosolic superoxide dismutase 1) and *TARDBP* (TAR DNA-binding protein 43 [TDP-43]) (107). Similar to other neurodegenerative diseases, protein misfolding and inclusion formation are among the hallmarks of ALS.

Mutant SOD1 and TDP-43 proteins form aberrant, non-native disulfide bonds and ultimately undergo aggregation (21, 33). Alterations in nuclear localization or export of TDP-43 result in protein aggregation and recapitulate the biochemical profile of pathological TDP-43-associated findings in ALS (191). The findings from a recent study demonstrated that the isomerase activity of PDI protects neuronal cells from inclusion formation, protein unfolding, and aberrant cytoplasmic localization of mutant forms of TDP-43, as well as ER stress induced by mutant TDP-43 or SOD1 and ER-Golgi transport dysfunction (127).

Of particular note, both oxidoreductase and chaperone activities of PDI protect against apoptosis in neuronal cells that express mutant forms of TDP-43 or SOD1. Further, Asp292Asn and Arg300His mutations in the b' domain of PDI have been linked to ALS; the expression of these PDI variants in zebrafish results in the disruption of motor neuron connectivity and impairs dendrite outgrowth (193).

Overexpression of PDI in the motor neuron-like cell line, NSC-34, results in reduced aggregation and inclusion formation due to mutant SOD1 as well as reductions in ER stress, whereas knockdown of PDI promotes mutant SOD1 inclusion formation (179). Similar to findings reported in the brains of patients with AD and PD, S-nitrosylated PDI has been detected in spinal cord tissues of patients with ALS as well as in transgenic mice that express the SOD1 mutation Gly93Ala (26). Collectively, these findings suggest that PDI protects against ER stress and protein misfolding in patients with AD, PD, or ALS and that this function is impaired by S-nitrosylation.

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by the degeneration and loss of neurons in the striatum (178). This genetic disorder is caused by the expansion of cytosine-adenine-guanine repeats within the *huntingtin* gene. This genetic aberration leads to the misfolding and aggregation of huntingtin protein and apoptotic cell death in the striatum and cortex. Currently, there is no effective therapy available to prevent or slow the progression of this disease.

A recent report documented upregulated expression of both PDI and ERp57 in the brains of patients with HD and a

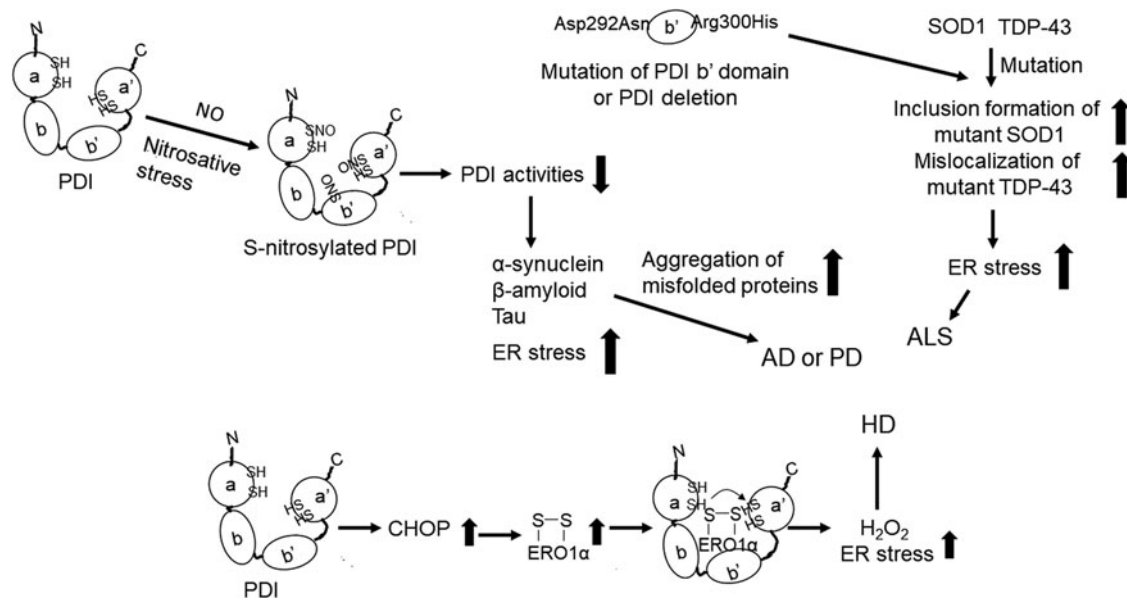


FIG. 3. The role of PDI and ERO1 α in neurodegenerative diseases. S-nitrosylation of PDI in association with nitrosative stress of neurons impairs its chaperone and enzymatic activities and induces the aggregation and accumulation of misfolded proteins (*e.g.*, tau), thereby leading to neurotoxicity and various sequelae characteristic of AD and PD. The ER stress in brain cells results in upregulated expression of PDI and CHOP and may enhance PDI-ERO1 α redox cycling and induce H₂O₂ overproduction and ER stress, thereby contributing to the pathology of HD. Further, mutations in the PDI b' domain and PDI deletion may also be linked to the pathogenesis of ALS. AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; C/EBP, CCAAT/enhancer-binding protein; CHOP, C/EBP-homologous protein; HD, Huntington's disease; PD, Parkinson's disease.

mouse model of HD; inhibition of PDI in this mouse model suppressed ER stress in the brain and results in improved survival (213). These results suggest that PDI might be a therapeutic target for the treatment of HD patients. Although it is not yet clear how PDI contributes to the pathology of HD, one group has suggested that this may result from the upregulation of CCAAT/enhancer-binding protein (C/EBP)-homologous protein (CHOP), which is an ER stress protein that modulates ERO1 α expression after activation (93).

As the ERO1 α -PDI redox cycle produces H₂O₂, accumulation of mutant huntingtin protein may lead to overactivation of the ERO1 α -PDI cycle and the overproduction of H₂O₂, leading to ER stress in cells. Therefore, unlike its protective role *vis-à-vis* the pathogenesis of AD and ALS, PDI may aggravate the pathology associated with HD. It is interesting to note that PDI inhibitors were capable of attenuating ER stress, huntingtin-associated toxicity, and motor dysfunction in both *in vitro* and preclinical studies (55, 72, 213). The results of these studies will be discussed later in this review.

No published studies have focused on the contributions of ERO1 α to the pathogenesis of neurodegenerative diseases. One study showed that inhibition of ERO1 α or PDI reduces neurotoxicity and accumulation of α -synuclein in the ER of the human SH-SY5Y neuroblastoma cell line and improves cell survival after treatment with the PD-associated neurotoxin, 1-methyl-4-phenylpyridinium (92). However, it is not clear whether ERO1 α modulates α -synuclein accumulation directly or indirectly *via* its actions on PDI. Future studies aimed at identifying the specific function of PDI and ERO1 α in neurodegenerative diseases may provide insights into potential therapeutic inventions that address the mechanisms underlying ER stress and the UPR.

Cancer

Cancer is the second leading cause of death worldwide (205). Hypoxia is the most common characteristic of the tumor microenvironment. Hypoxia plays a critical role in the initiation and progression of solid tumors and leads to resistance to radiation and chemotherapeutic interventions (64). Numerous proteins, including growth factors, extracellular matrix proteins, and proteases, are involved in promoting tumor growth. Since O₂ is required for disulfide bond formation, hypoxia significantly compromises this process. There is now substantial evidence for ERO1 α and PDI overexpression in numerous cancers; these enzymes constitute a key pathway for oxidative protein folding in the disease state. Figure 4 summarizes our current knowledge regarding the roles of PDI and ERO1 α in cancer.

Studies using human cancer cell lines have demonstrated a role for ERO1 α as an endogenous marker of hypoxia in the liver, pancreatic, colon, and breast cancer tissues (85, 161). ERO1 α was upregulated in the mouse Hepa-1c1c7 hepatocellular carcinoma cell line in response to hypoxia in a manner that was dependent on HIF-1, but not p53 (105). Knockdown of ERO1 α reduced the secretion of vascular endothelial growth factor (VEGF) and resulted in cell cycle arrest and apoptosis (105). In another study that featured both cell lines and patient-derived hepatocellular carcinoma tissue, cancer-associated ERO1 α was found to promote tumor cell angiogenesis, migration, and invasion *via* upregulation of VEGF-A expression (200). Collectively, these findings suggest that ERO1 α might be an actionable target for the development of strategies to control VEGF-driven angiogenesis and tumor growth.

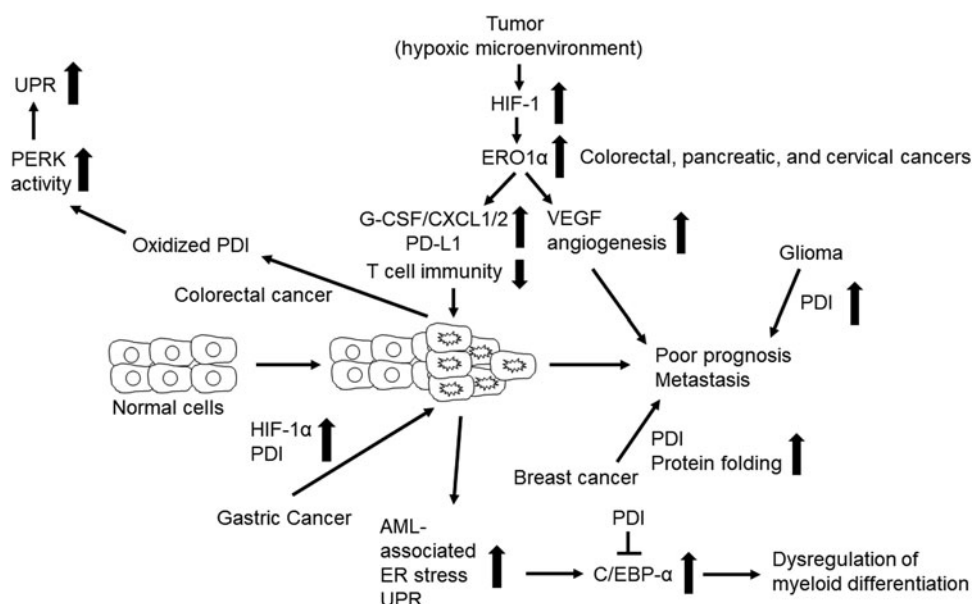


FIG. 4. The role of ERO1 α and PDI in cancer. The hypoxic tumor microenvironment induces the upregulation of ERO1 α *via* HIF-1, suppresses T cell immunity, and promotes tumor angiogenesis, thereby facilitating metastasis and resulting in poor survival. The PDI expression may be upregulated by HIF-1 α in gastric cancer cells. In glioma and breast cancer, upregulation of PDI may be a prognostic biomarker. Oxidized PDI increases PERK activity and the UPR in colorectal cancer. In AML, dysregulation of C/EBP- α results in aberrant myeloid differentiation. Upregulated levels of PDI may serve to suppress C/EBP function. AML, acute myeloid leukemia; HIF-1 α , hypoxia-inducible factor-1 α ; PERK, protein kinase R-like endoplasmic reticulum kinase; VEGF, vascular endothelial growth factor.

Proteomic analysis revealed that ERO1 α is highly upregulated in pancreatic cancer cells under hypoxia and that it serves as a potential biomarker predicting the survival of patients with pancreatic cancer (49). Kutomi *et al.* reported that deletion of ERO1 α in murine 4T1 breast cancer cells markedly reduces tumor growth and lung metastasis after their injection into mice (86). Importantly, these authors found that patients with ERO1 α -positive breast cancer exhibit poorer survival than those who are negative for this marker. These results suggest that ERO1 α might be developed as a novel biomarker to predict the survival of patients with breast cancer.

Mechanistically, ERO1 α promotes increased production of granulocyte colony-stimulating factor and chemokine CXC motif ligand 1/2 (CXCL1/2) by facilitating the formation of intramolecular disulfide bonds, recruiting polymorphonuclear myeloid-derived suppressor cells and inhibiting T cell-mediated immune responses (163).

Further, ERO1 α facilitates oxidative protein folding and enhances the expression of programmed death-ligand 1 (PD-L1) in MDA-MB-231 breast cancer cells, impairing the functional activity of cytotoxic T cells (164). By contrast, other studies revealed that hypoxia-induced expression of ERO1 α in a human SW480 colorectal cancer cell line promotes the oxidative folding and expression of MHC class I molecules *via* the actions of oxidized PDI, increasing their susceptibility to cytotoxic T cells (66, 85). Future studies will be needed to determine whether ERO1 α interacts with and alters responses to immunotherapy that are currently in use as treatments for various cancers.

It was reported that PDI plays a role in the pathology of breast cancer (3, 192), gastric cancer (206), gliomas (215),

acute myeloid leukemia (AML) (50), and pancreatic adenocarcinoma (43). The growth and ongoing survival of rapidly growing, highly invasive metastatic breast cancer cells would most likely require more active oxidative protein folding machinery than that found in nonmetastatic cells. One recent study revealed that PDI is overexpressed in nonadherent breast cancer cells and that PDI knockdown inhibits anchorage-independent cell proliferation and mammosphere growth (192). These results suggest that cancer-associated PDI may contribute to breast cancer cell metastasis.

Bioinformatics and biochemical studies revealed that PDI is a target gene of HIF-1 α and that both proteins are upregulated in human gastric cancer cell lines (206). Knockdown of HIF-1 α impairs invasion and metastasis of gastric cancer cells, an effect that was partially reversed by overexpression of PDI. Although the detailed underlying mechanism remains to be determined, these findings suggest the importance of the HIF-1 α -PDI pathway in promoting gastric cancer metastasis.

Analyses of Gene Expression Omnibus and the Human Protein Atlas showed upregulated expression of PDI in gliomas; elevated PDI expression has been recognized as a novel predictor for poor prognosis in patients diagnosed with glioma (215). Inhibition of cell surface PDI with an anti-PDI antibody blocks the adhesive function of glioma cells (48). Collectively, these results suggest that PDI might be a useful prognostic biomarker and therapeutic target for gliomas. Further, a recent study demonstrated that ERO1 α expression is upregulated in human cervical cancer and correlates with the grade of malignancy and poor prognosis and that blocking ERO1 α -PDI signaling by mutating the Val101 residue in ERO1 α reduces the migration, invasion, and growth of a human cervical carcinoma cell line (HeLa) (209).

Many cancers are associated with increased ER stress and activation of the UPR, which have a significant effect on protein expression (155). The transcription factor C/EBP- α plays a crucial role in the production of granulocyte/monocyte progenitors from common myeloid progenitors (145). Dysregulation of C/EBP- α function is a common finding in AML (145). PDI interacts with calreticulin binding to the stem-loop region of the C/EBP- α mRNA and blocks C/EBP- α translation (50). Further, other studies revealed that PDI expression is enhanced in cells from patients with AML on activation of the UPR. Collectively, these findings suggest that PDI suppresses C/EBP- α function and perturbs myeloid differentiation in AML.

Activation of protein kinase R-like endoplasmic reticulum kinase (PERK) is a critical step in the initiation of the UPR (144). A study using the HCT116 human colorectal carcinoma cell line suggested that oxidized PDI acts to activate PERK and that deletion or inhibition of PDI reduces PERK-mediated signaling and sensitizes cancer cells to hypoxia and ER stress (84). These results provide evidence suggesting that PDI might be a therapeutic target for the treatment of patients with colon cancer. Overall, ERO1 α and PDI expression is upregulated by tumor hypoxia, and these enzymes function to promote tumor growth and metastasis.

Cardiovascular diseases

The CVDs are a leading cause of death and contribute to substantial disability worldwide (1, 65). In addition to inherited genetic risk factors, hypoxia, cell death, and ongoing activation of intravascular cells and the coagulation cascade are crucial for the initiation and progression of CVDs (59, 153, 157, 166).

Hypoxia activates the UPR in cardiomyocytes and induces apoptosis (167). Figure 5 summarizes the roles of ERO1 α and PDI in myocardial infarction. A study using the ERO1 inhibitor, QM295, and mice with *Ero1-l* loss-of-function mutations showed that ERO1 α is upregulated in fibroblasts under hypoxic conditions and that the enzyme plays an important role in calcium homeostasis in cardiomyocytes and progressive heart failure (27).

The PDI upregulation was detected in the infarcted area after ligation of the left anterior descending artery (81). The

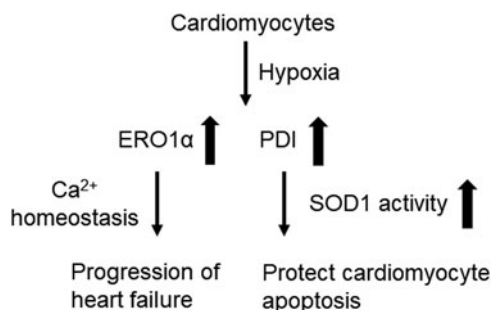


FIG. 5. The role of ERO1 α and PDI in myocardial infarction. Hypoxia in cardiomyocytes induces expression of ERO1 α and upregulation of the UPR. These responses enhance Ca²⁺ homeostasis and lead to progressive heart failure. Myocardial infarction results in the upregulated expression of PDI, thereby enhancing SOD1 activity and protection against cardiomyocyte apoptosis. SOD1, cytosolic superoxide dismutase 1.

expression of both ERO1 and PDI was enhanced in the murine HL1 cardiomyocyte cell line in response to acute hypoxia. The PDI protects these cells from undergoing apoptosis *via* an activity-dependent mechanism (153). Postmortem analysis revealed that PDI is a key factor underlying cardiomyocyte survival in patients with ischemic cardiomyopathy (153). Although an injection of adenoviral vectors expressing PDI into heart tissue results in reduced infarct size and cardiac remodeling in a mouse model of myocardial infarction (153), it is not clear whether the expression of ERO1 α results in similar effects.

Another study demonstrated that the effects of PDI on preventing cardiomyocyte apoptosis during acute myocardial infarction disappear in patients with diabetes and mice, in which most of the PDI was in reduced form (171). Although the detailed mechanism is unknown, the beneficial effects of PDI may result from the increased activity of SOD1 (172, 185). In contrast to acute hypoxia, chronic hypoxia (3 weeks) before myocardial infarction protects cardiac function in mice (168). This discrepancy may relate to the upregulation of myocardial endothelial cell (EC) PDI, which induces angiogenesis and protects cardiomyocytes against myocardial infarction. These results suggest that each of the known cellular functions of PDI has a distinct role in mitigating the progression of myocardial infarction.

Atherosclerosis is a chronic inflammatory disease of large and medium-sized arteries, and it is associated with elevated levels of low-density lipoproteins (LDLs) (99). During the disease process, ER stress induces phenotypic switching and apoptosis of vascular smooth muscle cells (VSMCs) (60, 160) and it promotes foam cell formation, pro-inflammatory cytokine production in macrophages (122), and EC apoptosis (2). Elevated LDL levels and cytokines can further activate ER stress.

Proteomic analysis revealed PDI upregulation in rat aortic VSMCs after stimulation with growth factors (128). The ROS produced *via* the actions of NADPH oxidase 1 (NOX1) are crucial factors underlying the proliferation and migration of VSMCs in atherosclerosis (187). One study using an siRNA approach suggested that PDI enhances the expression of NOX1 but not NOX4 and ROS production in VSMCs in response to stimulation with platelet-derived growth factor and that PDI positively regulates the activities of both Rac1 and RhoA, thereby promoting VSMC migration (131). Stretch stress and advanced glycosylation end products also upregulate PDI and NOX1 in VSMCs; the PDI produced by this mechanism enhances proliferation and apoptosis of VSMCs, thereby accelerating the atherosclerosis of diabetic vein grafts (133).

One recent paper using biochemical analysis and proximity ligation assays showed that PDI interacts with p47^{phox} through Cys residues found in its two active sites and that PDI interacts with p47^{phox} in thrombin-stimulated VSMCs and the carotid artery after wire-mediated injury in mice (46).

Fernandes *et al.* used an inducible PDI overexpression system in VSMCs to show that PDI overexpression modulates the expression of both NOX1 and NOX4 (41). At an early time point (24–48 h), PDI overexpression resulted in upregulated expression of NOX1, elevated levels of H₂O₂, and VSMC migration; however, at a later time point (72–96 h), PDI overexpression resulted in upregulated expression of NOX4 and VSMC differentiation, which was associated

with sustained high levels of NOX1. Further, the authors demonstrated that NOX1 expression is downregulated whereas NOX4 expression is enhanced in the carotid arteries of PDI-overexpressing transgenic mice. This result suggests that PDI can regulate both NOX1 and NOX4 activity and VSMC function.

Elevation plasma LDLs are strongly associated with the increased risk of atherosclerosis (113). Oxidized LDLs inhibit PDI activity in human microvascular ECs by inducing the formation of 4-hydroxynonenal (4-HNE)-PDI adducts, which were detected in advanced atherosclerotic plaques obtained from patients undergoing carotid endarterectomy (115). However, these results relied on the use of diosin glutathione disulfide (Di-E-GSSG) in cell-based assays, which raises questions about the specificity of these assays for the assessment of PDI activity.

Treatment of human umbilical vein ECs with oxidized LDLs induces the binding of the RNA-binding protein, heterogeneous nuclear ribonucleoprotein E1, to the *PDI 5'UTR*, upregulating PDI expression (108). Hyperhomocysteinemia, another risk factor for atherosclerosis (106), increases ER stress in hepatocytes of ApoE KO mice and downregulates both mRNA and protein levels of ERO1 α (201). Overexpression of ERO1 α in hepatocytes reduces the expressions of ER stress-related proteins, including GRP78, PERK, activating transcription factor 6 (ATF6), and X-box binding protein 1 (XBP-1). These results imply that ERO1 α limits the impact of hyperhomocysteinemia-induced ER stress during atherosclerosis. Figure 6 presents the roles of PDI and ERO1 α in the pathology of atherosclerosis.

Ischemia is an underlying factor in ~80% of all strokes. Ischemia results from occlusion of the cerebral artery, which blocks blood flow and O₂ delivery to the brain, leading to the damage and death of brain cells (156). Figure 7 summarizes

the role of PDI and ERO1 α in ischemic stroke. One study using a rat model of ischemic stroke showed that both CHOP and ERO1 α are upregulated in the hippocampus in response to ischemic injury (135). Although CHOP regulates ERO1 α expression in ER-stressed cells (93), hypothermia results in downregulated CHOP expression and augmented ERO1 α expression in hypoxic cells. These results suggest that HIF-1 α may induce the upregulation of ERO1 α . PDI expression is also enhanced in rat primary astrocytes in response to hypoxia (2% O₂ for 48 h) and subsequent reoxygenation (20% O₂ for 48 h) (162).

Another study revealed that PDI is upregulated in the cerebral cortex of the ischemic brain and that its oxidoreductase activity is a critical factor in promoting protection against hypoxia-induced cell death (162). Although the molecular mechanism remains elusive, the cytoprotective effects of p-hydroxy benzyl alcohol and tanshinone IIA in ischemic stroke may be derived from their capacity to upregulate PDI expression in brain tissue (68, 190).

Using megakaryocyte-specific *Pdi* CKO mice, we demonstrated that platelet-derived PDI promotes platelet-neutrophil aggregation and contributes to the pathogenesis of ischemic stroke (97). Platelet-neutrophil interactions are mainly mediated by the binding of P-selectin and GPIb α , which are found on the platelet surface to neutrophil P-selectin glycoprotein ligand-1 and α M β 2 integrin, respectively (94, 95).

Adherent platelet-neutrophil aggregates can form thrombi in the inflamed endothelium, which will ultimately result in microvessel occlusion (79, 96). We found that PDI cleaves two allosteric disulfide bonds in GPIb α , thereby enhancing its ligand-binding function. Importantly, although the inhibition of extracellular PDI or platelet GPIb α results in decreased infarct volume and protects against the development of

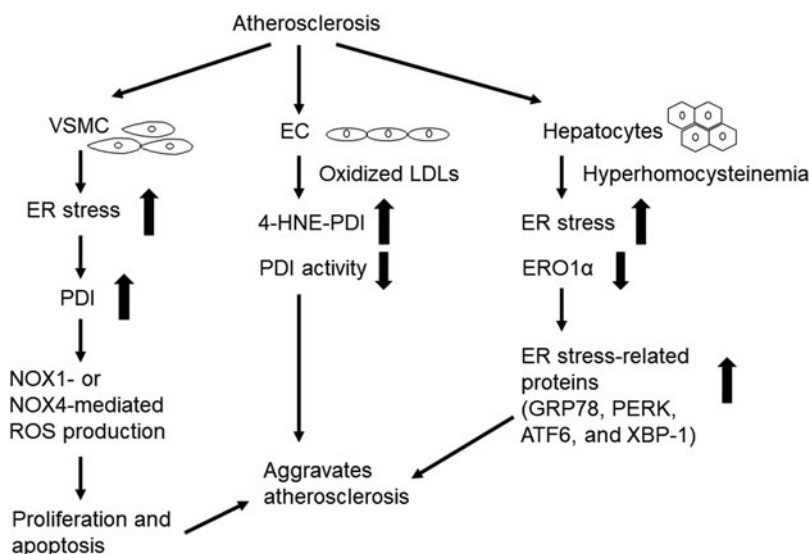


FIG. 6. The role of PDI and ERO1 α in the progression of atherosclerosis. PDI and ERO1 α function differently in the various cell types during the progression of atherosclerosis. PDI is upregulated in VSMCs, where it promotes ROS production through NOX1 or NOX4, which leads to cellular proliferation or apoptosis. In ECs, oxidized LDLs form 4-HNE-PDI, which impairs its activity. Hyperhomocysteinemia in hepatocytes induces ER stress, upregulates ER stress-related proteins (GRP78, PERK, ATF6, and XBP-1), and downregulates ERO1 α expression. All of these pathways serve to aggravate the pathology associated with atherosclerosis. 4-HNE, 4-hydroxynonenal; ATF6, activating transcription factor 6; LDLs, low-density lipoproteins; NOX1, NADPH oxidase 1; ROS, reactive oxygen species; VSMC, vascular smooth muscle cell; XBP-1, X-box binding protein 1.

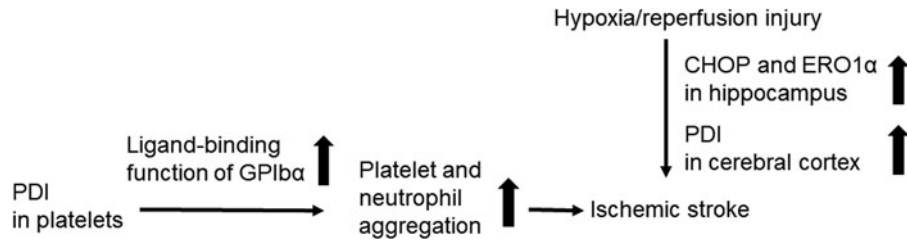


FIG. 7. The role of PDI and ERO1 α in ischemic stroke. CHOP and ERO1 α are upregulated in the hippocampus, and PDI is upregulated in the cerebral cortex during ischemic stroke. Platelet-derived PDI binds to GPIb α and enhances its ligand-binding function, thereby contributing to platelet–neutrophil aggregation and vascular occlusion. GPIb α , glycoprotein Ib α .

neurological deficits in a mouse model of ischemic stroke, no additive effects were observed (97). These results suggest that a specific blockade of PDI–GPIb α signaling might be an attractive strategy for the treatment of thromboinflammatory diseases.

Platelets adhere to extracellular matrix proteins, such as collagen and von Willebrand factor, at the site of arterial injury where they undergo receptor-mediated activation and aggregation (120). Although this process is essential for hemostasis, excessive accumulation of platelets will result in thrombosis and vascular occlusion. Since current antiplatelet therapies that target signaling molecules or receptor–ligand interactions increase the risk of major bleeding (13), many efforts have been made to develop potent but safer antithrombotic agents that do not impair hemostasis.

Modification of thiol–disulfide bonds identified in platelet receptors induces conformational changes and modulates their ligand-binding activity (22). Extracellular PDI has been identified as a target for novel treatments of thrombotic disease. Figure 8 illustrates the role of extracellular PDI in the

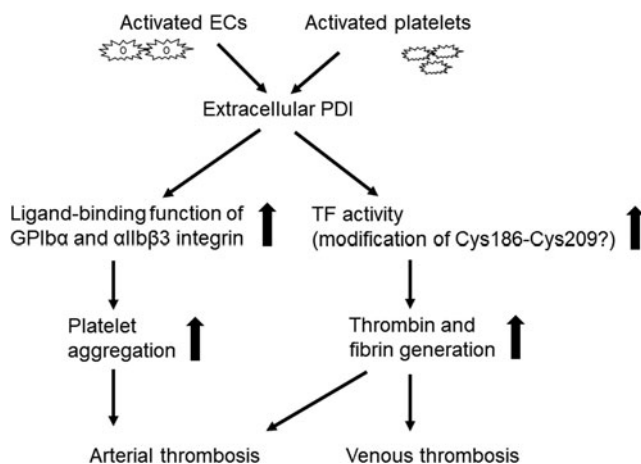


FIG. 8. The role of extracellular PDI in arterial and venous thrombosis. PDI is released from activated intravascular cells, including activated (adherent) platelets and ECs, in response to vascular injury. Extracellular PDI promotes the ligand-binding activity of GPIb α and α IIb β 3 by modifying their disulfide bonds, thereby inducing the formation of platelet thrombi. PDI also enhances TF activity, which leads to the generation of thrombin and fibrin. Both platelet aggregation and fibrin clots contribute to arterial/venous thrombosis and vascular occlusion. TF, tissue factor.

pathology of thrombosis. Several studies that used thiol-reacting agents and PDI inhibitors suggested that platelet-derived PDI enhances platelet adhesive function (39, 40, 88, 89).

Circulating PDI detected on the surface of platelet-derived microparticles also promotes aggregation (140). Reinhardt *et al.* demonstrated that extracellular PDI plays a crucial role in regulating the activity of tissue factor (TF) and fibrin generation in a mouse model of FeCl₃-induced arterial thrombosis (141). Further, the inhibition of extracellular PDI with a blocking antibody perturbs arterial thrombus formation and prolongs bleeding times at the site of vascular injury (30), suggesting that extracellular PDI plays an important role in both thrombosis and hemostasis.

Studies using megakaryocyte-specific *Pdi* CKO and β 3 KO mice demonstrated that platelet-derived PDI binds directly to the α IIb β 3 integrin and is required for its full activation (31, 78, 212). This interaction promoted platelet accumulation, with only a minimal effect on fibrin generation at the site of vascular injury. Although EC-specific *Pdi* CKO mice have not yet been generated, one study using an α IIb β 3 antagonist suggested that EC PDI contributes to the generation of fibrin (62).

The EC microparticles isolated from diabetic mice contain PDI, and the treatment of mouse platelets with the microparticles activates the α IIb β 3 integrin (137). Further, EC-derived PDI exposes an ArgGlyAsp sequence in thrombospondin-1, thereby promoting its binding to α v β 3 integrin (56), which may also contribute to thrombosis.

An *in vitro* study suggested that platelet ERO1 α may regulate the function of PDI and α IIb β 3 and platelet aggregation (159). However, detailed molecular mechanisms and the function of ERO1 α in thrombosis *in vivo* remain elusive.

Venous thromboembolism is the third most common CVD. This condition is the direct result of blood stasis, hypercoagulability, and vascular wall injury (189). TF-initiated coagulation is a critical feature of this disease process. Mass spectrometric analysis using purified TF and PDI revealed that the Cys209 residue in TF is constitutively conjugated with GSH and that PDI deglutathionylates TF and catalyzes oxidation of the Cys186–Cys209 disulfide bond (141). In contrast, PDI is found to enhance the pro-coagulant activity of TF detected on EC-derived microparticles *via* a mechanism that is independent of its oxidoreductase activity (177). These results imply that both oxidoreductase and chaperone activities of PDI may be required to regulate TF activity.

Another study revealed that ATP-induced P2X7 receptor signaling induces the release of TF-positive microparticles

via thiol- and PDI-dependent mechanisms (44). Of the two blocking anti-PDI antibodies (RL90 and BD34), only RL90 effectively reduced the release of these microparticles. Another study revealed that the inhibition of extracellular PDI with high concentrations of RL90 (50 $\mu\text{g}/\text{mL}$) or quercetin-3-rutinoside (rutin; 100 μM) interferes with the pro-coagulant activity of TF in the human THP-1 monocytic cell line treated with antithymocyte globulin (91). However, since RL90 and rutin inhibit the activity of both PDI and ERp57 (197, 212), these results may implicate the contribution of ERp57 to microparticle release and TF activity.

The TF activation in myelomonocytic cells induced by antiphospholipid antibodies is inhibited by treatment with the PDI inhibitor, 16F16, or by blocking TF-PDI binding (116). A study featuring a rat model of venous thrombosis documented upregulation and colocalization of PDI and TF in leukocytes and ECs in the inferior vena cava (211). Similarly, Subramaniam *et al.* demonstrated that blocking PDI with PACMA 31 attenuates platelet accumulation and TF-dependent fibrin formation in a mouse model involving partial stenosis of the inferior vena cava (158). Although the aforementioned studies provide evidence for PDI-mediated modulation of TF activity, several published reports have challenged this concept (8, 83, 129).

Diabetes

Diabetes is a metabolic disease that results in high blood sugar. Glucose homeostasis is tightly regulated by insulin, which is a hormone secreted from β cells found in pancreatic islets (52). The production and secretion of insulin depend on the synthesis and appropriate folding of its proinsulin precursor in the ER. Chronic elevation in proinsulin synthesis results in the accumulation of misfolded protein due to incorrect pairing of the intramolecular disulfide bonds (101). Increased ER stress and UPR result in the dysfunction and apoptosis of β cells, which can cause diabetes. There are two main subtypes of this disorder. Type 1 diabetes results from a defect in insulin secretion, and type 2 diabetes is characterized by insulin resistance and defective insulin secretion (149). Figure 9 illustrates the role of PDI and ERO1 α in diabetes.

Higher levels of PDI-bearing platelet microparticles are detected in patients with diabetes compared with healthy

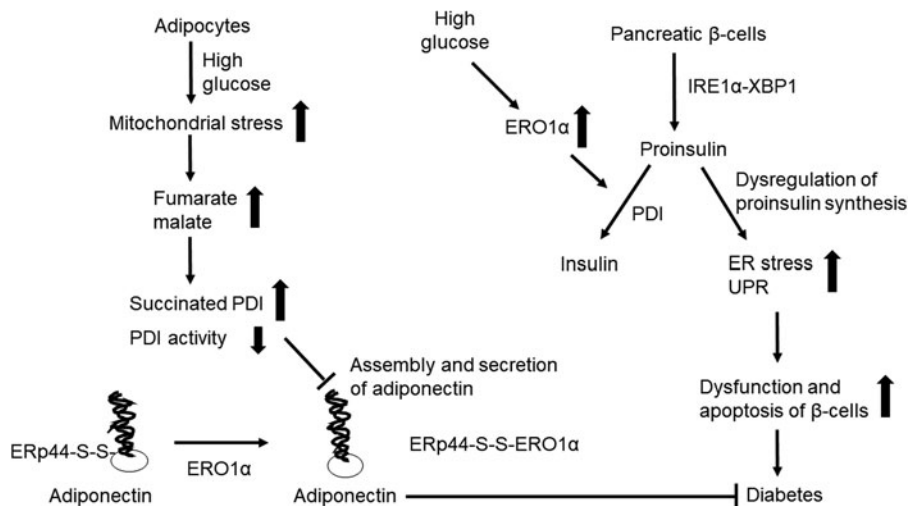
control subjects (140). One study revealed that both the redox states and enzymatic activities of PDI and ERO1 α are altered in liver microsomes isolated from streptozotocin-treated rats (117). Proteomic analysis revealed that insulin- and TNF- α -induced oxidative stress in differentiated 3T3-L1 adipocyte-like cells induces PDI upregulation (25). Overexpression of wild-type but not an activity-null mutant form of ERO1 α prevented proinsulin misfolding and reduced ER stress; co-expression of PDI impaired the beneficial effects observed in response to ERO1 α overexpression (196).

As ERO1 β is the dominant isoform expressed in pancreatic β cells (7, 36), Zito *et al.* generated homozygous ERO1 β mutant mice and demonstrated a key role for this protein in oxidative folding of proinsulin and glucose tolerance (214). ERO1 β is upregulated in mouse islets treated with high glucose, and pancreatic and duodenal homeobox 1 promote transcriptional regulation of ERO1-L β expression, thereby facilitating oxidative protein folding (77). The knockdown of ERO1 β reduces insulin content and secretion in the mouse MIN6 insulinoma cell line and promotes tunicamycin-induced cell death (77). In contrast to ERO1 β , knockdown of PDI augments proinsulin folding in the rat INS-1 β -cell line (138). The PDI can interact directly with proinsulin *via* the formation of a mixed disulfide bond, which results in its retention in the ER.

One study performed in β cell-specific *Pdi* CKO mice on a high-fat diet demonstrated that PDI is crucial for optimal insulin production and glucose tolerance under conditions of metabolic stress (61). The IRE1 α -XBP1 signaling pathway is constitutively activated in pancreatic β cells under homeostatic conditions (174). Tsuchiya *et al.* (174) showed that β cell-specific deletion of *Ire1 α* results in reduced insulin secretion, decreased insulin and proinsulin content, and down-regulated expression of PDI family isomerases, including PDI, ERp5, and ERp44. Taken together, PDI is a downstream effector of IRE1 α -XBP1 signaling and it plays a critical role in oxidative folding of proinsulin. Overall, these results suggest that expression and/or activity levels of PDI and ERO1 are altered under diabetic conditions and that these enzymes facilitate appropriate proinsulin folding and insulin secretion and thus are critical regulators of glucose homeostasis.

The plasma level of adiponectin, an antidiabetic and anti-atherogenic hormone secreted from adipocytes, is decreased in

FIG. 9. The role of ERO1 α and PDI in diabetes. Insulin production is tightly regulated in the β cells of the pancreas and relies on the appropriate folding of proinsulin by ERO1 β and PDI. Misfolded proinsulin leads to increased UPR and ER stress and, ultimately, β cell dysfunction and death, which characterizes the progression of diabetes. High glucose levels in adipocytes result in mitochondrial stress. PDI activity is reduced due to succinylation, which impairs the synthesis and secretion of the anti-diabetic hormone, adiponectin. ERO1 α also plays a crucial role in releasing adiponectin from ERp44.



patients diagnosed with diabetes (194). ERO1 α regulates the assembly and secretion of adiponectin by releasing Erp44 thiol-mediated retention (186).

Mitochondrial stress in adipocytes due to high glucose levels results in increased levels of both fumarate and malate in diabetes; this leads to succinylation of the PDI active site Cys residues and impaired assembly and secretion of adiponectin (103). This article also noted that succinylation perturbs the oxidoreductase activity of PDI in adipose tissue of diabetic mice. Restoring glucose levels from high to normal concentrations also restores PDI reductase activity and reduces ER stress in adipocytes. Although it remains to be determined whether PDI regulates adiponectin function, these results suggest that PDI succinylation could be a novel regulatory mechanism by which altered mitochondrial metabolism induces ER stress in adipocytes in patients with diabetes.

The deposition of islet amyloid polypeptide (IAPP) is a common feature of type 2 diabetes (18). Misfolding and aggregation of IAPP lead to β cell dysfunction and death. The administration of adenovirus encoding *P4HB* to transgenic mice expressing human IAPP specifically in β cells resulted in amelioration of insulin secretion and inhibition of β cell apoptosis by PDI under glucolipotoxic conditions (112).

Inhibitors

ERO1 inhibitors

A high-throughput screening study identified two compounds, EN460 and QM295 as ERO1 inhibitors, both with an IC₅₀ value of 1.9 μ M (Fig. 10A, B) (19). Both inhibitors activate the UPR in 293T cells and protect hypersensitive PERK-null fibroblasts against severe ER stress. However, EN460 and QM295 are weak inhibitors when evaluated in cell-based assays, and they also exhibit cellular toxicity, potentially due to poor solubility and cell permeability, nonspecific interactions with free thiols, and/or inhibition of other FAD-binding enzymes.

As ERO1 α is upregulated in response to tumor hypoxia, the inhibitors have been tested for their efficacy in numerous cancer models. Among these findings, the treatment of multiple myeloid cells with EN460 reduced cell proliferation and induced apoptosis (54). Treatment with EN460 inhibited the proliferation of pancreatic ductal adenocarcinoma cells *in vitro* and also impeded tumor growth *in vivo* (207).

Costanzo *et al.* evaluated a network of genetic interaction profiles and reported that erodoxin interacts with yeast genes involved in protein folding, glycosylation, and cell wall

biosynthesis, and it also inhibits ERO1 activity (Fig. 10C) (34). However, it is unknown whether erodoxin has *in vivo* efficacy in animal models. Due to the high (65%) amino acid sequence identity shared between ERO1 α and ERO1 β , no isoform-specific inhibitor has been identified.

PDI inhibitors

Given its contributions to pathology associated with a variety of diseases, PDI has received considerable attention as a promising druggable target. Studies using a high-throughput screen identified rutin and the bepristats as potential PDI inhibitors (Fig. 11A–E) (14, 63). As flavonoid-based antioxidant compounds, both quercetin and rutin are known to inhibit thrombosis (12, 32, 114), inflammation (37, 146), neurodegenerative diseases (38, 102), and cancer (57, 73). Rutin binds to the b' domain of PDI and exerts an inhibitory effect on platelet aggregation and thrombus formation at the site of arteriolar injury (63, 100). Nevertheless, several studies have addressed the nonspecific effects of rutin at the same concentration as that used to inhibit PDI activity (78, 97, 212).

The results of a Phase II clinical trial revealed that quercetin-3-glucoside (isoquercetin) reduces hypercoagulability in patients with advanced cancer (216). Similar to rutin, isoquercetin interacts with the PDI b' domain *via* the formation of hydrogen bonds with Glu239, Asp297, and Asn298 (Fig. 11B). The bepristats also bind to the substrate-binding pocket of the PDI b' domain, blocking substrate binding and inhibiting platelet aggregation and thrombus formation (15). Although PDI-null platelets exhibit a moderate defect (~50% inhibition) in agonist-induced aggregation (78, 97, 212), treatment with bepristats abrogates platelet aggregation (14), implicating their off-target effects.

ML359 (Fig. 11F) was identified as a potent PDI inhibitor with an IC₅₀ value of 250 nM, but treatment with 30 μ M of ML359 had only a minimal inhibitory effect on platelet aggregation (76). Although its binding site has not been reported, given its structural similarity with bepristat 1a (Fig. 11D), ML359 is likely to bind to the b' domain.

A recent study using *in silico* molecular docking reported that tannic acid (Fig. 11G) binds to both active sites and the b' domain of PDI. In contrast to cell-permeable small-molecule inhibitors, given its molecular mass of 1.7 kDa, tannic acid is likely to inhibit the activity of extracellular but not intracellular PDI (142). When injected intraperitoneally, tannic acid reduced thrombus formation without prolonging bleeding times *in vivo*. Another study revealed that the hexamer, LysPheTrpTrpPheSer, interacts with the substrate-binding site of PDI and eliminates ERO1 α activity (109). This result

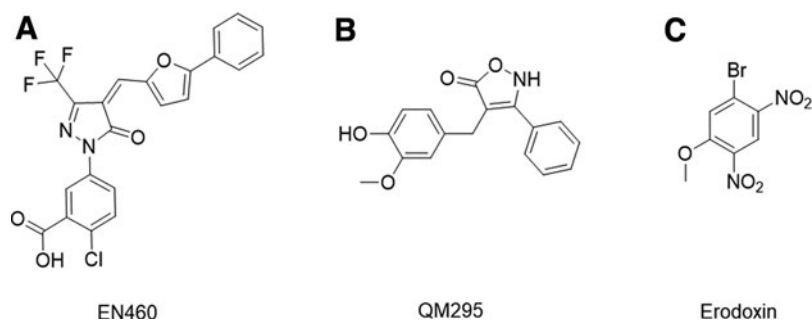
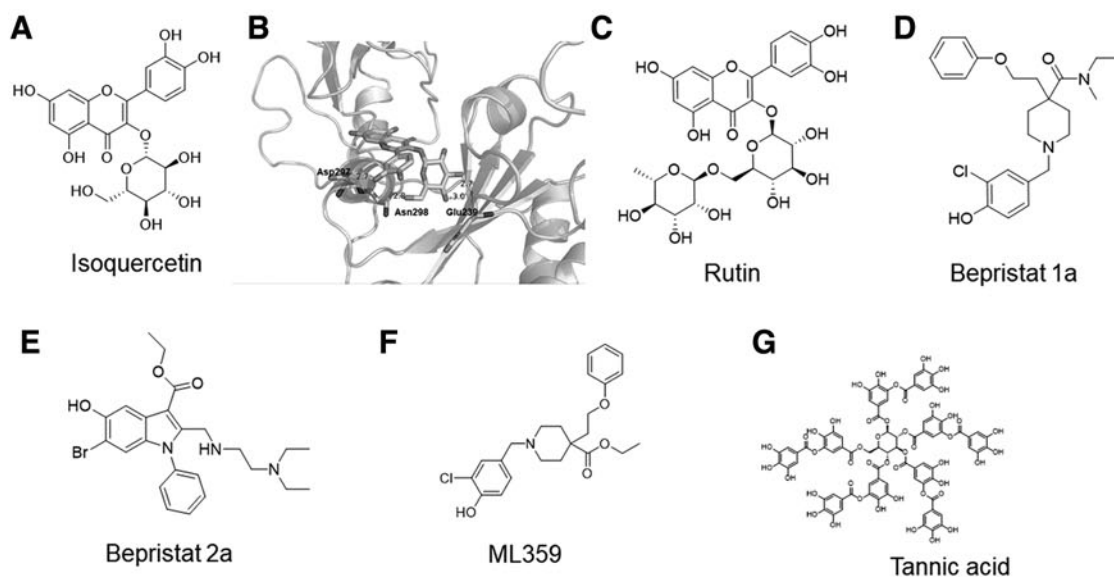
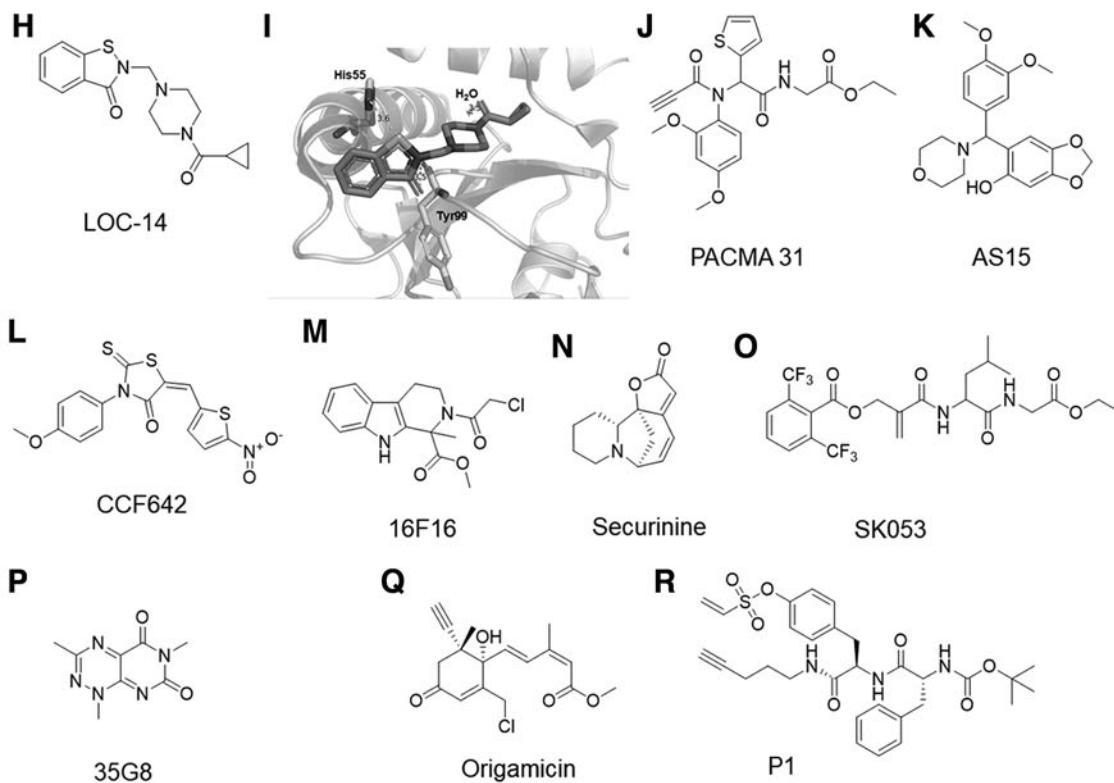


FIG. 10. The structures of ERO1 α inhibitors. (A) EN460, (B) QM295, and (C) Erodoxin.

PDI inhibitors that bind to the b'-domain



PDI inhibitors that bind to the active site(s)



A PDI inhibitor whose binding site is not identified

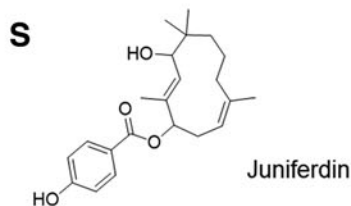


FIG. 11. The structures of PDI inhibitors and their binding site. (A–G) Inhibitors that bind to the b' domain of PDI. (H–R) Inhibitors that bind to the active site(s) of PDI. (B, H) Glide docking was performed to confirm the binding site of isoquercetin and LOC14 to PDI. (S) No binding site has been reported for this inhibitor.

suggests that ERO1 α binding to the substrate-binding pocket in the PDI b' domain is a critical step that promotes both ERO1 α activation and the ERO1 α -PDI redox cycle.

Most PDI inhibitors bind to the N- or C-terminal active site or both. As these interactions are typically thiol mediated, inhibition associated with molecules in this category is likely to be irreversible. Among those inhibitors, LOC14 (Fig. 11H) is the most potent PDI inhibitor with a K_d value of 62 nM (71). This compound was reported as binding to reduced PDI only, suggesting that the free thiols in the PDI active site are required for LOC14 binding. Using glide docking, we found that LOC14 fits well within the a domain of reduced PDI and interacts with His55 and Tyr99 (Fig. 11I). The compound is stable in mouse liver microsomes; blood plasma undergoes low rates of clearance while within microsomes, exhibits low binding affinity to plasma proteins, and penetrates the blood-brain barrier (71). Treatment with LOC14 improves the viability of PC12 cells expressing the mutant huntingtin protein (71) and suppresses ER stress in a mouse model of HD (213).

PACMA 31 (Fig. 11J) was identified as an orally available PDI inhibitor (199). Since PDI is overexpressed in ovarian tumors, it has been associated with poor prognosis in patients with ovarian cancer (148). PACMA 31 covalently binds to the Cys397 and Cys400 residues in the PDI a' domain and irreversibly inhibits its activity with an IC_{50} value of 10 μ M (199). This compound is cytotoxic and can suppress the growth of multiresistant ovarian cancer cells. PACMA 31 also exhibited a significant inhibitory effect on human ovarian cancer cell growth in a mouse xenograft model while displaying no substantial toxicity to normal tissues (199). Further, *in vitro* and *in vivo* studies revealed the beneficial effects of PACMA 31 in multiple myeloma (176), hepatocellular carcinoma (195), and breast cancer (136).

It is recently reported that similar to PACMA 31, the irreversible PDI inhibitor, AS15 (IC_{50} value of 300 nM; Fig. 11K), forms a covalent bond to the C-terminal active site of PDI (154). AS15 and its derivatives synergistically inhibit the growth of human glioblastoma cells (A-172 and U-118 MG lines) when treated in combination with a GSH synthesis inhibitor.

Unbiased screening experiments identified CCF642 (Fig. 11L) as a PDI inhibitor (176). Computational modeling suggested that the inhibitory effect is based on covalent binding between the carbonyl group of CCF642 and the Lys residue found at the two active sites of PDI (176). Compared with bortezomib, which is a U.S. Food and Drug Administration-approved drug for myeloma, CCF642 exhibits similar inhibitory effects on multiple myeloma in studies carried out *in vivo*. Another study showed that CCF642 reduces the expression of ER stress markers and neuronal apoptosis in a mouse model of experimental autoimmune encephalomyelitis, resulting in neuroprotection (69).

Hoffstrom *et al.* identified the compound 16F16 (Fig. 11M) as an irreversible inhibitor for both PDI and ERp57 (55). PDI accumulates at mitochondria-associated ER membranes in PC12 cells that express polyglutamine-expanded huntingtin exon 1; treatment with 16F16 suppressed apoptosis of the PC12 cells (55), suggesting mechanisms underlying PDI-mediated protection against apoptotic cell death in HD. In addition, 16F16 inhibits the migration of breast cancer cells (136) and the replication of influenza viruses (80). However, this inhibitor did not affect the pro-coagulant activity of TF (204). Although the sites of

the 16F16-PDI interaction remain unclear, cystamine, which is a simple organic disulfide, competes with 16F16A for binding to PDI (55), suggesting that 16F16 is likely to bind to the active site of PDI.

One recent study in which the reactive group in 16F16 was replaced with an NO donor suggested that 16F16-NO S-nitrosylates PDI by releasing NO, resulting in the reversible inhibition of PDI activity (98). Securinine (Fig. 11N) was identified as another irreversible PDI inhibitor (72). Similar to the effects of phenylarsine oxide, which is a compound that reacts with vicinal thiols, securinine binds irreversibly to the N-terminal active site of PDI. Treatment with securinine results in improved viability of PC12 cells that express the mutant form of huntingtin (72). In addition, securinine inhibits SH-SY5Y cell viability with an IC_{50} value of 37 μ M (123).

Several other PDI inhibitors have been characterized. SK053 (Fig. 11O) is an inhibitor of the thioredoxin-thioredoxin reductase system, and it also binds to the C-terminal active site of PDI and inhibits its enzymatic activity with an IC_{50} value of 10 μ M (28, 82). Treatment with SK053 results in elevated C/EBP- α levels in the human HL-60 promyelocytic leukemia cell line and impairs cell growth and differentiation (28). A computational docking study revealed that compound 35G8 (Fig. 11P) binds to Cys397 in the C-terminal active site of PDI (87). This compound inhibits PDI activity with an IC_{50} value of 170 nM and is cytotoxic to glioblastoma cell lines at concentrations of 1–5 μ M.

Origamicin (Fig. 11Q) was also identified as a PDI inhibitor that covalently binds to Cys residues in the active sites (124). Microarray analysis revealed that treatment with origamicin alters the gene expression profiles of both SH-SY5Y and Huh7 cells infected with the hepatitis C virus (124, 125). Origamicin inhibits hepatitis C virus replication and modulates the viability of SH-SY5Y cells at concentrations of 25–50 μ M. Screening of the human MCF-7 breast cancer cell line identified a phenyl vinyl sulfonate-containing small compound (P1) (Fig. 11R) as a cell-permeable PDI inhibitor with an IC_{50} value of 1.7 μ M, although it cross-reacts with both ERp72 and ERp5 (45). Treatment with P1 inhibits the proliferation of various cancer cells. The phenyl vinyl sulfonate group of P1 probably binds to Cys 397 in the C-terminal active site of PDI (45).

Juniferdin (Fig. 11S) is a natural compound that inhibits PDI reductase activity with an IC_{50} value of 156 nM and has no effect on the activities of ERp57 and ERp72 (74). The binding site in PDI has not been reported. Juniferdin perturbs PDI-catalyzed reduction of the human immunodeficiency virus type 1 protein, gp120, which is crucial for virus entry into cells (74, 147). Treatment with juniferdin also reduces the rates of intracellular replication of influenza A and B viruses (80). Further studies are required to determine whether this inhibitor interferes with other viral infections.

Concluding Remarks

ERO1 and PDI are upregulated in response to hypoxia and the ER stress-activated UPR that has been associated with numerous diseases. In particular, ERO1 α has an emerging role in the hypoxic tumor microenvironment. Along with its critical function in the ER, PDI released from cells in the peripheral vasculature also regulates cell adhesive functions associated with thrombosis and inflammation.

Although the ERO1 α -PDI redox cycle facilitates oxidative protein folding and disulfide bond modification, the target molecules and regulatory mechanisms might vary between individual cell types. Thus, future studies will be required to determine the answers to additional remaining questions. Among these issues, although absolute PDI deficiency would be lethal in humans, PDI that has undergone posttranslational modification (e.g., S-nitrosylation) has been found in patients diagnosed with neurodegenerative diseases. Therefore, it will be important to determine which posttranslational modifications or mutations occur in PDI and ERO1 under specific disease conditions.

Clinical and research data associated with altered expression or mutation of *PDI* and *ERO1-L* genes will greatly advance our understanding of their pathophysiological roles. Likewise, although most of the protein is retained in the ER, a small fraction of the total PDI is found in other organelles. The role and function of PDI detected outside the ER should be elucidated. Upregulated expression of both PDI and ERO1 might lead to their release under specific disease conditions. It would be important to understand whether and how PDI and ERO1 are released from cells as well as their individual roles in specific disease states.

Finally, as described earlier, PDI and ERO1 synthesized by and released from individual cell types might subservise unique functions in health and disease. The molecular and cellular mechanisms underlying the contributions of ERO1 α and PDI to the initiation and progression of various diseases remain to be determined. Both specific inhibitors and tissue-specific CKO mice will be critical resources to answer the aforementioned questions.

There are several issues related to the identification and development of ERO1 α and PDI inhibitors that remain of concern. First, due to the high amino acid sequence identity and the need to target the FAD-binding pocket, it may be challenging to identify ERO1 α -specific inhibitors. Similarly, the selectivity and physicochemical properties (e.g., membrane-permeability, solubility, and others) of most PDI inhibitors have not been fully evaluated. For example, given that extracellular PDI activity is a critical feature contributing to the pathology of thrombotic diseases, cell-permeable inhibitors should be evaluated with caution as they might impair the indispensable function of PDI in the ER.

PDI is upregulated in areas of myocardial infarction in mouse models of left anterior descending artery ligation. The expression of ERO1 and PDI is enhanced by acute hypoxia in cells of the murine HL1 cardiomyocyte line, and PDI plays a protective role in limiting apoptosis *via* its activity-dependent mechanism (150). Further, postmortem analysis revealed that PDI is a key factor promoting cardiomyocyte survival in patients with ischemic cardiomyopathy.

Second, many PDI inhibitors are capable of forming covalent bonds with Cys residues in the active sites, thereby enabling them to inhibit PDI activity irreversibly. Since PDI inhibitors are likely to be administered for long periods of time, any adverse effects associated with these compounds should be carefully examined. Third, the administration of specific inhibitors of either PDI or ERO1 α may not exhibit potent inhibitory effects *in vivo*, as other isoforms may compensate for diminished function. Thus, it would be interesting to test the combined effects of both sets of inhibitors in diseases such as cancer in which both PDI and ERO1 α are upregulated.

Authors' Contributions

V.J., T.K., V.M., and J-K.M. wrote a draft; Z.A. and K.L.O. provided important comments; and J.C. wrote the article.

Author Disclosure Statement

Z.A. and K.L.O. are employees of Cayman Chemical Company Inc. The other authors declare no competing financial interests.

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References

1. Abad Rico JI, Llau Pitarch JV, and Rocha E. Overview of venous thromboembolism. *Drugs* 70 Suppl 2: 3–10, 2010.
2. Abe JI, Ko KA, Kotla S, Wang Y, Paez-Mayorga J, Shin IJ, Imanishi M, Vu HT, Tao Y, Leiva-Juarez MM, Thomas TN, Medina JL, Won JH, Fujii Y, Giancursio CJ, McBeath E, Shin JH, Guzman L, Abe RJ, Taunton J, Mochizuki N, Faubion W, Cooke JP, Fujiwara K, Evans SE, and Le NT. MAGI1 as a link between endothelial activation and ER stress drives atherosclerosis. *JCI Insight* 4: e125570, 2019.
3. Alhammad R, Khunchai S, Tongmuang N, Limjindaporn T, Yenchitsomanus PT, Mutti L, Krstic-Demonacos M, and Demonacos C. Protein disulfide isomerase A1 regulates breast cancer cell immunorecognition in a manner dependent on redox state. *Oncol Rep* 44: 2406–2418, 2020.
4. Appenzeller-Herzog C, Riemer J, Christensen B, Sorensen ES, and Ellgaard L. A novel disulphide switch mechanism in Ero1alpha balances ER oxidation in human cells. *EMBO J* 27: 2977–2987, 2008.
5. Araki K, Iemura S, Kamiya Y, Ron D, Kato K, Natsume T, and Nagata K. Ero1-alpha and PDIs constitute a hierarchical electron transfer network of endoplasmic reticulum oxidoreductases. *J Cell Biol* 202: 861–874, 2013.
6. Araki K and Nagata K. Functional in vitro analysis of the ERO1 protein and protein-disulfide isomerase pathway. *J Biol Chem* 286: 32705–32712, 2011.
7. Awazawa M, Futami T, Sakada M, Kaneko K, Ohsugi M, Nakaya K, Terai A, Suzuki R, Koike M, Uchiyama Y, Kadowaki T, and Ueki K. Deregulation of pancreas-specific oxidoreductin ERO1beta in the pathogenesis of diabetes mellitus. *Mol Cell Biol* 34: 1290–1299, 2014.
8. Bach RR and Monroe D. What is wrong with the allosteric disulfide bond hypothesis? *Arterioscler Thromb Vasc Biol* 29: 1997–1998, 2009.
9. Baker KM, Chakravarthi S, Langton KP, Sheppard AM, Lu H, and Bulleid NJ. Low reduction potential of Ero1alpha regulatory disulphides ensures tight control of substrate oxidation. *EMBO J* 27: 2988–2997, 2008.
10. Bartels AK, Gottert S, Desel C, Schafer M, Krossa S, Scheidig AJ, Grotzinger J, and Lorenzen I. KDEL receptor 1 contributes to cell surface association of protein disulfide isomerases. *Cell Physiol Biochem* 52: 850–868, 2019.

11. Bastos-Aristizabal S, Kozlov G, and Gehring K. Structural insight into the dimerization of human protein disulfide isomerase. *Protein Sci* 23: 618–626, 2014.
12. Becker CG, Dubin T, and Glenn F. Induction of acute cholecystitis by activation of factor XII. *J Exp Med* 151: 81–90, 1980.
13. Becker RC, Sexton T, and Smyth SS. Translational implications of platelets as vascular first responders. *Circ Res* 122: 506–522, 2018.
14. Bekendam RH, Bendapudi PK, Lin L, Nag PP, Pu J, Kennedy DR, Feldenzer A, Chiu J, Cook KM, Furie B, Huang M, Hogg PJ, and Flaumenhaft R. A substrate-driven allosteric switch that enhances PDI catalytic activity. *Nat Commun* 7: 12579, 2016.
15. Bellisola G, Fracasso G, Ippoliti R, Menestrina G, Rosen A, Solda S, Udali S, Tomazzolli R, Tridente G, and Colombatti M. Reductive activation of ricin and ricin A-chain immunotoxins by protein disulfide isomerase and thioredoxin reductase. *Biochem Pharmacol* 67: 1721–1731, 2004.
16. Benham AM. The protein disulfide isomerase family: key players in health and disease. *Antioxid Redox Signal* 16: 781–789, 2012.
17. Bertoli G, Simmen T, Anelli T, Molteni SN, Fesce R, and Sitia R. Two conserved cysteine triads in human Ero1 α cooperate for efficient disulfide bond formation in the endoplasmic reticulum. *J Biol Chem* 279: 30047–30052, 2004.
18. Bishoyi AK, Roham PH, Rachineni K, Save S, Hazari MA, Sharma S, and Kumar A. Human islet amyloid polypeptide (hIAPP)—a curse in type II diabetes mellitus: insights from structure and toxicity studies. *Biol Chem* 402: 133–153, 2020.
19. Blais JD, Chin KT, Zito E, Zhang Y, Heldman N, Harding HP, Fass D, Thorpe C, and Ron D. A small molecule inhibitor of endoplasmic reticulum oxidation 1 (ERO1) with selectively reversible thiol reactivity. *J Biol Chem* 285: 20993–21003, 2010.
20. Booth C and Koch GL. Perturbation of cellular calcium induces secretion of luminal ER proteins. *Cell* 59: 729–737, 1989.
21. Bosco DA, Morfini G, Karabacak NM, Song Y, Gros-Louis F, Pasinelli P, Goolsby H, Fontaine BA, Lemay N, McKenna-Yasek D, Frosch MP, Agar JN, Julien JP, Brady ST, and Brown RH, Jr. Wild-type and mutant SOD1 share an aberrant conformation and a common pathogenic pathway in ALS. *Nat Neurosci* 13: 1396–1403, 2010.
22. Butera D, Cook KM, Chiu J, Wong JW, and Hogg PJ. Control of blood proteins by functional disulfide bonds. *Blood* 123: 2000–2007, 2014.
23. Cabibbo A, Pagani M, Fabbri M, Rocchi M, Farmery MR, Bulleid NJ, and Sitia R. ERO1-L, a human protein that favors disulfide bond formation in the endoplasmic reticulum. *J Biol Chem* 275: 4827–4833, 2000.
24. Capitani M and Sallese M. The KDEL receptor: new functions for an old protein. *FEBS Lett* 583: 3863–3871, 2009.
25. Chan H, Bhide KP, Vaidyam A, Hedrick V, Sobreira TJP, Sors TG, Grant RW, and Aryal UK. Proteomic analysis of 3T3-L1 adipocytes treated with insulin and TNF- α . *Proteomes* 7: 35, 2019.
26. Chen X, Zhang X, Li C, Guan T, Shang H, Cui L, Li XM, and Kong J. S-nitrosylated protein disulfide isomerase contributes to mutant SOD1 aggregates in amyotrophic lateral sclerosis. *J Neurochem* 124: 45–58, 2013.
27. Chin KT, Kang G, Qu J, Gardner LB, Coetzee WA, Zito E, Fishman GI, and Ron D. The sarcoplasmic reticulum luminal thiol oxidase ERO1 regulates cardiomyocyte excitation-coupled calcium release and response to hemodynamic load. *FASEB J* 25: 2583–2591, 2011.
28. Chlebowska-Tuz J, Sokolowska O, Gaj P, Lazniewski M, Firczuk M, Borowiec K, Sas-Nowosielska H, Bajor M, Malinowska A, Muchowicz A, Ramji K, Stawinski P, Sobczak M, Pilch Z, Rodziewicz-Lurzynska A, Zajac M, Giannopoulos K, Juszczynski P, Basak GW, Plewczynski D, Ploski R, Golab J, and Nowis D. Inhibition of protein disulfide isomerase induces differentiation of acute myeloid leukemia cells. *Haematologica* 103: 1843–1852, 2018.
29. Cho J. Protein disulfide isomerase in thrombosis and vascular inflammation. *J Thromb Haemost* 11: 2084–2091, 2013.
30. Cho J, Furie BC, Coughlin SR, and Furie B. A critical role for extracellular protein disulfide isomerase during thrombus formation in mice. *J Clin Invest* 118: 1123–1131, 2008.
31. Cho J, Kennedy DR, Lin L, Huang M, Merrill-Skoloff G, Furie BC, and Furie B. Protein disulfide isomerase capture during thrombus formation in vivo depends on the presence of β 3 integrins. *Blood* 120: 647–655, 2012.
32. Choi JH, Kim DW, Park SE, Lee HJ, Kim KM, Kim KJ, Kim MK, Kim SJ, and Kim S. Anti-thrombotic effect of rutin isolated from *Dendropanax morbifera* Leveille. *J Biosci Bioeng* 120: 181–186, 2015.
33. Cohen TJ, Hwang AW, Unger T, Trojanowski JQ, and Lee VM. Redox signalling directly regulates TDP-43 via cysteine oxidation and disulphide cross-linking. *EMBO J* 31: 1241–1252, 2012.
34. Costanzo M, Baryshnikova A, Bellay J, Kim Y, Spear ED, Sevier CS, Ding H, Koh JL, Toufighi K, Mostafavi S, Prinz J, St Onge RP, VanderSluis B, Makhnevych T, Vizeacoumar FJ, Alizadeh S, Bahr S, Brost RL, Chen Y, Cokol M, Deshpande R, Li Z, Lin ZY, Liang W, Marback M, Paw J, San Luis BJ, Shuteriqi E, Tong AH, van Dyk N, Wallace IM, Whitney JA, Weirauch MT, Zhong G, Zhu H, Houry WA, Brudno N, Ragibzadeh S, Papp B, Pal C, Roth FP, Gjaever G, Nislow C, Troyanskaya OG, Bussey H, Bader GD, Gingras AC, Morris QD, Kim PM, Kaiser CA, Myers CL, Andrews BJ, and Boone C. The genetic landscape of a cell. *Science* 327: 425–431, 2010.
35. Delic M, Rebnegger C, Wanka F, Puxbaum V, Haberhauer-Troyer C, Hann S, Kollensperger G, Mattanovich D, and Gasser B. Oxidative protein folding and unfolded protein response elicit differing redox regulation in endoplasmic reticulum and cytosol of yeast. *Free Radic Biol Med* 52: 2000–2012, 2012.
36. Dias-Gunasekara S, Gubbens J, van Lith M, Dunne C, Williams JA, Katakly R, Scoones D, Laphorn A, Bulleid NJ, and Benham AM. Tissue-specific expression and dimerization of the endoplasmic reticulum oxidoreductase Ero1 β . *J Biol Chem* 280: 33066–33075, 2005.
37. Domitrovic R, Jakovac H, Vasiljev Marchesi V, Vladimirovic Knezevic S, Cvijanovic O, Tadic Z, Romic Z, and Rahelic D. Differential hepatoprotective mechanisms of rutin and quercetin in CCl $_4$ -intoxicated BALB/cN mice. *Acta Pharmacol Sin* 33: 1260–1270, 2012.

38. Enogieru AB, Haylett W, Hiss DC, Bardien S, and Ekpo OE. Rutin as a potent antioxidant: implications for neurodegenerative disorders. *Oxid Med Cell Longev* 2018: 6241017, 2018.
39. Essex DW, Chen K, and Swiatkowska M. Localization of protein disulfide isomerase to the external surface of the platelet plasma membrane. *Blood* 86: 2168–2173, 1995.
40. Essex DW and Li M. Protein disulphide isomerase mediates platelet aggregation and secretion. *Br J Haematol* 104: 448–454, 1999.
41. Fernandes DC, Wosniak J, Jr., Goncalves RC, Tanaka LY, Fernandes CG, Zanatta DB, de Mattos ABM, Strauss BE, Laurindo FRM. PDIA1 acts as master organizer of NOX1/NOX4 balance and phenotype response in vascular smooth muscle. *Free Radic Biol Med* 162: 603–614, 2021.
42. Frand AR and Kaiser CA. The ERO1 gene of yeast is required for oxidation of protein dithiols in the endoplasmic reticulum. *Mol Cell* 1: 161–170, 1998.
43. Fu XM, Dai X, Ding J, and Zhu BT. Pancreas-specific protein disulfide isomerase has a cell type-specific expression in various mouse tissues and is absent in human pancreatic adenocarcinoma cells: implications for its functions. *J Mol Histol* 40: 189–199, 2009.
44. Furlan-Freguia C, Marchese P, Gruber A, Ruggeri ZM, and Ruf W. P2X7 receptor signaling contributes to tissue factor-dependent thrombosis in mice. *J Clin Invest* 121: 2932–2944, 2011.
45. Ge J, Zhang CJ, Li L, Chong LM, Wu X, Hao P, Sze SK, and Yao SQ. Small molecule probe suitable for in situ profiling and inhibition of protein disulfide isomerase. *ACS Chem Biol* 8: 2577–2585, 2013.
46. Gimenez M, Verissimo-Filho S, Wittig I, Schickling BM, Hahner F, Schurmann C, Netto LES, Rosa JC, Brandes RP, Sartoretto S, De Lucca Camargo L, Abdulkader F, Miller FJ, Jr., and Lopes LR. Redox activation of Nox1 (NADPH Oxidase 1) involves an intermolecular disulfide bond between protein disulfide isomerase and p47(phox) in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 39: 224–236, 2019.
47. Goldberger RF, Epstein CJ, and Anfinsen CB. Acceleration of reactivation of reduced bovine pancreatic ribonuclease by a microsomal system from rat liver. *J Biol Chem* 238: 628–635, 1963.
48. Goplen D, Wang J, Enger PO, Tysnes BB, Terzis AJ, Laerum OD, and Bjerkvig R. Protein disulfide isomerase expression is related to the invasive properties of malignant glioma. *Cancer Res* 66: 9895–9902, 2006.
49. Gupta N, Park JE, Tse W, Low JK, Kon OL, McCarthy N, and Sze SK. ERO1alpha promotes hypoxic tumor progression and is associated with poor prognosis in pancreatic cancer. *Oncotarget* 10: 5970–5982, 2019.
50. Haefliger S, Klebig C, Schaubitzer K, Schardt J, Timchenko N, Mueller BU, and Pabst T. Protein disulfide isomerase blocks CEBPA translation and is up-regulated during the unfolded protein response in AML. *Blood* 117: 5931–5940, 2011.
51. Hahm E, Li J, Kim K, Huh S, Rogelj S, and Cho J. Extracellular protein disulfide isomerase regulates ligand-binding activity of alphaMbeta2 integrin and neutrophil recruitment during vascular inflammation. *Blood* 121: 3789–3800, 2013.
52. Hassler JR, Scheuner DL, Wang S, Han J, Kodali VK, Li P, Nguyen J, George JS, Davis C, Wu SP, Bai Y, Sartor M, Cavalcoli J, Malhi H, Baudouin G, Zhang Y, Yates JR, III, Itkin-Ansari P, Volkmann N, and Kaufman RJ. The IRE1alpha/XBP1s pathway is essential for the glucose response and protection of beta cells. *PLoS Biol* 13: e1002277, 2015.
53. Hatahet F and Ruddock LW. Protein disulfide isomerase: a critical evaluation of its function in disulfide bond formation. *Antioxid Redox Signal* 11: 2807–2850, 2009.
54. Hayes KE, Batsomboon P, Chen WC, Johnson BD, Becker A, Eschrich S, Yang Y, Robart AR, Dudley GB, Geldenhuys WJ, and Hazlehurst LA. Inhibition of the FAD containing ER oxidoreductin 1 (Ero1) protein by EN-460 as a strategy for treatment of multiple myeloma. *Bioorg Med Chem* 27: 1479–1488, 2019.
55. Hoffstrom BG, Kaplan A, Letso R, Schmid RS, Turmel GJ, Lo DC, and Stockwell BR. Inhibitors of protein disulfide isomerase suppress apoptosis induced by misfolded proteins. *Nat Chem Biol* 6: 900–906, 2010.
56. Hotchkiss KA, Matthias LJ, and Hogg PJ. Exposure of the cryptic Arg-Gly-Asp sequence in thrombospondin-1 by protein disulfide isomerase. *Biochim Biophys Acta* 1388: 478–488, 1998.
57. Imani A, Maleki N, Bohlouli S, Kouhsoltani M, Sharifi S, and Maleki Dizaj S. Molecular mechanisms of anticancer effect of rutin. *Phytother Res* 2020 [Epub ahead of print]; DOI: 10.1002/ptr.6977.
58. Inaba K, Masui S, Iida H, Vavassori S, Sitia R, and Suzuki M. Crystal structures of human Ero1alpha reveal the mechanisms of regulated and targeted oxidation of PDI. *EMBO J* 29: 3330–3343, 2010.
59. Jackson SP, Darbousset R, and Schoenwaelder SM. Thromboinflammation: challenges of therapeutically targeting coagulation and other host defense mechanisms. *Blood* 133: 906–918, 2019.
60. Jaminon A, Reesink K, Kroon A, and Schurgers L. The role of vascular smooth muscle cells in arterial remodeling: focus on calcification-related processes. *Int J Mol Sci* 20: 5694, 2019.
61. Jang I, Pottekat A, Poothong J, Yong J, Lagunas-Acosta J, Charbono A, Chen Z, Scheuner DL, Liu M, Itkin-Ansari P, Arvan P, and Kaufman RJ. PDIA1/P4HB is required for efficient proinsulin maturation and β cell health in response to diet induced obesity. *Elife* 8: e44528, 2019.
62. Jasuja R, Furie B, and Furie BC. Endothelium-derived but not platelet-derived protein disulfide isomerase is required for thrombus formation in vivo. *Blood* 116: 4665–4674, 2010.
63. Jasuja R, Passam FH, Kennedy DR, Kim SH, van Hessem L, Lin L, Bowley SR, Joshi SS, Dilks JR, Furie B, Furie BC, and Flaumenhaft R. Protein disulfide isomerase inhibitors constitute a new class of antithrombotic agents. *J Clin Invest* 122: 2104–2113, 2012.
64. Jing X, Yang F, Shao C, Wei K, Xie M, Shen H, and Shu Y. Role of hypoxia in cancer therapy by regulating the tumor microenvironment. *Mol Cancer* 18: 157, 2019.
65. Joseph P, Leong D, McKee M, Anand SS, Schwalm JD, Teo K, Mente A, and Yusuf S. Reducing the global burden of cardiovascular disease, part 1: the epidemiology and risk factors. *Circ Res* 121: 677–694, 2017.
66. Kajiwara T, Tanaka T, Kukita K, Kutomi G, Saito K, Okuya K, Takaya A, Kochin V, Kanaseki T, Tsukahara T, Hirohashi Y, Torigoe T, Hirata K, Sato N, and Tamura Y. Hypoxia augments MHC class I antigen presentation via facilitation of ERO1-alpha-mediated oxidative folding in murine tumor cells. *Eur J Immunol* 46: 2842–2851, 2016.

67. Kalia LV and Lang AE. Parkinson's disease. *Lancet* 386: 896–912, 2015.
68. Kam KY, Yu SJ, Jeong N, Hong JH, Jalin AM, Lee S, Choi YW, Lee CK, and Kang SG. p-Hydroxybenzyl alcohol prevents brain injury and behavioral impairment by activating Nrf2, PDI, and neurotrophic factor genes in a rat model of brain ischemia. *Mol Cells* 31: 209–215, 2011.
69. Kamarehei M, Pejman S, Kaboudanian Ardestani S, Zahednasab H, Firouzi M, and Harirchian MH. Inhibition of protein disulfide isomerase has neuroprotective effects in a mouse model of experimental autoimmune encephalomyelitis. *Int Immunopharmacol* 82: 106286, 2020.
70. Kanemura S, Sofia EF, Hirai N, Okumura M, Kadokura H, and Inaba K. Characterization of the endoplasmic reticulum-resident peroxidases GPx7 and GPx8 shows the higher oxidative activity of GPx7 and its linkage to oxidative protein folding. *J Biol Chem* 295: 12772–12785, 2020.
71. Kaplan A, Gaschler MM, Dunn DE, Colligan R, Brown LM, Palmer AG, 3rd, Lo DC, and Stockwell BR. Small molecule-induced oxidation of protein disulfide isomerase is neuroprotective. *Proc Natl Acad Sci U S A* 112: E2245–E2252, 2015.
72. Kaplan A and Stockwell BR. Structural elucidation of a small molecule inhibitor of protein disulfide isomerase. *ACS Med Chem Lett* 6: 966–971, 2015.
73. Kerimi A and Williamson G. Differential impact of flavonoids on redox modulation, bioenergetics, and cell signaling in normal and tumor cells: a comprehensive review. *Antioxid Redox Signal* 29: 1633–1659, 2018.
74. Khan MM, Simizu S, Lai NS, Kawatani M, Shimizu T, and Osada H. Discovery of a small molecule PDI inhibitor that inhibits reduction of HIV-1 envelope glycoprotein gp120. *ACS Chem Biol* 6: 245–251, 2011.
75. Khodagholi F, Digaleh H, Motamedi F, Foolad F, and Shaerzadeh F. Nitric oxide and protein disulfide isomerase explain the complexities of unfolded protein response following intra-hippocampal abeta injection. *Cell Mol Neurobiol* 36: 873–881, 2016.
76. Khodier C, VerPlank L, Nag PP, Pu J, Wurst J, Pilyugina T, Dockendorff C, Galinski CN, Scalise AA, Passam F, van Hessem L, Dilks J, Kennedy DR, Flaumenhaft R, Palmer MAJ, Dandapani S, Munoz B, and Schrieber SL. Identification of ML359 as a small molecule inhibitor of protein disulfide isomerase. In: *Probe Reports from the NIH Molecular Libraries Program*. Bethesda, MD, 2010.
77. Khoo C, Yang J, Rajpal G, Wang Y, Liu J, Arvan P, and Stoffers DA. Endoplasmic reticulum oxidoreductin-1-like beta (ERO1beta) regulates susceptibility to endoplasmic reticulum stress and is induced by insulin flux in beta-cells. *Endocrinology* 152: 2599–2608, 2011.
78. Kim K, Hahn E, Li J, Holbrook LM, Sasikumar P, Stanley RG, Ushio-Fukai M, Gibbins JM, and Cho J. Platelet protein disulfide isomerase is required for thrombus formation but not essential for hemostasis in mice. *Blood* 122: 1052–1061, 2013.
79. Kim K, Li J, Tseng A, Andrews RK, and Cho J. NOX2 is critical for heterotypic neutrophil-platelet interactions during vascular inflammation. *Blood* 126: 1952–1964, 2015.
80. Kim Y and Chang KO. Protein disulfide isomerases as potential therapeutic targets for influenza A and B viruses. *Virus Res* 247: 26–33, 2018.
81. Kiouptsi K, Finger S, Garlapati VS, Knorr M, Brandt M, Walter U, Wenzel P, and Reinhardt C. Hypoxia evokes increased PDI and PDIA6 expression in the infarcted myocardium of ex-germ-free and conventionally raised mice. *Biol Open* 8: bio038851, 2019.
82. Klossowski S, Muchowicz A, Firczuk M, Swiech M, Redzej A, Golab J, and Ostaszewski R. Studies toward novel peptidomimetic inhibitors of thioredoxin-thioredoxin reductase system. *J Med Chem* 55: 55–67, 2012.
83. Kothari H, Nayak RC, Rao LV, and Pendurthi UR. Cystine 186-cystine 209 disulfide bond is not essential for the procoagulant activity of tissue factor or for its de-encryption. *Blood* 115: 4273–4283, 2010.
84. Kranz P, Neumann F, Wolf A, Classen F, Pompsch M, Ocklenburg T, Baumann J, Janke K, Baumann M, Goepelt K, Riffkin H, Metzen E, and Brockmeier U. PDI is an essential redox-sensitive activator of PERK during the unfolded protein response (UPR). *Cell Death Dis* 8: e2986, 2017.
85. Kukita K, Tamura Y, Tanaka T, Kajiwara T, Kutomi G, Saito K, Okuya K, Takaya A, Kanaseki T, Tsukahara T, Hirohashi Y, Torigoe T, Furuhashi T, Hirata K, and Sato N. Cancer-associated oxidase ERO1-alpha regulates the expression of MHC Class I molecule via oxidative folding. *J Immunol* 194: 4988–4996, 2015.
86. Kutomi G, Tamura Y, Tanaka T, Kajiwara T, Kukita K, Ohmura T, Shima H, Takamaru T, Satomi F, Suzuki Y, Torigoe T, Sato N, and Hirata K. Human endoplasmic reticulum oxidoreductin 1-alpha is a novel predictor for poor prognosis of breast cancer. *Cancer Sci* 104: 1091–1096, 2013.
87. Kyani A, Tamura S, Yang S, Shergalis A, Samanta S, Kuang Y, Ljungman M, and Neamati N. Discovery and mechanistic elucidation of a class of protein disulfide isomerase inhibitors for the treatment of glioblastoma. *ChemMedChem* 13: 164–177, 2018.
88. Lahav J, Jurk K, Hess O, Barnes MJ, Farndale RW, Luboshitz J, and Kehrel BE. Sustained integrin ligation involves extracellular free sulfhydryls and enzymatically catalyzed disulfide exchange. *Blood* 100: 2472–2478, 2002.
89. Lahav J, Wijnen EM, Hess O, Hamaia SW, Griffiths D, Makris M, Knight CG, Essex DW, and Farndale RW. Enzymatically catalyzed disulfide exchange is required for platelet adhesion to collagen via integrin alpha2beta1. *Blood* 102: 2085–2092, 2003.
90. LaMantia M, Miura T, Tachikawa H, Kaplan HA, Lennarz WJ, and Mizunaga T. Glycosylation site binding protein and protein disulfide isomerase are identical and essential for cell viability in yeast. *Proc Natl Acad Sci U S A* 88: 4453–4457, 1991.
91. Langer F, Spath B, Fischer C, Stolz M, Ayuk FA, Kroger N, Bokemeyer C, and Ruf W. Rapid activation of monocyte tissue factor by antithymocyte globulin is dependent on complement and protein disulfide isomerase. *Blood* 121: 2324–2335, 2013.
92. Lehtonen S, Jaronen M, Vehvilainen P, Lakso M, Rudgalvyte M, Keksa-Goldsteine V, Wong G, Courtney MJ, Koistinaho J, and Goldsteins G. Inhibition of excessive oxidative protein folding is protective in MPP(+) toxicity-induced Parkinson's disease models. *Antioxid Redox Signal* 25: 485–497, 2016.

93. Li G, Mongillo M, Chin KT, Harding H, Ron D, Marks AR, and Tabas I. Role of ERO1- α -mediated stimulation of inositol 1,4,5-triphosphate receptor activity in endoplasmic reticulum stress-induced apoptosis. *J Cell Biol* 186: 783–792, 2009.
94. Li J and Cho J. Ser/Thr protein kinase B β -NADPH oxidase 2 signaling in thromboinflammation. *Curr Opin Hematol* 24: 460–466, 2017.
95. Li J, Kim K, Barazia A, Tseng A, and Cho J. Platelet-neutrophil interactions under thromboinflammatory conditions. *Cell Mol Life Sci* 72: 2627–2643, 2015.
96. Li J, Kim K, Hahm E, Molokie R, Hay N, Gordeuk VR, Du X, and Cho J. Neutrophil AKT2 regulates heterotypic cell-cell interactions during vascular inflammation. *J Clin Invest* 124: 1483–1496, 2014.
97. Li J, Kim K, Jeong SY, Chiu J, Xiong B, Petukhov PA, Dai X, Li X, Andrews RK, Du X, Hogg PJ, and Cho J. Platelet protein disulfide isomerase promotes glycoprotein I α -mediated platelet-neutrophil interactions under thromboinflammatory conditions. *Circulation* 139: 1300–1319, 2019.
98. Li L, Liu J, Ding Y, Shi Z, Peng B, Yang N, Hong D, Zhang C, Yao C, Ge J, and Huang W. Design, synthesis and evaluation of protein disulfide isomerase inhibitors with nitric oxide releasing activity. *Bioorg Med Chem Lett* 30: 126898, 2020.
99. Libby P, Buring JE, Badimon L, Hansson GK, Deanfield J, Bittencourt MS, Tokgozoglu L, and Lewis EF. Atherosclerosis. *Nat Rev Dis Primers* 5: 56, 2019.
100. Lin L, Gopal S, Sharda A, Passam F, Bowley SR, Stopa J, Xue G, Yuan C, Furie BC, Flaumenhaft R, Huang M, and Furie B. Quercetin-3-rutinoside inhibits protein disulfide isomerase by binding to its b'x domain. *J Biol Chem* 290: 23543–23552, 2015.
101. Liu M, Li Y, Cavener D, and Arvan P. Proinsulin disulfide maturation and misfolding in the endoplasmic reticulum. *J Biol Chem* 280: 13209–13212, 2005.
102. Magalingam KB, Radhakrishnan A, and Haleagrahara N. Rutin, a bioflavonoid antioxidant protects rat pheochromocytoma (PC-12) cells against 6-hydroxydopamine (6-OHDA)-induced neurotoxicity. *Int J Mol Med* 32: 235–240, 2013.
103. Manuel AM, Walla MD, Faccenda A, Martin SL, Tanis RM, Piroli GG, Adam J, Kantor B, Mutus B, Townsend DM, and Frizzell N. Succination of protein disulfide isomerase links mitochondrial stress and endoplasmic reticulum stress in the adipocyte during diabetes. *Antioxid Redox Signal* 27: 1281–1296, 2017.
104. Masui S, Vavassori S, Fagioli C, Sitia R, and Inaba K. Molecular bases of cyclic and specific disulfide interchange between human ERO1 α protein and protein-disulfide isomerase (PDI). *J Biol Chem* 286: 16261–16271, 2011.
105. May D, Itin A, Gal O, Kalinski H, Feinstein E, and Keshet E. Ero1-L α plays a key role in a HIF-1-mediated pathway to improve disulfide bond formation and VEGF secretion under hypoxia: implication for cancer. *Oncogene* 24: 1011–1020, 2005.
106. McCully KS. Homocysteine and the pathogenesis of atherosclerosis. *Expert Rev Clin Pharmacol* 8: 211–219, 2015.
107. Mejzini R, Flynn LL, Pitout IL, Fletcher S, Wilton SD, and Akkari PA. ALS genetics, mechanisms, and therapeutics: where are we now? *Front Neurosci* 13: 1310, 2019.
108. Meng N, Peng N, Huang S, Wang SQ, Zhao J, Su L, Zhang Y, Zhang S, Zhao B, and Miao J. Heterogeneous nuclear ribonucleoprotein E1 regulates protein disulfide isomerase translation in oxidized low-density lipoprotein-activated endothelial cells. *Acta Physiol (Oxf)* 213: 664–675, 2015.
109. Moilanen A and Ruddock LW. Non-native proteins inhibit the ER oxidoreductin 1 (Ero1)-protein disulfide-isomerase relay when protein folding capacity is exceeded. *J Biol Chem* 295: 8647–8655, 2020.
110. This reference has been deleted.
111. Molteni SN, Fassio A, Ciriolo MR, Filomeni G, Pasqualeto E, Fagioli C, and Sitia R. Glutathione limits Ero1-dependent oxidation in the endoplasmic reticulum. *J Biol Chem* 279: 32667–32673, 2004.
112. Montane J, de Pablo S, Obach M, Cadavez L, Castano C, Alcarraz-Vizan G, Visa M, Rodriguez-Comas J, Parrizas M, Servitja JM, and Novials A. Protein disulfide isomerase ameliorates beta-cell dysfunction in pancreatic islets overexpressing human islet amyloid polypeptide. *Mol Cell Endocrinol* 420: 57–65, 2016.
113. Mortensen MB and Nordestgaard BG. Elevated LDL cholesterol and increased risk of myocardial infarction and atherosclerotic cardiovascular disease in individuals aged 70–100 years: a contemporary primary prevention cohort. *Lancet* 396: 1644–1652, 2020.
114. Mozzicafreddo M, Cuccioloni M, Bonfili L, Eleuteri AM, Fioretti E, and Angeletti M. Antiplasmin activity of natural occurring polyphenols. *Biochim Biophys Acta* 1784: 995–1001, 2008.
115. Muller C, Bandemer J, Vindis C, Camare C, Mucher E, Gueraud F, Larroque-Cardoso P, Bernis C, Auge N, Salvayre R, and Negre-Salvayre A. Protein disulfide isomerase modification and inhibition contribute to ER stress and apoptosis induced by oxidized low density lipoproteins. *Antioxid Redox Signal* 18: 731–742, 2013.
116. Muller-Calleja N, Ritter S, Hollerbach A, Falter T, Lackner KJ, and Ruf W. Complement C5 but not C3 is expendable for tissue factor activation by cofactor-independent antiphospholipid antibodies. *Blood Adv* 2: 979–986, 2018.
117. Nardai G, Stadler K, Papp E, Korcsmaros T, Jakus J, and Csermely P. Diabetic changes in the redox status of the microsomal protein folding machinery. *Biochem Biophys Res Commun* 334: 787–795, 2005.
118. Nguyen VD, Saaranen MJ, Karala AR, Lappi AK, Wang L, Raykhel IB, Alanen HI, Salo KE, Wang CC, and Ruddock LW. Two endoplasmic reticulum PDI peroxidases increase the efficiency of the use of peroxide during disulfide bond formation. *J Mol Biol* 406: 503–515, 2011.
119. Nguyen VD, Wallis K, Howard MJ, Haapalainen AM, Salo KE, Saaranen MJ, Sidhu A, Wierenga RK, Freedman RB, Ruddock LW, and Williamson RA. Alternative conformations of the x region of human protein disulfide-isomerase modulate exposure of the substrate binding b' domain. *J Mol Biol* 383: 1144–1155, 2008.
120. Nieswandt B, Pleines I, and Bender M. Platelet adhesion and activation mechanisms in arterial thrombosis and ischaemic stroke. *J Thromb Haemost* 9 Suppl 1: 92–104, 2011.
121. Niu Y, Zhang L, Yu J, Wang CC, and Wang L. Novel Roles of the Non-catalytic elements of yeast protein-disulfide

- isomerase in its interplay with endoplasmic reticulum oxidoreductin 1. *J Biol Chem* 291: 8283–8294, 2016.
122. Oh J, Riek AE, Weng S, Petty M, Kim D, Colonna M, Cella M, and Bernal-Mizrachi C. Endoplasmic reticulum stress controls M2 macrophage differentiation and foam cell formation. *J Biol Chem* 287: 11629–11641, 2012.
 123. Ozcelik D. Treatment of neuroblastoma cells with inhibitors of protein disulfide isomerase upregulates NQO1 activity. *Chem Res Toxicol* 33: 2099–2107, 2020.
 124. Ozcelik D and Pezacki JP. Small molecule inhibition of protein disulfide isomerase in neuroblastoma cells induces an oxidative stress response and apoptosis pathways. *ACS Chem Neurosci* 10: 4068–4075, 2019.
 125. Ozcelik D, Seto A, Rakic B, Farzam A, Supek F, and Pezacki JP. Gene expression profiling of endoplasmic reticulum stress in hepatitis C virus-containing cells treated with an inhibitor of protein disulfide isomerases. *ACS Omega* 3: 17227–17235, 2018.
 126. Pagani M, Fabbri M, Benedetti C, Fassio A, Pilati S, Bulleid NJ, Cabibbo A, and Sitia R. Endoplasmic reticulum oxidoreductin 1-beta (ERO1-Lbeta), a human gene induced in the course of the unfolded protein response. *J Biol Chem* 275: 23685–23692, 2000.
 127. Parakh S, Shadfar S, Perri ER, Ragagnin AMG, Piattoni CV, Fogolin MB, Yuan KC, Shahheydari H, Don EK, Thomas CJ, Hong Y, Comini MA, Laird AS, Spencer DM, and Atkin JD. The redox activity of protein disulfide isomerase inhibits ALS phenotypes in cellular and zebrafish models. *iScience* 23: 101097, 2020.
 128. Patton WF, Erdjument-Bromage H, Marks AR, Tempst P, and Taubman MB. Components of the protein synthesis and folding machinery are induced in vascular smooth muscle cells by hypertrophic and hyperplastic agents. Identification by comparative protein phenotyping and microsequencing. *J Biol Chem* 270: 21404–21410, 1995.
 129. Pendurthi UR, Ghosh S, Mandal SK, and Rao LV. Tissue factor activation: is disulfide bond switching a regulatory mechanism? *Blood* 110: 3900–3908, 2007.
 130. Perri ER, Thomas CJ, Parakh S, Spencer DM, and Atkin JD. The unfolded protein response and the role of protein disulfide isomerase in neurodegeneration. *Front Cell Dev Biol* 3: 80, 2015.
 131. Pescatore LA, Bonatto D, Forti FL, Sadok A, Kovacic H, and Laurindo FR. Protein disulfide isomerase is required for platelet-derived growth factor-induced vascular smooth muscle cell migration, Nox1 NADPH oxidase expression, and RhoGTPase activation. *J Biol Chem* 287: 29290–29300, 2012.
 132. Pihlajaniemi T, Helaakoski T, Tasanen K, Myllyla R, Huhtala ML, Koivu J, and Kivirikko KI. Molecular cloning of the beta-subunit of human prolyl 4-hydroxylase. This subunit and protein disulphide isomerase are products of the same gene. *EMBO J* 6: 643–649, 1987.
 133. Ping S, Liu S, Zhou Y, Li Z, Li Y, Liu K, Bardeesi AS, Wang L, Chen J, Deng L, Wang J, Wang H, Chen D, Zhang Z, Sheng P, and Li C. Protein disulfide isomerase-mediated apoptosis and proliferation of vascular smooth muscle cells induced by mechanical stress and advanced glycosylation end products result in diabetic mouse vein graft atherosclerosis. *Cell Death Dis* 8: e2818, 2017.
 134. Pollard MG, Travers KJ, and Weissman JS. Ero1p: a novel and ubiquitous protein with an essential role in oxidative protein folding in the endoplasmic reticulum. *Mol Cell* 1: 171–182, 1998.
 135. Poone GK, Hasseldam H, Munkholm N, Rasmussen RS, Gronberg NV, and Johansen FF. The hypothermic influence on CHOP and Ero1-alpha in an endoplasmic reticulum stress model of cerebral ischemia. *Brain Sci* 5: 178–187, 2015.
 136. Popielarski M, Ponamarczuk H, Stasiak M, Watala C, and Swiatkowska M. Modifications of disulfide bonds in breast cancer cell migration and invasiveness. *Am J Cancer Res* 9: 1554–1582, 2019.
 137. Qin RR, Zhu H, Wang F, Song M, Lin PL, Xing YQ, Zhang W, Zhong M, and Wang ZH. Platelet activation in diabetic mice models: the role of vascular endothelial cell-derived protein disulfide isomerase-mediated GP IIb/IIIa receptor activation. *Aging (Albany NY)* 11: 6358–6370, 2019.
 138. Rajpal G, Schuiki I, Liu M, Volchuk A, and Arvan P. Action of protein disulfide isomerase on proinsulin exit from endoplasmic reticulum of pancreatic beta-cells. *J Biol Chem* 287: 43–47, 2012.
 139. Ramming T, Hansen HG, Nagata K, Ellgaard L, and Appenzeller-Herzog C. GPx8 peroxidase prevents leakage of H₂O₂ from the endoplasmic reticulum. *Free Radic Biol Med* 70: 106–116, 2014.
 140. Raturi A, Miersch S, Hudson JW, and Mutus B. Platelet microparticle-associated protein disulfide isomerase promotes platelet aggregation and inactivates insulin. *Biochim Biophys Acta* 1778: 2790–2796, 2008.
 141. Reinhardt C, von Bruhl ML, Manukyan D, Grahl L, Lorenz M, Altmann B, Dlugai S, Hess S, Konrad I, Orschiedt L, Mackman N, Ruddock L, Massberg S, and Engelmann B. Protein disulfide isomerase acts as an injury response signal that enhances fibrin generation via tissue factor activation. *J Clin Invest* 118: 1110–1122, 2008.
 142. Ren L, You T, Li Q, Chen G, Liu Z, Zhao X, Wang Y, Wang L, Wu Y, Tang C, and Zhu L. Molecular docking-assisted screening reveals tannic acid as a natural protein disulphide isomerase inhibitor with antiplatelet and antithrombotic activities. *J Cell Mol Med*, 24:14257–14269, 2020.
 143. Rojas P, Ramirez AI, Fernandez-Albarral JA, Lopez-Cuenca I, Salobrar-Garcia E, Cadena M, Elvira-Hurtado L, Salazar JJ, de Hoz R, and Ramirez JM. Amyotrophic lateral sclerosis: a neurodegenerative motor neuron disease with ocular involvement. *Front Neurosci* 14: 566858, 2020.
 144. Ron D and Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* 8: 519–529, 2007.
 145. Rosenbauer F and Tenen DG. Transcription factors in myeloid development: balancing differentiation with transformation. *Nat Rev Immunol* 7: 105–117, 2007.
 146. Rotelli AE, Guardia T, Juarez AO, de la Rocha NE, and Pelzer LE. Comparative study of flavonoids in experimental models of inflammation. *Pharmacol Res* 48: 601–606, 2003.
 147. Ryser HJ, Levy EM, Mandel R, and DiSciullo GJ. Inhibition of human immunodeficiency virus infection by agents that interfere with thiol-disulfide interchange upon virus-receptor interaction. *Proc Natl Acad Sci U S A* 91: 4559–4563, 1994.
 148. Samanta S, Tamura S, Dubeau L, Mhaweck-Fauceglia P, Miyagi Y, Kato H, Lieberman R, Buckanovich RJ, Lin

- YG, and Neamati N. Expression of protein disulfide isomerase family members correlates with tumor progression and patient survival in ovarian cancer. *Oncotarget* 8: 103543–103556, 2017.
149. Sapra A and Bhandari P. Diabetes mellitus. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021.
150. Sato Y, Kojima R, Okumura M, Hagiwara M, Masui S, Maegawa K, Saiki M, Horibe T, Suzuki M, and Inaba K. Synergistic cooperation of PDI family members in peroxiredoxin 4-driven oxidative protein folding. *Sci Rep* 3: 2456, 2013.
151. Scheltens P, Blennow K, Breteler MM, de Strooper B, Frisoni GB, Salloway S, and Van der Flier WM. Alzheimer's disease. *Lancet* 388: 505–517, 2016.
152. Schwaller M, Wilkinson B, and Gilbert HF. Reduction-reoxidation cycles contribute to catalysis of disulfide isomerization by protein-disulfide isomerase. *J Biol Chem* 278: 7154–7159, 2003.
153. Severino A, Campioni M, Straino S, Salloum FN, Schmidt N, Herbrand U, Frede S, Toietta G, Di Rocco G, Bussani R, Silvestri F, Piro M, Liuzzo G, Biasucci LM, Mellone P, Feroce F, Capogrossi M, Baldi F, Fandrey J, Ehrmann M, Crea F, Abbate A, and Baldi A. Identification of protein disulfide isomerase as a cardiomyocyte survival factor in ischemic cardiomyopathy. *J Am Coll Cardiol* 50: 1029–1037, 2007.
154. Shergalis A, Xue D, Gharbia FZ, Driks H, Shrestha B, Tanweer A, Cromer K, Ljungman M, and Neamati N. Characterization of aminobenzylphenols as protein disulfide isomerase inhibitors in glioblastoma cell lines. *J Med Chem* 63: 10263–10286, 2020.
155. Sriram A, Bohlen J, and Teleman AA. Translation acrobatics: how cancer cells exploit alternate modes of translational initiation. *EMBO Rep* 19: e45947, 2018.
156. Stegner D, Klaus V, and Nieswandt B. Platelets as modulators of cerebral ischemia/reperfusion injury. *Front Immunol* 10: 2505, 2019.
157. Stevens H and McFadyen JD. Platelets as central actors in thrombosis-reprising an old role and defining a new character. *Semin Thromb Hemost* 45: 802–809, 2019.
158. Subramaniam S, Jurk K, Hobohm L, Jackel S, Saffarzadeh M, Schwierczek K, Wenzel P, Langer F, Reinhardt C, and Ruf W. Distinct contributions of complement factors to platelet activation and fibrin formation in venous thrombus development. *Blood* 129: 2291–2302, 2017.
159. Swiatkowska M, Padula G, Michalec L, Stasiak M, Skurzynski S, and Cierniewski CS. Ero1alpha is expressed on blood platelets in association with protein-disulfide isomerase and contributes to redox-controlled remodeling of alphaIIb beta3. *J Biol Chem* 285: 29874–29883, 2010.
160. Takaguri A, Kubo T, Mori M, and Satoh K. The protective role of YAP1 on ER stress-induced cell death in vascular smooth muscle cells. *Eur J Pharmacol* 815: 470–477, 2017.
161. Takei N, Yoneda A, Kosaka M, Sakai-Sawada K, and Tamura Y. Ero1alpha is a novel endogenous marker of hypoxia in human cancer cell lines. *BMC Cancer* 19: 510, 2019.
162. Tanaka S, Uehara T, and Nomura Y. Up-regulation of protein-disulfide isomerase in response to hypoxia/brain ischemia and its protective effect against apoptotic cell death. *J Biol Chem* 275: 10388–10393, 2000.
163. Tanaka T, Kajiwara T, Torigoe T, Okamoto Y, Sato N, and Tamura Y. Cancer-associated oxidoreductase Ero1-alpha drives the production of tumor-promoting myeloid-derived suppressor cells via oxidative protein folding. *J Immunol* 194: 2004–2010, 2015.
164. Tanaka T, Kutomi G, Kajiwara T, Kukita K, Kochin V, Kanaseki T, Tsukahara T, Hirohashi Y, Torigoe T, Okamoto Y, Hirata K, Sato N, and Tamura Y. Cancer-associated oxidoreductase Ero1-alpha promotes immune escape through up-regulation of PD-L1 in human breast cancer. *Oncotarget* 8: 24706–24718, 2017.
165. Tavender TJ and Bulleid NJ. Peroxiredoxin IV protects cells from oxidative stress by removing H2O2 produced during disulphide formation. *J Cell Sci* 123: 2672–2679, 2010.
166. Thalín C, Hisada Y, Lundström S, Mackman N, and Wallén H. Neutrophil extracellular traps: villains and targets in arterial, venous, and cancer-associated thrombosis. *Arterioscler Thromb Vasc Biol* 39: 1724–1738, 2019.
167. Thuerauf DJ, Marcinko M, Gude N, Rubio M, Sussman MA, and Glembotski CC. Activation of the unfolded protein response in infarcted mouse heart and hypoxic cultured cardiac myocytes. *Circ Res* 99: 275–282, 2006.
168. Tian F, Zhou X, Wikstrom J, Karlsson H, Sjöland H, Gan LM, Boren J, and Akyurek LM. Protein disulfide isomerase increases in myocardial endothelial cells in mice exposed to chronic hypoxia: a stimulatory role in angiogenesis. *Am J Physiol Heart Circ Physiol* 297: H1078–H1086, 2009.
169. Tian G, Xiang S, Noiva R, Lennarz WJ, and Schindelin H. The crystal structure of yeast protein disulfide isomerase suggests cooperativity between its active sites. *Cell* 124: 61–73, 2006.
170. Tian R, Li SJ, Wang DL, Zhao Z, Liu Y, and He RQ. The acidic C-terminal domain stabilizes the chaperone function of protein disulfide isomerase. *J Biol Chem* 279: 48830–48835, 2004.
171. Toldo S, Boccellino M, Rinaldi B, Seropian IM, Mezzaroma E, Severino A, Quagliuolo L, Van Tassell BW, Marfella R, Paolisso G, Rossi F, Natarajan R, Voelkel N, Abbate A, Crea F, and Baldi A. Altered oxidoreductive state in the diabetic heart: loss of cardioprotection due to protein disulfide isomerase. *Mol Med* 17: 1012–1021, 2011.
172. Toldo S, Severino A, Abbate A, and Baldi A. The role of PDI as a survival factor in cardiomyocyte ischemia. *Methods Enzymol* 489: 47–65, 2011.
173. Trychta KA, Back S, Henderson MJ, and Harvey BK. KDEL receptors are differentially regulated to maintain the ER proteome under calcium deficiency. *Cell Rep* 25: 1829–1840.e6, 2018.
174. Tsuchiya Y, Saito M, Kadokura H, Miyazaki JI, Tashiro F, Imagawa Y, Iwawaki T, and Kohno K. IRE1-XBP1 pathway regulates oxidative proinsulin folding in pancreatic beta cells. *J Cell Biol* 217: 1287–1301, 2018.
175. Uehara T, Nakamura T, Yao D, Shi ZQ, Gu Z, Ma Y, Masliah E, Nomura Y, and Lipton SA. S-nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration. *Nature* 441: 513–517, 2006.
176. Vatolin S, Phillips JG, Jha BK, Govindgari S, Hu J, Grabowski D, Parker Y, Lindner DJ, Zhong F, Distelhorst CW, Smith MR, Cotta C, Xu Y, Chilakala S, Kuang RR, Tall S, and Reu FJ. Novel protein disulfide isomerase inhibitor with anticancer activity in multiple myeloma. *Cancer Res* 76: 3340–3350, 2016.

177. Versteeg HH and Ruf W. Tissue factor coagulant function is enhanced by protein-disulfide isomerase independent of oxidoreductase activity. *J Biol Chem* 282: 25416–25424, 2007.
178. Vidal R, Caballero B, Couve A, and Hetz C. Converging pathways in the occurrence of endoplasmic reticulum (ER) stress in Huntington's disease. *Curr Mol Med* 11: 1–12, 2011.
179. Walker AK, Farg MA, Bye CR, McLean CA, Horne MK, and Atkin JD. Protein disulphide isomerase protects against protein aggregation and is S-nitrosylated in amyotrophic lateral sclerosis. *Brain* 133: 105–116, 2010.
180. Wang C, Chen S, Wang X, Wang L, Wallis AK, Freedman RB, and Wang CC. Plasticity of human protein disulfide isomerase: evidence for mobility around the X-linker region and its functional significance. *J Biol Chem* 285: 26788–26797, 2010.
181. Wang C, Li W, Ren J, Fang J, Ke H, Gong W, Feng W, and Wang CC. Structural insights into the redox-regulated dynamic conformations of human protein disulfide isomerase. *Antioxid Redox Signal* 19: 36–45, 2013.
182. Wang K, Liu JQ, Zhong T, Liu XL, Zeng Y, Qiao X, Xie T, Chen Y, Gao YY, Tang B, Li J, Zhou J, Pang DW, Chen J, Chen C, and Liang Y. Phase separation and cytotoxicity of tau are modulated by protein disulfide isomerase and S-nitrosylation of this molecular chaperone. *J Mol Biol* 432: 2141–2163, 2020.
183. Wang L, Li SJ, Sidhu A, Zhu L, Liang Y, Freedman RB, and Wang CC. Reconstitution of human Ero1-L α /protein-disulfide isomerase oxidative folding pathway in vitro. Position-dependent differences in role between the a and a' domains of protein-disulfide isomerase. *J Biol Chem* 284: 199–206, 2009.
184. Wang L, Zhang L, Niu Y, Sitia R, and Wang CC. Glutathione peroxidase 7 utilizes hydrogen peroxide generated by Ero1 α to promote oxidative protein folding. *Antioxid Redox Signal* 20: 545–556, 2014.
185. Wang P, Chen H, Qin H, Sankarapandi S, Becher MW, Wong PC, and Zweier JL. Overexpression of human copper, zinc-superoxide dismutase (SOD1) prevents postischemic injury. *Proc Natl Acad Sci U S A* 95: 4556–4560, 1998.
186. Wang ZV, Schraw TD, Kim JY, Khan T, Rajala MW, Follenzi A, and Scherer PE. Secretion of the adipocyte-specific secretory protein adiponectin critically depends on thiol-mediated protein retention. *Mol Cell Biol* 27: 3716–3731, 2007.
187. Weber DS, Taniyama Y, Rocic P, Seshiah PN, Dechert MA, Gerthoffer WT, and Griendling KK. Phosphoinositide-dependent kinase 1 and p21-activated protein kinase mediate reactive oxygen species-dependent regulation of platelet-derived growth factor-induced smooth muscle cell migration. *Circ Res* 94: 1219–1226, 2004.
188. Wei PC, Hsieh YH, Su MI, Jiang X, Hsu PH, Lo WT, Weng JY, Jeng YM, Wang JM, Chen PL, Chang YC, Lee KF, Tsai MD, Shew JY, and Lee WH. Loss of the oxidative stress sensor NPGPx compromises GRP78 chaperone activity and induces systemic disease. *Mol Cell* 48: 747–759, 2012.
189. Weitz JI and Chan NC. Novel antithrombotic strategies for treatment of venous thromboembolism. *Blood* 135: 351–359, 2020.
190. Wen PY, Li J, Lu BL, Liu J, Yang FZ, Zhou L, Luo H, Li WW, and Zhou J. Tanshinone IIA increases levels of NeuN, protein disulfide isomerase, and Na⁺/K⁺-ATPase and decreases evidence of microglial activation after cerebral ischemic injury. *Neuroreport* 27: 435–444, 2016.
191. Winton MJ, Igaz LM, Wong MM, Kwong LK, Trojanowski JQ, and Lee VM. Disturbance of nuclear and cytoplasmic TAR DNA-binding protein (TDP-43) induces disease-like redistribution, sequestration, and aggregate formation. *J Biol Chem* 283: 13302–13309, 2008.
192. Wise R, Duhachek-Muggy S, Qi Y, Zolkiewski M, and Zolkiewska A. Protein disulfide isomerases in the endoplasmic reticulum promote anchorage-independent growth of breast cancer cells. *Breast Cancer Res Treat* 157: 241–252, 2016.
193. Woehlbier U, Colombo A, Saaranen MJ, Perez V, Ojeda J, Bustos FJ, Andreu CI, Torres M, Valenzuela V, Medinas DB, Rozas P, Vidal RL, Lopez-Gonzalez R, Salameh J, Fernandez-Collemani S, Munoz N, Matus S, Armisen R, Sagredo A, Palma K, Irrazabal T, Almeida S, Gonzalez-Perez P, Campero M, Gao FB, Henny P, van Zundert B, Ruddock LW, Concha ML, Henriquez JP, Brown RH, and Hetz C. ALS-linked protein disulfide isomerase variants cause motor dysfunction. *EMBO J* 35: 845–865, 2016.
194. Wolf G. New insights into thiol-mediated regulation of adiponectin secretion. *Nutr Rev* 66: 642–645, 2008.
195. Won JK, Yu SJ, Hwang CY, Cho SH, Park SM, Kim K, Choi WM, Cho H, Cho EJ, Lee JH, Lee KB, Kim YJ, Suh KS, Jang JJ, Kim CY, Yoon JH, and Cho KH. Protein disulfide isomerase inhibition synergistically enhances the efficacy of sorafenib for hepatocellular carcinoma. *Hepatology* 66: 855–868, 2017.
196. Wright J, Birk J, Haataja L, Liu M, Ramming T, Weiss MA, Appenzeller-Herzog C, and Arvan P. Endoplasmic reticulum oxidoreductin-1 α (Ero1 α) improves folding and secretion of mutant proinsulin and limits mutant proinsulin-induced endoplasmic reticulum stress. *J Biol Chem* 288: 31010–31018, 2013.
197. Wu Y, Ahmad SS, Zhou J, Wang L, Cully MP, and Essex DW. The disulfide isomerase ERp57 mediates platelet aggregation, hemostasis, and thrombosis. *Blood* 119: 1737–1746, 2012.
198. Xu LR, Liu XL, Chen J, and Liang Y. Protein disulfide isomerase interacts with tau protein and inhibits its fibrillization. *PLoS One* 8: e76657, 2013.
199. Xu S, Butkevich AN, Yamada R, Zhou Y, Debnath B, Duncan R, Zandi E, Petasis NA, and Neamati N. Discovery of an orally active small-molecule irreversible inhibitor of protein disulfide isomerase for ovarian cancer treatment. *Proc Natl Acad Sci U S A* 109: 16348–16353, 2012.
200. Yang S, Yang C, Yu F, Ding W, Hu Y, Cheng F, Zhang F, Guan B, Wang X, Lu L, and Rao J. Endoplasmic reticulum resident oxidase ERO1-L α promotes hepatocellular carcinoma metastasis and angiogenesis through the S1PR1/STAT3/VEGF-A pathway. *Cell Death Dis* 9: 1105, 2018.
201. Yang X, Xu H, Hao Y, Zhao L, Cai X, Tian J, Zhang M, Han X, Ma S, Cao J, and Jiang Y. Endoplasmic reticulum oxidoreductin 1 α mediates hepatic endoplasmic reticulum stress in homocysteine-induced atherosclerosis. *Acta Biochim Biophys Sin (Shanghai)* 46: 902–910, 2014.
202. Yoshimori T, Semba T, Takemoto H, Akagi S, Yamamoto A, and Tashiro Y. Protein disulfide-isomerase in rat exocrine pancreatic cells is exported from the endoplasmic reticulum despite possessing the retention signal. *J Biol Chem* 265: 15984–15990, 1990.
203. Yu J, Li T, Liu Y, Wang X, Zhang J, Wang X, Shi G, Lou J, Wang L, Wang CC, and Wang L. Phosphorylation switches

protein disulfide isomerase activity to maintain proteostasis and attenuate ER stress. *EMBO J* 39: e103841, 2020.

204. Yu Y, Boing AN, Hau CM, Hajji N, Ruf W, Sturk A, and Nieuwland R. Tissue factor coagulant activity is regulated by the plasma membrane microenvironment. *Thromb Haemost* 118: 990–1000, 2018.

205. Zaorsky NG, Churilla TM, Egleston BL, Fisher SG, Ridge JA, Horwitz EM, and Meyer JE. Causes of death among cancer patients. *Ann Oncol* 28: 400–407, 2017.

206. Zhang J, Guo S, Wu Y, Zheng ZC, Wang Y, and Zhao Y. P4HB, a novel hypoxia target gene related to gastric cancer invasion and metastasis. *Biomed Res Int* 2019: 9749751, 2019.

207. Zhang J, Yang J, Lin C, Liu W, Huo Y, Yang M, Jiang SH, Sun Y, and Hua R. Endoplasmic reticulum stress-dependent expression of ERO1L promotes aerobic glycolysis in pancreatic cancer. *Theranostics* 10: 8400–8414, 2020.

208. Zhang L, Niu Y, Zhu L, Fang J, Wang X, Wang L, and Wang CC. Different interaction modes for protein-disulfide isomerase (PDI) as an efficient regulator and a specific substrate of endoplasmic reticulum oxidoreductin-1alpha (Ero1alpha). *J Biol Chem* 289: 31188–31199, 2014.

209. Zhang Y, Li T, Zhang L, Shanguan F, Shi G, Wu X, Cui Y, Wang X, Wang X, Liu Y, Lu B, Wei T, Wang CC, and Wang L. Targeting the functional interplay between endoplasmic reticulum oxidoreductin-1alpha and protein disulfide isomerase suppresses the progression of cervical cancer. *EBioMedicine* 41: 408–419, 2019.

210. Zhang Y, Liu R, Ni M, Gill P, and Lee AS. Cell surface relocalization of the endoplasmic reticulum chaperone and unfolded protein response regulator GRP78/BiP. *J Biol Chem* 285: 15065–15075, 2010.

211. Zhou J, May L, Liao P, Gross PL, and Weitz JI. Inferior vena cava ligation rapidly induces tissue factor expression and venous thrombosis in rats. *Arterioscler Thromb Vasc Biol* 29: 863–869, 2009.

212. Zhou J, Wu Y, Wang L, Rauova L, Hayes VM, Poncz M, and Essex DW. The C-terminal CGHC motif of protein disulfide isomerase supports thrombosis. *J Clin Invest* 125: 4391–4406, 2015.

213. Zhou X, Li G, Kaplan A, Gaschler MM, Zhang X, Hou Z, Jiang M, Zott R, Cremers S, Stockwell BR, and Duan W. Small molecule modulator of protein disulfide isomerase attenuates mutant huntingtin toxicity and inhibits endoplasmic reticulum stress in a mouse model of Huntington's disease. *Hum Mol Genet* 27: 1545–1555, 2018.

214. Zito E, Chin KT, Blais J, Harding HP, and Ron D. ERO1-beta, a pancreas-specific disulfide oxidase, promotes insulin biogenesis and glucose homeostasis. *J Cell Biol* 188: 821–832, 2010.

215. Zou H, Wen C, Peng Z, Shao Y, Hu L, Li S, Li C, and Zhou HH. P4HB and PDIA3 are associated with tumor progression and therapeutic outcome of diffuse gliomas. *Oncol Rep* 39: 501–510, 2018.

216. Zwicker JI, Schlechter BL, Stopa JD, Liebman H, Aggarwal A, Puligandla M, Caughey T, Bauer KA, Kuemmerle N, Wong E, Wun T, McLaughlin M, Hidalgo M, Neuberger D, Furie B, and Flaumenhaft R. Targeting protein disulfide isomerase with the flavonoid isoquercetin to improve hypercoagulability in advanced cancer. *JCI Insight* 4: e125851, 2019.

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Abbreviations Used

- 4-HNE = 4-hydroxynonenal
 AD = Alzheimer's disease
 ALS = amyotrophic lateral sclerosis
 AML = acute myeloid leukemia
 ATF6 = activating transcription factor 6
 C/EBP = CCAAT/enhancer-binding protein
 CHOP = C/EBP-homologous protein
 CKO = conditional knockout
 CVD = cardiovascular disease
 CXCL1/2 = chemokine CXC motif ligand 1/2
 Di-E-GSSG = diosin glutathione disulfide
 EC = endothelial cell
 ER = endoplasmic reticulum
 ERO1 = ER oxidoreductase 1
 FAD = flavin adenine dinucleotide
 GPIb α = glycoprotein Ib α
 GPx7 = GSH peroxidase 7
 GRP78 = glucose-regulated protein 78
 GSH = glutathione
 HD = Huntington's disease
 HIF-1 α = hypoxia-inducible factor-1 α
 IAPP = islet amyloid polypeptide
 LDL = low-density lipoprotein
 NADPH = nicotinamide adenine dinucleotide phosphate
 NO = nitric oxide
 NOX1 = NADPH oxidase 1
 P4HB = prolyl 4-hydroxylase subunit β
 PD = Parkinson's disease
 PDI = protein disulfide isomerase
 PD-L1 = programmed death-ligand 1
 PERK = protein kinase R-like endoplasmic reticulum kinase
 ROS = reactive oxygen species
 SOD1 = cytosolic superoxide dismutase 1
 TDP-43 = TAR DNA-binding protein 43
 TF = tissue factor
 UPR = unfolded protein response
 VEGF = vascular endothelial growth factor
 VSMC = vascular smooth muscle cell
 XBP-1 = X-box binding protein 1