HHS Public Access

Author manuscript

J Cell Physiol. Author manuscript; available in PMC 2016 July 01.

Published in final edited form as:

J Cell Physiol. 2015 July ; 230(7): 1413–1420. doi:10.1002/jcp.24923.

Role of the Unfolded Protein Response, GRP78 and GRP94 in Organ Homeostasis

GENYUAN ZHU[1] and **AMY S. LEE**[1,*]

[1]Department of Biochemistry and Molecular Biology, USC Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, California

Abstract

The endoplasmic reticulum (ER) is a cellular organelle where secretory and membrane proteins, as well as lipids, are synthesized and modified. When cells are subjected to ER stress, an adaptive mechanism referred to as the Unfolded Protein Response (UPR) is triggered to allow the cells to restore homeostasis. Evidence has accumulated that the UPR pathways provide specialized and unique roles in diverse development and metabolic processes. The glucose regulated proteins (GRPs) are traditionally regarded as ER proteins with chaperone and calcium binding properties. The GRPs are constitutively expressed at basal levels in all organs, and as stress-inducible ER chaperones, they are major players in protein folding, assembly and degradation. This conventional concept is augmented by recent discoveries that GRPs can be actively translocated to other cellular locations such as the cell surface, where they assume novel functions that regulate signaling, proliferation, apoptosis and immunity. Recent construction and characterization of mouse models where the gene encoding for the UPR components and the GRPs is genetically altered provide new insights on the physiological contribution of these proteins in vivo. This review highlights recent progress towards the understanding of the role of the UPR and two major GRPs (GRP78 and GRP94) in regulating homeostasis of organs arising from the endoderm, mesoderm and ectoderm. GRP78 and GRP94 exhibit shared and unique functions, and in specific organs their depletion elicits adaptive responses with physiological consequences.

Keywords

Endoplasmic reticulum; GRP78/BiP; GRP94; UPR; Mouse models; Organs; Cancer

Since the initial discovery of the glucose regulated proteins (GRPs) about 40 years ago, the knowledge of these proteins has undergone rapid expansion after they have been identified as stress-inducible molecular chaperones belonging to the heat shock protein (HSP) family (Lee, 2001). In contrast to cytosolic HSPs, the GRPs contain signal peptides that target them to the endoplasmic reticulum (ER) where they perform critical functions in maintaining ER homeostasis and assist in protein folding and degradation (Ma and Hendershot, 2004; Ni and Lee, 2007; Marzec et al., 2012). The recent discovery that these GRPs can localize to the cell surface and regulate novel functions beyond the ER adds to the importance of these

[*]Correspondence to: Amy S. Lee, Department of Biochemistry and Molecular Biology, USC Norris Comprehensive Cancer, Room 5308, 1441 Eastlake Ave., Los Angeles, CA 90089-9176; Telephone: (323) 865-0507; Fax: (323) 865-0094; amylee@usc.edu.

proteins in health and disease (Gonzalez-Gronow et al., 2009; Luo and Lee, 2013; Ni et al., 2011). Additionally, the unfolded protein response (UPR), which allows the cells to adapt to ER stress, plays a major role in maintaining ER function when the protein production exceeds the folding capacity of the ER. This review focuses on the contributions of the UPR and the GRPs in regulating organ homeostasis and integrity, and summarizes important insights derived from conventional and tissue specific knockout mouse models.

Basic Functions of the GRPs

The ER is a specialized perinuclear organelle where secretory and membrane proteins, as well as lipids, are synthesized. Within the lumen of the ER, protein chaperones assist in folding of newly synthesized polypeptides and prevent aggregation of unfolded or misfolded proteins (Ni and Lee, 2007). Quality control exists in the ER to avoid accumulation of unfolded or misfolded proteins. Abnormal protein conformations are a major cause for disturbed cellular homeostasis; therefore, perturbations in the ER are thought to be the origin of many diseases and developmental abnormalities (Schroder and Kaufman, 2005). ER stress occurs when there is an excess influx of nascent and unfolded polypeptides or the protein folding machinery is impaired. Signal transduction cascades, termed the UPR, are activated to restore the ER to its normal physiological state. The UPR turns on a cascade of pathways to restore homeostasis through transient arrest of protein translation, and at the same time, increases the folding capacity and decreases the burden of misfolded protein through degradation. However, if ER stress is too severe to be rescued, the UPR can also induce apoptotic pathways (Ron and Walter, 2007). In addition to secreted and membrane protein synthesis and secretion, the ER is a critical site for maintaining lipid metabolism, including lipid synthesis, modification and export (Fu et al., 2012).

The most abundant ER chaperone is the 78-kilodalton glucose regulated protein (GRP78/BiP), which is responsible for maintaining the permeability barrier of the ER during protein translocation, guiding protein folding and assembly, and targeting misfolded proteins for degradation (Ma and Hendershot, 2004). GRP78 is also an ER calcium binding protein. Importantly, GRP78 is a gatekeeper of the mammalian UPR. In non-stressed cells, GRP78 associates with the transmembrane ER stress sensors inositol-requiring kinase 1 (IRE1), activating transcription factor 6 (ATF6) and PKR-like eukaryotic initiation factor 2α kinase (PERK). Upon stress, malfolded proteins titrate GRP78 away from associations with these proteins, freeing them for activation of the UPR (Rutkowski and Kaufman, 2004). The UPR can also be triggered when GRP78 is knockdown in non-stressed mammalian cells, confirming that GRP78 is a negative regulator of the UPR in multiple models (Pyrko et al., 2007; Li et al., 2008).

Analogous to its suppressing activity of the UPR, GRP78 also acts as a potent suppressor of apoptosis through repressing the activation of the pro-apoptotic machinery (such as BIK and caspase-7) localized to the outer surface of the ER (Reddy et al., 2003; Fu et al., 2007; Zhou et al., 2011). Upon titration of GRP78, not only is the pro-apoptotic CHOP being induced as a consequence of UPR activation, intrinsic apoptosis is also triggered (Lee, 2014). GRP78 maintains ER integrity. GRP78 depletion leads to ER expansion and failure to form autophagosomes, therefore suppressing stress-induced autophagy (Li et al., 2008).

Furthermore, it is now established that GRP78 can be relocalized to the cell surface, as well as associated with the mitochondria and nucleus, and even being actively secreted in specific cell lines (Ni et al., 2011). In these new cellular localizations, GRP78 assumes additional functions that control signaling, proliferation, invasion, apoptosis, inflammation and immunity (Lee, 2014). Of particular interest is that cell surface GRP78 is both an upstream regulator and downstream target of the PI3K/AKT pathway, which is important for development and tumorigenesis (Shani et al., 2008; Gray et al., 2013; Liu et al., 2013b; Zhang et al., 2013).

Another major ER chaperone is the 94-kilodalton glucose regulated protein (GRP94/Gp96), which is the most abundant glycoprotein in the ER. Interestingly, unlike GRP78 which is conserved from yeast to human, GRP94 exists only in multi-cellular organisms. GRP94 is instrumental in the initiation of both the innate and adaptive immune response (Yang and Li, 2005). GRP94, like GRP78, participates in protein folding, interacts with other components of the ER protein folding machinery, stores ER calcium and assists in the targeting of misfolded proteins for ER associated degradation (ERAD) (Eletto et al., 2010; Marzec et al., 2012). Compared to GRP78, the client proteins of GRP94 are more selective with critical roles in immunity, growth signaling and cell adhesion. Examples of GRP94 client proteins include histocompatibility class I (MHC I), insulin-like growth factor II (IGF II), Toll like receptor I (TLR1) and a subset of integrins (Liu and Li, 2008; Eletto et al., 2010; Staron et al., 2010). GRP94 also exhibits cell surface and secreted forms that facilitate antigen presentation and immune responses (Luo and Lee, 2013).

With the creation and characterization of conventional and conditional knockout models, the role of UPR, GRP78 and GRP94 in mouse embryonic development and adult organ homeostasis and integrity can be directly investigated. Below, the major findings from these studies are discussed and the phenotypes of each mouse model are summarized in Table 1 for the UPR components, Table 2 for GRP78 and Table 3 for GRP94.

Whole Body

The essential role of GRP78 in cell proliferation and survival was first demonstrated in 2006 in a mouse model where both *Grp78* alleles were deleted. The *Grp78*−/− embryos failed to survive beyond the peri-implantation stage (around E3.5), associating with reduced proliferation and massive apoptosis of the inner cell mass, highlighting the fact that no other protein can substitute for the function of GRP78 (Luo et al., 2006). In contrast, *Grp78* heterozygous (*Grp78*+/−) mice, which express half of the wild-type level of the GRP78 protein, are viable and demonstrate normal organ development. Furthermore, through compensatory mechanisms in response to partial loss of GRP78, *Grp78* heterozygosity protects against high fat diet (HFD)-induced obesity and insulin resistance, as well as cerulein-induced acute pancreatitis in the exocrine pancreas of HFD fed mice (Ye et al., 2010a; Ye et al., 2010b).

Grp94−/− embryos survived up to E8.5, while *Grp94*+/− pups were born in expected Mendelian ratio and displayed normal development (Wanderling et al., 2007; Mao et al., 2010). The survival of *Grp94*−/− embryos past the blastocyst stage allows for the

establishment of *Grp94−/−* embryonic stem cell (ESC) lines. Nonetheless, since GRP94 is important for IGF-1 maturation and secretion, the growth of these ESCs exhibit dependence on exogenous growth factors. Whereas *Grp94−/−* ESCs can differentiate into cells of all three germ layers: ectoderm (neurons), mesoderm (adipocytes) and endoderm (hepatocytes), they cannot differentiate into cardiomyocyte-like cells (Wanderling et al., 2007; Mao et al., 2010).

Liver

The liver is a central organ for whole body homeostasis, including carbohydrate metabolism, glycogen storage, biosynthesis of amino acids and nucleotides, production of plasma proteins and hormones, and lipid metabolism (Tanaka et al., 2011). The levels of triglycerides in the liver are regulated by diverse mechanisms such as de novo lipid synthesis, lipolysis, dietary lipid uptake, and delivery/secretion of lipoprotein particles (Jo et al., 2013). As noted earlier, ER is the primary organelle for lipid metabolism, due to the residency of many enzymes involved in intermediary and complex lipid metabolism (Fu et al., 2012). ER stress and UPR signaling have been well established to link to the excessive accumulation of triglycerides in the liver, which is termed as hepatic steatosis (Fu et al., 2012). ER stress in liver can be induced by oxidative stress, chemical toxicity, hepatic viral infection, metabolic disorders, and abuse of drugs and alcohol (Liu et al., 2010). When the ER cannot restore its functionality in a timely manner under these conditions, it enters into a pathological state, perturbing the balance of lipid homeostasis. Some major components of the UPR regulate lipid metabolism in liver. For example, conditional knockout of *Xbp1* in the liver led to marked hypocholesterolemia and hypotriglyceridemia, as a result of decreased production of lipids in the liver (Lee et al., 2008). When mice received intraperitoneal injection of the ER-stress inducing reagent tunicamycin, wild-type mice were able to recover from the insult, whereas ATF6α−/− mice accumulated neutral lipids in the liver and developed steatosis (Yamamoto et al., 2010). Liver specific PERK-null mice showed impaired UPR activation following tunicamycin-injection (Bunpo et al., 2009).

As expected from the generally protective role of GRP78, liver-specific *Grp78* knockout mice (*Grp78f/f; Alb-Cre*) exhibited mild fatty liver and liver injury, as well as UPR activation (Ji et al., 2011; Chen et al., 2014c). However, the hepatocytes of the *Grp78f/f; Alb-Cre* mice still expressed about 30% of wild-type GRP78 level even at 6 months of age, and the ductal cells always maintained GRP78 expression. At around 14 months, the surface of *Grp78f/f; Alb-Cre* livers displayed some small benign nodules showing fat accumulation accompanied by inflammation (Chen et al., 2014c). GRP78 deficiency in liver exacerbated liver injury induced by alcohol, HFD, drugs and toxins, which could be partially alleviated by simultaneous treatment of the chemical chaperone 4-phenylbutyrate (4-PBA) (Ji et al., 2011). For male *Grp78f/f; Alb-Cre* mice, no sign of malignancy was evident in aged mice (Chen et al., 2014c). However, it was reported that older, long-term alcohol-fed female *Grp78* liver knockout mice showed alterations in DNA methylation and ERAD gene expression, with appearance of tumor-like masses in the liver (Han et al., 2013). In delineating the progression of hepatocellular carcinoma (HCC) which often arises from liver injury, recent experiments on mouse tumor models revealed both tumor suppressing and promoting roles in the same molecule (Feng, 2012). In the context of double knockout of

GRP78 and the tumor suppressor PTEN in the liver, increased hepatomegaly, steatosis, liver injury and progenitor cell proliferation, and acceleration of both HCC and cholangiocarcinoma (CC) were observed, compared to the single PTEN knockout mice (Chen et al., 2014c). Nonetheless, GRP78 is unlikely a tumor suppressor since all the HCC and CC in these mice showed strong re-expression of GRP78. Rather, GRP78 appears to be required for promotion of liver tumorigenesis, as it is also highly elevated in human liver cancer (Lim et al., 2005).

Depletion of GRP94 in the liver by *Alb-Cre* was more efficient compared to GRP78. Livers of the young *Grp94f/f; Alb-Cre* mice showed minor liver injury and focal steatosis, but strikingly, liver progenitor cells expansion and disorganization of cell adhesion molecules were evident, which is consistent with the role of GRP94 in maintaining cell-cell interaction (Chen et al., 2014b). G*rp94f/f; Alb-Cre* livers developed abnormal small nodules at 15 months, and HCC and ductular reactions (DRs) by 21 months (Chen et al., 2014a). Strikingly, *Grp94f/f; Alb-Cre* livers were gradually repopulated with GRP94-positive hepatocytes, while the DRs maintained GRP94-negative (Chen et al., 2014a). In the context of double knockout of GRP94 and PTEN in the liver, accelerated development of HCC and CC was observed, with selective activation of the ERK pathway, disruption of cell adhesion and activation of liver progenitor cells at the premalignant stage (Chen et al., 2014b). Nonetheless, heterogeneous GRP94 expression from very low to wild-type level was observed in the HCC formed in the double GRP94 and PTEN knockout mice, whereas the CC cells remained mostly GRP94 negative (Lee and Chen, 2014). Thus, while GRP94 maintains cell adhesion and stem cell quiescence which are tumor suppressing factors, it can also support tumorigenesis as a stress chaperone and regulator of pro-oncogenic signaling pathways. Recently, in another model of liver specific knockout of GRP94, elevation of long chain ceramides and upregulation of several enzymes in the biogenesis of ceramides were observed, indicating a novel role of GRP94 in sphingolipid homeostasis (Rachidi et al., 2014). This same study also showed repopulation of the liver with GRP94-positive hepatocytes as the mice aged, and upon treatment with carcinogen, the knockout mice developed hepatic hyperplasia and cancer that were GRP94-positive.

Pancreas

The bulk of the pancreas is composed of exocrine tissue, which is highly specialized for the production and secretion of digestive enzymes. In response to meal stimulation, acinar cells of the exocrine pancreas exhibit the highest protein synthesis rate among human tissues (Case, 1978). Acinar cells adapt to these functions by expansion of the ER. Additionally, ER stress caused by misfolding of mutant digestive enzymes has been linked to hereditary chronic pancreatitis in humans, suggesting that the UPR and ER chaperones are important for maintaining pancreatic homeostasis (Kereszturi et al., 2009). In agreement with this, inducible conditional knockout of *Perk* in adult pancreata resulted in hyperglycemia associated with loss of islet and β−cell architecture, intracellular accumulation of proinsulin and Glut 2, massive ER expansion and onset of adaptive UPR in pancreatic tissue (Gao et al., 2012). Thus, PERK is essential for secretory homeostasis and β-cell survival in adult mice, implying that development of PERK inhibitor in human subjects needs to be cautiously pursued (Atkins et al., 2013). Diet-induced obese ATF6α−/− mice exhibited

glucose intolerance due to pancreatic β-cell failure, associating with dilated ER, but are partially resistant to diet-induced insulin resistance (Usui et al., 2012). Conditional knockout of XBP1 in adult pancreatic acinar cells induced extensive apoptosis, followed by pancreas regeneration of acinar cells from the surviving XBP1 positive cells (Hess et al., 2011).

For ER chaperones, *Grp78+/−* acinar cells exhibited partial ER dilation and fared worse in cerulein-induced pancreatitis associated with CHOP induction (Ye et al., 2010b). Interestingly, in HFD-fed mice, *Grp78* heterozygosity triggered compensatory upregulation of other ER chaperones in the pancreas, corresponding with mitigated pancreatitis upon cerulein treatment. However, these responses are dependent on the genetic background of the mice, underscoring the complexity of these physiological adaptations. In human and murine models of pancreatic cancer, GRP78 expression is highly elevated in the ductal structures of pre-cancer, as well as in pancreatic adenocarcinoma (PDAC) (Hill et al., 2012). In the mouse PDAC lesions, GRP78 is present in both the cytoplasm and the membranes, where it co-localized with activated AKT. These studies suggest that GRP78 could have the dual function of maintaining ER integrity as well as mediating PI3K/AKT oncogenic signaling at the cell surface in pancreatic cancer.

Intestine and Esophagus

The mammalian intestine is covered by a single layer of epithelial cells that are replenished every 4-5 days through proliferation and differentiation of intestinal stem cells within the intestinal crypts (van der Flier and Clevers, 2009). The interplay between ER stress and inflammation contribute to the pathogenesis of intestinal diseases (Guo and Li, 2014). XBP1 deletion in intestinal epithelial cells results in UPR activation, spontaneous enteritis and increased susceptibility to induced colitis (Kaser et al., 2008). Abrogation of Wnt signaling resulted in loss of proliferation, whereas activation of Wnt pathway led to the phenotypes associated with early colorectal lesions (Kuhnert et al., 2004; Sansom et al., 2004). Mouse models harboring intestinal knockout of GRP94 led to Wnt signaling defect through loss of the Wnt co-receptor LRP6, postnatal death, loss of intestinal barrier function, decreased number of villi and significant reduction of crypt (Liu et al., 2013a). The rescue of these phenotypes via β-catenin activation indicated a critical role of GRP94 in regulation of canonical Wnt-signaling in intestine. In another study, *Ah1-Cre* mediated GRP78 knockout in intestine led to induction of ER stress, loss of self-renewal capacity and rapid repopulation of wild-type stem cells (Heijmans et al., 2013). This same study also showed *Ah1-Cre* activity in esophagus, and GRP78 null esophageal epithelial cells lost their proliferative capacity and were replaced by unrecombined GRP78-positive cells (Rosekrans et al., 2014).

Hematopoietic System

Hematopoiesis, which occurs during embryo development and adulthood, is the formation of various lineages of mature blood cells derived from hematopoietic stem cells (HSC) with self-renewal capability (Doulatov et al., 2012). Complex and dynamic molecular crosstalk occurs between HSCs and the niche cells, which involves secreted ligands and their receptors, extracellular matrix proteins and adhesion molecules (Lo Celso and Scadden,

2011; Boisset et al., 2013). As a major organelle for synthesis and trafficking of these secretory and surface molecules, a well-organized and functional ER is critical for maintenance of HSCs homeostasis. IRE1α/XBP-1 axis has been shown to be involved in many aspects of the hematopoietic system. For example, XBP-1 is needed for transition of mature activated B cells to antibody secreting plasma cells, as well as the formation of dendritic cells (Reimold et al., 2001; Iwakoshi et al., 2007; Todd et al., 2009). Upon stress and damage, human HSCs pool maintains clonal integrity through PERK induced apoptosis of damaged cells, whereas downstream progenitors mount an adaptive response leading to their survival (van Galen et al., 2014).

As the immunoglobulin heavy chain binding protein BiP, GRP78 facilitates antibody production in plasma cells, and retains unassembled heavy chains in the ER (Hendershot et al., 1987). GRP78 is a proteolytic target of subtilase cytotoxin (SubAB), which blocks antibody secretion in the B cells as a result of the cleaved C-terminal fragment of GRP78 binding and retaining the light chains in the ER (Hu et al., 2009). *Mx1-Cre* mediated acute GRP78 ablation in the mouse bone marrow led to activation of the UPR and significant reduction of HSCs, common lymphoid and myeloid progenitors via a cell intrinsic effect, at least in part due to increased apoptosis (Wey et al., 2012a). Acute induction of *Grp78* heterozygosity by *Mx1-Cre* is sufficient to suppress PTEN-null induced AKT/S6K activation in bone marrow cells and leukemogenesis, with minimal effect on normal hematopoiesis (Wey et al., 2012b). This effect is mediated at least in part through cell surface GRP78, since treatment with a monoclonal antibody targeting GRP78 also suppressed PTEN-null induced AKT activation and leukemic blast production in the bone marrow (Liu et al., 2013b). Acting through cell surface GRP78, Cripto maintains HSCs in the hypoxic-niche as an intermediary of the hypoxia-inducible factor-1α (Miharada et al., 2011). Furthermore, GRP78 secreted to extracellular environment induces cytokine secretion and displays immunomodulatory properties (Panayi and Corrigall, 2006; Li and Li, 2012).

Many client proteins of GRP94 are key players in immune responses, including hematopoietic system-specific integrins, as well as several TLRs (Liu and Li, 2008). GRP94 is required at defined stages of early T- and B-cell development and GRP94 deficient bone marrow chimeras exhibited increase in HSCs, associating with AKT activation (Staron et al., 2010; Luo et al., 2013). The expansion of the HSC pool could result from impaired interaction of HSCs and the niche, as GRP94-null primitive hematopoietic cells showed loss of integrin α4 expression, correlating with enhanced mobilization and compromised homing and lodging ability (Luo et al., 2011). Cell surface GRP94 has been shown to participate in innate immune responses through interaction with TLR2/TLR4 and in adaptive immune response by escorting GRP94-associated peptides into MHC class I cross-presentation pathway (Jockheck-Clark et al., 2010). Overexpression of cell surface GRP94 induced dendritic cell activation and spontaneous lupus-like autoimmune diseases (Liu et al., 2003). Recombinant GRP94 is able to activate macrophages (Huang et al., 2009), polymorphonuclear neutrophils and monocytes, suggesting a novel function of GRP94 as a danger signal to the innate immune system (Radsak et al., 2003).

Muscle

PERK is important in maintaining muscle calcium homeostasis such that muscle cells isolated from *Perk*−/− mice showed high levels of ER stress and disruption of the calcium signaling complex (Huang et al., 2006). GRP94 has been studied extensively in muscle physiology. GRP94 is essential for muscle differentiation such that G*rp94*−/− ESCs cannot differentiate into any of the muscle sublineages (Wanderling et al., 2007). In skeletal and cardiac muscle, GRP94 is completely contained within the sarcoplasmic reticulum lumen (Vitadello et al., 1998), and reduction in GRP94 levels in skeletal myoblasts leads to loss of myocyte fusion competence (Ostrovsky et al., 2010). GRP94 functions as a protective stress protein in muscle, such that GRP94 level is transiently increased in fibrillating atrial myocytes, and cell death of stressed cardiomyocytes can be rescued through overexpression of GRP94 (Vitadello et al., 2003). IGF-I and IGF-II are essential for development and growth of skeletal muscles and their production is dependent on GRP94. Ablation of GRP94 in striated muscle of *Grp94f/f; MCK-Cre* mice resulted in smaller skeletal muscles with decreased IGF contents, and growth defect associating with diminished circulating IGF-1 (Barton et al., 2012).

Adipose Tissue

In addition to regulating fat mass and nutrient homeostasis, adipocytes participate in the immune response, blood pressure control, bone mass regulation and reproductive function through synthesis and release of hormones (Rosen and Spiegelman, 2006). White adipose tissue (WAT) functions as an endocrine organ, as it integrates metabolic signals and regulates energy balance by secreting adipokines such as leptin, adiponectin, resistin, and tumor necrosis factor α (TNFα) (Rosen and Spiegelman, 2006). Specific functions of ER in adipocytes include adipokine synthesis and secretion, triglyceride (TG) droplet synthesis and cholesterol sensing via release of Sterol Regulatory Element-Binding Protein (SREBP) from the ER membrane to nucleus (Maxfield and Tabas, 2005; Rosen and MacDougald, 2006). All three branches of the UPR contribute to adipogenesis. 3T3-L1 cells with XBP1 or IRE1α knockdown demonstrated profound defects in adipogenesis, and only the spliced form of XBP1 can rescue the impairment (Sha et al., 2009). The differentiation of MEFs into adipocytes induced by transduction with Myc-SREBP1 was attenuated by loss of PERK, with reduced expression of lipogenic enzymes (Bobrovnikova-Marjon et al., 2008). In C3H10T1/2 cells induced to adipogenesis, reduction of ATF6α expression led to decrease of key adipogenic genes and reduced lipid accumulation (Lowe et al., 2012).

Knockdown of GRP78 significantly delayed adipocyte differentiation from MEFs or preadipocytes (Zhu et al., 2013). In contrast, *Grp94*−/− ESCs are capable of differentiating into adipocytes (Mao et al., 2010). *Grp78f/f; Ap2-Cre* mice with *Cre*-activity in adipocytes as well as in brain and bone, showed dramatic reduction in adipose tissue, growth retardation, bone reduction, early mortality and altered profile of glucose homeostasis and adipokine secretion (Zhu et al., 2013). It is notable that dyslipidemia, fatty liver and type 2 diabetes phenotypes observed in other lipodystrophic mouse models were absent in the *Grp78f/f; Ap2-Cre* mice. As mentioned above, *Grp78* heterozygosity in a specific genetic

background showed improvement of insulin sensitivity, and this was mediated by adaptive UPR in the WAT (Ye et al., 2010a).

Mammary Gland

The mammary gland is composed of different cell types, including epithelial cells that form a branched structure, adipocytes, vascular endothelial cells, fibroblasts and immune cells (Macias and Hinck, 2012). Mammary gland development and function are regulated under different and interrelated components, such as growth factors, hormones and the extracellular matrix (ECM). Postnatal growth of mammary gland is influenced by growth hormone (GH), estrogen and insulin-like growth factor-1 (IGF1) (Macias and Hinck, 2012). Mammary gland branching morphogenesis is partially dependent on the ECM, ECM receptors and ECM degrading enzymes, which affect cell survival, polarity, proliferation, differentiation and adhesion (Fata et al., 2004). It demands a highly functional ER to accommodate for the production of all these secreted and surface proteins. In support that the integrity of the PERK pathway is important for mammary homeostasis, targeted deletion of *Perk* in mammary epithelium inhibited the sustained expression of lipogenic enzymes, leading to altered lipid composition in milk (Bobrovnikova-Marjon et al., 2008). PERK also facilitates survival of ECM-detached mammary epithelial cells by autophagy induction, ATP production and an antioxidant response, which may enhance survival of ECM-detached tumor cells in ductal carcinomas in situ (DCIS) (Avivar-Valderas et al., 2011).

For ER chaperones, GRP78, but not GRP94, is required for mammary gland development. Intriguingly, GRP78 knockdown in the mammary epithelium using *MMTV-Cre* did not succeed, however, could be achieved in the isolated *Grp78f/f* epithelial cells in vitro via infection with adenovirus *Cre* (Zhu et al., 2014). GRP78 ablated mammary epithelial stem/progenitor cells failed to regenerate the mammary glands in deepithelialized recipient mice. GRP78 that translocates to cell surface contributes to the regulation of Cripto signaling in mammary stem cells (Kelber et al., 2009; Spike et al., 2014). Targeted perturbation of cell surface Cripto/GRP78 complex blocked Cripto-mediated regulation of downstream AKT and TGFβ signaling pathways (Kelber et al., 2009). *Grp78* heterozygosity impeded transgene-induced mammary tumor development, with the tumors showing reduced proliferation, increased apoptosis, and dramatic reduction of tumor angiogenesis (Dong et al., 2008). Early-phase mammary tumor growth and metastatic growth of melanoma to the lung was suppressed in syngeneic tumor models using *Grp78*+/− host mice (Dong et al., 2011). Furthermore, endothelial cell-specific *Grp78* heterozygous knockout mice (*Grp78f/+; Tie2-Cre*) showed profound reduction of tumor angiogenesis and metastatic lesions with minor effect on normal tissue microvessel density (Dong et al., 2011). Collectively, these models established that GRP78 is not only required for tumor progression, but also for tumor neovascularization.

Prostate

The prostate is a compound tubuloalveolar exocrine gland and the prostate epithelium is comprised of tall columnar cells and basal cells which are supported by a fibroelastic stroma containing randomly oriented smooth muscle bundles. Homozygous deletion of *Grp78*

specifically in prostate epithelium of *Grp78f/f; Probasin-Cre* mice did not affect postnatal prostate development or the fertility of the mouse (Fu et al., 2008). It is possible that the prostate epithelium function can be sustained by the low level of residual GRP78 in these cells, or these cells are being sufficiently replenished by non-recombined GRP78-positive stem cells with self-renewal properties. In contrast, in mouse models, Pten null prostate tumorigenesis and AKT activation are potently suppressed by targeted knockout of GRP78 in the prostate epithelium (Fu et al., 2008).

Cerebellum

Neurodegenerative diseases are often associated with dysfunction in protein quality control, leading to accumulation of misfolded proteins that triggers ER stress (Rao and Bredesen, 2004). The function and potential of GRP78 as a therapeutic target for neurodegenerative disorders have been previously reviewed (Muchowski and Wacker, 2005; Wang et al., 2009; Gorbatyuk and Gorbatyuk, 2013). ER protein quality control is critical for cerebellar Purkinje cell (PC) survival, and is linked to the Marinesco-Sjogren syndrome in humans (Anttonen et al., 2005). PCs in the cerebellum are a class of GABAergic neurons and they are necessary for controlling movements with precision and convey the entire output of the cerebellar cortex (Mizuhara et al., 2010; Heiney et al., 2014). In a mouse model where GRP78 was specifically eliminated in the PCs, reduction of cytosolic ubiquitin and prominent dilatation of the ER were observed, accompanied by the activation of the UPR and compensatory upregulation of other ER chaperones (Wang et al., 2010). These mice exhibited growth retardation, severe motor coordination defect and cerebellar atrophy. In contrast, PCs deficient in GRP94 showed none of these cerebellar degeneration phenotypes, underscoring the specific requirement of GRP78 for the integrity of this organ (Wang et al., 2010). In agreement with these findings, a knockin mouse model expressing heterozygous mutant GRP78 also revealed motor disabilities in aging and degeneration of some motoneurons in the spinal cord (Jin et al., 2014). In another model with knockin of a mutant Grp78 gene, the mice exhibited disordered cerebral cortex and cerebellum, and smaller brain (Mimura et al., 2008). In mice harboring a hypomorph mutant of GRP78, neonatal respiratory failure, abnormal cerebellum and decrease in axon projection were observed (Favero et al., 2013).

Conclusions

Taken together, the ER is a crucial command center through participation in diverse essential cellular activities and potential influences on function of various organs. Evidence is accumulating that the UPR modulates a wide variety of physiological pathways that are critical for specific organ function. ER chaperones including GRP78 and GRP94 play major roles in ER integrity and their functions could be tissue-specific. The mouse models elegantly demonstrate the consequences of their deficiency or altered function in mouse development, tissue differentiation and organ maintenance. One important aspect learned from these in vivo studies is that while normal organs including the endothelium are not majorly affected when GRP78 expression level is reduced by half or lower, the ability for cancers to grow and metastasize can be severely compromised. In some cancers, GRP94 is also pro-oncogenic. The addiction of tumors and other pathologies to high levels of GRPs

provides a therapeutic window to target GRPs with minimal harm to normal organs. Despite these advances, there are substantial gaps in our knowledge on how the UPR pathways and the GRPs exert their physiological effects. For example, their interacting partners as well as the intermediary steps in specific tissues remain to be elucidated. Drugs specifically targeting the UPR and the GRPs need to be further developed and their efficacy validated in clinical trials. Nonetheless, the revelation of the general and specific functions of the UPR pathways and GRPs in organ maintenance and tumorigenesis will provide new insight in designing therapeutic agents to maximize the benefits while minimizing the undesirable side effects on normal organs.

Acknowledgments

Contract grant sponsor: National Institutes of Cancer and National Institutes of Aging; Contract grant numbers: R01-CA027607, P01-AG034906.

Literature Cited

Anttonen AK, Mahjneh I, Hamalainen RH, Lagier-Tourenne C, Kopra O, Waris L, Anttonen M, Joensuu T, Kalimo H, Paetau A, Tranebjaerg L, Chaigne D, Koenig M, Eeg-Olofsson O, Udd B, Somer M, Somer H, Lehesjoki AE. The gene disrupted in Marinesco-Sjögren syndrome encodes SIL1, an HSPA5 cochaperone. Nat Genet. 2005; 37:1309–1311. [PubMed: 16282978]

Atkins C, Liu Q, Minthorn E, Zhang SY, Figueroa DJ, Moss K, Stanley TB, Sanders B, Goetz A, Gaul N, Choudhry AE, Alsaid H, Jucker BM, Axten JM, Kumar R. Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. Cancer Res. 2013; 73:1993–2002. [PubMed: 23333938]

Avivar-Valderas A, Salas E, Bobrovnikova-Marjon E, Diehl JA, Nagi C, Debnath J, Aguirre-Ghiso JA. PERK integrates autophagy and oxidative stress responses to promote survival during extracellular matrix detachment. Mol Cell Biol. 2011; 31:3616–3629. [PubMed: 21709020]

Barton ER, Park S, James JK, Makarewich CA, Philippou A, Eletto D, Lei H, Brisson B, Ostrovsky O, Li Z, Argon Y. Deletion of muscle GRP94 impairs both muscle and body growth by inhibiting local IGF production. FASEB J. 2012; 26:3691–3702. [PubMed: 22649033]

Bobrovnikova-Marjon E, Hatzivassiliou G, Grigoriadou C, Romero M, Cavener DR, Thompson CB, Diehl JA. PERK-dependent regulation of lipogenesis during mouse mammary gland development and adipocyte differentiation. Proc Natl Acad Sci USA. 2008; 105:16314–16319. [PubMed: 18852460]

Boisset JC, Clapes T, Van Der Linden R, Dzierzak E, Robin C. Integrin alphaIIb (CD41) plays a role in the maintenance of hematopoietic stem cell activity in the mouse embryonic aorta. Biol Open. 2013; 2:525–532. [PubMed: 23789102]

Bunpo P, Brueck D, Cavener DR, Wek RC, Anthony TG. Deletion of PERK in liver impairs the hepatic ER stress response to tunicamycin. FASEB J. 2009; 23:850, 811.

Case RM. Synthesis, intracellular transport and discharge of exportable proteins in the pancreatic acinar cell and other cells. Biol Rev Camb Philos Soc. 1978; 53:211–354. [PubMed: 208670]

Chen WT, Ha D, Kanel G, Lee AS. Targeted deletion of ER chaperone GRP94 in the liver results in injury, repopulation of GRP94-positive hepatocytes, and spontaneous hepatocellular carcinoma development in aged mice. Neoplasia. 2014a (in press).

Chen WT, Tseng CC, Pfaffenbach K, Kanel G, Luo B, Stiles BL, Lee AS. Liver-specific knockout of GRP94 in mice disrupts cell adhesion, activates liver progenitor cells, and accelerates liver tumorigenesis. Hepatology. 2014b; 59:947–957. [PubMed: 24027047]

Chen WT, Zhu G, Pfaffenbach K, Kanel G, Stiles B, Lee AS. GRP78 as a regulator of liver steatosis and cancer progression mediated by loss of the tumor suppressor PTEN. Oncogene. 2014c [Epub ahead of print].

Dong D, Ni M, Li J, Xiong S, Ye W, Virrey JJ, Mao C, Ye R, Wang M, Pen L, Dubeau L, Groshen S, Hofman FM, Lee AS. Critical role of the stress chaperone GRP78/BiP in tumor proliferation, survival, and tumor angiogenesis in transgene-induced mammary tumor development. Cancer Res. 2008; 68:498–505. [PubMed: 18199545]

Dong D, Stapleton C, Luo B, Xiong S, Ye W, Zhang Y, Jhaveri N, Zhu G, Ye R, Liu Z, Bruhn KW, Craft N, Groshen S, Hofman FM, Lee AS. A critical role for GRP78/BiP in the tumor microenvironment for neovascularization during tumor growth and metastasis. Cancer Res. 2011; 71:2848–2857. [PubMed: 21467168]

Doulatov S, Notta F, Laurenti E, Dick JE. Hematopoiesis: a human perspective. Cell Stem Cell. 2012; 10:120–136. [PubMed: 22305562]

Eletto D, Dersh D, Argon Y. GRP94 in ER quality control and stress responses. Semin Cell Dev Biol. 2010; 21:479–485. [PubMed: 20223290]

Fata JE, Werb Z, Bissell MJ. Regulation of mammary gland branching morphogenesis by the extracellular matrix and its remodeling enzymes. Breast Cancer Res. 2004; 6:1–11. [PubMed: 14680479]

Favero CB, Henshaw RN, Grimsley-Myers CM, Shrestha A, Beier DR, Dwyer ND. Mutation of the BiP/GRP78 gene causes axon outgrowth and fasciculation defects in the thalamocortical connections of the mammalian forebrain. J Comp Neurol. 2013; 521:677–696. [PubMed: 22821687]

Feng GS. Conflicting roles of molecules in hepatocarcinogenesis: paradigm or paradox. Cancer Cell. 2012; 21:150–154. [PubMed: 22340589]

Fu S, Watkins SM, Hotamisligil GS. The role of endoplasmic reticulum in hepatic lipid homeostasis and stress signaling. Cell Metab. 2012; 15:623–634. [PubMed: 22560215]

Fu Y, Li J, Lee AS. GRP78/BiP inhibits endoplasmic reticulum BIK and protects human breast cancer cells against estrogen-starvation induced apoptosis. Cancer Res. 2007; 67:3734–3740. [PubMed: 17440086]

Fu Y, Wey S, Wang M, Ye R, Liao CP, Roy-Burman P, Lee AS. Pten null prostate tumorigenesis and AKT activation are blocked by targeted knockout of ER chaperone GRP78/BiP in prostate epithelium. Proc Natl Acad Sci USA. 2008; 105:19444–19449. [PubMed: 19033462]

Gao Y, Sartori DJ, Li C, Yu QC, Kushner JA, Simon MC, Diehl JA. PERK is required in the adult pancreas and is essential for maintenance of glucose homeostasis. Mol Cell Biol. 2012; 32:5129–5139. [PubMed: 23071091]

Gonzalez-Gronow M, Selim MA, Papalas J, Pizzo SV. GRP78: a multifunctional receptor on the cell surface. Antioxid Redox Signal. 2009; 11:2299–2306. [PubMed: 19331544]

Gorbatyuk MS, Gorbatyuk OS. The Molecular Chaperone GRP78/BiP as a Therapeutic Target for Neurodegenerative Disorders: A Mini Review. J Genet Syndr Gene Ther. 2013; 4pii:128. [PubMed: 23750325]

Gray MJ, Mhawech-Fauceglia P, Yoo E, Yang W, Wu E, Lee AS, Lin YG. AKT inhibition mitigates GRP78 (glucose-regulated protein) expression and contribution to chemoresistance in endometrial cancers. Int J Cancer. 2013; 133:21–30. [PubMed: 23280503]

Guo B, Li Z. Endoplasmic reticulum stress in hepatic steatosis and inflammatory bowel diseases. Front Genet. 2014; 5:242. [PubMed: 25120559]

Han H, Hu J, Lau MY, Feng M, Petrovic LM, Ji C. Altered methylation and expression of ER-associated degradation factors in long-term alcohol and constitutive ER stress-induced murine hepatic tumors. Front Genet. 2013; 4:224. [PubMed: 24198826]

Heijmans J, van Lidth de Jeude JF, Koo BK, Rosekrans SL, Wielenga MC, van de Wetering M, Ferrante M, Lee AS, Onderwater JJ, Paton JC, Paton AW, Mommaas AM, Kodach LL, Hardwick JC, Hommes DW, Clevers H, Muncan V, van den Brink GR. ER stress causes rapid loss of intestinal epithelial stemness through activation of the unfolded protein response. Cell Rep. 2013; 3:1128–1139. [PubMed: 23545496]

Heiney SA, Kim J, Augustine GJ, Medina JF. Precise control of movement kinematics by optogenetic inhibition of Purkinje cell activity. J Neurosci. 2014; 34:2321–2330. [PubMed: 24501371]

Hendershot L, Bole D, Kohler G, Kearney JF. Assembly and secretion of heavy chains that do not associate posttranslationally with immunoglobulin heavy chain-binding protein. J Cell Biol. 1987; 104:761–767. [PubMed: 3102505]

Hess DA, Humphrey SE, Ishibashi J, Damsz B, Lee AH, Glimcher LH, Konieczny SF. Extensive pancreas regeneration following acinar-specific disruption of Xbp1 in mice. Gastroenterology. 2011; 141:1463–1472. [PubMed: 21704586]

Hill R, Li Y, Tran LM, Dry S, Calvopina JH, Garcia A, Kim C, Wang Y, Donahue TR, Herschman HR, Wu H. Cell intrinsic role of COX-2 in pancreatic cancer development. Mol Cancer Ther. 2012; 11:2127–2137. [PubMed: 22784710]

Hu CC, Dougan SK, Winter SV, Paton AW, Paton JC, Ploegh HL. Subtilase cytotoxin cleaves newly synthesized BiP and blocks antibody secretion in B lymphocytes. J Exp Med. 2009; 206:2429–2440. [PubMed: 19808260]

Huang G, Yao J, Zeng W, Mizuno Y, Kamm KE, Stull JT, Harding HP, Ron D, Muallem S. ER stress disrupts Ca2+-signaling complexes and Ca2+ regulation in secretory and muscle cells from PERK-knockout mice. J Cell Sci. 2006; 119:153–161. [PubMed: 16352659]

Huang QQ, Sobkoviak R, Jockheck-Clark AR, Shi B, Mandelin AM 2nd, Tak PP, Haines GK 3rd, Nicchitta CV, Pope RM. Heat shock protein 96 is elevated in rheumatoid arthritis and activates macrophages primarily via TLR2 signaling. J Immunol. 2009; 182:4965–4973. [PubMed: 19342676]

Iwakoshi NN, Pypaert M, Glimcher LH. The transcription factor XBP-1 is essential for the development and survival of dendritic cells. J Exp Med. 2007; 204:2267–2275. [PubMed: 17875675]

Ji C, Kaplowitz N, Lau MY, Kao E, Petrovic LM, Lee AS. Liver-specific loss of glucose-regulated protein 78 perturbs the unfolded protein response and exacerbates a spectrum of liver diseases in mice. Hepatology. 2011; 54:229–239. [PubMed: 21503947]

Jin H, Mimura N, Kashio M, Koseki H, Aoe T. Late-Onset of Spinal Neurodegeneration in Knock-In Mice Expressing a Mutant BiP. PLoS One. 2014; 9:e112837. [PubMed: 25405877]

Jo H, Choe SS, Shin KC, Jang H, Lee JH, Seong JK, Back SH, Kim JB. Endoplasmic reticulum stress induces hepatic steatosis via increased expression of the hepatic very low-density lipoprotein receptor. Hepatology. 2013; 57:1366–1377. [PubMed: 23152128]

Jockheck-Clark AR, Bowers EV, Totonchy MB, Neubauer J, Pizzo SV, Nicchitta CV. Re-examination of CD91 function in GRP94 (glycoprotein 96) surface binding, uptake, and peptide cross-presentation. J Immunol. 2010; 185:6819–6830. [PubMed: 21048103]

Kaser A, Lee AH, Franke A, Glickman JN, Zeissig S, Tilg H, Nieuwenhuis EE, Higgins DE, Schreiber S, Glimcher LH, Blumberg RS. XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. Cell. 2008; 134:743–756. [PubMed: 18775308]

Kelber JA, Panopoulos AD, Shani G, Booker EC, Belmonte JC, Vale WW, Gray PC. Blockade of Cripto binding to cell surface GRP78 inhibits oncogenic Cripto signaling via MAPK/PI3K and Smad2/3 pathways. Oncogene. 2009; 28:2324–2336. [PubMed: 19421146]

Kereszturi E, Szmola R, Kukor Z, Simon P, Weiss FU, Lerch MM, Sahin-Toth M. Hereditary pancreatitis caused by mutation-induced misfolding of human cationic trypsinogen: a novel disease mechanism. Hum Mutat. 2009; 30:575–582. [PubMed: 19191323]

Kuhnert F, Davis CR, Wang HT, Chu P, Lee M, Yuan J, Nusse R, Kuo CJ. Essential requirement for Wnt signaling in proliferation of adult small intestine and colon revealed by adenoviral expression of Dickkopf-1. Proc Natl Acad Sci USA. 2004; 101:266–271. [PubMed: 14695885]

Lee AH, Scapa EF, Cohen DE, Glimcher LH. Regulation of hepatic lipogenesis by the transcription factor XBP1. Science. 2008; 320:1492–1496. [PubMed: 18556558]

Lee AS. The glucose-regulated proteins: stress induction and clinical applications. Trends Biochem Sci. 2001; 26:504–510. [PubMed: 11504627]

Lee AS. Glucose-regulated proteins in cancer: molecular mechanisms and therapeutic potential. Nat Rev Cancer. 2014; 14:263–276. [PubMed: 24658275]

Lee AS, Chen WT. The Yin and Yang of GRP94 in liver tumorigenesis. Hepatology. 2014 [Epub ahead of print].

Li J, Ni M, Lee B, Barron E, Hinton DR, Lee AS. The unfolded protein response regulator GRP78/BiP is required for endoplasmic reticulum integrity and stress-induced autophagy in mammalian cells. Cell Death Differ. 2008; 15:1460–1471. [PubMed: 18551133]

Li Z, Li Z. Glucose regulated protein 78: a critical link between tumor microenvironment and cancer hallmarks. Biochim Biophys Acta. 2012; 1826:13–22. [PubMed: 22426159]

Lim SO, Park SG, Yoo JH, Park YM, Kim HJ, Jang KT, Cho JW, Yoo BC, Jung GH, Park CK. Expression of heat shock proteins (HSP27, HSP60, HSP70, HSP90, GRP78, GRP94) in hepatitis B virus-related hepatocellular carcinomas and dysplastic nodules. World J Gastroenterol. 2005; 11:2072–2079. [PubMed: 15810071]

Liu B, Dai J, Zheng H, Stoilova D, Sun S, Li Z. Cell surface expression of an endoplasmic reticulum resident heat shock protein gp96 triggers MyD88-dependent systemic autoimmune diseases. Proc Natl Acad Sci USA. 2003; 100:15824–15829. [PubMed: 14668429]

Liu B, Li Z. Endoplasmic reticulum HSP90b1 (gp96, grp94) optimizes B-cell function via chaperoning integrin and TLR but not immunoglobulin. Blood. 2008; 112:1223–1230. [PubMed: 18509083]

Liu B, Staron M, Hong F, Wu BX, Sun S, Morales C, Crosson CE, Tomlinson S, Kim I, Wu D, Li Z. Essential roles of grp94 in gut homeostasis via chaperoning canonical Wnt pathway. Proc Natl Acad Sci USA. 2013a; 110:6877–6882. [PubMed: 23572575]

Liu J, Jin X, Yu CH, Chen SH, Li WP, Li YM. Endoplasmic reticulum stress involved in the course of lipogenesis in fatty acids-induced hepatic steatosis. J Gastroenterol Hepatol. 2010; 25:613–618. [PubMed: 19929925]

Liu R, Li X, Gao W, Zhou Y, Wey S, Mitra SK, Krasnoperov V, Dong D, Liu S, Li D, Zhu G, Louie S, Conti PS, Li Z, Lee AS, Gill PS. Monoclonal antibody against cell surface GRP78 as a novel agent in suppressing PI3K/AKT signaling, tumor growth and metastasis. Clin Cancer Res. 2013b; 19:6802–6811. [PubMed: 24048331]

Lo Celso C, Scadden DT. The haematopoietic stem cell niche at a glance. J Cell Sci. 2011; 124:3529–3535. [PubMed: 22083139]

Lowe CE, Dennis RJ, Obi U, O'Rahilly S, Rochford JJ. Investigating the involvement of the ATF6alpha pathway of the unfolded protein response in adipogenesis. Int J Obes (Lond). 2012; 36:1248–1251. [PubMed: 22124452]

Luo B, Lam BS, Lee SH, Wey S, Zhou H, Wang M, Chen SY, Adams GB, Lee AS. The endoplasmic reticulum chaperone protein GRP94 is required for maintaining hematopoietic stem cell interactions with the adult bone marrow niche. PLoS One. 2011; 6:e20364. [PubMed: 21647226]

Luo B, Lee AS. The critical roles of endoplasmic reticulum chaperones and unfolded protein response in tumorigenesis and anticancer therapies. Oncogene. 2013; 32:805–818. [PubMed: 22508478]

Luo B, Tseng CC, Adams GB, Lee AS. Deficiency of GRP94 in the hematopoietic system alters proliferation regulators in hematopoietic stem cells. Stem Cells Dev. 2013; 22:3062–3073. [PubMed: 23859598]

Luo S, Mao C, Lee B, Lee AS. GRP78/BiP is required for cell proliferation and protecting the inner cell mass from apoptosis during early mouse embryonic development. Mol Cell Biol. 2006; 26:5688–5697. [PubMed: 16847323]

Ma Y, Hendershot LM. The role of the unfolded protein response in tumour development: friend or foe? Nat Rev Cancer. 2004; 4:966–977. [PubMed: 15573118]

Macias H, Hinck L. Mammary gland development. Wiley Interdiscip Rev Dev Biol. 2012; 1:533–557. [PubMed: 22844349]

Mao C, Wang M, Luo B, Wey S, Dong D, Wesselschmidt R, Rawlings S, Lee AS. Targeted mutation of the mouse Grp94 gene disrupts development and perturbs endoplasmic reticulum stress signaling. PLoS One. 2010; 5:e10852. [PubMed: 20520781]

Marzec M, Eletto D, Argon Y. GRP94: An HSP90-like protein specialized for protein folding and quality control in the endoplasmic reticulum. Biochimica et Biophysica Acta (BBA) - Mol Cell Res. 2012; 1823:774–787.

Maxfield FR, Tabas I. Role of cholesterol and lipid organization in disease. Nature. 2005; 438:612–621. [PubMed: 16319881]

Miharada K, Karlsson G, Rehn M, Rorby E, Siva K, Cammenga J, Karlsson S. Cripto regulates hematopoietic stem cells as a hypoxic-niche-related factor through cell surface receptor GRP78. Cell Stem Cell. 2011; 9:330–344. [PubMed: 21982233]

Mimura N, Yuasa S, Soma M, Jin H, Kimura K, Goto S, Koseki H, Aoe T. Altered quality control in the endoplasmic reticulum causes cortical dysplasia in knock-in mice expressing a mutant BiP. Mol Cell Biol. 2008; 28:293–301. [PubMed: 17954555]

Mizuhara E, Minaki Y, Nakatani T, Kumai M, Inoue T, Muguruma K, Sasai Y, Ono Y. Purkinje cells originate from cerebellar ventricular zone progenitors positive for Neph3 and E-cadherin. Dev Biol. 2010; 338:202–214. [PubMed: 20004188]

Muchowski PJ, Wacker JL. Modulation of neurodegeneration by molecular chaperones. Nat Rev Neurosci. 2005; 6:11–22. [PubMed: 15611723]

Ni M, Lee AS. ER chaperones in mammalian development and human diseases. FEBS Lett. 2007; 581:3641–3651. [PubMed: 17481612]

Ni M, Zhang Y, Lee AS. Beyond the endoplasmic reticulum: atypical GRP78 in cell viability, signalling and therapeutic targeting. Biochem J. 2011; 434:181–188. [PubMed: 21309747]

Ostrovsky O, Eletto D, Makarewich C, Barton ER, Argon Y. Glucose regulated protein 94 is required for muscle differentiation through its control of the autocrine production of insulin-like growth factors. Biochim Biophys Acta. 2010; 1803:333–341. [PubMed: 19914304]

Panayi GS, Corrigall VM. BiP regulates autoimmune inflammation and tissue damage. Autoimmun Rev. 2006; 5:140–142. [PubMed: 16431346]

Pyrko P, Schonthal AH, Hofman FM, Chen TC, Lee AS. The unfolded protein response regulator GRP78/BiP as a novel target for increasing chemosensitivity in malignant gliomas. Cancer Res. 2007; 67:9809–9816. [PubMed: 17942911]

Rachidi S, Sun S, Wu BX, Jones E, Drake RR, Ogretmen B, Cowart A, Clarke CJ, Hannun YA, Chiosis G, Liu B, Li Z. Endoplasmic reticulum heat shock protein gp96 maintains liver homeostasis and promotes hepatocellular carcinogenesis. J Hepatol. 2014 [Epub ahead of print].

Radsak MP, Hilf N, Singh-Jasuja H, Braedel S, Brossart P, Rammensee HG, Schild H. The heat shock protein Gp96 binds to human neutrophils and monocytes and stimulates effector functions. Blood. 2003; 101:2810–2815. [PubMed: 12446445]

Rao RV, Bredesen DE. Misfolded proteins, endoplasmic reticulum stress and neurodegeneration. Curr Opin Cell Biol. 2004; 16:653–662. [PubMed: 15530777]

Reddy RK, Mao C, Baumeister P, Austin RC, Kaufman RJ, Lee AS. Endoplasmic reticulum chaperone protein GRP78 protects cells from apoptosis induced by topoisomerase inhibitors: role of ATP binding site in suppression of caspase-7 activation. J Biol Chem. 2003; 278:20915–20924. [PubMed: 12665508]

Reimold AM, Iwakoshi NN, Manis J, Vallabhajosyula P, Szomolanyi-Tsuda E, Gravallese EM, Friend D, Grusby MJ, Alt F, Glimcher LH. Plasma cell differentiation requires the transcription factor XBP-1. Nature. 2001; 412:300–307. [PubMed: 11460154]

Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol. 2007; 8:519–529. [PubMed: 17565364]

Rosekrans SL, Heijmans J, Büller NV, Westerlund J, Lee AS, Muncan V, van den Brink GR. ER stress induces epithelial differentiation in the mouse oesophagus. Gut. 2014 [Epub ahead of print].

Rosen ED, MacDougald OA. Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol. 2006; 7:885–896. [PubMed: 17139329]

Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. Nature. 2006; 444:847–853. [PubMed: 17167472]

Rutkowski DT, Kaufman RJ. A trip to the ER: coping with stress. Trends Cell Biol. 2004; 14:20–28. [PubMed: 14729177]

Sansom OJ, Reed KR, Hayes AJ, Ireland H, Brinkmann H, Newton IP, Batlle E, Simon-Assmann P, Clevers H, Nathke IS, Clarke AR, Winton DJ. Loss of Apc in vivo immediately perturbs Wnt signaling, differentiation, and migration. Genes Dev. 2004; 18:1385–1390. [PubMed: 15198980]

Schroder M, Kaufman RJ. The mammalian unfolded protein response. Annu Rev Biochem. 2005; 74:739–789. [PubMed: 15952902]

Sha H, He Y, Chen H, Wang C, Zenno A, Shi H, Yang X, Zhang X, Qi L. The IRE1alpha-XBP1 pathway of the unfolded protein response is required for adipogenesis. Cell Metab. 2009; 9:556–564. [PubMed: 19490910]

Shani G, Fischer WH, Justice NJ, Kelber JA, Vale W, Gray PC. GRP78 and Cripto form a complex at the cell surface and collaborate to inhibit transforming growth factor beta signaling and enhance cell growth. Mol Cell Biol. 2008; 28:666–677. [PubMed: 17991893]

Spike BT, Kelber JA, Booker E, Kalathur M, Rodewald R, Lipianskaya J, La J, He M, Wright T, Klemke R, Wahl GM, Gray PC. CRIPTO/GRP78 signaling maintains fetal and adult mammary stem cells ex vivo. Stem Cell Reports. 2014; 2:427–439. [PubMed: 24749068]

Staron M, Yang Y, Liu B, Li J, Shen Y, Zuniga-Pflucker JC, Aguila HL, Goldschneider I, Li Z. gp96, an endoplasmic reticulum master chaperone for integrins and Toll-like receptors, selectively regulates early T and B lymphopoiesis. Blood. 2010; 115:2380–2390. [PubMed: 19965672]

Tanaka M, Itoh T, Tanimizu N, Miyajima A. Liver stem/progenitor cells: their characteristics and regulatory mechanisms. J Biochem. 2011; 149:231–239. [PubMed: 21217146]

Todd DJ, McHeyzer-Williams LJ, Kowal C, Lee AH, Volpe BT, Diamond B, McHeyzer-Williams MG, Glimcher LH. XBP1 governs late events in plasma cell differentiation and is not required for antigen-specific memory B cell development. J Exp Med. 2009; 206:2151–2159. [PubMed: 19752183]

Usui M, Yamaguchi S, Tanji Y, Tominaga R, Ishigaki Y, Fukumoto M, Katagiri H, Mori K, Oka Y, Ishihara H. Atf6alpha-null mice are glucose intolerant due to pancreatic beta-cell failure on a high-fat diet but partially resistant to diet-induced insulin resistance. Metabolism. 2012; 61:1118–1128. [PubMed: 22386934]

van der Flier LG, Clevers H. Stem cells, self-renewal, and differentiation in the intestinal epithelium. Annu Rev Physiol. 2009; 71:241–260. [PubMed: 18808327]

van Galen P, Kreso A, Mbong N, Kent DG, Fitzmaurice T, Chambers JE, Xie S, Laurenti E, Hermans K, Eppert K, Marciniak SJ, Goodall JC, Green AR, Wouters BG, Wienholds E, Dick JE. The unfolded protein response governs integrity of the haematopoietic stem-cell pool during stress. Nature. 2014; 510:268–272. [PubMed: 24776803]

Vitadello M, Colpo P, Gorza L. Rabbit cardiac and skeletal myocytes differ in constitutive and inducible expression of the glucose-regulated protein GRP94. Biochem J. 1998; 332:351–359. [PubMed: 9601063]

Vitadello M, Penzo D, Petronilli V, Michieli G, Gomirato S, Menabo R, Di Lisa F, Gorza L. Overexpression of the stress protein Grp94 reduces cardiomyocyte necrosis due to calcium overload and simulated ischemia. FASEB J. 2003; 17:923–925. [PubMed: 12670879]

Wanderling S, Simen BB, Ostrovsky O, Ahmed NT, Vogen SM, Gidalevitz T, Argon Y. GRP94 is essential for mesoderm induction and muscle development because it regulates insulin-like growth factor secretion. Mol Biol Cell. 2007; 18:3764–3775. [PubMed: 17634284]

Wang M, Wey S, Zhang Y, Ye R, Lee AS. Role of the unfolded protein response regulator GRP78/BiP in development, cancer, and neurological disorders. Antioxid Redox Signal. 2009; 11:2307–2316. [PubMed: 19309259]

Wang M, Ye R, Barron E, Baumeister P, Mao C, Luo S, Fu Y, Luo B, Dubeau L, Hinton DR, Lee AS. Essential role of the unfolded protein response regulator GRP78/BiP in protection from neuronal apoptosis. Cell Death Differ. 2010; 17:488–498. [PubMed: 19816510]

Wey S, Luo B, Lee AS. Acute inducible ablation of GRP78 reveals its role in hematopoietic stem cell survival, lymphogenesis and regulation of stress signaling. PLoS One. 2012a; 7:e39047. [PubMed: 22723926]

Wey S, Luo B, Tseng CC, Ni M, Zhou H, Fu Y, Bhojwani D, Carroll WL, Lee AS. Inducible knockout of GRP78/BiP in the hematopoietic system suppresses Pten-null leukemogenesis and AKT oncogenic signaling. Blood. 2012b; 119:817–825. [PubMed: 21937694]

Yamamoto K, Takahara K, Oyadomari S, Okada T, Sato T, Harada A, Mori K. Induction of liver steatosis and lipid droplet formation in ATF6alpha-knockout mice burdened with pharmacological endoplasmic reticulum stress. Mol Biol Cell. 2010; 21:2975–2986. [PubMed: 20631254]

Yang Y, Li Z. Roles of heat shock protein gp96 in the ER quality control: redundant or unique function? Mol Cells. 2005; 20:173–182. [PubMed: 16267390]

Ye R, Jung DY, Jun JY, Li J, Luo S, Ko HJ, Kim JK, Lee AS. Grp78 heterozygosity promotes adaptive unfolded protein response and attenuates diet-induced obesity and insulin resistance. Diabetes. 2010a; 59:6–16. [PubMed: 19808896]

Ye R, Mareninova OA, Barron E, Wang M, Hinton DR, Pandol SJ, Lee AS. Grp78 heterozygosity regulates chaperone balance in exocrine pancreas with differential response to cerulein-induced acute pancreatitis. Am J Pathol. 2010b; 177:2827–2836. [PubMed: 20971738]

Zhang Y, Tseng CC, Tsai YL, Fu X, Schiff R, Lee A. Cancer cells resistant to therapy promote cell surface relocalization of GRP78 which complexes with PI3K and enhances PI(3,4,5)P3 production. PLoS One. 2013; 8:e80071. [PubMed: 24244613]

Zhou H, Zhang Y, Fu Y, Chan L, Lee AS. Novel mechanism of anti-apoptotic function of 78-kDa glucose-regulated protein (GRP78): endocrine resistance factor in breast cancer, through release of B-cell lymphoma 2 (BCL-2) from BCL-2-interacting killer (BIK). J Biol Chem. 2011; 286:25687–25696. [PubMed: 21622563]

Zhu G, Ye R, Jung DY, Barron E, Friedline RH, Benoit VM, Hinton DR, Kim JK, Lee AS. GRP78 plays an essential role in adipogenesis and postnatal growth in mice. FASEB J. 2013; 27:955–964. [PubMed: 23180827]

Zhu G, Wang M, Spike B, Gray PC, Shen J, Lee SH, Chen SY, Lee AS. Differential requirement of GRP94 and GRP78 in mammary gland development. Sci Rep. 2014; 4:5390. [PubMed: 24953136]

Table 1

Mutant mouse models of genes encoding UPR components

UPR			
Targeted KO tissues	**Mouse model**	**Major phenotypes**	**Reference**
PERK			
hepatocytes, cholangiocytes	*Perkf/f, Alb-Cre*	impaired UPR activation following tunicamycin injection	Bunpo et al., 2009
inducible, adult bone marrow cells and other organs	*Perkf/f, R26R$^{ERT2\text{-}Cre}$*	hyperglycemia, death of β cells, ER malfunction, UPR↑	Gao et al., 2012
mammary epithelium	*Perkf/f, MMTV-Cre*	lipogeneic enzymes↓, lipid content↓, autophagy↓ and cell death↑ in lactating mammary gland	Bobrovnikova-Marjon et al., 2008; Avivar-Valderas et al., 2011
ATF6			
whole body	*Atf6α−/−*	excerbate liver dysfunction and steatosis induced by tunicamycin injection	Yamamoto et al., 2010
		glucose tolerance↓ and insulin sensitivity↑ on HFD, swollen ER in β cells	Usui et al., 2012
XBP1			
inducible, adult bone marrow cells and other organs	*Xbp1f/f, Mx1-Cre*	hypocholesterolemia, hypotriglyceridemia, lipid production↓ from liver	Lee et al., 2008
inducible, adult acinar cells	*Xbp1f/f, Mist1-Cre^{ER}*	extensive apoptosis, UPR↑, regeneration of acinar cells from residual XBP1+ cells	Hess et al., 2011
intestinal epithelium	*Xbp1f/f, Villin-Cre*	spontaneous enteritis, ER stress, paneth and goblet cells↓, antimicrobial function↓	Liu et al., 2013
inducible, intestinal epithelium	*Xbp1f/f, Villin-Cre^{ERT2}*	apoptosis of paneth cells, small intestinal inflammation, regenerative response	Kaser et al., 2008
B cells	*Xbp1f/f, CD19-Cre*	defect in plasma cell differentiation, normal memory B cell development	Todd et al., 2009

Table 2

GRP78 mutant mouse models

GRP78			
Targeted KO tissues	**Mouse model**	**Major phenotypes**	**Reference**
whole body	*Grp78−/−*	Lethality at E3.5, proliferation↓, apoptosis↑	Luo et al., 2006
	Grp78+/−	normal, GPR94 and PDI↑	Luo et a1., 2006
		energy expenditure↑, HFD-induced obesity↓, UPR↑ in WAT	Ye et al., 2010a
		exacerbate acute pancreatitis under RD, improve acute pancreatitis under HFD	Ye et al., 2010b
	Bip(ΔKDEL)-HA*	disordered cerebral cortex and cerebellum, smaller brain size	Mimura et al., 2008
	baffled#	abnormal cerebellum, defect in axon projection	Favero et al., 2013
	*mutantGrp78/+**	motor disabilites, degeneration of motoneurons in aged mice	Jin et al., 2014
Endoderm			
hepatocytes, cholangiocytes	*Grp78f/f, Alb-Cre*	fatty liver, UPR↑, exacerbate acute and chronic hepatic disorders	Chen et al., 2014c; Ji et al., 2011
intestinal and oesophageal epithelium	*Grp78f/f, Ah1-Cre*	**Intestine**: ER stress↑, intestinal epithelial stemness↓, repopulation by WT cells	Heijmans et al., 2013
		Oesophagus: differentiation of mutant epithelial cells, repopulation by WT cells	Rosekrans et al., 2014
Mesoderm			
inducible, adult bone marrow cells and other organs	*Grp78f/f, Mx1-Cre*	HSC↑, common lymphoid and myeloid progenitors↑, UPR↑	Wey et al., 2012
adipocytes, brain, bone	*Grp78f/f, Ap2-Cre*	postnatal death, growth retardation, lipoatrophy, insulin sensitivity↑	Zhu et al., 2013
endothelial cells	*Grp78f/+, Tie2-Cre*	tumor growth and metastasis↓, minimal effect on normal tissue MVD	Dong et al., 2011
Ectoderm			
mammary epithelium	*Grp78f/f, MMTV-Cre* §	normal mammary epithelium, minimal reduction of GRP78	Zhu et al., 2014
prostate epithelium	*Grp78f/f, PB-Cre4*	normal prostate, fertile	Fu et al., 2008
purkinje cells of cerebellum	*Grp78f/−, pc-Cre*	cerebellar degeneration, retarded growth, UPR↑, ER dilation	Wang et al., 2009

*knockin

#hypomorph mutant of GRP78

§knockdown of GRP78 was not achieved in this mouse model. The function of GRP78 in mammary epithelium was validated by transplatation assay

Table 3

GRP94 mutant mouse models

GRP94			
targeted KO tissues	**mouse model**	**major phenotypes**	**Reference**
whole body	*Grp94−/−* (exon 2 disruption)	lethality from E7.5 to E10.5, ER chaperones↑, differentiation to cardiomyocytes↓	Mao et al., 2010
	Grp94−/− (exon 3 disruption)	lethality at E7, IGF II production↓, fail to develop mesoderm	Wangderling et al., 2007
Endoderm			
hepatocytes, cholangiocytes	*Grp94f/f, Alb-Cre*	liver progenitor cells↑, HCC and DRs, repopulation by GRP94+ hepatocytes, disorganized adhesion molecules, oncogenic signaling↑, long chain ceramides↑	Chen et al., 2014a; Chen et al., 2014b; Rachidi et al., 2014
intestinal epithelium	*Grp94f/f, Villin-Cre*	postnatal death, intestine proliferation↓, Wnt signaling↓	Liu et al., 2013a
inducible, adult bone marrow cells and other organs	*Grp94f/f, R26R$^{ERT\text{-}Cre}$*	loss of intestinal homeostasis, β-Catenin nuclear translocation↓, postnatal death	Liu et al., 2013a
Mesoderm			
inducible, adult bone marrow cells and other organs	*Grp94f/f, Mx1-Cre*	interaction of HSC with niche↓, HSC pool↑, cell surface integrin α4↓	Luo et al., 2011
	Grp94f/f, R26R$^{ERT2\text{-}Cre}$	defective lymphopoiesis, interaction of progenitor with niche↓	Staron et al., 2010
B cell	*Grp94f/f, CD19-Cre*	antibody production↓ upon TLR stimulation, normal B cell development	Liu et al., 2008
Macrophage	*Grp94f/−, Lys-Cre*	TLR response↓, endotoxin shock↓, normal macrophage development	Yang et al., 2007
muscle	*Grp94f/f, MCK-Cre*	growth retardation, skeletal muscles↓, local and circulating IGF-I↓	Barton et al., 2012
Ectoderm			
mammary epithelium	*Grp94f/f, MMTV-Cre*	normal mammary gland development	Zhu et al., 2014
purkinje cells of cerebellum	*Grp94f/−, pc-Cre*	no defect	Wang et al., 2009