Published in final edited form as:

Pharmacol Ther. 2018 January ; 181: 34–48. doi:10.1016/j.pharmthera.2017.07.011.

Multifunctional Molecule ERp57: From Cancer To Neurodegenerative Diseases

Aubryanna Hettinghouse^{a,*}, Ronghan Liu^{a,*}, and Chuan-ju Liu^{a,b,†}

^aDepartment of Orthopaedic Surgery, New York University Medical Center, New York, NY, 10003, USA

^bDepartment of Cell Biology, New York University School of Medicine, New York, NY 10016, USA

Abstract

The protein disulfide isomerase (PDI) gene family is a protein family classically characterized by endoplasmic reticulum (ER) localization and isomerase and redox activity. ERp57, a prominent multifunctional member of the PDI family, is detected at various levels in multiple cellular localizations outside of the ER. ERp57 has been functionally linked to a host of physiological processes and numerous studies have demonstrated altered expression and aberrant functionality of ERp57 in association with diverse pathological states. Here, we summarize available knowledge of ERp57's functions in subcellular compartments and the roles of dysregulated ERp57 in various diseases toward an emphasis on the potential utility of therapeutic development of ERp57.

Keywords

ERp57/PDIA3; clinical biomarker; cancer; neurodegeneration; musculoskeletal system; immune response

1. Introduction

The protein disulfide isomerase (*PDI*) gene family includes 21 genes; *PDI* genes belong to the thioredoxin (*TRX*) superfamily and each human PDI family member contains a TRX-like domain, which may be present in catalytically active or inactive forms (Freedman, et al., 1994; Galligan and Petersen, 2012; Hatahet and Ruddock, 2009). PDI proteins with active TRX-domains are generally localized to the endoplasmic reticulum (ER) where they mediate thiol-disulfide interchanges critical during post-translational protein folding (Galligan and Petersen, 2012). The first identified member and family namesake, PDI, was initially

[†]To whom correspondence should be addressed: Rm 1608, HJD, Department of Orthopaedic Surgery, New York University Medical Center, 301 East 17th Street, New York, NY 10003. Tel: 212-598-6103; Fax: 212-598-6096; chuanju.liu@med.nyu.edu.

*Authors contribute equally.

6. Conflict of Interest statement

The authors declare that there are no conflicts of interest

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

characterized through its role in redox signaling and isomerization of disulphide bonds; further study of PDI revealed additional functionality as a component of enzyme systems prolyl-4-hydroxylase and triacylglycerol transfer protein (Freedman, et al., 1994). As the PDI family has expanded, the study of additional members has revealed that PDI proteins comprise a diverse family with members varying in localization and functionality.

ERp57 (also known as PDIA3, ERp60, GRP58, and 1,25D3-MARRS; encoded by *PDIA3*) is a prominent member of the PDI family that has attracted significant attention from researchers. ERp57 was first detected as a stress-responsive protein with upregulated expression following glucose depletion-induced cellular stress (Lee, 1981). Originally erroneously identified as a phospholipase C (Bennett, et al., 1988), ERp57 was later demonstrated exhibit redox and protein disulfide isomerase activity (Bourdi, et al., 1995; Charnock-Jones, et al., 1996). ERp57, unlike most PDI family members, does not contain a C-terminal ER retention motif capable of effectively limiting localization to the ER lumen and ERp57 has been found in many different subcellular locations (Hatahet and Ruddock, 2009; Raykhel, et al., 2007).

ERp57 is expressed ubiquitously at an organ systems level but with variability in physiological expression levels within different tissues. Available evidence indicates multiple distinct functional roles of ERp57 under physiological and disease states. De-regulation of ERp57 has been implicated in multiple pathologies including neurodegenerative diseases (Altmann, et al., 2016; Gonzalez-Perez, et al., 2015; Hetz, et al., 2005; Nardo, et al., 2011; Tohda, et al., 2012; Torres, et al., 2015; Xu, et al., 2013), metabolic diseases (Dihazi, et al., 2013; Nardai, et al., 2005; Wilding, et al., 2015), musculoskeletal system conditions (Doroudi, et al., 2015; Wang, et al., 2010; Yang, et al., 2016), airway inflammation (Hoffman, et al., 2016), and cancer (Choe, et al., 2015; Gaucchi, et al., 2013; Wise, et al., 2016) and ERp57 expression level has been evaluated as a useful biomarker for diagnosis and/or prognosis in several conditions (Choe, et al., 2015; Leys, et al., 2007; Liao, et al., 2011; Nardo, et al., 2011; Torres, et al., 2015). This review aims to provide a summation of current knowledge relating to multiple functions of ERp57 in various cellular compartments, highlight the possible clinical applications of targeting ERp57, and provide perspectives on the studies currently necessary to further understanding of this protein.

2. The structure and localization of ERp57

TRX-like domains of the PDI proteins are present as catalytically active domains (a or a') or inactive domains (b or b'). Each domain contains a TRX-like fold with alternating α -helices and β -strands (Ferrari and Soling, 1999; Kozlov, et al., 2006; Silvennoinen, et al., 2004). ERp57 is structurally similar to PDI, containing four TRX-like domains (a-b-b'-a'; Figure 1), with matching redox active CGHC motifs and similar reduction potentials of the enzymes' dual catalytic domains (Hatahet and Ruddock, 2009; Kozlov, et al., 2006). The catalytically inactive central domains, b and b', have a vital role in the specific functionality of ERp57 in binding and protein folding.

Flanking the TRX-like domains of ERp57 are an N-terminal amino acid signal sequence and a C-terminal sequence. The N-terminal signal sequence directs initial ER localization while the C-terminus contains a QDEL ER retention/retrieval motif (Khanal and Nemere, 2007). ERp57 is classically considered an ER resident protein but also contains a nuclear location sequence and stimulation with various macrophage differentiation-inducing agents and cellular stressors is able to induce ERp57 transfer from cytoplasm to nucleus (Grillo, et al., 2006; Grindel, et al., 2011; Wu, et al., 2010). ERp57 has also been detected on cell surface (Khanal and Nemere, 2007), as well as in mitochondria (He, et al., 2014; Ozaki, et al., 2008). Proposed explanations for presentation of ERp57 outside of the ER include proteolysis of the retention sequence, formation of complexes that block recognition of the retention sequence, and saturation of the ER retention machinery (Turano, et al., 2002). Regardless of the mechanism(s) underlying ERp57's subcellular localization, it is clear that the enzyme's activity is not limited to those attributed to classical ER-resident proteins.

3. The biological functions of ERp57

The multiple functions and the binding partners of ERp57 in various cellular compartments are briefly summarized in Table 1 and Figure 2, and described in detail below.

In ER

The ER has multiple functions critical to cell survival and signaling including quality control during protein maturation as the site of sequester and modification of newly synthesized and misfolded proteins, steroid and phospholipid synthesis, maintenance of Ca^{2+} homeostasis, endoplasmic-reticulum-associated protein degradation (ERAD) and modulation of proliferation and apoptosis through the unfolded protein response (UPR). ERp57 participates in many of the functions carried out within this organelle. Implementation of murine loss-of-function models has powerfully demonstrated the significance of ERp57's function in the ER. Homozygous knockout of *PDIA3*, the gene that encodes ERp57, is embryonic lethal; heterozygous *PDIA3* knockouts are viable but exhibit increased ER stress and activation of the UPR leading to elevated ER-stress-induced apoptosis and inflammation (Linz, et al., 2015; Wang, et al., 2010).

ERp57 participates in protein folding through its protein disulfide isomerase activity in association with the P-domain of paralogous molecular chaperone molecules calreticulin and calnexin (CRT and CLNX respectively) (Bourdi, et al., 1995; Charnock-Jones, et al., 1996; Molinari and Helenius, 1999; Oliver, et al., 1999; Oliver, et al., 1997; Torres, et al., 2015; Wise, et al., 2016). CRT/CLNX assists in protein quality control by selectively binding misfolded proteins and preventing their secretion from the ER. Characterization of ERp57's b-b' domains has revealed the structure of ERp57's lectin P-domain binding site responsible for interaction between ERp57 and CRT/CLNX (Kozlov, et al., 2006). Lysine and arginine residues in the b' domain of ERp57, essential for interaction with CRT/CLNX, lend to an overall positive surface charge facilitatory to the binding of the negatively charged lectin P-domain; the b domain provides an additional surface-accessible lysine residue contributory to the stability of interaction (Kozlov, et al., 2006). The binding of CRT/CLNX and ERp57

brings CRT/CLNX-bound substrates in contact with the ERp57 active isomerase sites (Kozlov, et al., 2006).

ERp57 also plays an indirect but important role in infection and immune response as a component of the major histocompatibility complex (MHC) class I peptide-loading complex (PLC) (Lindquist, et al., 1998; Wearsch and Cresswell, 2008). ERp57 appears to serve a structural function in the PLC core through disulfide linkage of Cys57, the N-terminal residue in the catalytic motif of the ERp57 a domain, with Cys95 of tapasin; structural characterization has further revealed that non-covalent interactions at the a and a' active sites of ERp57 stabilize the heterodimer (Dick, et al., 2002; Dong, et al., 2009). Notably, however, the redox activity of ERp57 is not required for the interaction (Peaper and Cresswell, 2008). Significant reduction in stable interactions of MHC class I molecules with the loading complex and reduced presentation of antigens following targeted deletion of ERp57 in murine B cells illustrate the importance of ERp57 to MHC PLC formation and function (Garbi, et al., 2006). These structural and in vitro studies indicate that ERp57 indirectly participates in peptide-loading, MHC class I compound binding, and glycoprotein folding through association with tapasin and stabilization of the MHC class I PLC.

ERp57 has also been implicated in viral infection. Independent of CRT/CLNX binding, ERp57 isomerizes disulfide bonds of the Simian virus 40 (SV40) capsid proteins, which constitutes an initial uncoating of the virus preceding translocation to the cytosol (Schelhaas, et al., 2007). Similarly, ERp57 mediated isomerization of disulphide bonds has been proposed as a key step of viral infection by murine polyomavirus (Walczak, 2011).

Ca^{2+} is an integral second messenger that regulates a wide variety of cellular processes through rapid signal integration and response as well as changes to gene transcription on a longer time scale (Berridge, et al., 2003). As the primary internal storage site of calcium ions, ER Ca^{2+} homeostasis is integral to calcium signaling. At the ER membrane, ERp57 regulates ER Ca^{2+} flux through binding to the luminal domain of the Ca^{2+} sensor stromal interaction molecule 1 (STIM1) and consequent inhibition of store-operated Ca^{2+} entry (Prins, et al., 2011). Further, ERp57 negatively regulates the activity of sarco/endoplasmic reticulum Ca^{2+} -ATPase 2b (SERCA2b) through a mechanism dependent upon Ca^{2+} and the catalytic activity of the ERp57 active domains (Li and Camacho, 2004). The dissociation of ERp57 from SERCA2b upon internal calcium store depletion is accompanied by reduction of SERCA2b and increased activation of the pump. These interactions demonstrate ERp57's regulatory role in ER Ca^{2+} flux and homeostasis.

In sum, ERp57 has well-established functionality as a resident, oxidoreductive chaperone in the ER. ERp57 participates in protein folding mediated through interaction with CRT/CLNX; loss of ERp57 leads to activation of the UPR and ER-stress induced inflammation and cell death. Recently, studies have indicated that the physiological role of ERp57 in protein folding may be hijacked to facilitate viral infection in certain conditions. Further, through CRT-mediated interaction with SERCA2b and through binding STIM1, ERp57 functions in regulation of internal Ca^{2+} stores. ERp57 is also a vital structural component of the MHC class I PLC. These functions, tying ERp57 to multiple crucial processes within the ER, have been widely reported and are relatively well-understood; in other cellular locations,

the enzyme's redox and binding properties are thought to similarly drive functionality, however, the effects of ERp57 outside of the ER are not yet fully defined.

At the Membrane

The thiol-disulfide interchange capability of ERp57 also plays an important role in aspects of ERp57 function in membrane localities through establishment of inter- and intramolecular disulfide bonds. For example, ERp57 is upregulated during gamete maturation and is necessary for efficient fusion of sperm and egg; likely through thiol-disulfide exchange-mediated conformational activation of membrane proteins (Ellerman, et al., 2006; Liu, et al., 2015; Liu, et al., 2014). Protein:protein interactions that do not involve the catalytic sites of ERp57 are likewise important at the membrane. Complex formation between ERp57 and CRT on the cytosolic side of the plasma membrane is required for CRT translocation to the membrane; exposure of CRT on the cell membrane is a required for the immunogenic death observed in tumor cells (Obeid, 2008; Panaretakis, et al., 2008).

ERp57 also acts as a direct binding partner of proteins at the cell membrane to mediate signaling cascades in multiple cell types. Identification of ERp57 as a membrane-associated receptor for the active form of vitamin D3, 1 α , 25(OH)2D3 (also known as 1 α ,25-dihydroxyvitamin D3 or calcitriol) has implicated ERp57 in regulation of bone-related gene transcription and mineralization (Boyan, et al., 2007; Chen, et al., 2016), chondrocyte extracellular matrix (ECM) (Chen, et al., 2016; Linz, et al., 2015), protection from UV-induced damage (Sequeira, et al., 2012; Song, et al., 2013), and hormone induced cellular apoptosis and differentiation (Richard, et al., 2010). Similar to other steroidal hormones, 1 α , 25(OH) 2D3 is capable of regulating gene expression through binding to its nuclear receptor (vitamin D receptor; nVDR), but can also activate a fast response pathway through ERp57 interaction (Nemere, et al., 2012b). A recent study has isolated the a' domain of ERp57 is the probable primary domain involved in 1 α , 25(OH)2D3 binding (Gaucci, et al., 2016).

The rapid response pathway initiated through membrane associated ERp57: 1 α , 25(OH)2D3 binding is carried out through modulation of transmembrane ion transport and activation of several signal transduction cascades, the full physiological implications of which have not been conclusively characterized (Boyan, et al., 2007; Chen, et al., 2016). Upon binding to ERp57, 1 α , 25(OH)2D3-dependent activation of phospholipase A2 (PLA2) signaling stimulates protein kinase C (PKC) activation. ERp57 overexpression promotes 1 α , 25(OH)2D3-induced stimulation of PLA as evidenced by rapid prostaglandin E2 (PGE2) production (Boyan, et al., 2007). In turn, PGE2-mediated phospholipase C (PLC) activation advances inositol-trisphosphate (IP3) and diacylglycerol (DAG) release, triggering calcium release from the ER and activation of membrane associated PKC and phosphorylation of mitogen-activated protein kinases/extracellular signal-regulated kinases (MAPK/ERK). MAPK/ERK modulates gene expression; knockdown of ERp57 abrogates alterations in gene expression associated with the 1 α , 25(OH)2D3 rapid response pathway (Gaucci, et al., 2016).

Apart from directly activating a signal transduction cascade as a receptor, ERp57's binding to additional substrates indirectly regulates the activity of further signals originating at the membrane, including signal transducer and activator of transcription 3 (STAT3) (Guo, et al.,

2002; Sehgal, et al., 2002), epidermal growth factor (EGF) (Gauci, et al., 2013) and tumor necrosis factor α (TNF- α) signaling (Yang, et al., 2015; Yang, et al., 2016). The influx of Ca^{2+} ions and activation of PKC prompted through interaction between ERp57 and 1α , 25(OH)2D3 activates ADAM10 translocation to the cell surface where the metalloproteinase mediates ectodomain shedding of tumor necrosis factor receptor 1 (TNFR1). The consequent release of soluble TNFR1 restrains inflammatory TNF α signaling through reduction in receptor presentation at the membrane (Yang, et al., 2015).

STATs are cytoplasmic transcription factors responsible for transcriptional regulation of genes involved in proliferation, differentiation, immune response, apoptosis and oncogenesis when activated through binding to a membrane bound receptor (Calo, et al., 2003; Mui, 1999; Yu, et al., 2009). ERp57 associates with STAT3 plasma membrane rafts and cytosolic statosome complexes, hypothesized to constitute sites of assembly and signal integration for multiple STAT co-factors responsible for activation and shuttling STATs from the plasma membrane through the cytosol and to the nucleus (Guo, et al., 2002; Sehgal, et al., 2002). Although the function(s) of ERp57 in these plasma membrane lipid rafts and statosome complexes remains indefinite, the inhibition of cytokine activated STAT3 DNA-binding by addition of recombinant ERp57 indicates that ERp57 may be involved in inhibition of activated STAT3 signaling, an effect that could be exerted at the level of the plasma membrane (Sehgal, et al., 2002).

ERp57 impacts EGF through post-translational modification of membrane bound epidermal growth factor receptor (EGFR). EGFR activation is generally associated with anti-apoptotic signaling and de-regulation of EGFR correlates with tumorigenesis (Guo, et al., 2015; Herbst, 2004). Knockdown of ERp57 in cancer cells results in impaired phosphorylation of EGFR and increased internalization and degradation of the receptor (Gauci, et al., 2013). These observations suggest a role of ERp57 in EGFR endocytosis and activation, hypothesized to occur through ERp57's catalytic activity; however, the mechanism underlying ERp57's effect on EGF signaling remains incompletely described (Gauci, et al., 2013).

Maintenance of ionic homeostasis and signaling is critical for appropriate cellular responses and developmental processes. ERp57 has established involvement in the regulation of ionic oscillations across cellular membranes through indirect downstream effects as well as direct interactions with proton pumps. These interactions occur on the plasma membrane as well as the ER and mitochondrial membranes. In murine intestinal epithelial cells, enhanced calcium and phosphate uptake occurs upon binding of 1α , 25(OH)2D3 and ERp57 and plays a role in driving PKC and MAPK/ERK activation during membrane associated receptor mediated rapid response to 1α , 25(OH)2D3 (Nemere, et al., 2012a; Nemere, et al., 2012b; Wang, et al., 2010). ERp57 is highly expressed and colocalizes with H^+ , K^+ -ATPase α subunit in the apical canalicular membrane of human gastric parietal cells. ERp57 positively modulates basal activity of H^+ , K^+ -ATPase through a mechanism independent of isomerase activity, chaperoning function, or expression level effects (Fujii, et al., 2013).

An evolving understanding of manifold functionality for ERp57 in the endomembrane system is well attested by the literature. ERp57 interacts with a host of membrane proteins

directly and indirectly to affect transient signaling as well as gene transcription and expression. The full mechanistic details and functional implications of relationships between ERp57 and membrane associated binding partners have not been fully characterized and may be cell type and/or co-factor dependent; regardless, existing evidence distinguishes ERp57 as a critical participant in numerous signal transduction cascades initiated at the membrane.

In Mitochondria

Several laboratories have reported critical function of ERp57 in calcium flux mediated function and apoptotic signaling in mitochondria. Mitochondrial Ca^{2+} uptake is regulated, in part, by ERp57's stimulatory effect on mitochondrial calcium uniporter (MCU) transcription; however, transcription of regulatory components of the MCU complex are not responsive to knockdown of ERp57 and the exact mechanism of the ERp57:MCU relationship remains to be elucidated (He, et al., 2014). Further, ERp57 associates with mitochondrial μ -calpain, a calcium-dependent cysteine protease, to cleave and activate apoptosis-inducing factor (AIF) (Ozaki, et al., 2008).

In cytosol

Complex formation between ERp57 and CRT on the cytosolic side of the plasma membrane is required for CRT translocation to the membrane; exposure of CRT on the cell membrane is a required for the immunogenic death observed in tumor cells (Obeid, 2008; Panaretakis, et al., 2008). As briefly reviewed above, ERp57 is an accessory scaffolding protein of both membrane rafts and cytosolic statosome complexes involved in STAT3's movement from the cell membrane, through the cytoplasm, to the nuclear membrane (Guo, et al., 2002; Ndubuisi, et al., 1999; Sehgal, et al., 2002). Similarly, ERp57 has been shown to co-localize with additional transcription factors during translocation between the cytoplasm and nucleus, including nuclear factor kappa beta (NF- κ B) (Wu, et al., 2010), retinoic acid receptor (RAR- α) (Zhu, et al., 2010), mechanistic/mammalian target of rapamycin (mTOR) (Fingar and Blenis, 2004; Ramirez-Rangel, et al., 2011; Sarbassov and Sabatini, 2005), and tumor suppressor protein p53 (Green and Kroemer, 2009; Hussmann, et al., 2015).

ERp57 has been found co-localize with NF- κ B, a transcription factor heavily implicated in immunoresponsive signaling, in the cytoplasm and nucleus of leukemia cells stimulated to undergo monocyte/macrophage differentiation (Wu, et al., 2010). This finding suggests that cytosolic ERp57 may function as a chaperone through either direct interaction with NF- κ B or indirect interaction via conformational regulation within the NF- κ B complex (Wu, et al., 2010). Similarly, cytoplasmic co-localization and complex formation between ERp57, RAR- α and all-trans retinoic acid (ATRA) is required for nuclear appearance of RAR- α , a transcriptional regulator implicated in multiple developmental processes (Zhu, et al., 2010). Presumably, the thiol oxidoreductase activity of ERp57 at cysteine residues of RAR- α is critical for ATRA-RAR- α binding and subsequent nuclear import of the complex (Zhu, et al., 2010). ERp57 also promotes assembly and modifies the activity of mTOR complexes, showing preferential interaction with the mTOR complex 1 (mTORC1). A reduction in mTORC1 response to insulin in ERp57 knockdown cells indicates a role of ERp57 in upstream signal detection, probably through function in the redox-sensing mechanism of

mTOR (Ramirez-Rangel, et al., 2011; Sarbassov and Sabatini, 2005). ERp57 also interacts with cytoplasmic p53 via an indeterminate mechanism to inhibit p53-mediated stimulation of mitochondrial outer membrane permeabilization (MOMP) and consequent apoptosis (Fingar and Blenis, 2004; Green and Kroemer, 2009). These findings support a role of ERp57 in modulation of mTOR and p53 mediated processes including apoptosis, cellular proliferation, redox sensing, and protein production (Fingar and Blenis, 2004; Green and Kroemer, 2009).

These cytosolic functions of ERp57 are apparent under physiological conditions and during cellular stress in which ERp57's chaperone, redox sensing and protein folding modalities are critical to a variety of cellular processes and responses. Through cytosolic interactions, ERp57 directly and indirectly modulates the activity of multiple pathways involved in cellular stress response, cellular differentiation, autophagy, apoptosis, and immune response. Although we have reviewed only a subset of the interactions observed in the cytoplasm, it is clear that the effects of ERp57's presence in the cytoplasm are manifold.

In the nucleus

In addition to the C-terminal QEDL ER retention/retrieval sequence, ERp57 also contains a KKKK nuclear location sequence (Adikesavan, et al., 2008). The presence of ERp57 in the nuclear compartment was first reported by immunofluorescence in 1993 (Ohtani, et al., 1993) and has since been independently verified by multiple investigators. Stimulation of various mammalian cell lines with TNF- α , 1 α , 25(OH)2D3, or phorbol 12-myristate 13-acetate has been demonstrated to induce nuclear translocation of cytoplasmic and/or membrane localized ERp57 to the nucleus (Grindel, et al., 2011; Rohe, et al., 2005; Wu, et al., 2010). Nuclear ERp57 has been shown to directly interact with DNA, influence the activation and DNA binding of various transcription factors and protein complexes, and participate in the nuclear import and export of proteins (Aureli, et al., 2013; Coppari, et al., 2002; Grillo, et al., 2006).

In DNA binding, ERp57 targets to regulatory regions for genes involved in cellular adhesion, intracellular traffic, and cellular stress response (Aureli, et al., 2013; Chichiarelli, et al., 2007; Grillo, et al., 2006). The presence of nuclear ERp57 does affect the transcription of target regions; however, ERp57 does not bind to DNA with the affinity typical of a transcription factor (Aureli, et al., 2013). Importantly, ERp57 has been isolated as a component of several multiprotein nuclear complexes engaged in DNA binding which may dictate nuclear functionality. ERp57 associates with a nuclear protein complex that targets to modified DNA in cells with defective DNA mismatch repair (Krynetski, et al., 2003) wherein ERp57 is able to phosphorylate H2AX, indicating a role of ERp57 in early response to the appearance of double-strand breaks, potentially involved in targeting DNA repair machinery to sites of damage and/or activating apoptosis (Krynetskaia, et al., 2009; Podhorecka, et al., 2010). ERp57 is present in STAT3–DNA complexes and inhibition of ERp57 can reduce STAT3–DNA binding (Eufemi, et al., 2004). ERp57 is also capable of indirectly influencing gene expression by participating in redox-mediated activation of transcription factors. DNA repair enzyme and transcriptional regulator APE/Ref-1 associates with ERp57 in the nucleus and the putative functional implications of ERp57:APE/Ref-1

association include cooperative reduction of transcription factors including activator protein 1 (AP-1) and related downstream effects including protection against oxidative stress induced apoptosis (Grillo, et al., 2006). ERp57 also has negative regulatory effects on transcriptional activators exemplified through its reduction of E2A proteins, promoting heterodimerization of the transcription factor and thereby preventing DNA binding and transcription of B cell differentiation genes (Markus and Benezra, 1999). These studies suggest that stress-responsive ERp57 may function to affect transcription of genes relevant to cell survival and differentiation either through direct or indirect interaction with DNA targets and/or transcriptional regulators.

4. Associations of ERp57 Dysregulation and Pathologies

The evidence for importance of ERp57 in multiple regulatory pathways at the cellular and molecular levels, reviewed above, supports supposition that de-regulation of ERp57 could be a feature of multiple disease etiologies in which these pathways are disrupted. In fact, altered expression levels of ERp57 have been associated multiple diseases affecting diverse organ systems. Here, we have briefly reviewed the relationships between ERp57 physiological functions and proposed and/or evidenced dysfunction in disease (summarized in Table 2).

Cancer

ERp57 is variably dysregulated in many forms of cancer and aberrant ERp57 expression has been evaluated clinically as a prognostic marker; either upregulation or downregulation of ERp57 can correlate with poor prognosis, depending on the affected tissues (Celli and Jaiswal, 2003; Choe, et al., 2015; Chung, et al., 2013; Gaucchi, et al., 2013; Leys, et al., 2007; Liao, et al., 2011; Ogino, et al., 2003; Wise, et al., 2016; Zhu, et al., 2010; Zhu, et al., 2016). The association between bidirectional alteration in ERp57 expression and development and prognosis of many different cancers indicates potential involvement in the complex cellular and molecular processes underlying the etiological heterogeneity of cancer.

ERp57 has been evaluated mechanistically and as a therapeutic target in cancer progression and treatment and knockdown of ERp57 in various cancer cell lines instigates diverse alterations to the pathways involved in ERp57's physiological functions. ERp57 has been implicated as a potential target for therapeutic modulation of apoptotic signaling and metastasis via its indirect regulation of EGFR (Gaucchi, et al., 2013; Porter, et al., 2010), mediation of rapid response to $1\alpha, 25(\text{OH})_2\text{D}_3$ (Richard, et al., 2010), association with the MHC PLC (Santana-Codina, et al., 2013), binding CRT (Panaretakis, et al., 2008), activation of the UPR (Husmann, et al., 2015), mTOR signaling (Fingar and Blenis, 2004; Ramirez-Rangel, et al., 2011; Sarbassov and Sabatini, 2005), and direct DNA binding (Aureli, et al., 2013; Grillo, et al., 2006).

ERp57's receptor-ligand interactions, as well as its associations with protein complexes, have been implicated in cancer cell proliferation. Overexpression of ERp57 promotes proliferative signaling due, in part, to ERp57-mediated stabilization of mTORC1 and ERp57 may have a further role as a component of mTORC1's redox sensing machinery (Ramirez-Rangel, et al., 2011). EGFR is overexpressed in triple negative breast cancer cells and activation of EGFR is associated with anti-apoptotic signaling (Gaucchi, et al., 2013).

Knockdown of ERp57 in MDA-MB-231 cells and in cervical cancer cells results in impaired phosphorylation and increased degradation of the receptor (Gauci, et al., 2013; Porter, et al., 2010). Similarly, knockdown of ERp57 in MCF-7 breast cancer cells reveals increased cellular sensitivity to vitamin D-mediated anti-proliferative signaling (Richard, et al., 2010). The shared effects of ERp57 overexpression on cell survival via interactions with EGFR, 1 α , 25(OH)2D3, and mTOR- mediated proliferative signaling in multiple cancerous cell lines indicates that targeted depletion of ERp57 may be a candidate for expedient development of new therapies.

Study of correlations between ERp57 expression levels and prognoses has revealed that ERp57 also participates in disease progression and metastasis of multiple cancers. ERp57 is upregulated during anchorage-independent growth and metastasis of estrogen receptor negative cells and epithelial ovarian cancer cells (Wise, et al., 2016; Zhu, et al., 2016). ERp57 overexpression also coincides with underexpression of MHC class I molecules resulting in a negative effect upon MHC PLC stability and antigen presentation, a key event in breakdown of immune surveillance and tumor cell invasion (Garrido, et al., 1993; Marincola, et al., 1999; Santana-Codina, et al., 2013). Further, ERp57 is required for immunologic cell death through mediating exposure of CRT on the cell surface (Panaretakis, et al., 2008). In epithelial ovarian cancer, low expression of Dicer, an RNAase essential for RNA interference, is a marker of metastasis and poor prognosis and has been linked to increased ERp57 expression (Zhu, et al., 2016). Knockdown of ERp57 in Dicer-deficient cells is able to abrogate increased cellular proliferation and migration, presumably through loss of ERp57's downstream effects on STAT3 and Wnt signaling (Liao, et al., 2014; Xie, et al., 2004; Zhu, et al., 2016). These observations indicate that ERp57 may participate in changes to cancer cell adhesion, immunogenicity and invasion through multiple pathways.

ERp57 has been singled out as a marker of chemoresistance in several cancers (Choe, et al., 2015; Cicchillitti, et al., 2009; Hussmann, et al., 2015). The requirement of ERp57 for CRT presentation and efficient MHC PLC activity suggests that a defect in these functions could underlie the establishment of chemoresistent cell populations. ERp57's associations with various nuclear complexes have also been demonstrated to correlate with chemoresistance. In ovarian cancer cells, ERp57 complexes with and exerts conformational modification on β -actin and is associated with mitotic defects and the development of drug resistance (Cicchillitti, et al., 2010). Moreover, sensitization of radioresistant laryngeal cancer cells through inhibiting ERp57-STAT3 complex mediated activation of myeloid leukemia cell differentiation protein (Mcl-1) has been demonstrated (Choe, et al., 2015). Similarly, inhibition of ERp57 in HCT116 colon cancer cells has been shown to bolster ER-stress induced apoptosis with concomitant chemotherapy and irradiation treatment through p53-mediated PKR-like ERkinase (PERK) UPR activation. This study further revealed that ERp57 activates proliferative signaling via mTOR in colon and MDA-MB-231 breast cancer cells. Notably, however, basal-like breast cancer cells did not display increased apoptosis following ERp57 knockdown (Hussmann, et al., 2015). Accordingly, the involvement of ERp57 in the development of drug resistance warrants further study to more fully describe mechanisms and disease specificity.

ERp57 also engages in direct interactions with DNA at sequences compatible with regulatory function (Aureli, et al., 2013; Grillo, et al., 2006). Silencing of ERp57 in melanoma cells results in significantly downregulated expression of target genes involved in DNA repair and stress response (Aureli, et al., 2013). However, ERp57 does not bind to DNA with the affinity typical of a transcription factor and the roles of ERp57's DNA binding capability in transcriptional regulation are not entirely clear. Current evidence suggests that ERp57 takes part in the activation of transcription factors, including APE/Ref-1-ERp57 mediated activation of AP-1 (a gene implicated in protection against oxidative stress induced apoptosis) through participation in nuclear protein complexes. (Aureli, et al., 2013; Grillo, et al., 2006). The observations of ERp57-Ref-1 nuclear translocation following oxidative stress (Grillo, et al., 2006) and of APE/Ref-1's association with *MSH6*, a target of ERp57 producing a component of the DNA repair machinery, offer support for a role of ERp57-containing protein complexes in transcriptional regulation through cooperative activation of transcriptional regulators. A role of ERp57 in early response to DNA modification has also been unveiled through observation of ERp57-mediated phosphorylation of H2AX following chemotherapy, generating γ H2AX - a sensitive marker of genotoxicity (Chichiarelli, et al., 2007; Krynetski, et al., 2003). Accordingly, ERp57 may directly and indirectly participate in activation of cell survival, DNA repair, and/or pro-apoptotic signals through DNA binding and transcriptional modulation in conditions of oxidative stress and DNA damage.

The role of ERp57 in cancer is complex and disease dependent. ERp57 is expressed at levels outside of the physiological range in several cancers and targeted knockdown or overexpression of ERp57 in cancerous cell lines has a variable impact on many of pathways involved in apoptosis, proliferation, and motility. Further, ERp57 is an upstream mediator of key signaling pathways involved in tumorigenesis, invasion, cell proliferation, apoptosis etc. and has also been shown to modulate activity of pro-survival transcription factors in melanoma and cervical cancer cell lines. ERp57's value as a marker for disease, prognosis, and chemoresistance in various cancers may highlight the utility of ERp57 expression levels in evaluation and/or supplementation of therapeutic agents for cancer treatment. For example, upregulated expression of ERp57 has been reported in acute myeloid leukemia cells and treatment with shikonin, a plant extract with anticancer effects, results in downregulation of ERp57 and inhibition of ERp57 increases shikonin-induced apoptosis in primary and established human leukemia cell lines (Trivedi, et al., 2016). Collectively, ERp57 may be a powerful molecular target in the development of new cancer therapies. However, as indicated by the association between ERp57's varied expression levels and different prognoses in different carcinomas, targeted modulation of ERp57 can have contrasting effects upon various signaling pathways implicated in disease establishment and progression and these multi-level effects should be considered during therapeutic development.

Neurodegenerative diseases

Aggregation of misfolded proteins is a central feature in the pathology of many neurodegenerative diseases including frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's Disease

(HD) and Creutzfeldt–Jakob disease (CJD). ER stress and activation of the UPR upon accumulation of misfolded and/or nascent proteins are common features implicated in the cellular pathology of these diseases. However, the cumulative effect of UPR activation is dependent on the duration and integration of cell survival and apoptotic signals and the role of the UPR in neurodegeneration is complex; with evidence for both protective and detrimental effects of UPR activation commonly observed in the same disease. Notably, activation of the UPR features increased recruitment of PDI family members to facilitate protein folding or trigger apoptosis. An increasing number of studies have focused on the role of ERp57, revealing potential dual functionality as a protective and pro-apoptotic factor, in neurodegeneration. The identification of ERp57 as a progranulin (PGRN)-interacting protein (Almeida, et al., 2011) has drawn attention to a role of ERp57 in the mechanisms underlying the relationship between progranulin insufficiency and neurodegenerative and peripheral proteopathies including FTD, a common form of dementia that has been strongly linked to mutations in the PGRN gene (*GRN*) (Baker, et al., 2006; Cruys, et al., 2006; van der Zee, et al., 2007). ERp57 directly interacts with PGRN to regulate PGRN secretion and may be a powerful target for therapeutic modulation in conditions that feature PGRN deficiency (Almeida, et al., 2011). Further, Gaucher's disease (GD), a lysosomal storage disease with variable neurological involvement, has recently been associated with the appearance of several *GRN* variants and low serum levels of PGRN (Jian, et al., 2016a; Jian, et al., 2016b). Mechanistically, PGRN recruits HSP70 to form a complex which binds to and acts as a chaperone of lysosomal enzyme β -glucocerebrosidase (GCase), mutations of which are causative of GD, and impedes aggregation of mutated GCase (Jian, et al., 2016a). PGRN's ability to interact with and disaggregate lysosomal enzymes suggests that modulation of PGRN secretion through targeting ERp57 might also represent a new therapeutic strategy in lysosomal storage diseases.

ALS is characterized by degeneration of motor neurons in the brain and spinal cord (Kent-Braun, et al., 1998). Several gene variants have been isolated as contributory or causative factors in cases of familial and sporadic ALS and the genes most heavily implicated include *C9orf72* and superoxide dismutase 1 (*SOD1*); revealing a complex role of elevated ER stress response in the cellular pathology of ALS (Dafinca, et al., 2016; Nishitoh, et al., 2008; Wang, et al., 2011). Moreover, a recent screen for relevant mutations in PDIs among ALS patients revealed several missense mutations and single nucleotide polymorphisms (SNPs) in *PDIA3*/ERp57 potentially affecting CRT/CLNX binding (Gonzalez-Perez, et al., 2015). Longitudinal analysis of protein expression during ALS disease course reveals that ERp57 is strongly upregulated in the cerebrospinal fluid and peripheral blood of ALS patients and ERp57 level is highly correlated with disease severity (Atkin, et al., 2008; Nardo, et al., 2011). These findings suggest that ERp57 may be upregulated to combat disrupted protein folding in ALS and that sustained elevation and/or dysfunction of ERp57 may aggravate neuronal apoptosis.

ERp57 is also demonstrates a stimulatory effect on axonal regrowth in several neurodegenerative diseases. AD is a proteopathy in which extracellular aggregates of β -amyloid exert a cytotoxic effect on surrounding neurons. Under physiological conditions, ERp57 is believed to bind amyloid β ($A\beta$) through interaction with CRT and thereby prevent aggregation (Erickson, et al., 2005). Pharmacological stimulation of cortical and

hippocampal axonal regrowth in transgenic mice co-expressing multiple AD related mutations is dependent upon activation of ERp57 and activation of ERp57 results in an overall reduction of AD neuropathology and increased performance on object recognition tasks in these murine models (Tohda, et al., 2012). Potential evidence for ERp57's role in axonal regeneration has also been indicated in degenerative disease affecting the peripheral nervous system. Notably, peripheral neuropathy is variably reported as an aspect of PD and overexpression of ERp57 in transgenic mice facilitates peripheral nerve regeneration after injury (Castillo, et al., 2015).

Additional roles of ERp57 in aspects of neurodegeneration have been described. Autoantibodies against glial fibrillary acidic protein (GFAP), a protein that is upregulated during reactive gliosis in AD (Muramori, et al., 1998; Pike, et al., 1995) and glaucoma (Wang, et al., 2002; Wang, et al., 2000; Wilding, et al., 2015), have been shown to exert a protective effect against oxidative stress via cell-surface interaction with ERp57 (Wilding, et al., 2015). In CJD, upregulated ERp57 is believed to be a defensive response against misfolded prion protein toxicity (Hetz, et al., 2005). However, recent characterization of the role of ERp57 in mediating steady state levels of both wild-type and prion-related disorder (PrD)-related mutant protein has suggested that ERp57 deficiency may contribute to prion protein folding but may not contribute to protective effect against ER stress susceptibility (Sepulveda, et al., 2016; Torres, et al., 2015). Importantly, the ability of several molecules to inhibit mutant huntingtin protein mediated toxicity through inhibition of ERp57 and/or PDI highlights that PDIs can exert pro-apoptotic effects through stimulation of MOMP in neurodegenerative diseases that feature aberrant cytosolic protein aggregation (Hoffstrom, et al., 2010).

In summation, ERp57 has been proposed as an important mediator of cellular response to endoplasmic reticulum stress during the disease course of several neurodegenerative disorders. The conventional role of ERp57 as a disulfide isomerase and chaperone, as well as its role in signal transduction at the cell membrane, has been implicated in mediation of ERp57's protective effects. Upon ER stress, ERp57 may be upregulated as part of a protective response to facilitate folding of aggregated proteins and restore cellular proteostasis. At the same time, concentrated ERp57 is able to stimulate apoptosis through MOMP. Notably, ERp57 is a selective foldase responsible for interaction with only a subset of potential protein aggregates. Additional studies are required to more fully define the in vivo importance of ERp57's activity in response to ER stress as well as other subcellular localizations in neurodegenerative disease. ERp57 could prove a useful target for modulation of apoptosis in the nervous system.

Musculoskeletal System

Little is known about the direct involvement of ERp57 in the cellular pathology of musculoskeletal diseases. Notably however, ERp57's physiological role as a vitamin D receptor indicates a critical role in skeletal development. Further, ERp57's interactions with SERCA2b and indirect influence on TNFR1 signaling suggest that ERp57 activity could be therapeutically modulated in musculoskeletal disorders.

1 α , 25(OH) $_2$ D $_3$ is a key factor in musculoskeletal development and homeostasis through promotion of chondrocyte proliferation, maturation, hypertrophy and osteoblast differentiation (Enishi, et al., 2014; Linz, et al., 2015; Yang, et al., 2014). As reviewed above, ERp57 acts as a rapid response 1 α , 25(OH) $_2$ D $_3$ receptor. Heterozygous (*PDIA3*^{+/-}) *PDIA3* knockout mice exhibit abnormalities in bone morphometry and quality similar to those observed in mice deficient in nuclear steroid hormone vitamin D receptor (nVDR^{-/-}), the canonical 1 α , 25(OH) $_2$ D $_3$ receptor involved in transcriptional regulation during bone development (Wang, et al., 2014). Further, chondrocyte-specific *PDIA3* knockout mice exhibit increased ER stress and activation of the UPR leading to elevated ER-stress-induced apoptosis and inflammation (Linz, et al., 2015). Expression of ERp57 and nVDR are both believed to participate in osteogenic commitment and expression levels exhibit opposing changes during osteogenic differentiation in murine embryoid bodies, suggesting that differential modulation of 1 α , 25(OH) $_2$ D $_3$ receptors may work in concert and/or independently to dictate stage specific differentiation signals (Olivares-Navarrete, et al., 2012).

The ERp57:1 α , 25(OH) $_2$ D $_3$ interaction can further influence bone and cartilage development via additional mechanisms. In matrix vesicles of growth zone chondrocytes, ERp57: 1 α , 25(OH) $_2$ D $_3$ binding similarly results in activation of PLA2, consequent degradation of membrane integrity and release of matrix metalloproteinase 3 (MMP-3) and activation of TGF- β (Boyan, et al., 2012; Boyan, et al., 2007; Chen, et al., 2010). TGF- β is able to transcriptionally regulate ERp57 expression through the canonical mothers against decapentaplegic homolog 3 (SMAD3) signaling (Rohe, et al., 2007). A recent study has further revealed that ERp57 participates in mediation of crosstalk between bone morphogenetic protein-2 (BMP-2) and 1 α , 25(OH) $_2$ D $_3$ in directing mineralization and osteoblastic differentiation (Chen, et al., 2016). The association of ERp57 with TGF- β and BMP-2, vital pathways capable of coordinating signal integration during skeletal development (Chen, et al., 2012), suggests that the dependence of rapid response to 1 α , 25(OH) $_2$ D $_3$ upon ERp57 is may not be limited to the latter's function in initial signal transduction. Additionally, the observation of ERp57:1 α , 25(OH) $_2$ D $_3$ mediated regulation of Ca²⁺ in mammalian intestinal cells (Nemere, et al., 2012a; Nemere, et al., 2012b) raises the need for further research into a role of ERp57 in calcium metabolism and potential effects in bone. The relationship between ERp57, bioactive vitamin D, and activation of bone regulatory pathways supports additional study concerning the involvement of ERp57 in bone development, particularly in vitamin D-related disorders.

ERp57 may also indirectly restrain inflammatory TNF α signaling through release of soluble TNFR1. TNF α signaling is heavily implicated in numerous inflammatory disorders affecting multiple organs systems including bone and muscle. ERp57:1 α , 25(OH) $_2$ D $_3$ mediated activation Ca²⁺ influx triggers ADAM10 translocation to the cell surface where the metalloproteinase mediates cleavage of the extracellular portion of TNFR1. The release of soluble TNFR1 restrains inflammatory signaling through neutralization of TNF α and reduction in receptor presentation at the membrane (Yang, et al., 2015). Notably, TNFR2, a TNF α receptor demonstrated to mediate a protective pathway in several physiological and disease processes (Defer, et al., 2007; Dong, et al., 2016; Wei, et al., 2014), including musculoskeletal diseases (Tang, et al., 2011; Williams, et al., 2016; Zhao, et al., 2013), does

not undergo cleavage in this process (Yang, et al., 2015; Yang, et al., 2016). Several anti-TNF α therapies are used clinically for treatment of musculoskeletal diseases including rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis (Atzeni, et al., 2015; Braun, et al., 2006; Gorman, et al., 2002; Maksymowych, et al., 2012). However, TNF α inhibitors are expensive, not all patients appropriately respond to available anti-TNF α therapies and some approaches target both inflammatory TNFR1 and protective TNFR2 signaling (Van Hauwermeiren, et al., 2011; Wei, et al., 2016a). Accordingly, the development of alternative biologics is imperative and further study of ERp57 may present new targets for restraining inflammatory TNF α signaling. Importantly, our group has demonstrated effective amelioration of several musculoskeletal conditions via treatment with a non-oncogenic progranulin derivative, termed Atsttrin, capable of competitively inhibiting TNFR1:TNF α signaling coincident with targeted activation of TNFR2 (Tang, et al., 2011; Wei, et al., 2016b; Zhao, et al., 2015). Moreover, direct interaction between ERp57 and PGRN has been reported (Almeida, et al., 2011) and the overall significance of the PGRN:ERp57 in inflammatory signaling observed in musculoskeletal conditions remains to be thoroughly expounded. ERp57 represents another potentially powerful molecular target that could be co-opted to provide a new avenue for selective inhibition of inflammatory TNFR1 signaling while the interaction between PGRN and ERp57 could be exploited in stimulation of protective TNFR2 signaling.

Digestive system and accessory organs

The importance of ERp57's role as a modulator of ionic flux has been particularly attested within the digestive system. 1α , 25(OH)2D3:ERp57-mediated regulation of ion pump activity has been implicated as a key regulator of calcium and phosphate flux in intestinal cells in response to vitamin D stimulation (Nemere, et al., 2012a; Nemere, et al., 2015; Prins, et al., 2011). In murine intestinal cells, Ca²⁺ transport initiated upon 1α , 25(OH)2D3:ERp57 binding is dependent upon downstream activation of the PKA pathway while phosphate uptake is believed to be partially dependent on PKA activation in mammalian cells (Nemere, et al., 2012b). Calcium uptake is a major mediator of cellular and subcellular signaling and calcium balance is necessary for the maintenance of multiple systems. The participation of ERp57 in 1α , 25(OH)2D3-induced Ca²⁺ movement and downstream effects suggests that ERp57 may have some role in maintaining calcium homeostasis however more research is warranted to determine the overall importance of ERp57-mediated rapid response to intestinal calcium absorption and calcium metabolism.

ERp57 has also been proposed to modulate basal activity of H⁺, K⁺-ATPase (Fujii, et al., 2013). H⁺, K⁺-ATPase activity in the stomach regulates acid secretion H⁺, K⁺ ATPase is therapeutically targeted by protein pump inhibitors used in the treatment of various acid diseases (Sachs, et al., 2007). Potassium-chloride cotransporter 4 (KCC4), another ion pump implicated in gastric acid secretion, is co-expressed with and K⁺–Cl[–] cotransport via KCC4 has been functionally linked to H⁺, K⁺-ATPase activity (Fujii, et al., 2013; Fujii, et al., 2009) ERp57's potential role in the modulation of KCC4 has not been elucidated. ERp57 also stimulates sodium uptake through interaction with the cytoplasmic C-terminal domain of plasma membrane of the Na⁺-Cl[–] cotransporter (NCC) in mammalian kidney cells and

ERp57 deficiency co-occurs with reduced sodium uptake, demonstrating functional significance (Wyse, et al., 2002).

ERp57 has been implicated in models of several metabolic diseases including diabetes (Feng, et al., 2009; Nardai, et al., 2003; Yamamoto, et al., 2014), nonalcoholic fatty liver disease (NAFLD) (Zhang, et al., 2015), and obesity (Bravo, et al., 2011). Knockdown of ERp57 is associated with increased autophagic failure-induced cell death in pancreatic β cells (Yamamoto, et al., 2014). Elevated ERp57 secretion is strongly associated with ECM production in animal models of renal fibrosis and inhibition of ERp57 impairs accumulation of ECM (Dihazi, et al., 2013). Altered redox state distributions have been reported in streptozotocin-induced diabetic rats, which exhibit a reductive shift in and downregulated function of ERp57 (Nardai, et al., 2003); knockdown of *EIF2AK3*, encoding PERK, in a rat β cell model of permanent neonatal diabetes is also associated with altered redox state of ERp57, defective insulin secretion, and reduced proliferation (Feng, et al., 2009). In NAFLD, ERp57 serves a protective role against fatty acid induced steatosis and apoptosis (Zhang, et al., 2015). Further, in fasting rats gastric mucosa protein level of ERp57 is downregulated and is predominately dephosphorylated; this redox shift could be inhibited by leptin, a hunger-inhibiting hormone associated with obesity, and leptin could induce gastric mucosa ERp57 mRNA expression (Bravo, et al., 2011). Leptin activity is at least partially dependent on STAT3 activation and ERp57 is believed to regulate STAT3 (Choe, et al., 2015; Guo, et al., 2002; Ndubuisi, et al., 1999; Villanueva and Myers, 2008). Interestingly, inducible knockout of ERp57 in mice correlates with reduced body fat, coincident with an extended lifespan exhibiting some sexual dimorphism, relative to wild-type littermates (Nemere, et al., 2015). The effect of ERp57 upon lifespan and body fat homeostasis may be, at least in part, attributable to estradiol mediated competitive inhibition of 1α , 25(OH)2D3:ERp57 binding leading to lipid droplet accumulation in particular cellular environments of wild-type mammals, however, the estradiol:ERp57 interaction requires further study (Nemere, et al., 2015). These studies demonstrate that ERp57 redox state may be differentially altered during ER stress associated with metabolic diseases affecting the digestive system accessory organs. More broadly, ERp57 may have a role in energy modulation and nutrient availability, clarification of which is necessary to better understand the comprehensive role of ER proteins in metabolic disease.

Respiratory system

Pathological features of allergic asthma, including inflammation, airway fibrosis, and airway hyper-responsiveness, are in part attributable to allergen-induced upregulation of protein synthesis contributory to activation of the UPR and ER stress induced cell death (Gregory and Lloyd, 2011; Hoffman, et al., 2013; Lambrecht and Hammad, 2012; Ryu, et al., 2013; Tabas and Ron, 2011). ERp57 and activating transcription factor 6 (ATF6), an ER stress transducer (Hetz, 2012), are upregulated upon *in vitro* stimulation of human bronchiolar and nasal epithelial cells with house dust mite (HDM), a common allergen (Hoffman, et al., 2013). Knockdown of ATF6 in these cells alleviates allergen-induced ERp57 upregulation and cell death associated with HDM exposure (Hoffman, et al., 2016). Knockdown of ERp57 in asthmatic mice is correlated to reduced ER stress in response to allergen challenge (Hoffman, et al., 2013). A recent study employing targeted deletion of ERp57 in lung

epithelial cells has further demonstrated diminished immune response upon HDM stimulation coincident with a reduction in multiple relevant inflammatory T cell activators, diminished functionality of pro-fibrotic growth factors, and reduced disulfide bridges and oligomerization of chemotactic proteins and apoptotic factors, respectively (Hoffman, et al., 2016). These findings provide strong evidence for the involvement of ER stress-dependent responses in at least some asthmatic pathologies and that ERp57 is heavily involved in regulation of allergen response. Accordingly, ERp57 may be a useful target in the development of new biologics for treating allergic asthma.

ERp57 has also recently been revealed as a potential facilitator of *Burkholderia cenocepacia*, a pathogen connected to poor prognosis of cystic fibrosis, infection and adhesion (Pacello, et al., 2016). In this study, ERp57-depleted epithelial cells were more resistant to bacterial infection, ERp57 antibody reduced adhesion and infection, and inhibition of ERp57 was associated with reduced inflammatory response in infected cells (Pacello, et al., 2016). Notably, however, the mechanism of *Burkholderia* infection is not fully understood and may be strain specific. Regardless, ERp57 inhibition may present a promising avenue for preventing or treating pathogenic lung infection.

Other diseases and conditions

Platelets express number of PDI family proteins including PDI, ERp57, ERp72, and ERp29 (Holbrook, et al., 2010). ERp57 is involved in regulation of normal platelet aggregation, integrin activation and signaling (Essex and Li, 1999; Lahav, et al., 2002). Upon vascular injury, ERp57 is secreted and localizes to the platelet thrombus to recruit platelets and facilitate fibrinogen binding and fibrin deposition (Cui, et al., 2015; Zhou, et al., 2014). The overlap in expression patterns and potential functional redundancy of PDI proteins regarding platelet function suggests that targeting one or more of these proteins could provide useful intervention in pathological thrombosis. The roles of PDIs in platelet function must be further elucidated in order to identify the optimal PDI target for therapeutic modulation, however, recent evaluation of a ERp57-inhibitor has generated promising results (Cui, et al., 2015).

5. Conclusions and perspectives

An increasing number of studies have demonstrated that ERp57 is a multifunctional protein implicated in numerous physiological processes and disease states. ERp57's unique structure, multiple subcellular localizations, and ubiquitous but variable and specific expression patterns in different tissues, each vitally contribute to the complexity of ERp57's physiologic functionality. Accordingly, alteration in ERp57 through structural and/or regulatory modification may promote development of various diseases. In fact, available literature has linked dysregulation of ERp57 expression to developmental abnormalities of bone and cartilage, cancer, neurodegeneration, asthma, bacterial infection, and metabolic disorders; recent work has also indicated the ability of viral appropriation of ERp57's isomerase functionality during infection. Currently, the mechanisms underlying ERp57's associations with numerous pathological states are not fully understood and additional work

is required to optimize the extensive therapeutic potential of targeting ERp57. However, ERp57 is clearly represents a promising target in translational research.

Acknowledgments

We apologize to the colleagues whose publications are not included due to the space limitation. Studies in C.J. Liu laboratory were supported partly by NIH research grants R01AR062207, R01AR061484, and a DOD research grant W81XWH-16-1-0482.

Abbreviations

1α,25(OH)2D3	1,25-dihydroxyvitamin D3
ADAM10	a disintegrin and metalloproteinase 10
AIF	apoptosis-inducing factor
AP-1	activator protein 1
ATRA	all-trans retinoic acid
BMP-2	bone morphogenic protein 2
CLNX	calnexin
CRT	calreticulin
ECM	extracellular matrix
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ER	endoplasmic reticulum
ERp57	endoplasmic reticulum protein 57
GFAP	glial fibrillary acidic protein
HDM	house dust mite
KCC4	potassium-chloride cotransporter 4
p53	tumor suppressor p53
MAPK/ERK	mitogen-activated protein kinases/extracellular signal-regulated kinases
MCU	mitochondrial calcium uniporter
MHC	major histocompatibility complex
MOMP	mitochondrial outer membrane permeabilization
mTOR	mechanistic target of rapamycin

mTORc1	mTOR complex 1
NCC	sodium-chloride cotransporter
NF-κB	nuclear factor kappa beta
nVDR	nuclear vitamin D receptor
PDI	protein disulfide isomerase
PDIA3	protein disulfide-isomerase A3
PKC	protein kinase C
PLA2	phospholipase A2
PLC	peptide loading complex
RAR-α	retinoic acid receptor
SECRA2b	sarco/endoplasmic reticulum Ca ²⁺ -ATPase 2b
STAT3	signal transducer and activator of transcription 3
STIM1	sensor stromal interaction molecule 1
TGF-β	transforming growth factor β
TNFR1	tumor necrosis factor receptor 1
TNFR2	tumor necrosis factor receptor 2
TNFα	tumor necrosis factor α
TRX	thioredoxin
UPR	unfolded protein response

References Cited

- Adikesavan AK, Unni E, Jaiswal AK. Overlapping signal sequences control nuclear localization and endoplasmic reticulum retention of GRP58. *Biochem Biophys Res Commun.* 2008; 377:407–412. [PubMed: 18851953]
- Almeida S, Zhou L, Gao FB. Progranulin, a Glycoprotein Deficient in Frontotemporal Dementia, Is a Novel Substrate of Several Protein Disulfide Isomerase Family Proteins. *PLoS One.* 2011; 6:e26454. [PubMed: 22028881]
- Altmann C, Hardt S, Fischer C, Heidler J, Lim HY, Häussler A, et al. Progranulin overexpression in sensory neurons attenuates neuropathic pain in mice: Role of autophagy. *Neurobiology of Disease.* 2016; 96:294–311. [PubMed: 27629805]
- Atkin JD, Farg MA, Walker AK, McLean C, Tomas D, Horne MK. Endoplasmic reticulum stress and induction of the unfolded protein response in human sporadic amyotrophic lateral sclerosis. *Neurobiol Dis.* 2008; 30:400–407. [PubMed: 18440237]
- Atzeni F, Gianturco L, Talotta R, Varisco V, Ditto MC, Turiel M, et al. Investigating the potential side effects of anti-TNF therapy for rheumatoid arthritis: cause for concern? *Immunotherapy.* 2015; 7:353–361. [PubMed: 25917627]

- Aureli C, Gaucci E, Arcangeli V, Grillo C, Eufemi M, Chichiarelli S. ERp57/PDIA3 binds specific DNA fragments in a melanoma cell line. *Gene*. 2013; 524:390–395. [PubMed: 23587917]
- Baker M, Mackenzie IR, Pickering-Brown SM, Gass J, Rademakers R, Lindholm C, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome. *Nature*. 2006; 17:442.
- Bennett CF, Balcarek JM, Varrichio A, Crooke ST. Molecular cloning and complete aminoacid sequence of form-I phosphoinositide-specific phospholipase C. *Nature*. 1988; 334:268–270. [PubMed: 3398923]
- Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol*. 2003; 4:517–529. [PubMed: 12838335]
- Bourdi M, Demady D, Martin JL, Jabbour SK, Martin BM, George JW, et al. cDNA cloning and baculovirus expression of the human liver endoplasmic reticulum P58: characterization as a protein disulfide isomerase isoform, but not as a protease or a carnitine acyltransferase. *Arch Biochem Biophys*. 1995; 323:397–403. [PubMed: 7487104]
- Boyan BD, Chen J, Schwartz Z. Mechanism of Pdia3-dependent 1 α ,25-dihydroxy vitamin D3 signaling in musculoskeletal cells. *Steroids*. 2012; 77:892–896. [PubMed: 22569272]
- Boyan BD, Wong KL, Fang M, Schwartz Z. 1 α ,25(OH) $_2$ D $_3$ is an autocrine regulator of extracellular matrix turnover and growth factor release via ERp60 activated matrix vesicle metalloproteinases. *J Steroid Biochem Mol Biol*. 2007; 103:467–472. [PubMed: 17224270]
- Braun J, Davis J, Dougados M, Sieper J, van der Linden S, van der Heijde D. First update of the international ASAS consensus statement for the use of anti-TNF agents in patients with ankylosing spondylitis. *Annals of the Rheumatic Diseases*. 2006; 65:316–320. [PubMed: 16096329]
- Bravo SB, Caminos JE, Gonzalez CR, Vazquez MJ, Garces MF, Cepeda LA, et al. Leptin and fasting regulate rat gastric glucose-regulated protein 58. *Int J Pept*. 2011; 2011:969818. [PubMed: 22121381]
- Calo V, Migliavacca M, Bazan V, Macaluso M, Buscemi M, Gebbia N, et al. STAT proteins: from normal control of cellular events to tumorigenesis. *J Cell Physiol*. 2003; 197:157–168. [PubMed: 14502555]
- Castillo V, Onate M, Woehlbier U, Rozas P, Andreu C, Medinas D, et al. Functional Role of the Disulfide Isomerase ERp57 in Axonal Regeneration. *PLoS One*. 2015; 10:e0136620. [PubMed: 26361352]
- Celli CM, Jaiswal AK. Role of GRP58 in mitomycin C-induced DNA cross-linking. *Cancer Res*. 2003; 63:6016–6025. [PubMed: 14522930]
- Charnock-Jones DS, Day K, Smith SK. Cloning, expression and genomic organization of human placental protein disulfide isomerase (previously identified as phospholipase C α). *Int J Biochem Cell Biol*. 1996; 28:81–89. [PubMed: 8624847]
- Chen G, Deng C, Li YP. TGF- β and BMP signaling in osteoblast differentiation and bone formation. *Int J Biol Sci*. 2012; 8:272–288. [PubMed: 22298955]
- Chen J, Dosier CR, Park JH, De S, Guldborg RE, Boyan BD, et al. Mineralization of three-dimensional osteoblast cultures is enhanced by the interaction of 1 α ,25-dihydroxyvitamin D $_3$ and BMP2 via two specific vitamin D receptors. *Journal Of Tissue Engineering And Regenerative Medicine*. 2016; 10:40–51. [PubMed: 23784946]
- Chen J, Olivares-Navarrete R, Wang Y, Herman TR, Boyan BD, Schwartz Z. Protein-disulfide Isomerase-associated 3 (Pdia3) Mediates the Membrane Response to 1,25-Dihydroxyvitamin D(3) in Osteoblasts. *J Biol Chem*. 2010; 285:37041–37050. [PubMed: 20843786]
- Chichiarelli S, Ferraro A, Altieri F, Eufemi M, Coppari S, Grillo C, et al. The stress protein ERp57/GRP58 binds specific DNA sequences in HeLa cells. *J Cell Physiol*. 2007; 210:343–351. [PubMed: 17061245]
- Choe MH, Min JW, Jeon HB, Cho DH, Oh JS, Lee HG, et al. ERp57 modulates STAT3 activity in radioresistant laryngeal cancer cells and serves as a prognostic marker for laryngeal cancer. *Oncotarget*. 2015; 6:2654–2666. [PubMed: 25605256]
- Chung H, Cho H, Perry C, Song J, Ylaya K, Lee H, et al. Downregulation of ERp57 expression is associated with poor prognosis in early-stage cervical cancer. *Biomarkers*. 2013; 18:573–579. [PubMed: 23957851]

- Cicchillitti L, Della Corte A, Di Michele M, Donati MB, Rotilio D, Scambia G. Characterisation of a multimeric protein complex associated with ERp57 within the nucleus in paclitaxel-sensitive and -resistant epithelial ovarian cancer cells: The involvement of specific conformational states of β -actin. *International Journal of Oncology*. 2010; 37:445–454. [PubMed: 20596672]
- Cicchillitti L, Di Michele M, Urbani A, Ferlini C, Donat MB, Scambia G, et al. Comparative proteomic analysis of paclitaxel sensitive A2780 epithelial ovarian cancer cell line and its resistant counterpart A2780TC1 by 2D-DIGE: the role of ERp57. *J Proteome Res*. 2009; 8:1902–1912. [PubMed: 19714814]
- Coppari S, Altieri F, Ferraro A, Chichiarelli S, Eufemi M, Turano C. Nuclear localization and DNA interaction of protein disulfide isomerase ERp57 in mammalian cells. *J Cell Biochem*. 2002; 85:325–333. [PubMed: 11948688]
- Cruts M, Gijselink I, van der Zee J, Engelborghs S, Wils H, Pirici D, et al. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature*. 2006:442.
- Cui G, Shan L, Guo L, Chu IK, Li G, Quan Q, et al. Novel anti-thrombotic agent for modulation of protein disulfide isomerase family member ERp57 for prophylactic therapy. *Sci Rep*. 2015; 5:10353. [PubMed: 26037049]
- Dafinca R, Scaber J, Ababneh N, Lalic T, Weir G, Christian H, et al. C9orf72 Hexanucleotide Expansions Are Associated with Altered Endoplasmic Reticulum Calcium Homeostasis and Stress Granule Formation in Induced Pluripotent Stem Cell-Derived Neurons from Patients with Amyotrophic Lateral Sclerosis and Frontotemporal Dementia. *Stem Cells*. 2016; 34:2063–2078. [PubMed: 27097283]
- Defer N, Azroyan A, Pecker F, Pavoiné C. TNFR1 and TNFR2 signaling interplay in cardiac myocytes. *J Biol Chem*. 2007; 282:35564–35573. [PubMed: 17913704]
- Dick TP, Bangia N, Peaper DR, Cresswell P. Disulfide bond isomerization and the assembly of MHC class I-peptide complexes. *Immunity*. 2002; 16:87–98. [PubMed: 11825568]
- Dihazi H, Dihazi GH, Bibi A, Eltoweissy M, Mueller CA, Asif AR, et al. Secretion of ERP57 is important for extracellular matrix accumulation and progression of renal fibrosis, and is an early sign of disease onset. *J Cell Sci*. 2013; 126:3649–3663. [PubMed: 23781031]
- Dong G, Wearsch PA, Peaper DR, Cresswell P, Reinisch KM. Insights into MHC class I peptide loading from the structure of the tapasin-ERp57 thiol oxidoreductase heterodimer. *Immunity*. 2009; 30:21–32. [PubMed: 19119025]
- Dong Y, Fischer R, Naude PJ, Maier O, Nyakas C, Duffey M, et al. Essential protective role of tumor necrosis factor receptor 2 in neurodegeneration. *Proc Natl Acad Sci U S A*. 2016; 113:12304–12309. [PubMed: 27791020]
- Doroudi M, Plaisance MC, Boyan BD, Schwartz Z. Membrane actions of 1 α ,25(OH) $_2$ D $_3$ are mediated by Ca(2+)/calmodulin-dependent protein kinase II in bone and cartilage cells. *J Steroid Biochem Mol Biol*. 2015; 145:65–74. [PubMed: 25263660]
- Ellerman DA, Myles DG, Primakoff P. A role for sperm surface protein disulfide isomerase activity in gamete fusion: evidence for the participation of ERp57. *Dev Cell*. 2006; 10:831–837. [PubMed: 16740484]
- Enishi T, Yukata K, Takahashi M, Sato R, Sairyo K, Yasui N. Hypertrophic chondrocytes in the rabbit growth plate can proliferate and differentiate into osteogenic cells when capillary invasion is interposed by a membrane filter. *PLoS One*. 2014; 9:e104638. [PubMed: 25121501]
- Erickson RR, Dunning LM, Olson DA, Cohen SJ, Davis AT, Wood WG, et al. In cerebrospinal fluid ER chaperones ERp57 and calreticulin bind beta-amyloid. *Biochem Biophys Res Commun*. 2005; 332:50–57. [PubMed: 15896298]
- Essex DW, Li M. Protein disulphide isomerase mediates platelet aggregation and secretion. *Br J Haematol*. 1999; 104:448–454. [PubMed: 10086777]
- Eufemi M, Coppari S, Altieri F, Grillo C, Ferraro A, Turano C. ERp57 is present in STAT3-DNA complexes. *Biochem Biophys Res Commun*. 2004; 323:1306–1312. [PubMed: 15451439]
- Feng D, Wei J, Gupta S, McGrath BC, Cavener DR. Acute ablation of PERK results in ER dysfunctions followed by reduced insulin secretion and cell proliferation. *BMC Cell Biol*. 2009; 10:61. [PubMed: 19732428]

- Ferrari DM, Soling HD. The protein disulphide-isomerase family: unravelling a string of folds. *Biochem J.* 1999; 339(Pt 1):1–10. [PubMed: 10085220]
- Fingar DC, Blenis J. Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. *Oncogene.* 2004; 23:3151–3171. [PubMed: 15094765]
- Freedman RB, Hirst TR, Tuite MF. Protein disulphide isomerase: building bridges in protein folding. *Trends Biochem Sci.* 1994; 19:331–336. [PubMed: 7940678]
- Fujii T, Awaka SY, Takahashi Y, Fujita K, Tsuji H, Shimizu T, et al. Modulation of H(+), K(+)-ATPase activity by the molecular chaperone ERp57 highly expressed in gastric parietal cells. *FEBS Lett.* 2013; 587:3898–3905. [PubMed: 24188822]
- Fujii T, Takahashi Y, Ikari A, Morii M, Tabuchi Y, Tsukada K, et al. Functional association between K⁺-Cl⁻ cotransporter-4 and H⁺, K⁺-ATPase in the apical canalicular membrane of gastric parietal cells. *J Biol Chem.* 2009; 284:619–629. [PubMed: 18984587]
- Galligan JJ, Petersen DR. The human protein disulfide isomerase gene family. *Hum Genomics.* 2012; 6:6. [PubMed: 23245351]
- Garbi N, Tanaka S, Momburg F, Hammerling GJ. Impaired assembly of the major histocompatibility complex class I peptide-loading complex in mice deficient in the oxidoreductase ERp57. *Nat Immunol.* 2006; 7:93–102. [PubMed: 16311600]
- Garrido F, Cabrera T, Concha A, Glew S, Ruiz-Cabello F, Stern PL. Natural history of HLA expression during tumour development. *Immunology Today.* 1993; 14:491–499. [PubMed: 8274189]
- Gauci E, Altieri F, Turano C, Chichiarelli S. The protein ERp57 contributes to EGF receptor signaling and internalization in MDA-MB-468 breast cancer cells. *J Cell Biochem.* 2013; 114:2461–2470. [PubMed: 23696074]
- Gauci E, Raimondo D, Grillo C, Cervoni L, Altieri F, Nittari G, et al. Analysis of the interaction of calcitriol with the disulfide isomerase ERp57. *Sci Rep.* 2016; 6:37957. [PubMed: 27897272]
- Gonzalez-Perez P, Woehlbier U, Chian RJ, Sapp P, Rouleau GA, Leblond CS, et al. Identification of rare protein disulfide isomerase gene variants in amyotrophic lateral sclerosis patients. *Gene.* 2015; 566:158–165. [PubMed: 25913742]
- Gorman JD, Sack KE, Davis JCJ. Treatment of Ankylosing Spondylitis by Inhibition of Tumor Necrosis Factor α . *New England Journal of Medicine.* 2002; 346:1349–1356. [PubMed: 11986408]
- Green DR, Kroemer G. Cytoplasmic Functions of the Tumor Suppressor p53. *Nature.* 2009; 458:1127. [PubMed: 19407794]
- Gregory LG, Lloyd CM. Orchestrating house dust mite-associated allergy in the lung. *Trends in immunology.* 2011; 32:402–411. [PubMed: 21783420]
- Grillo C, D'Ambrosio C, Scaloni A, Maceroni M, Merluzzi S, Turano C, et al. Cooperative activity of Ref-1/APE and ERp57 in reductive activation of transcription factors. *Free Radic Biol Med.* 2006; 41:1113–1123. [PubMed: 16962936]
- Grindel BJ, Rohe B, Safford SE, Bennett JJ, Farach-Carson MC. Tumor necrosis factor- α treatment of HepG2 cells mobilizes a cytoplasmic pool of ERp57/1.25D(3)-MARRS to the nucleus. *J Cell Biochem.* 2011; 112:2606–2615. [PubMed: 21598303]
- Guo G, Gong K, Wohlfeld B, Hatanpaa KJ, Zhao D, Habib AA. Ligand-Independent EGFR Signaling. *Cancer Res.* 2015; 75:3436–3441. [PubMed: 26282175]
- Guo GG, Patel K, Kumar V, Shah M, Fried VA, Etlinger JD, et al. Association of the chaperone glucose-regulated protein 58 (GRP58/ER-60/ERp57) with Stat3 in cytosol and plasma membrane complexes. *J Interferon Cytokine Res.* 2002; 22:555–563. [PubMed: 12060494]
- Hatahet F, Ruddock LW. Protein disulfide isomerase: a critical evaluation of its function in disulfide bond formation. *Antioxid Redox Signal.* 2009; 11:2807–2850. [PubMed: 19476414]
- He J, Shi W, Guo Y, Chai Z. ERp57 modulates mitochondrial calcium uptake through the MCU. *FEBS Lett.* 2014; 588:2087–2094. [PubMed: 24815697]
- Herbst RS. Review of epidermal growth factor receptor biology. *International Journal of Radiation Oncology*Biophysics.* 2004; 59:S21–S26.
- Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nat Rev Mol Cell Biol.* 2012; 13. [PubMed: 23212477]

- Hetz C, Russelakis-Carneiro M, Walchli S, Carboni S, Vial-Knecht E, Maundrell K, et al. The disulfide isomerase Grp58 is a protective factor against prion neurotoxicity. *J Neurosci*. 2005; 25:2793–2802. [PubMed: 15772339]
- Hoffman SM, Chapman DG, Lahue KG, Cahoon JM, Rattu GK, Daphtary N, et al. Protein disulfide isomerase-endoplasmic reticulum resident protein 57 regulates allergen-induced airways inflammation, fibrosis, and hyperresponsiveness. *J Allergy Clin Immunol*. 2016; 137:822–832. e827. [PubMed: 26435004]
- Hoffman SM, Tully JE, Nolin JD, Lahue KG, Goldman DH, Daphtary N, et al. Endoplasmic reticulum stress mediates house dust mite-induced airway epithelial apoptosis and fibrosis. *Respiratory Research*. 2013; 14:141. [PubMed: 24364984]
- Hoffstrom BG, Kaplan A, Letso R, Schmid RS, Turmel GJ, Lo DC, et al. Inhibitors of protein disulfide isomerase suppress apoptosis induced by misfolded proteins. *Nat Chem Biol*. 2010; 6:900–906. [PubMed: 21079601]
- Holbrook LM, Watkins NA, Simmonds AD, Jones CI, Ouweland WH, Gibbins JM. Platelets release novel thiol isomerase enzymes which are recruited to the cell surface following activation. *Br J Haematol*. 2010; 148:627–637. [PubMed: 19995400]
- Husmann M, Janke K, Kranz P, Neumann F, Mersch E, Baumann M, et al. Depletion of the thiol oxidoreductase ERp57 in tumor cells inhibits proliferation and increases sensitivity to ionizing radiation and chemotherapeutics. *Oncotarget*. 2015; 6:39247–39261. [PubMed: 26513173]
- Jian J, Tian QY, Hettinghouse A, Zhao S, Liu H, Wei J, et al. Progranulin Recruits HSP70 to β -Glucocerebrosidase and Is Therapeutic Against Gaucher Disease. *EBioMedicine*. 2016a; 13:212–224. [PubMed: 27789271]
- Jian J, Zhao S, Tian QY, Liu H, Zhao Y, Chen WC, et al. Association Between Progranulin and Gaucher Disease. *EBioMedicine*. 2016b; 11:127–137. [PubMed: 27515686]
- Kent-Braun JA, Walker CH, Weiner MW, Miller RG. Functional significance of upper and lower motor neuron impairment in amyotrophic lateral sclerosis. *Muscle Nerve*. 1998; 21:762–768. [PubMed: 9585330]
- Khanal RC, Nemere I. The ERp57/Grp58/1,25D3-MARRS receptor: multiple functional roles in diverse cell systems. *Curr Med Chem*. 2007; 14:1087–1093. [PubMed: 17456022]
- Kozlov G, Maattanen P, Schrag JD, Pollock S, Cygler M, Nagar B, et al. Crystal structure of the bb' domains of the protein disulfide isomerase ERp57. *Structure*. 2006; 14:1331–1339. [PubMed: 16905107]
- Krynetskaia NF, Phadke MS, Jadhav SH, Krynetskiy EY. Chromatin-associated proteins HMGB1/2 and PDIA3 trigger cellular response to chemotherapy-induced DNA damage. *Mol Cancer Ther*. 2009; 8:864–872. [PubMed: 19372559]
- Krynetski EY, Krynetskaia NF, Bianchi ME, Evans WE. A nuclear protein complex containing high mobility group proteins B1 and B2, heat shock cognate protein 70, ERp60, and glyceraldehyde-3-phosphate dehydrogenase is involved in the cytotoxic response to DNA modified by incorporation of anticancer nucleoside analogues. *Cancer Res*. 2003; 63:100–106. [PubMed: 12517784]
- Lahav J, Jurk K, Hess O, Barnes MJ, Farndale RW, Luboshitz J, et al. Sustained integrin ligation involves extracellular free sulfhydryls and enzymatically catalyzed disulfide exchange. *Blood*. 2002; 100:2472–2478. [PubMed: 12239158]
- Lambrecht BN, Hammad H. The airway epithelium in asthma. *Nat Med*. 2012; 18:684–692. [PubMed: 22561832]
- Lee AS. The accumulation of three specific proteins related to glucose-regulated proteins in a temperature-sensitive hamster mutant cell line K12. *J Cell Physiol*. 1981; 106:119–125. [PubMed: 7204505]
- Leys CM, Nomura S, LaFleur BJ, Ferrone S, Kaminishi M, Montgomery E, et al. Expression and prognostic significance of prothymosin- α and ERp57 in human gastric cancer. *Surgery*. 2007; 141:41–50. [PubMed: 17188166]
- Li Y, Camacho P. Ca(2+)-dependent redox modulation of SERCA 2b by ERp57. *The Journal of Cell Biology*. 2004; 164:35–46. [PubMed: 14699087]

- Liao CJ, Wu TI, Huang YH, Chang TC, Lai CH, Jung SM, et al. Glucose-regulated protein 58 modulates β -catenin protein stability in a cervical adenocarcinoma cell line. *BMC Cancer*. 2014; 14:555. [PubMed: 25081282]
- Liao CJ, Wu TI, Huang YH, Chang TC, Wang CS, Tsai MM, et al. Glucose-regulated protein 58 modulates cell invasiveness and serves as a prognostic marker for cervical cancer. *Cancer Sci*. 2011; 102:2255–2263. [PubMed: 21917082]
- Lindquist JA, Jensen ON, Mann M, Hämmerling GJ. ER-60, a chaperone with thiol-dependent reductase activity involved in MHC class I assembly. *The EMBO Journal*. 1998; 17:2186–2195. [PubMed: 9545232]
- Linz A, Knieper Y, Gronau T, Hansen U, Aszodi A, Garbi N, et al. ER Stress During the Pubertal Growth Spurt Results in Impaired Long-Bone Growth in Chondrocyte-Specific ERp57 Knockout Mice. *J Bone Miner Res*. 2015; 30:1481–1493. [PubMed: 25704664]
- Liu Y, Guo Y, Song N, Fan Y, Li K, Teng X, et al. Proteomic pattern changes associated with obesity-induced asthenozoospermia. *Andrology*. 2015; 3:247–259. [PubMed: 25293813]
- Liu Y, Zhu Y, Wu X, Li Y, Guo Q, Li W, et al. Increased expression of ERp57 in rat oocytes during meiotic maturation is associated with sperm-egg fusion. *Mol Reprod Dev*. 2014; 81:315–325. [PubMed: 24415168]
- Maksymowycz WP, Russell AS, Chiu P, Yan A, Jones N, Clare T, et al. Targeting tumour necrosis factor alleviates signs and symptoms of inflammatory osteoarthritis of the knee. *Arthritis Res Ther*. 2012; 14:R206. [PubMed: 23036475]
- Marincola FM, Jaffee EM, Hicklin DJ, Ferrone S. Escape of Human Solid Tumors from T-Cell Recognition: Molecular Mechanisms and Functional Significance. *Advances in Immunology*. 1999; 74:181–273.
- Markus M, Benezra R. Two isoforms of protein disulfide isomerase alter the dimerization status of E2A proteins by a redox mechanism. *J Biol Chem*. 1999; 274:1040–1049. [PubMed: 9873049]
- Molinari M, Helenius A. Glycoproteins form mixed disulphides with oxidoreductases during folding in living cells. *Nature*. 1999; 402:90–93. [PubMed: 10573423]
- Mui AL. The role of STATs in proliferation, differentiation, and apoptosis. *Cell Mol Life Sci*. 1999; 55:1547–1558. [PubMed: 10526572]
- Muramori F, Kobayashi K, Nakamura I. A quantitative study of neurofibrillary tangles, senile plaques and astrocytes in the hippocampal subdivisions and entorhinal cortex in Alzheimer's disease, normal controls and non-Alzheimer neuropsychiatric diseases. *Psychiatry Clin Neurosci*. 1998; 52:593–599. [PubMed: 9895207]
- Nardai G, Korcsmaros T, Papp E, Csermely P. Reduction of the endoplasmic reticulum accompanies the oxidative damage of diabetes mellitus. *Biofactors*. 2003; 17:259–267. [PubMed: 12897447]
- Nardai G, Stadler K, Papp E, Korcsmaros T, Jakus J, Csermely P. Diabetic changes in the redox status of the microsomal protein folding machinery. *Biochem Biophys Res Commun*. 2005; 334:787–795. [PubMed: 16023999]
- Nardo G, Pozzi S, Pignataro M, Lauranzano E, Spano G, Garbelli S, et al. Amyotrophic lateral sclerosis multiprotein biomarkers in peripheral blood mononuclear cells. *PLoS One*. 2011; 6:e25545. [PubMed: 21998667]
- Ndubuisi MI, Guo GG, Fried VA, Etlinger JD, Sehgal PB. Cellular physiology of STAT3: Where's the cytoplasmic monomer? *J Biol Chem*. 1999; 274:25499–25509. [PubMed: 10464281]
- Nemere I, Garbi N, Hammerling G, Hintze KJ. Role of the 1,25D3-MARRS receptor in the 1,25(OH)2D3-stimulated uptake of calcium and phosphate in intestinal cells. *Steroids*. 2012a; 77:897–902. [PubMed: 22546984]
- Nemere I, Garbi N, Winger Q. The 1,25D3 -MARRS receptor/PDIA3/ERp57 and lifespan. *J Cell Biochem*. 2015; 116:380–385. [PubMed: 25283641]
- Nemere I, Garcia-Garbi N, Hammerling GJ, Winger Q. Intestinal cell phosphate uptake and the targeted knockout of the 1,25D3-MARRS receptor/PDIA3/ERp57. *Endocrinology*. 2012b; 153:1609–1615. [PubMed: 22334724]
- Nishitoh H, Kadowaki H, Nagai A, Maruyama T, Yokota T, Fukutomi H, et al. ALS-linked mutant SOD1 induces ER stress- and ASK1-dependent motor neuron death by targeting Derlin-1. *Genes Dev*. 2008; 22:1451–1464. [PubMed: 18519638]

- Obeid M. ERP57 membrane translocation dictates the immunogenicity of tumor cell death by controlling the membrane translocation of calreticulin. *J Immunol.* 2008; 181:2533–2543. [PubMed: 18684944]
- Ogino T, Bandoh N, Hayashi T, Miyokawa N, Harabuchi Y, Ferrone S. Association of tapasin and HLA class I antigen down-regulation in primary maxillary sinus squamous cell carcinoma lesions with reduced survival of patients. *Clin Cancer Res.* 2003; 9:4043–4051. [PubMed: 14519625]
- Ohtani H, Wakui H, Ishino T, Komatsuda A, Miura AB. An isoform of protein disulfide isomerase is expressed in the developing acrosome of spermatids during rat spermiogenesis and is transported into the nucleus of mature spermatids and epididymal spermatozoa. *Histochemistry.* 1993; 100:423–429. [PubMed: 8163386]
- Olivares-Navarrete R, Sutha K, Hyzy SL, Hutton DL, Schwartz Z, McDevitt T, et al. Osteogenic Differentiation of Stem Cells Alters Vitamin D Receptor Expression. *Stem Cells and Development.* 2012; 21:1726–1735. [PubMed: 22034957]
- Oliver JD, Roderick HL, Llewellyn DH, High S. ERp57 functions as a subunit of specific complexes formed with the ER lectins calreticulin and calnexin. *Mol Biol Cell.* 1999; 10:2573–2582. [PubMed: 10436013]
- Oliver JD, van der Wal FJ, Bulleid NJ, High S. Interaction of the thiol-dependent reductase ERp57 with nascent glycoproteins. *Science.* 1997; 275:86–88. [PubMed: 8974399]
- Ozaki T, Yamashita T, Ishiguro S. ERp57-associated mitochondrial mu-calpain truncates apoptosis-inducing factor. *Biochim Biophys Acta.* 2008; 1783:1955–1963. [PubMed: 18559257]
- Pacello F, D'Orazio M, Battistoni A. An ERp57-mediated disulphide exchange promotes the interaction between Burkholderia cenocepacia and epithelial respiratory cells. *Sci Rep.* 2016; 6:21140. [PubMed: 26879174]
- Panaretakis T, Joza N, Modjtahedi N, Tesniere A, Vitale I, Durchschlag M, et al. The co-translocation of ERp57 and calreticulin determines the immunogenicity of cell death. *Cell Death Differ.* 2008; 15:1499–1509. [PubMed: 18464797]
- Peaper DR, Cresswell P. The redox activity of ERp57 is not essential for its functions in MHC class I peptide loading. *Proc Natl Acad Sci U S A.* 2008; 105:10477–10482. [PubMed: 18650385]
- Pike CJ, Cummings BJ, Cotman CW. Early association of reactive astrocytes with senile plaques in Alzheimer's disease. *Exp Neurol.* 1995; 132:172–179. [PubMed: 7789457]
- Podhorecka M, Skladanowski A, Bozko P. H2AX Phosphorylation: Its Role in DNA Damage Response and Cancer Therapy. *Journal of Nucleic Acids.* 2010; 2010:9.
- Porter JR, Fritz CC, Depew KM. Discovery and development of Hsp90 inhibitors: a promising pathway for cancer therapy. *Current Opinion in Chemical Biology.* 2010; 14:412–420. [PubMed: 20409745]
- Prins D, Groenendyk J, Touret N, Michalak M. Modulation of STIM1 and capacitative Ca²⁺ entry by the endoplasmic reticulum luminal oxidoreductase ERp57. *EMBO Rep.* 2011; 12:1182–1188. [PubMed: 21941299]
- Ramirez-Rangel I, Bracho-Valdes I, Vazquez-Macias A, Carretero-Ortega J, Reyes-Cruz G, Vazquez-Prado J. Regulation of mTORC1 complex assembly and signaling by GRp58/ERp57. *Mol Cell Biol.* 2011; 31:1657–1671. [PubMed: 21321085]
- Raykhel I, Alanen H, Salo K, Jurvansuu J, Nguyen VD, Latva-Ranta M, et al. A molecular specificity code for the three mammalian KDEL receptors. *The Journal of Cell Biology.* 2007; 179:1193–1204. [PubMed: 18086916]
- Richard CL, Farach-Carson MC, Rohe B, Nemere I, Meckling KA. Involvement of 1,25D3-MARRS (membrane associated, rapid response steroid-binding), a novel vitamin D receptor, in growth inhibition of breast cancer cells. *Exp Cell Res.* 2010; 316:695–703. [PubMed: 20036234]
- Rohe B, Safford SE, Nemere I, Farach-Carson MC. Identification and characterization of 1,25D3-membrane-associated rapid response, steroid (1,25D3-MARRS)-binding protein in rat IEC-6 cells. *Steroids.* 2005; 70:458–463. [PubMed: 15862831]
- Rohe B, Safford SE, Nemere I, Farach-Carson MC. Regulation of expression of 1,25D3-MARRS/ERp57/PDIA3 in rat IEC-6 cells by TGF beta and 1,25(OH)2D3. *Steroids.* 2007; 72:144–150. [PubMed: 17188725]

- Ryu JH, Yoo JY, Kim MJ, Hwang SG, Ahn KC, Ryu JC, et al. Distinct TLR-mediated pathways regulate house dust mite-induced allergic disease in the upper and lower airways. *J Allergy Clin Immunol*. 2013;131. [PubMed: 23726261]
- Sachs G, Shin JM, Vagin O, Lambrecht N, Yakubov I, Munson K. The Gastric H, K ATPase as a Drug Target: Past, Present, and Future. *Journal of Clinical Gastroenterology*. 2007; 41:S226–S242. [PubMed: 17575528]
- Santana-Codina N, Carretero R, Sanz-Pamplona R, Cabrera T, Guney E, Oliva B, et al. A transcriptome-proteome integrated network identifies endoplasmic reticulum thiol oxidoreductase (ERp57) as a hub that mediates bone metastasis. *Mol Cell Proteomics*. 2013; 12:2111–2125. [PubMed: 23625662]
- Sarbassov DD, Sabatini DM. Redox regulation of the nutrient-sensitive raptor-mTOR pathway and complex. *J Biol Chem*. 2005; 280:39505–39509. [PubMed: 16183647]
- Schelhaas M, Malmstrom J, Pelkmans L, Haugstetter J, Ellgaard L, Grunewald K, et al. Simian Virus 40 depends on ER protein folding and quality control factors for entry into host cells. *Cell*. 2007; 131:516–529. [PubMed: 17981119]
- Sehgal PB, Guo GG, Shah M, Kumar V, Patel K. Cytokine signaling: STATS in plasma membrane rafts. *J Biol Chem*. 2002; 277:12067–12074. [PubMed: 11815625]
- Sepulveda M, Rozas P, Hetz C, Medinas DB. ERp57 as a novel cellular factor controlling prion protein biosynthesis: Therapeutic potential of protein disulfide isomerases. *Prion*. 2016; 10:50–56. [PubMed: 26864548]
- Sequeira VB, Rybchyn MS, Tongkao-On W, Gordon-Thomson C, Malloy PJ, Nemere I, et al. The role of the vitamin D receptor and ERp57 in photoprotection by 1alpha,25-dihydroxyvitamin D3. *Mol Endocrinol*. 2012; 26:574–582. [PubMed: 22322599]
- Silvennoinen L, Myllyharju J, Ruoppolo M, Orru S, Caterino M, Kivirikko KI, et al. Identification and characterization of structural domains of human ERp57: association with calreticulin requires several domains. *J Biol Chem*. 2004; 279:13607–13615. [PubMed: 14732712]
- Song EJ, Gordon-Thomson C, Cole L, Stern H, Halliday GM, Damian DL, et al. 1alpha,25-Dihydroxyvitamin D3 reduces several types of UV-induced DNA damage and contributes to photoprotection. *J Steroid Biochem Mol Biol*. 2013; 136:131–138. [PubMed: 23165145]
- Tabas I, Ron D. Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. *Nat Cell Biol*. 2011;13. [PubMed: 21151131]
- Tang W, Lu Y, Tian QY, Zhang Y, Guo FJ, Liu GY. The growth factor progranulin binds to TNF receptors and is therapeutic against inflammatory arthritis in mice. *Science*. 2011;332. [PubMed: 21659569]
- Tohda C, Urano T, Umezaki M, Nemere I, Kuboyama T. Diosgenin is an exogenous activator of 1,25D(3)-MARRS/Pdia3/ERp57 and improves Alzheimer's disease pathologies in 5XFAD mice. *Sci Rep*. 2012; 2:535. [PubMed: 22837815]
- Torres M, Medinas DB, Matamala JM, Woehlbier U, Cornejo VH, Solda T, et al. The Protein-disulfide Isomerase ERp57 Regulates the Steady-state Levels of the Prion Protein. *J Biol Chem*. 2015; 290:23631–23645. [PubMed: 26170458]
- Trivedi R, Muller GA, Rathore MS, Mishra DP, Dihazi H. Anti-Leukemic Activity of Shikonin: Role of ERP57 in Shikonin Induced Apoptosis in Acute Myeloid Leukemia. *Cell Physiol Biochem*. 2016; 39:604–616. [PubMed: 27415599]
- Turano C, Coppari S, Altieri F, Ferraro A. Proteins of the PDI family: Unpredicted non-ER locations and functions. *J Cell Physiol*. 2002; 193:154–163. [PubMed: 12384992]
- van der Zee J, Le Ber I, Maurer-Stroh S, Engelborghs S, Gijssels I, Camuzat A, et al. Mutations other than null mutations producing a pathogenic loss of progranulin in frontotemporal dementia. *Hum Mutat*. 2007; 28:416.
- Van Hauwermeiren F, Vandenbroucke RE, Libert C. Treatment of TNF mediated diseases by selective inhibition of soluble TNF or TNFR1. *Cytokine & Growth Factor Reviews*. 2011; 22:311–319. [PubMed: 21962830]
- Villanueva EC, Myers MG. Leptin receptor signaling and the regulation of mammalian physiology. *Int J Obes*. 2008; 32:S8–S12.

- Walczak CP, Tsai B. A PDI Family Network Acts Distinctly and Coordinately with ERp29 To Facilitate Polyomavirus Infection. *J Virol*. 2011; 85:2386–2396. [PubMed: 21159867]
- Wang L, Cioffi GA, Cull G, Dong J, Fortune B. Immunohistologic evidence for retinal glial cell changes in human glaucoma. *Invest Ophthalmol Vis Sci*. 2002; 43:1088–1094. [PubMed: 11923250]
- Wang L, Popko B, Roos RP. The unfolded protein response in familial amyotrophic lateral sclerosis. *Hum Mol Genet*. 2011; 20:1008–1015. [PubMed: 21159797]
- Wang X, Tay SS, Ng YK. An immunohistochemical study of neuronal and glial cell reactions in retinæ of rats with experimental glaucoma. *Exp Brain Res*. 2000; 132:476–484. [PubMed: 10912828]
- Wang Y, Chen J, Lee CS, Nizkorodov A, Riemenschneider K, Martin D, et al. Disruption of Pdia3 gene results in bone abnormality and affects 1 α ,25-dihydroxy-vitamin D3-induced rapid activation of PKC. *J Steroid Biochem Mol Biol*. 2010; 121:257–260. [PubMed: 20576531]
- Wang Y, Nizkorodov A, Riemenschneider K, Lee CSD, Olivares-Navarrete R, Schwartz Z, et al. Impaired Bone Formation in Pdia3 Deficient Mice. *PLoS One*. 2014; 9:e112708. [PubMed: 25405762]
- Wearsch PA, Cresswell P. The quality control of MHC class I peptide loading. *Curr Opin Cell Biol*. 2008; 20:624–631. [PubMed: 18926908]
- Wei F, Zhang Y, Jian J, Mundra JJ, Tian Q, Lin J, et al. PGRN protects against colitis progression in mice in an IL-10 and TNFR2 dependent manner. *Sci Rep*. 2014; 4:7023. [PubMed: 25387791]
- Wei J, Buza J, Liu C. Does Progranulin Account for the Opposite Effects of Etanercept and Infliximab/Adalimumab in Osteoarthritis? *Journal of Orthopaedic Research*. 2016a; 34:12–14. [PubMed: 26519939]
- Wei J, Hettinghouse A, Liu C. The role of progranulin in arthritis. *Ann N Y Acad Sci*. 2016b; 1383:5–20. [PubMed: 27505256]
- Wilding C, Bell K, Funke S, Beck S, Pfeiffer N, Grus FH. GFAP antibodies show protective effect on oxidatively stressed neuroretinal cells via interaction with ERP57. *Journal of Pharmacological Sciences*. 2015; 127:298–304. [PubMed: 25837926]
- Williams A, Wang ECY, Thurner L, Liu C-j. Review: Novel Insights Into Tumor Necrosis Factor Receptor, Death Receptor 3, and Progranulin Pathways in Arthritis and Bone Remodeling. *Arthritis & Rheumatology*. 2016; 68:2845–2856. [PubMed: 27428882]
- Wise R, Duhachek-Muggy S, Qi Y, Zolkiewski M, Zolkiewska A. Protein disulfide isomerases in the endoplasmic reticulum promote anchorage-independent growth of breast cancer cells. *Breast Cancer Res Treat*. 2016; 157:241–252. [PubMed: 27161215]
- Wu W, Beilhartz G, Roy Y, Richard CL, Curtin M, Brown L, et al. Nuclear translocation of the 1,25D3-MARRS (membrane associated rapid response to steroids) receptor protein and NF κ B in differentiating NB4 leukemia cells. *Exp Cell Res*. 2010; 316:1101–1108. [PubMed: 20064506]
- Wyse B, Ali N, Ellison DH. Interaction with grp58 increases activity of the thiazide-sensitive Na-Cl cotransporter. *American Journal of Physiology - Renal Physiology*. 2002; 282:F424–F430. [PubMed: 11832422]
- Xie, T-x, Wei, D., Liu, M., Gao, AC., Ali-Osman, F., Sawaya, R., et al. Stat3 activation regulates the expression of matrix metalloproteinase-2 and tumor invasion and metastasis. *Oncogene*. 2004; 23:3550–3560. [PubMed: 15116091]
- Xu LR, Liu XL, Chen J, Liang Y. Protein disulfide isomerase interacts with tau protein and inhibits its fibrillization. *PLoS One*. 2013; 8:e76657. [PubMed: 24098548]
- Yamamoto E, Uchida T, Abe H, Taka H, Fujimura T, Komiya K, et al. Increased expression of ERp57/GRP58 is protective against pancreatic beta cell death caused by autophagic failure. *Biochem Biophys Res Commun*. 2014; 453:19–24. [PubMed: 25241190]
- Yang L, Tsang KY, Tang HC, Chan D, Cheah KSE. Hypertrophic chondrocytes can become osteoblasts and osteocytes in endochondral bone formation. *Proc Natl Acad Sci U S A*. 2014; 111:12097–12102. [PubMed: 25092332]

- Yang WS, Kim HW, Lee JM, Han NJ, Lee MJ, Park SK. 1,25-Dihydroxyvitamin D3 Causes ADAM10-Dependent Ectodomain Shedding of Tumor Necrosis Factor Receptor 1 in Vascular Smooth Muscle Cells. *Molecular Pharmacology*. 2015; 87:533–542. [PubMed: 25556238]
- Yang WS, Yu H, Kim JJ, Lee MJ, Park SK. Vitamin D-induced ectodomain shedding of TNF receptor 1 as a nongenomic action: D3 vs D2 derivatives. *J Steroid Biochem Mol Biol*. 2016; 155:18–25. [PubMed: 26385608]
- Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat Rev Cancer*. 2009; 9:798–809. [PubMed: 19851315]
- Zhang XQ, Pan Y, Yu CH, Xu CF, Xu L, Li YM, et al. PDIA3 Knockdown Exacerbates Free Fatty Acid-Induced Hepatocyte Steatosis and Apoptosis. *PLoS One*. 2015; 10:e0133882. [PubMed: 26214517]
- Zhao YP, Liu B, Tian Q-y, Wei J-l, Richbourgh B, Liu C-j. Progranulin protects against osteoarthritis through interacting with TNF- α and β -Catenin signalling. *Annals of the Rheumatic Diseases*. 2015; 74:2244–2253. [PubMed: 25169730]
- Zhao YP, Tian QY, Frenkel S, Liu CJ. The promotion of bone healing by progranulin, a downstream molecule of BMP-2, through interacting with TNF/TNFR signaling. *Biomaterials*. 2013; 34:6412–6421. [PubMed: 23746860]
- Zhou J, Wu Y, Wang L, Rauova L, Hayes VM, Poncz M, et al. The disulfide isomerase ERp57 is required for fibrin deposition in vivo. *J Thromb Haemost*. 2014; 12:1890–1897. [PubMed: 25156521]
- Zhu L, Santos NC, Kim KH. Disulfide isomerase glucose-regulated protein 58 is required for the nuclear localization and degradation of retinoic acid receptor alpha. *Reproduction*. 2010; 139:717–731. [PubMed: 20130111]
- Zhu Y, Cai L, Guo J, Chen N, Yi X, Zhao Y, et al. Depletion of Dicer promotes epithelial ovarian cancer progression by elevating PDIA3 expression. *Tumour Biol*. 2016; 37:14009–14023. [PubMed: 27492604]

Fig. 1. The molecular structure of ERp57

A: ERp57 has a total 505 amino acids, and consists of 4 domains, a-b-b'-a', together with a N-terminal signal sequence and QDEL C-terminal ER retention/retrieval motif. The a and a' domains are shown in blue while b and b' are shown in yellow. Both a and a' domains hold a thioredoxin-like active site and each contains a redox active CGHC catalytic sequence. b and b' domains contain binding sites for calreticulin and calnexin. The amino acid positions of these domains and sequences are indicated in the figure. B: The X-ray crystal structure of the b and b' domains of Erp57 (Kozlov, et al., 2006) reveals a rigid asymmetrical three-dimensional structure. Each domain features four α helices that encase a central β sheet (from NCBI structure database MMDB: 41000 viewed using Cn3D).

Fig. 2. Multiple binding partners and functions of ERp57

ERp57 can be localized to the ER, nucleus, cytoplasm, mitochondria and plasma membrane. In the ER, ERp57's b-b' domains associate with the P-domain of CRT/CLNX and ERp57 a and a' active domains mediate the catalysis of CRT/CLNX bound substrates. The observation of ERp57-mediated isomerization and release of SV40 capsid proteins has implicated ERp57's activity in viral infection. ERp57 also structurally stabilizes the MHC I PLC through formation of a disulphide bond between ERp57 and tapasin. ERp57 directly interacts with specific glycoproteins, including the growth factor PGRN, and is able to modulate their secretion. ER Ca²⁺ homeostasis is regulated by ERp57's interactions with STIM1 and SERCA2b. ERp57 affects membrane proteins through both direct and indirect interactions. ERp57 mediates exposure of CRT on the cell surface, which is required for immunologic cell death. ERp57 acts as membrane receptor of 1 α , 25(OH)₂D₃ to mediate rapid responses to bioactive vitamin D including Ca²⁺ release through PLA mediated phospholipase C activation. Downstream effects of ERp57: 1 α , 25(OH)₂D₃ binding also include PLA2-mediated PKC driven ADAM10 translocation to the membrane where ADAM10 mediates ectodomain shedding of TNFR1. Ionic oscillations across the plasma membrane are controlled by ERp57's stimulatory interactions with the NCC and H⁺K⁺ ATPase. ERp57 is also associated with an inhibitory effect upon internalization and degradation of EGFR, though the mechanism underlying this relationship is unclear. ERp57 is also found in STAT3 membrane rafts and cytosolic statosome complexes proposed to sequester activated STAT3, though the role(s) of these complexes is indefinite. In mitochondria, ERp57 can regulate Ca²⁺ uptake and apoptotic signaling through binding with

the MCU and μ -calpain, respectively. Further, cytoplasmic ERp57 can interact with p53 to inhibit p53- induced MOMP and consequent apoptosis. ERp57 also promotes the formation of mTOR complexes and may participate in mTOR redox-sensing functions. ERp57 promotes RAR- α binding to ATRA and is required for the nuclear appearance of RAR- α . ERp57 is also capable of DNA binding at target sites either alone or as a member of several protein complexes. ERp57 associates with STAT3 and NF- κ B protein complexes during transit from the cytoplasm to the nucleus and participates in complex-DNA binding. ERp57 also associates with a nuclear protein complex which targets to sites of modified DNA and has been implicated in early signal for repair machinery. Nuclear ERp57 can be found bound with APE/Ref-1 and participates in reductive activation of transcription factors. Conversely, ERp57 reduction of E2A proteins promotes heterodimerization and inhibits transcriptional activity.

Table 1

ERp57's subcellular functions and mechanisms

Subcellular Localization	Biological Functions	Potential Mechanisms
Endoplasmic reticulum	Mediation of protein folding	Binding of the ERp57b-b' domains to the P-lectin domain of CRT/CLNX brings specific substrates in contact with ERp57 catalytic domains (Bourdi, et al., 1995; Charnock-Jones, et al., 1996; Molinari and Helenius, 1999; Oliver, et al., 1999; Oliver, et al., 1997; Torres, et al., 2015; Wise, et al., 2016)
Endoplasmic reticulum	Stabilization of the MHC class I PLC	Mediation of disulfide linkage between ERp57 and tapasin in the PLC core (Dick, et al., 2002; Dong, et al., 2009; Garbi, et al., 2006; Lindquist, et al., 1998; Peaper and Cresswell, 2008; Wearsch and Cresswell, 2008)
Endoplasmic reticulum	Viral peptide uncoating	Isomerization of disulfide bonds of caspid proteins (Schelhaas, et al., 2007; Walczak, 2011)
Endoplasmic reticulum	Modulation of store-operated Ca ²⁺ entry	ERp57 interacts with STIM1 C49 and C56 to inhibit store-operated Ca ²⁺ entry (Prins, et al., 2011)
Endoplasmic reticulum	Modulation of Ca ²⁺ oscillations	ERp57 is recruited to SERCA2b by CRT and inhibits pump activity through formation of disulfide bonds in L4 of SERCA. Upon Ca ²⁺ depletion, ERp57 dissociates and SERCA is reduced and active (Li and Camacho, 2004)
Endoplasmic reticulum	Participates in degradation of RAR- α	Unclear – ERp57 and RAR- α co-localize in the ER under inhibition of proteasome-mediated degradation. ERp57: RAR- α interaction in degradation may be mediated through CRT (Zhu, et al., 2010)
Plasma membrane	Participates in sperm and egg fusion	Unclear – ERp57 postulated to promote thiol-disulfide exchange mediated conformational activation of membrane proteins (Ellerman, et al., 2006; Liu, et al., 2015; Liu, et al., 2014)
Plasma Membrane	Immunogenicity signal	Cytosolic complex formation between ERp57 and CRT is required for CRT's presentation on the membrane (Obeid, 2008).
Plasma membrane	Rapid response to 1 α , 25(OH) ₂ D ₃	ERp57 acts as a membrane receptor for 1 α , 25(OH) ₂ D ₃ (Boyan, et al., 2012; Boyan, et al., 2007; Chen, et al., 2016; Gaucci, et al., 2016; Nemere, et al., 2012a; Nemere, et al., 2012b; Richard, et al., 2010; Sequeira, et al., 2012; Song, et al., 2013)
Plasma membrane	Indirect influence upon ectodomain shedding of TNFR1	ERp57: 1 α , 25(OH) ₂ D ₃ binding activates PKC which triggers ADAM10 translocation to the cell surface where the metalloproteinase mediates ectodomain shedding of TNFR1 (Yang, et al., 2015)
Plasma membrane	Associates with STAT3 plasma membrane rafts potentially to sequester STATs (Guo, et al., 2002; Sehgal, et al., 2002)	unknown
Plasma membrane	Protects EGF signaling	In MDA-MB-468 breast cancer cells and cervical cancer HeLa cells, ERp57 protects EGFR signaling through a positive effect on receptor autophosphorylation, possibly through ERp57-mediated conformational change of the receptor or through an indirect influence, though the mechanism remains undefined (Gauci, et al., 2013).
Plasma membrane	Modulation of gastric acid secretion	Positive modulates basal activity of H ⁺ , K ⁺ ATPase in gastric parietal cells through a incompletely described mechanism involving interaction with α HK and carried out independent of ERp57's catalytic sites and chaperone capacity (Fujii, et al., 2013)
Plasma membrane	Stimulates sodium and chloride uptake	In distal convoluted tubule kidney cells ERp57 associates with the NCC and positively effects NCC activity. The mechanism underlying ERp57's influence on NCC activity is unclear in mammalian cells. In yeast, this interaction occurs at the NCC COOH-terminal domain and a role of ERp57 in mediating insertion and removal of the NCC at the apical plasma membrane (Wyse, et al., 2002)
Mitochondria	Apoptotic signaling	Mitochondrial ERp57 associates with and may stabilize mitochondrial μ -calpain as a foldase of the large subunit of mitochondrial μ -calpain (Ozaki, et al., 2008)
Mitochondria	Stimulation of mitochondrial calcium uptake	ERp57 regulates expression of MCU (He, et al., 2014).

Subcellular Localization	Biological Functions	Potential Mechanisms
Cytosol	Associates with STAT3 complexes	ERp57 associates with cytosolic statosome complexes during traffic between the membrane and nucleus. The nature of ERp57's interaction with these complexes is unclear
Cytosol	Promotes assembly and activity of mTOR, potentially participates in upstream signal detection	ERp57 exerts a positive effect on the assembly of mTOR complexes, particularly mTORC1, through an undefined mechanism. ERp57 interacts with the kinase domain of mTORC1 and positively modulates basal activity of the complex – possibly due to ERp57's stimulation of mTORC1 catalytic activity. A loss of mTORC1 response to insulin following ERp57 knockdown suggests that ERp57 may also participate in upstream signal detection (Ramirez-Rangel, et al., 2011; Sarbassov and Sabatini, 2005).
Cytosol	Required for RAR- α 's translocation from the cytosol to the nucleus.	Complex formation between ERp57:RAR- α :ATRA-RAR- α is required for the nuclear appearance of RAR- α possibly through a mechanism dependent on ERp57's thiol oxidoreductase activity (Zhu, et al., 2010).
Cytosol	Potential role monocyte/macrophage differentiation	Correlative evidence suggests that ERp57 may associate with cytosolic NF κ B as a chaperone to mediate transport to the nucleus upon 1 α , 25(OH)2D3 stimulation of NB4 promyelocytic leukemia cells (Wu, et al., 2010).
Nucleus	Potential regulation of gene expression during monocyte/macrophage differentiation	Correlative evidence from cells stimulated to undergo monocyte/macrophage differentiation, wherein ERp57 and NF κ B rapidly translocate to the nucleus, suggests that ERp57 and NF κ B potentially act within a protein complex to drive non-genomic expression of ERp57 and NF κ B target genes (Grindel, et al., 2011; Rohe, et al., 2005; Wu, et al., 2010).
Nucleus	Regulating the transcriptional expression of the MSH6, TMEM126A and LRBA genes	Direct DNA binding and cooperative reductive activation of transcription factors in association with Ref-1/APE in M14 melanoma cells, Raji lymphoma cells, and HeLa cervical cancer cells (Aureli, et al., 2013; Grillo, et al., 2006).
Nucleus	Signaling sites of modified DNA	ERp57 associates with a nuclear protein complex that targets to modified DNA (Krynetski, et al., 2003) wherein ERp57 phosphorylates H2AX, indicating a role of ERp57 in early response to DNA damage and potentially targeting DNA repair machinery to sites of damage and/or activating apoptosis (Chichiarelli, et al., 2007; Krynetski, et al., 2003).
Nucleus	Participation in STAT3 complex-DNA Binding	Unclear – In cancer cells, ERp57 likely enhances the DNA-binding of STAT3 complex and may function as a chaperone and/or to activate proximal transcription factors (Eufemi, et al., 2004)
Nucleus	Activation of transcription factors	APE/Ref-1 associates with ERp57 in the nucleus to exert cooperative reduction of transcription factors including AP-1 (Grillo, et al., 2006).
Nucleus	Inhibition of E protein homodimerization	ERp57 mediates reduction of E2A proteins, promoting heterodimerization of the transcription factor and preventing DNA binding and transcription of B cell

Table 2
Clinical and Therapeutic Correlates of ERp57 expression and function

Disease or Model System	Observation of Clinical or Therapeutic Significance
Cancer	
Cervical cancer cells	Low ERp57 mRNA expression at early-stage serves as a predictor of recurrence and death (Chung, et al., 2013) High ERp57 protein expression has also been associated with invasiveness, recurrence and death; more heavily associated in adenocarcinoma as compared with squamous cell carcinoma (Liao, et al., 2011)
Gastric mucosa cancer cells	Reduced expression of ERp57 in patients with gastric cancer is associated with metastases and death (Leys, et al., 2007)
Laryngeal cancer cells	Increased expression of ERp57 associated with radioresistance of laryngeal cancer cells and increased ERp57-STAT3 complex is associated with poor prognosis in HEP-2, SNU899 and SNU1076 laryngeal cancer cells and patient tissues (Choe, et al., 2015)
Epithelial ovarian cancer	Low Dicer expression contributes to epithelial ovarian cancer progression by elevating ERp57 expression (Liao, et al., 2014; Xie, et al., 2004; Zhu, et al., 2016).
Ovarian cancer cells	Nuclear ERp57 interacts with β -actin to modulate a role of conformational states of actin in development of paclitaxel resistance in A2780 cells (Cicchillitti, et al., 2010)
Breast cancer cells	ERp57 modulates in vitro stimulation of anchorage-independent growth and bone metastasis in MDA-MB231 and BO2 breast cancer cell lines, in part, through stabilization of MCH class I PLC (Santana-Codina, et al., 2013; Wise, et al., 2016)
Breast cancer cells	Knockdown of ERp57 results in increased sensitivity of MCF-7 breast cancer cells to 1 α , 25(OH)2D3 mediated growth inhibitory signaling. (Richard, et al., 2010)
Breast and cervical cancer cells	ERp57 promotes internalization and phosphorylation of EGFR in MDA-MB-488 breast cancer and HeLa cervical cancer cell lines; EGFR overactivation is a common feature of tumor tissues (Gaucci, et al., 2013)
Melanoma, lymphoma and, cervical cancer cells	In M14, Raji, and HeLa cells, ERp57 modulates the expression of MSH6, TMEM126A and LRBA and participates in reductive activation of transcription factors in association with Ref-1/APE; overexpression of ERp57 is associated with a protective effect on cell survival (Aureli, et al., 2013; Grillo, et al., 2006)
B-lineage leukemia cells	ERp57 participates in a complex that identifies structural changes to DNA and phosphorylates H2AX in Nalm6 cells, suggesting a role in cell sensitivity to chemotherapeutic agents (Krynetskaia, et al., 2009; Krynetski, et al., 2003)
Leukemia cells	ERp57 is the primary target of anti-cancer plant extract shikonin and inhibition of ERp57 increases shikonin-induced apoptosis in in primary and established human leukemia cell lines (Trivedi, et al., 2016)
Colon cancer cells	Inhibition of ERp57 is associated with increased ER stress related apoptosis while overexpression correlates to increased proliferative mTOR signaling in HCT116 cells (Hussmann, et al., 2015)
Fibroblasts	In cells from patients with hereditary vitamin D-resistant rickets, ERp57 is required for 1 α , 25(OH)2D3- mediated photoprotection from DNA damage (Sequeira, et al., 2012).
Neurodegenerative Disease and Nerve damage	
Frontotemporal dementia	FTD is associated with halpoin sufficiency of GRN (Baker, et al., 2006; Cruts, et al., 2006; van der Zee, et al., 2007) and ERp57 mediates the secretion of progranulin in murine microglia (Almeida, et al., 2011). Recent studies also reveal potential association between PGRN deficiency and Gaucher's Disease (Jian, et al., 2016a; Jian, et al., 2016b) though the importance of ERp57 in modulation of PGRN secretion in GD has not yet been explored. These findings suggest that ERp57 may be protective in neurodegenerative proteopathies, particularly those associated with low activity of PGRN.
Amyotrophic lateral sclerosis	Several missense mutations and single nucleotide polymorphisms (SNPs) in PDIA3/ERP57 have been identified in ALS patients (Gonzalez-Perez, et al., 2015) ERp57 is strongly upregulated in the cerebrospinal fluid and peripheral blood of ALS patients and ERp57 level is highly correlated with disease severity (Atkin, et al., 2008; Nardo, et al., 2011)
Alzheimer's disease	ERp57 is believed prevent aggregation of amyloid β under physiological conditions (Erickson, et al., 2005). Activation of ERp57 results in axonal regrowth, an overall reduction of AD neuropathology and increased performance on object recognition tasks in murine models of AD (Tohda, et al., 2012). Autoantibodies against GFAP, a protein that is upregulated during reactive gliosis (Muramori, et al., 1998; Pike, et al., 1995; Wang, et al., 2002; Wang, et al., 2000; Wilding,

Disease or Model System	Observation of Clinical or Therapeutic Significance
	et al., 2015), have been shown to exert a protective effect against oxidative stress via cell-surface interaction with ERp57 (Wilding, et al., 2015).
Peripheral nerve injury	Overexpression of ERp57 in transgenic mice facilitates peripheral nerve regeneration after injury (Castillo, et al., 2015).
Prion-protein related disorders	ERp57 is upregulated, probably as a defensive response against misfolded prion protein toxicity, in CJD (Hetz, et al., 2005). ERp57 likely mediates steady state levels of both wild-type and prion-related disorder (PrD)- related mutant protein indicating a limited role of ERp57 deficiency to protective effect against ER stress susceptibility (Sepulveda, et al., 2016; Torres, et al., 2015).
Huntington's Disease	ERp57 stimulation of MOMP is implicated in neurodegenerative diseases that feature aberrant cytosolic protein aggregation and several molecules reduce huntingtin protein mediated toxicity through inhibition of ERp57 and/or PDI (Hoffstrom, et al., 2010)
Musculoskeletal System Development and Maintenance	
Heterozygous PDIA3 knockout mice	<i>PDIA3</i> ^{+/-} mice exhibit extensive abnormalities in bone morphometry and quality (Wang, et al., 2014).
Chondrocyte-specific PDIA3 knockout mice	Loss of ERp57 in chondrocytes causes increased ER stress and activation of the unfolded protein response (UPR) leading to elevated ER-stress- induced apoptosis and inflammation (Linz, et al., 2015).
Murine osteoblast precursor cells	In MC3T3-E1 cells, ERp57 participates in mediation of crosstalk between BMP-2 and 1 α , 25(OH)2D3 in directing mineralization and osteoblastic differentiation (Chen, et al., 2016). In murine embryoid bodies, ERp57 expression level changes are associated with osteogenic differentiation further supporting a role in osteoblastogenesis (Olivares-Navarrete, et al., 2012).
Murine enterocytes	The ERp57:1 α , 25(OH)2D3 interaction mediates regulation of Ca ²⁺ in mammalian intestinal cells (Nemere, et al., 2012a; Nemere, et al., 2012b), however the role of ERp57 in overall calcium metabolism and the potential effects in bone remain unexplored.
Human aortic smooth muscle cells	Ca ²⁺ influx following ERp57:1 α , 25(OH)2D3 binding induces cell surface translocation of ADAM10 and subsequent ectodomain cleavage of TNFR1 potentially inhibiting inflammatory signaling through neutralization of TNF α . (Yang, et al., 2015).
Digestive System and Accessory Organs	
HEK293 cells expressing H ⁺ , K ⁺ -ATPase	ERp57 controls basal activity of H ⁺ , K ⁺ -ATPase (Fujii, et al., 2013), which regulates gastric acid secretion. H ⁺ , K ⁺ -ATPase is therapeutically targeted by protein pump inhibitors used in the treatment of various acid diseases (Sachs, et al., 2007).
Murine intestinal cells and Sprague-Dawley rat kidney cells	1 α , 25(OH)2D3:ERp57-mediated regulation of ion pump activity has been implicated as a key regulator of calcium, sodium and phosphate flux in intestinal cells in response to vitamin D stimulation (Nemere, et al., 2012a; Nemere, et al., 2015; Prins, et al., 2011; Wyse, et al., 2002)
β cell -specific Atg7- deficient mice and rat insulinoma INS-1 cells	Knockdown of ERp57 is associated with increased autophagic failure- induced cell death in pancreatic β cells (Yamamoto, et al., 2014).
Human renal fibroblasts	In TK173 cells, elevated ERp57 secretion is strongly associated with ECM production and inhibition of ERp57 impairs accumulation of ECM (Dihazi, et al., 2013).
Human patients and Col4a3 knockout renal fibrosis model mice	Excretion of ERP57 in urine serves as a diagnostic marker for renal fibrosis during the early stage of the disease (Dihazi, et al., 2013).
Streptozotocin-induced Type 2 diabetic rats	Diabetic rats exhibit a reductive shift in and downregulated function of ERp57 (Nardai, et al., 2005).
Rat β cell model of permanent neonatal diabetes	Altered redox state of ERp57, co-occurring with defective insulin secretion and reduced proliferation occurs after knockdown of <i>EIF2AK3</i> in rat β cells (Feng, et al., 2009).
Human liver cells	In a L02 cell model of NAFLD, ERp57 protective against fatty acid induced steatosis and apoptosis (Zhang, et al., 2015).
Sprague Dawley rats and leptin-deficient mice	Gastric mucosa protein level of ERp57 is downregulated and ERp57 is dephosphorylated in fasting rats; this redox shift could be inhibited by leptin and leptin could induce gastric mucosa ERp57 mRNA expression in rats and fasted leptin-deficient mice (Bravo, et al., 2011) [143].
Inducible knockout of ERp57 in mice	Knockout of ERp57 correlates with reduced body fat, coincident with an extended lifespan. Estradiol mediated competitive inhibition of 1 α , 25(OH)2D3:ERp57 binding has been implicated in ERp57's role in body fat homeostasis (Nemere, et al., 2015) however, the estradiol:ERp57 interaction requires further study.
Respiratory system	

Disease or Model System	Observation of Clinical or Therapeutic Significance
Respiratory epithelial cells	ERp57 and activating transcription factor 6 (ATF6), an ER stress transducer (Hetz, 2012), are upregulated upon <i>in vitro</i> stimulation with house dust mite (Hoffman, et al., 2013). Knockdown of ATF6 in these cells alleviates allergen-induced ERp57 upregulation and cell death associated with HDM exposure (Hoffman, et al., 2016). Deletion of ERp57 in lung epithelial cells has further demonstrated diminished immune response upon HDM stimulation coincident with a reduction in multiple markers of immune response (Hoffman, et al., 2016).
ERp57 knockdown mice	Knockdown of ERp57 in asthmatic mice is correlated to reduced ER stress in response to allergen challenge (Hoffman, et al., 2013).
Respiratory epithelial cells	ERp57-depletion and inhibition confers resistance to <i>Burkholderia cenocepacia</i> infection in epithelial cells (Pacello, et al., 2016).
Other	
Sertoli cells	ERp57 may support spermatogenesis and germ cell survival through mediating nuclear localization of RAR α . (Zhu, et al., 2010)
Platelets	ERp57 is secreted and localizes to the platelet thrombus to recruit platelets and facilitate fibrinogen binding and fibrin deposition (Cui, et al., 2015; Zhou, et al., 2014). The overlap in expression patterns and potential functional redundancy of PDI proteins in platelet function suggests that targeting one or more of these proteins could provide useful intervention in pathological thrombosis.