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REVIEW

Novel interactions of GRP78: UPR and estrogen responses in the brain

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Abstract

Glucose-regulated protein 78 (GRP78; 78 kDa) belongs to a group of highly conserved heat shock proteins (Hsp) with important functions at the cellular level. The emerging interest for GRP78 relies on its different functions, both in normal and pathological circumstances. GRP78 regulates intracellular calcium, protein shaping, endoplasmic reticulum (ER) stress and cell survival by an immediate response to insults, and that its expression may also be regulated by estrogens. Although these roles are well explored, the mechanisms by which GRP78 induces these changes are not completely understood. In this review, we highlight various aspects related to the GRP78 functioning in cellular protection and repair in response to ER stress and unfolded protein response by the regulation of intracellular Ca^{2+} and other mechanisms. In this respect, the novel interactions between GRP78 and estrogens, such as estradiol and others, are analyzed in the context of the central nervous system (CNS). We also discuss the importance of GRP78 and estrogens in brain diseases including ischemia, Alzheimer's and Huntington's disorders. Finally, the main protective mechanisms of GRP78 and estrogens during ER dysfunction in the brain are described, and the prospective roles of GRP78 in therapeutic processes.

Keywords: calcium; estrogens; GRP78; neuroprotection; unfolded protein response

Introduction

Glucose-regulated protein 78 (GRP78), also known as HSPA5 or BiP, is a chaperone located in the endoplasmic reticulum (ER), nucleus and mitochondria that belongs to the Hsp70 (heat shock protein 70) highly conserved protein family and, for that reason, it is implicated in multiple functions, such as cell survival, protein homeostasis, embryonic cell growth and pluripotent cell survival (Stetler et al., 2010; Zhang and Zhang, 2010). To exert its protective functions under stress conditions, GRP78 should be translocated from the ER to other cell compartments, such as nucleus, mitochondria or cell membrane, where it acts as a membrane receptor transducing extracellular signals associated with cancer, cell survival, proliferation and metastasis (Sun et al., 2006; Gonzalez-Gronow et al., 2009; Liu et al., 2010; Misra and Pizzo, 2010a, b; Misra et al., 2011; Ni et al., 2011; Ouyang et al., 2011a). Furthermore, GRP78 directly binds to caspases 7 and 12 affecting the apoptotic downstream signalling (Zhang and Zhang, 2010; Dong et al., 2011).

Previous studies suggest a role for GRP78 in cell death regulation (Liu et al., 1997; Rao et al., 2002; Kadowaki et al., 2004; Kim et al., 2006), including apoptotic mechanisms in Purkinje cells in the cerebellum (Wang et al., 2010), and cerebral ischemia (Ouyang and Giffard, 2012). Expression of GRP78 tissues in the endometrium seems to be regulated by the expression of estrogen receptors, and increased estrogen concentration during the estrous cycle. Moreover, GRP78 regulates the efflux of ER Ca^{2+} to preserve the cytoplasmic calcium homeostasis (Coe and Michalak, 2009), both in normal and in pathological conditions (Coe and Michalak, 2009; Misra et al., 2009; Wang et al., 2010).

Taking into account the multiple biological-related functions of GRP78, such as calcium buffer and management (Coe and Michalak, 2009), and stress conditions such as heat shock, hypoxia, hypoglycaemia and electrolytic abnormalities, GRP78 might have neuroprotective functions in pathological states of the CNS, including ischemia, Alzheimer's and Huntington's disorders (Wang et al., 2010; Hara et al., 2011).

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Here, we will discuss the relationship of GRP78 with calcium management, its role in ER stress responses, and some potential neuroprotective mechanisms in the setting of the ER homeostasis, interaction with estrogenic compounds as a potential strategy in brain diseases.

GRP78 structure and cellular localization

Since the discovery of GRP78 in late 1970s, there has been a growing interest in its structure, function and location due to its downstream signalling mechanisms associated with cellular protection (Gething, 1999; Zhang and Zhang, 2010; Pfaffenbach and Lee, 2011). Human GRP78, also known as BiP or Hspa5, is a protein of 78 kDa of 654 residues that has been evolutionarily conserved from yeast to man (Figure 1; Lievremont et al., 1997; Lee, 2001). Its gene is located in the human chromosome 9 at position 9q33–9q34, with the 5′-end of the gene distal to the centromere. This gene spans 4,576 bp and contains eight exons, with a highly active promoter containing a TATA box, multiple CCAAT sequences, which are repetitive units of the ER stress response element (ERSE; Yoshida et al., 1998; Lee, 2001), and two potential binding sites for the transcriptional factor Sp1 (Ting and Lee, 1988). The human Grp78 promoter also has a conserved c-Myb binding site, which acts independently of sequences associated with the unfolded protein response (UPR) of GRP78. Thus, the binding of C-Myb causes transactivation of the Grp78 promoter and leads to the induction of endogenous GRP78 (Ramsay et al., 2005). GRP78 expression occurs in parallel with sex steroid receptors expression, as their amounts exhibit menstrual cycle-dependent changes in human endometrium (Guzel et al., 2011).

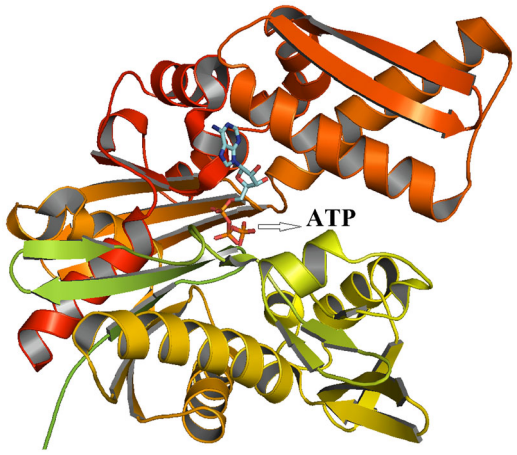

Figure 1 Crystallographic 3D structure of GRP78. The model was obtained using Pymol version 1.4.1. In order to predict the structure, the protein was assessed in its monomeric form, subsequently the red–green–blue (RGB) filter was changed to CMYK, and finally a raytracer of 1,200 * 1,200 was placed.

GRP78 belongs to the HspA/Hsp70 family of chaperones, the most widely studied group of heat shock proteins, currently known to be comprised of at least 13 highly related structures that are either constitutively expressed or induced following cellular stress (Stetler et al., 2010). Members of the Hsp70 family are found in multiple subcellular compartments including the mitochondria, ER, cytosol and lysosomes. Their activity is associated with a variety of cell functions (Stetler et al., 2010; Wisniewska et al., 2010), and this topic will be further discussed in this review.

GRP78, like other Hsp chaperones, has two conserved domains: (i) the 44-kDa NH_2-terminal domain (NBD), which is responsible for its ATPase activity (termed the Chaperone function domain), and (ii) a 20-kDa COOH-terminal polypeptide-binding domain. It also contains a highly helical and variable 10-kDa COOH-terminal tail of unknown function (Chevalier et al., 2000; Stetler et al., 2010). The N-terminal nucleotide-binding domain (NBD) is joined by a flexible linker to the C-terminal peptide substrate-binding domain (Wisniewska et al., 2010). There is an alternation between the ATP state, with low affinity and high exchange rates, and the ADP state, with high affinity and low exchange rates that is tightly regulated by several co-chaperones, which is critically dependent on the conformation of the NBD (Wisniewska et al., 2010).

GRP78 is mainly located in the perinuclear ER due to its C-terminal retention signal, KDEL (Zhang and Zhang, 2010). This KDEL domain (Lys–Asp–Glu–Leu, i.e. KDEL) identifies this protein as an ER resident. GRP78 is overexpressed following ER stress, leading to a re-localization of the protein on the cell surface (Ni et al., 2011). ER stress also enhances the retention of intron 1 of the protein transcript, leading to the translation of a novel isoform of GRP78 (Grp78va) that lacks the ER signal peptide and is localized in the cytosol, where GRP78 assumes novel functions in cell surface signalling (Ni et al., 2011). GRP78 can also be translocated to the mitochondrial inter-membrane space, inner membrane and matrix. However, the precise mechanism of this translocation process is not understood (Sun et al., 2006). Finally, GRP78 has also been observed in the nucleus after ER stress induction following treatment with capsaicin in HepG2 cells, suggesting a role of GRP78 against DNA damage and capsaicin-induced apoptosis (Zhai et al., 2005; Huang et al., 2009; Ni et al., 2011).

GRP78, as discussed above, can also exist on the cell surface of different cell types, especially in tumourigenic tissues and cell types under stress conditions, such as hypoxia or glucose starvation (Ni et al., 2011). Cell surface GRP78 can form complexes with many extracellular ligands and cell surface anchored proteins including α2-macroglobulin, Kringle 5, Par-4, Cripto and T-cadherin, regulating either pro-survival or pro-apoptotic pathways (Misra et al., 2005). For example, it is known that GRP78 associates with survival pathways,

Table 1 Roles of GRP78 at a cellular level. The table shows the main cell functions of GRP78, and cell type and/or compartments that it might be exerting any significant function or cellular process.

Function	Location and cell type (compartment)	References
Proliferation and cell survival	Membrane, tumour cells and normal cells	Gonzalez-Gronow et al. (2009), Misra et al. (2005), Misra and Pizzo (2010a, b), Ni et al. (2011), Quinones et al. (2008), Yu et al. (1999), Zhang and Zhang (2010)
Drives the unfolded protein response, protects the cell from stress conditions	Endoplasmic reticulum lumen and mitochondria	Austin (2009), Larner et al. (2006), Li et al. (2008), Naidoo (2011), Schroder (2006), Stetler et al. (2010), Sun et al. (2006), Wang et al. (2009), Wang et al. (2010), Zhang and Zhang (2010)
Antiapoptotic properties	Endoplasmic reticulum, mitochondria and neuronal cells	Fu et al. (2008), Miyake et al. (2000), Reddy et al. (2003), Shu et al. (2008), Suzuki et al. (2007), Wang et al. (2010), Yu et al. (1999), Zhang and Zhang (2010)
Calcium homeostasis	Endoplasmic reticulum lumen, mitochondria and neuronal cells	Coe and Michalak (2009), Jaepel and Blum (2011), Michalak et al. (2002), Ouyang et al. (2011b), Sammels et al. (2010), Treiman (2002)
Potential neuroprotection via calcium homeostasis, apoptotic signalling, reduction of oxidative damage	Endoplasmic reticulum, mitochondria and neuronal cells	Hara et al. (2011), Oida et al. (2008), Ouyang et al. (2011b, 2012), Suyama et al. (2011), Wang et al. (2010), Yu et al. (1999)

i.e. Akt, which could explain the potent anti-apoptotic properties of GRP78 (Dai et al., 2010; Lin et al., 2011; Nakajima et al., 2011; Zhang et al., 2011).

GRP78 functions as a cell surface receptor involved in mitogenesis and cellular proliferation (Luo et al., 2006; Ni et al., 2011), and its coexpression with major histocompatibility complex (MHC) I class antigens may act as a receptor for both dengue virus and coxsackievirus A9, an icosahedral single-stranded RNA virus that belongs to the genus Enterovirus of the family Picornaviridae (Triantafilou et al., 2002; Misra et al., 2004; Ni et al., 2011). However, the binding of GRP78 to the plasma membrane by extracellular ligands influences the activation of PI3K/Akt and MAPKs signal transduction pathways (Dong et al., 2011) inducing cell proliferation and survival.

In summary, GRP78 acts as a key integrator of multiple signalling pathways related with its specific functions as a chaperone, calcium-regulating protein, pro-survival factor or modulator of the ER stress (Sun et al., 2006; Cook et al., 2012). Recent evidence has also pointed out an interesting interaction between the estrogenic stimulation and the regulation of the GRP78 expression in different organs and physiological contexts, such as cancer and brain diseases, suggesting novel therapeutic approaches (Fu et al., 2007; Saleh et al., 2009; Andersson et al., 2010; Cook et al., 2012). We will next explore the specific functions of GRP78 in the brain and its possible modulation by estradiol and other estrogenic compounds.

GRP78 functions in the brain

GRP78 both in normal and pathological conditions has multiple functions critical for cellular homeostasis and physiological stress (Ouyang and Giffard, 2012). GRP78 is a major ER chaperone, and a master regulator of the Ca^{2+} efflux of the ER and the UPR (Coe and Michalak, 2009; Misra et al., 2009; Wang et al., 2009) by sequestering protein ER-like kinase (PERK) and inositol requiring enzyme 1 (IRE1; Misra et al., 2009; Pincus et al., 2010), and activating transcription factor 6 (ATF6), which are necessary proteins for initiating UPR in the ER (Schroder, 2006; Diehl et al., 2011). A role for GRP78 in cell death regulation has been mooted (Liu et al., 1997; Rao et al., 2002; Kadowaki et al., 2004; Kim et al., 2006), including apoptotic mechanisms in Purkinje cells of the cerebellum (Wang et al., 2010), cerebral ischemia (Ouyang and Giffard, 2012) and also has a possible function as a membrane receptor transducing intracellular ligands and extracellular signals associated with cancer, survival, proliferation and metastasis (Liu et al., 2010; Misra and Pizzo, 2010a; Misra et al., 2011; Ni et al., 2011). A summary of functions is shown in Table 1. Furthermore, GRP78 directly binds to caspases 7 and 12, affecting apoptotic downstream signalling (Zhang and Zhang, 2010; Dong et al., 2011). GRP78 facilitates the assembly of multimeric protein complexes inside the ER and transiently binds to newly synthesized proteins in the ER or proteins that have been misfolded due to stress conditions by recognizing unfolded polypeptides and preventing their aggregation (Gething, 1999). GRP78 has also been implicated in neuronal diseases, such as Parkinson's disease, cerebral ischemia, schizophrenia and transmissible spongiform encephalopathies (Figure 2; Wang et al., 2009; Ouyang and Giffard, 2012). In this context, we will show below the novel interactions of GRP78-estrogens, and its possible neuroprotective function in brain diseases.

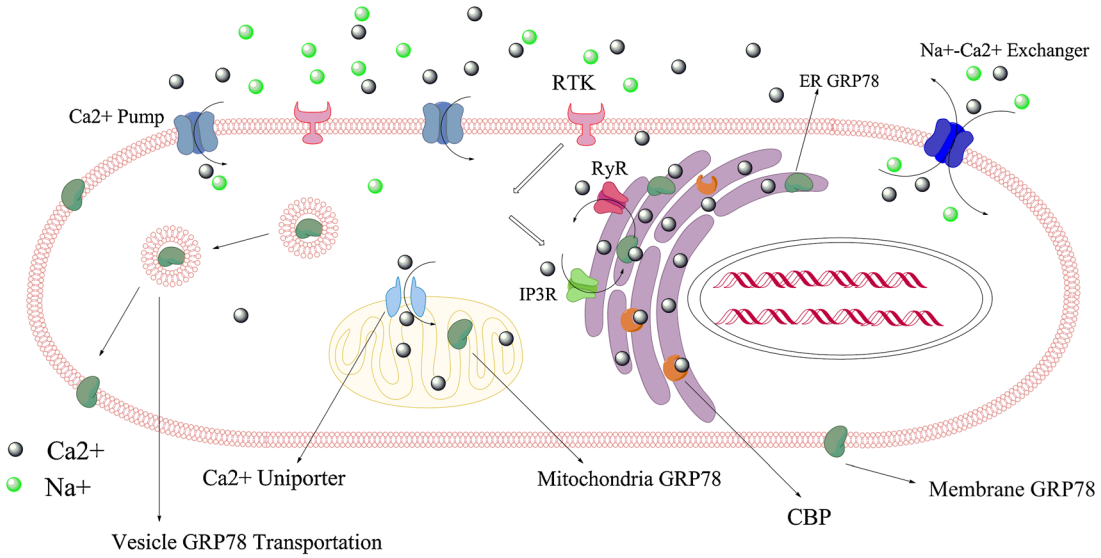

Figure 2 ER proteins contribute to the maintenance of Ca^{2+} homeostasis and signalling. Note Ca^{2+} concentrations, both outside and inside the cell, and in each cellular compartment. Ca^{2+} ion tends to enter the cell due to its high outside concentration in comparison to its low inside concentration, and this ion flux is controlled by Na^{+}–Ca^{2+} exchanger and Ca^{2+} pumps located in the membrane. Cytosolic Ca^{2+} concentration is low, however, RyR and IP3R regulate the ER Ca^{2+} concentrations resembling the Ca^{2+} concentration outside of the cell to create optimal conditions to the ER functions. The arrows indicate directions of Ca^{2+} ions flow. Inside the ER, CBPs buffer the Ca^{2+} concentration due to its ability to bind with low affinity to Ca^{2+} ions. Mitochondrial Ca^{2+} concentrations are regulated by transmembrane Ca^{2+} uniporter. Note the ability of GRP78 be located in different cellular compartments. The crooked thin arrows show ions (grey dots—Ca^{2+}, green dots—Na^{+}) movement through the channels and transporters. The straight arrow indicates different proteins such as GRP78, CBP and Ca^{2+} uniporter. '*' Indicates vesicle transportation of GRP78. CBP, calcium binding proteins; IP_3R, phosphatidil inositol receptor; RyR, ryanodine receptor; RTK, receptor tyrosine kinase.

ER and calcium management in the brain—the role of GRP78

Ca^{2+} signalling is essential in the CNS for learning and memory, neurotransmitter action, synaptic activity and gene expression; high concentrations of Ca^{2+} and/or its deregulation can induce excitotoxicity and cell death (Orrenius et al., 2003; Jaepel and Blum, 2011). Perturbations in Ca^{2+} signalling have also been thought to play a role in the pathogenesis of neurodegenerative disorders (Naidoo, 2009; Puzianowska-Kuznicka and Kuznicki, 2009; Sammels et al., 2010). Calcium concentration is highly regulated by several Ca^{2+} channels (Li et al., 2011), as well as by pumps, exchangers and buffers (Jaepel and Blum, 2011). For example, calcium pumps increase cytoplasmic Ca^{2+} concentration by influx of these ions through the plasma membrane in eukaryotic cells, whereas the IP3R (inositol-1,4,5-trisphosphate receptor) releases Ca^{2+} from intracellular ER stores (Marambaud et al., 2009; Sammels et al., 2010).

The ER is the main organelle involved in calcium management. Its dynamic environment, which resembles the Ca^{2+} conditions of the extracellular space, contains high concentrations of Ca^{2+} binding proteins (CBPs) that directly influence the functioning of the ER (Berridge, 2002; Michalak et al., 2002; Coe and Michalak, 2009). The ER calcium concentration is regulated by Ca^{2+} channels (Kostyuk and Verkhratsky, 1994; Mattson et al., 2000) and buffered by CBPs, which are also found in the cytosol (Malhotra and Kaufman, 2007; Coe and Michalak, 2009), whereas the cytoplasmic Ca^{2+} concentration is thought to be regulated via the IP3 (inositol-1,4,5-triphosphate) pathway (Berridge, 2002). As the ER luminal Ca^{2+} concentration affects several important functions, there is a strict control of Ca^{2+} concentration (Michalak et al., 2002). For instance, the SERCA-Ca^{2+} pumps (Sarco-ER Ca^{2+} ATPase) maintains Ca^{2+} intraluminal levels at 0.1–0.8 mM (Alvarez and Montero, 2002; Michalak et al., 2002; Jaepel and Blum, 2011), whereas free concentration of Ca^{2+} in the cytosol is ~100 nM at resting levels (Alvarez and Montero, 2002; Jaepel and Blum, 2011), hence the maximum variations of the Ca^{2+} levels are in the range of 2–5 μM when signalling takes place (Jaepel and Blum, 2011). Due to concentration differences, electrochemical forces drive Ca^{2+} fluxes from the extracellular space and the ER lumen into both cytosol and mitochondria (Michalak et al., 2002; Jaepel and Blum, 2011). An important concentration of total Ca^{2+} in ER is buffered by chaperones present in the ER lumen; the other significant concentration of Ca^{2+} is free and its fluctuations affect the functions inside and outside the ER (Michalak et al., 2002; Coe and Michalak, 2009). Among the various chaperones that exert a Ca^{2+} buffering function in ER homeostasis are calreticulin, calnexin, GRP94, protein

disulfide isomerase (PDI)/Calcistorin, ERp72 and GRP78. Interestingly, calreticulin mRNA is upregulated by estrogen in endometrial cells of bonnet monkeys (Parmar et al., 2009).

GRP78 binds Ca^{2+} at relatively low capacity (1–2 mol of Ca^{2+} per mol of protein), but is responsible for as much as 25% of the Ca^{2+} binding capacity of the ER (Lievremont et al., 1997). This binding capacity is mainly dependent on its abundant acidic amino acids, which are arranged in doublets or triplets (Lucero et al., 1994; Lievremont et al., 1997). Transfected cells overexpressing GRP78 have also shown considerable mt(Ca^{2+}) transients in response to stimulation, suggesting that the GRP78 calcium storage function is fundamental for mitochondrial physiology (McCormack et al., 1990; Lievremont et al., 1997).

Taking into account that the ER and mitochondria are physically and functionally linked through the mitochondria-associated membrane (MAM), there is increasing evidence of a crosstalk between the CBPs of both organelles that maintains mitochondrial functions; for example, Ca^{2+} transfer from ER to mitochondria can induce mitochondrial dysfunction and programmed cell death after stress (Ni et al., 2011). Regarding GRP78, its overexpression in astrocytes reduces the net flux of Ca^{2+} from ER to mitochondria and increases Ca^{2+} uptake capacity in isolated mitochondria, suggesting that GRP78 can help maintain mitochondrial homeostasis during ER stress (Shu et al., 2008; Ni et al., 2011). GRP78 can be recruited to the MAM by the sigma-1 receptor chaperone (Ouyang and Giffard, 2012), and can interact with other proteins, such as the voltage-dependent anion channel, the chaperone GRP75 and possibly the mitochondrial calcium uniporter, the primary influx pathway for Ca^{2+} into respiring mitochondria (Ouyang and Giffard, 2012). These results suggest that GRP78, and the other mentioned proteins of the MAM, are inter-organelle signalling modulators that can regulate Ca^{2+} signalling between the ER and the mitochondria, controlling death and survival of brain cells during ischemia and other processes, which are strongly related to signalling pathways activated by estrogens and other molecules (Saleh et al., 2009; Ouyang and Giffard, 2012).

GRP78 and the UPR

Due to the significant roles of calcium in the CNS (Gallego-Sandin et al., 2011), brain cells have strict control in the management of this ion (Landowne and Ritchie, 1971; Hidalgo and Carrasco, 2011; Sudhof, 2011). Calcium concentrations vary from intracellular and extracellular compartments due to the presence of ion channels in the cell membrane, Ca^{2+} ATPases and several other proteins that may regulate calcium (Puzianowska-Kuznicka and Kuznicki, 2009; Jaepel and Blum, 2011). However, deregulation of Ca^{2+} concentration and other disturbances, including glucose deprivation, hypoxia, oxidative stress, immune responses, heavy metals such as selenium and lead, ethanol, inflammation and viral infection, can cause the accumulation of unfolded proteins in the lumen of the ER and trigger the evolutionary conserved response known as UPR or unfolded protein response (Xu et al., 2005; Zu et al., 2006; Zhang et al., 2008; Sammels et al., 2010; Ke et al., 2011; Wang and Kaufman, 2012). UPR also acts as an adaptation mechanism of the ER to re-establish its normal functions caused by the high concentration of unfolded proteins (Malhotra and Kaufman, 2007; Coe and Michalak, 2009). These mechanisms include the transcriptional activation of genes that enhance the protein folding capacity of the ER and promote protein degradation of misfolded proteins, including chaperones and protein foldases, and to the induction of phospholipid synthesis that increases the size of the ER and dilutes its content (Xu et al., 2005; Schroder, 2006). Moreover, there is an inhibition of mRNA translation that reduces the influx of new proteins into the ER, until activation and production of UPR proteins, such as transcription factors and chaperones, including CHOP, IRE1, PERK, GRP94 and GRP78. However, when these processes fail, the UPR downstream proteins, IRE1 and PERK, can activate the c-jun N-terminal kinase (JNK) and NF-kB that promote inflammation and apoptotic mechanisms under excessive and prolonged ER stress (Xu et al., 2005; Wang and Kaufman, 2012). Interestingly, neurotoxins (e.g. 6-OHDA, MPP^+ and rotenone) also induce nuclear fragmentation, ER stress and UPR in catecholaminergic cells that are associated with changes in proteasomal and chaperone activities, suggesting that the expression of the UPR pathways allows neurons to maintain protein homeostasis even during augmented expression of reactive oxygen species (ROS; Betarbet et al., 2000; Greenamyre et al., 2001; Nagel et al., 2009; Bauereis et al., 2011; de Oliveira et al., 2011; Cabezas et al., 2012).

UPR is modulated by three molecular sensors, IRE, ATF6 and PERK, each one being involved in UPR signalling (Schroder, 2006; Coe and Michalak, 2009). For example, IRE1 modulates the transcription of chaperones, foldases, ER membrane biogenesis and protein secretion via the XBP1 transcription factor (Schroder, 2006; Wu and Kaufman, 2006; Pincus et al., 2010). ATF6 is an ER-associated transmembrane basic leucine zipper transcription factor that, following its release from GRP78, traffics to the Golgi apparatus for cleavage by specific proteases and thus becomes activated (Schindler and Schekman, 2009). Therefore, it can activate UPR target genes, alone or coupled with XBP1. Moreover, protein kinase-like ER kinase (PERK) is involved in the attenuation of the protein synthesis and apoptotic activation through the induction of the CCAAT enhancer-binding protein CHOP (Paschen and Frandsen, 2001; Schroder, 2006; Pincus et al., 2010; Diehl et al., 2011). Importantly, GRP78

plays a fundamental role in the modulation of the UPR mechanism and its signalling mechanisms, as it acts as a negative regulator of ATF6, IRE and PERK (Xu et al., 2005; Schroder, 2006; Wang and Kaufman, 2012). Under ER stress, GRP78 depletion is caused by an increase of unfolded proteins, which leads GRP78 to dissociate from PERK, ATF6 and IRE1, thus activating the UPR downstream signalling (Schroder, 2006; Zu et al., 2006). As a result, GRP78 acts as a key regulator of UPR and the processes related with the folding protein homeostasis, and both, its underexpression or overexpression, affects in a critical way cell functions due in part to its sensibility to calcium ions by regulating the downstream responses in the cell. A recent study suggests that the UPR signalling suppresses estrogenic responsiveness, indicating a possible crosstalk between estrogens and the UPR in the endometrium by a regulation of GRP78 levels (Guzel et al., 2011). However, more research is needed in order to precisely determine whether similar interaction is present in the brain during UPR events.

Current research has shown that the UPR is likely involved in neurodegenerative diseases that present accumulation and aggregation of misfolded proteins (Wang et al., 2009). For example, in vitro evidences have shown that deletion of *chop* attenuated neurotoxin-induced Parkinson's disease (Gow and Wrabetz, 2009), and deletion of *Xbp1* in a transgenic mice model delayed the onset of ALS disease and increased the life span, probably due to an enhanced clearance of mutant SOD1 aggregates by macroautophagy in motoneurons (Hetz et al., 2009). Moreover, a polymorphism in the *XBP1* promoter was linked with a risk factor for bipolar disorder and schizophrenia (Kakiuchi et al., 2004). Another feature associated with UPR, and the subsequent neurodegeneration, is the oxidative stress damage, which is a consequence of multiple events that lead to an increase in the production of ROS (LeDoux et al., 2007). These events can arise following either a decrease in the scavenging of ROS, a diminished repair system of oxidized macromolecules, or a combination of all these processes working concomitantly (LeDoux et al., 2007; Lee et al., 2012). In summary, further research is needed to uncover the specific functions of GRP78 during the UPR in neurodegenerative diseases, which in turn will possibly shed light on new potential therapeutic strategies.

GRP78 interaction with estrogens

There is an important relationship between the expression of heat shock proteins and the estrogenic stimulation in different tissues, such as mammary glands, endometrium and brain (Alvarez and Montero, 2002; Fu et al., 2007; Saleh et al., 2009; Cook et al., 2012; Luvsandagva et al., 2012). A tight association between nuclear steroid receptors and chaperones of the Hsp family, such as Hsp70 and Hsp90, facilitates the folding of the hormone-binding domain (HBD) of the receptors (Pratt and Toft, 1997). A possible estrogen-GRP78 interaction in cancer development raised considerable interest since GRP78 regulates various anti-apoptotic and pro-survival pathways such as TSC2/AMPK, mTOR or the inhibition of cytochrome *c* release (Lee, 2007; Cook et al., 2012). These observations were connected because estrogen receptor alpha (ERα) is expressed in many types of cancers with both anti-estrogen resistance and overexpressed GRP78 (Virrey et al., 2008; Cook et al., 2012; Luvsandagva et al., 2012). Furthermore, overexpression of GRP78 conferred resistance to chemotherapeutic agents like CPT-11, etoposide and temozolomide in endothelial cells derived from malignant glioma (TuBEC), whereas its silencing with siRNA restored their cytotoxicity drugs (Virrey et al., 2008) A similar result was observed in human endometrial explants treated with the estrogen receptor antagonist, tamoxifen, which showed an increased expression of GRP78, NF-kB and caspase 3, thereby inducing cell stress (Andersson et al., 2010). These results show that targeting of GRP78 sensitizes tumour cells to different chemotherapeutic drugs, including estrogen derivatives like tamoxifen, raloxifene and bazedoxifene, suggesting an important therapeutically approach (Virrey et al., 2008; Luvsandagva et al., 2012). We will now explore the interaction of GRP78 and estrogens in the context of brain pathologies as a possible protective strategy.

GRP78 and estrogens in brain diseases

Both GRP78 and estrogens, as mentioned above, have been associated with cell death regulation, cell survival, proliferation and metastasis, both in vitro and in epidemiological studies (Liu et al., 1997, 2010; Rao et al., 2002; Kadowaki et al., 2004; Kim et al., 2006; Misra et al., 2006; Gonzalez-Gronow et al., 2009; Misra and Pizzo, 2010a; Wang et al., 2010; Misra et al., 2011; Ni et al., 2011; Zhengqi, 2012). For example, in vitro studies have shown that malignant glioma cells present a greater overexpression of GRP78 compared with the normal adult brain; these cells are dependent on this protein for their fast proliferation rate. These cells thus have a higher resistance to chemotherapeutic agents, such as temozolomide, and the knockdown of GRP78 sensitizes glioma cells to this and other pharmacological agents (Pyrko et al., 2007; Wang et al., 2009). However, epidemiological studies have described a close correlation between an elevated GRP78 level and a higher recurrence with a poor prognosis in melanoma, breast, liver, prostate, kidney, colon, glioma and gastric cancers (Lee, 2007; Zhang et al., 2011). This suggests that GRP78 is fundamental for the survival of stressed cells such as those in cancer and other pathologies (Lee, 2007). GRP78 expression could serve as a biomarker of tumour behaviour and treatment response (Lee, 2007). Interestingly,

treatment of gastric cancer SGC7901 cells with low concentrations of 17ß estradiol increased expression of both GRP78 and estrogen receptor α-36 (a variant or ERα) suggesting that the development and progression of gastric cancer is related both with GRP78 expression and estrogen signalling (Zhengqi, 2012).

In astrocytomas and glioblastomas, GRP78 expression is inversely correlated with prognosis or recurrent patients (Zhang et al., 2011). GRP78 is expressed as a membrane protein receptor, which influences multiple signalling pathways in glioblastoma cells (Zhang et al., 2011). For example, silencing of GRP78 drastically attenuates the phosphorylation levels of both Akt and ERK1/2, thus affecting cell proliferation and anti-apoptotic functions (Sharma et al., 2006; Zhang et al., 2011). As suggested by Zhang et al. (2011), activated Akt via GRP78 increases procaspase-9 phosphorylation, which in turn decreases the level of cleaved caspase 7. Alternatively, GRP78 can form a complex with the pro-apoptotic protein BIK, caspases 7 and 12, thus preventing the release of cytochrome *c* from mitochondria and restraining apoptosis (Rao et al., 2002; Fu et al., 2007; Lee, 2007). However, the use of high concentrations (20 μM) of 17ß estradiol can induce apoptosis and inhibit cell growth of the glioblastoma cell line, T98G, and the glioma cell line, C6, by blocking JNK signalling, suggesting the potential of estradiol in the treatment of brain tumours (Altiok et al., 2011). GRP78 has also been implicated in neuronal diseases, such as Parkinson's disease, cerebral ischemia, schizophrenia and apoptosis of Purkinje cells (Wang et al., 2010; Ouyang and Giffard, 2012). Furthermore, taking into account that GRP78 has chaperone activity, it might prevent the aggregation and assist the proteasomal degradation of mutant prion proteins implicated in neurodegenerative disorders, such transmissible spongiform encephalopathies (Lee, 2001).

In ischemic models, increased levels of GRP78 remain elevated at 24 h of reperfusion (Lehotsky et al., 2009). This GRP78 overexpression can protect astrocytes against ischemic injury, reducing the net flux of Ca^{2+} from ER to mitochondria, and free radical production, preserving the mitochondrial membrane potential after stress (Ouyang et al., 2011a). Dysfunction of astrocytes occurs much earlier than delayed neuronal death (Ouyang et al., 2007; Xu et al., 2010) and overexpressing of the protective genes in astrocytes can protect neighbouring neurons, thus suggesting an important neuroprotective role for GRP78 in ischemia (Ouyang et al., 2011a). Finally, a reduction in the infarct area of rats pretreated with estradiol is associated with a decrease in cortical expression of Hsp70 and an increased expression of GRP78 in a rat ischemic model (Saleh et al., 2009). This suggests that the ischemic tolerance following estradiol pretreatment is mediated in part through cellular stress proteins such as GRP78 (Saleh et al., 2009).

Both estradiol and GRP78 have been implicated in neuroprotection of Parkinson's disease by regulating α-synuclein aggregation (Hirohata et al., 2009; Gorbatyuk et al., 2012). Overexpression of GRP78 diminished α-synuclein neurotoxicity by downregulating ER stress mediators and promoting the survival of nigral tyrosine hydroxylase-positive neurons in a Parkinson rat model, suggesting a neuroprotective role for GRP78 (Gorbatyuk et al., 2012). Similarly, not only 17ß estradiol protects dopaminergic neurons against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity (Simpkins et al., 2009), but estriol, estrone, androstenedione and testosterone inhibit formation of α-synuclein aggregates and destabilization of its fibrils (Hirohata et al., 2009).

An incomplete activation of the UPR, with a reduced expression of GRP78—a phenomenon observed in Alzheimer's disease (AD)—may be caused by mutations in presenilin-1, an important element in the proteolytic processing of amyloid precursor (Katayama et al., 1999). However, a transient transfection of GRP78 in HEK cells decreases secretion of the mutants Ab40 and Ab42 peptides, which are the major components of amyloid plaque in AD (Yang et al., 1998). These conflicting results suggest that further research is needed in order to clarify the importance of GRP78 in AD. However, numerous studies have shown the protective effect of estrogens in AD (Garcia-Segura et al., 2001; Wise et al., 2001; Behl, 2002; Simpkins et al., 2009) by exerting multiple effects, such as the reduction of amyloid peptide, the anti-oxidative activity of the hormone and the overexpression of ERα and ERβ in the brain (Simpkins et al., 2009). These results highlight the importance of GRP78 and estrogens in different pathologies, suggesting that GRP78 may be an important target against neurodegenerative diseases and other neurologic disorders.

Neuroprotective mechanisms of GRP78 and estrogens

Both overexpression or antisense modulation of Hsp70, GRP78 and HGRP94 can protect against cell death in different models, including prostate cancer, renal endothelial cancer, leukaemia, neurons and astrocytes, through the inhibition of caspases 7 and 12 (Little and Lee, 1995; Liu et al., 1997; Miyake et al., 2000; Lee, 2001; Suyama et al., 2011). GRP78 has also been reported to exert neuroprotective mechanisms following oxygen glucose deprivation (Ouyang et al., 2011b), ischemic-reperfusion injury (Ouyang et al., 2012) and cytotoxicity by 6-OHDA (Hara et al., 2011). Overexpression of GRP78 in astrocytes protects against ER stress (Suyama et al., 2011), but studies in this field are limited and there is insufficient evidence regarding the neuroprotection of GRP78 under overexpressing conditions. However, a fivefold increase in GRP78

expression in neurons, but not astrocytes, is observed under hypoxic conditions (Goldenberg-Cohen et al., 2012). Administration of GRP78 binding peptide, ADoPep, also prevents cardiomyocyte apoptosis under ischemic conditions. This protective mechanism seems to be exerted by the inhibition of hypoxia induced caspases 3 and 7 activity, and suggests the importance of GRP78 in the modulation of survival signals (Goldenberg-Cohen et al., 2012). Moreover, the inhibition of GRP78 by pharmacological compounds, such as salubrinal or by antisense oligonucleotides, protects rat hippocampal neurons against glutamate excitotoxicity, kainic acid and the oxidative insults of the amyloid beta-peptide through the blocking of caspases 3 and 12 (Yu et al., 1999; Sokka et al., 2007). Alternatively, it is possible that GRP78 exerts its neuprotective mechanism against excitotoxicity and apoptosis via stabilization of Ca^{2+} homeostasis and oxidative stress reduction (Liu et al., 1997; Reddy et al., 1999; Yu et al., 1999). Downregulation or knockdown of GRP78 has also been used in different cell models, for example cardiomiocytes and neurons to stimulate cell survival following insults like hypoxia (Hardy and Raiter, 2010; Goldenberg-Cohen et al., 2012) or ischemia (Ouyang et al., 2011a; Ouyang and Giffard, 2012). Taking into account that estrogens can regulate in vivo expression of GRP78 (Zhengqi, 2012), it is possible that a similar approach could be used to modulate GRP78 expression for cell protection in brain diseases models. It is also possible that estradiol could influence the calcium binding activities of GRP78, as estradiol affects calcium-dependent activity associated with cognition, which is a prominent component of brain aging and neurodegenerative disesases (Brewer et al., 2009). However, more research is needed in order to unravel the precise mechanism by which GRP78 exerts its neuroprotective functions, and how estradiol and other estrogens can regulate GRP78 expression in the brain.

Conclusions and perspectives

A growing body of evidence suggests that the malfunctioning of ER in the CNS may contribute to the massive loss of neurons during brain injury and other diseases. ER-related proteins, like GRP78, are central mediators of homeostasis in multiple processes that include protein homeostasis, cell growth and proliferation, apoptosis and inter-organellar modulation of calcium. Much research has explored the downregulation and overexpression of GRP78 in therapeutic models, including cancer, hypoxia and ischemia, mainly by the regulation of Akt-PI3K pathways (Dai et al., 2010; Misra and Pizzo, 2010a; Zhang et al., 2011; Chang et al., 2012; Wey et al., 2012). Downregulation of GRP78 by different methods (i.e. genetic, estrogenic chemical or environmental factors) is a promising approach to neuroprotective action during excitotoxic damage for both neurons and glial cells (Dorner et al., 1992; Wang et al., 2005; Suyama et al., 2011). However, a better understanding of the multiple interactions of GRP78 and the signalling crosstalk that allows the alternative activation of viability processes or apoptosis is required.

It seems important to explore the specific functions of the various forms taken by the GRP78 in different subcellular locations; the extracellular and soluble form of GRP78 is a candidate for the regulation of inflammatory responses (Shields et al., 2012), as this extracellular protein is possibly involved in the NF-kB inflammation-signalling pathway (Nakajima et al., 2011). This is a promising field in the regulation of inflammatory conditions in tissues. A better understanding of the downstream signalling mechanism of the UPR, such as the ones exerted by GRP78, and its modulation by estrogens may uncover new strategies of how ER stress is associated with brain diseases and the possible therapeutic approaches may so be applied.

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References

Altiok N, Ersoz M, Koyuturk M (2011) Estradiol induces JNK-dependent apoptosis in glioblastoma cells. Oncol Lett 2: 1281–5.

Alvarez J, Montero M (2002) Measuring [Ca^{2+}] in the endoplasmic reticulum with aequorin. Cell Calcium 32: 251–60.

Andersson H, Helmestam M, Zebrowska A, Olovsson M, Brittebo E (2010) Tamoxifen-induced adduct formation and cell stress in human endometrial glands. Drug metabolism and disposition: the biological fate of chemicals. Drug Metabol Dispos 38: 200–7.

Austin RC (2009) The unfolded protein response in health and disease. Antioxid Redox Sign 11: 2279–87.

Bauereis B, Haskins WE, Lebaron RG, Renthal R (2011) Proteomic insights into the protective mechanisms of an in vitro oxidative stress model of early stage Parkinson's disease. Neurosci Lett 488: 11–6.

Behl C (2002) Oestrogen as a neuroprotective hormone. Nat Rev Neurosci 3: 433–42.

Berridge MJ (2002) The endoplasmic reticulum: a multifunctional signaling organelle. Cell Calcium 32: 235–49.

Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT (2000) Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neurosci 3: 1301–6.

Brewer LD, Dowling AL, Curran-Rauhut MA, Landfield PW, Porter NM, Blalock EM (2009) Estradiol reverses a calcium-related biomarker of brain aging in female rats. J Neurosci 29: 6058–67.

Cabezas R, El-Bacha RS, Gonzalez J, Barreto GE (2012) Mitochondrial functions in astrocytes: neuroprotective implications from oxidative damage by rotenone. Neurosci Res 74: 80–90.

Chang YJ, Huang YP, Li ZL, Chen CH (2012) GRP78 knockdown enhances apoptosis via the down-regulation of oxidative stress and Akt pathway after epirubicin treatment in colon cancer DLD-1 cells. PLoS ONE 7: e35123.

Chevalier M, Rhee H, Elguindi EC, Blond SY (2000) Interaction of murine BiP/GRP78 with the DnaJ homologue MTJ1. J Biol Chem 275: 19620–7.

Coe H, Michalak M (2009) Calcium binding chaperones of the endoplasmic reticulum. Gen Physiol Biophys 28: 96–103.

Cook KL, Shajahan AN, Warri A, Jin L, Hilakivi-Clarke LA, Clarke R (2012) Glucose-regulated protein 78 controls cross-talk between apoptosis and autophagy to determine antiestrogen responsiveness. Cancer Res 72: 3337–49.

Dai RY, Chen SK, Yan DM, Chen R, Lui YP, Duan CY, Li J, He T, Li H (2010) PI3K/Akt promotes GRP78 accumulation and inhibits endoplasmic reticulum stress-induced apoptosis in HEK293 cells. Folia Biol 56: 37–46.

de Oliveira DM, Barreto G, Galeano P, Romero JI, Holubiec MI, Badorrey MS, Capani F, Alvarez LD (2011) *Paullinia cupana* Mart. var. Sorbilis protects human dopaminergic neuroblastoma SH-SY5Y cell line against rotenone-induced cytotoxicity. Hum Exp Toxicol 30: 1382–91.

Diehl JA, Fuchs SY, Koumenis C (2011) The cell biology of the unfolded protein response. Gastroenterology 141: 38–41, e1–2.

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 (2011) A critical role for GRP78/BiP in the tumor microenvironment for neovascularization during tumor growth and metastasis. Cancer Res 71: 2848–57.

Dorner AJ, Wasley LC, Kaufman RJ (1992) Overexpression of GRP78 mitigates stress induction of glucose regulated proteins and blocks secretion of selective proteins in Chinese hamster ovary cells. EMBO J 11: 1563–71.

Fu Y, Li J, Lee AS (2007) GRP78/BiP inhibits endoplasmic reticulum BIK and protects human breast cancer cells against estrogen starvation-induced apoptosis. Cancer Res 67: 3734–40.

Fu Y, Wey S, Wang M, Ye R, Liao CP, Roy-Burman P, Lee AS (2008) 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 105: 19444–9.

Gallego-Sandin S, Alonso MT, Garcia-Sancho J (2011) Calcium homoeostasis modulator 1 (CALHM1) reduces the calcium content of the endoplasmic reticulum (ER) and triggers ER stress. Biochem J 437: 469–75.

Garcia-Segura LM, Azcoitia I, DonCarlos LL (2001) Neuroprotection by estradiol. Prog Neurobiol 63: 29–60.

Gething MJ (1999) Role and regulation of the ER chaperone BiP. Semin Cell Dev Biol 10: 465–72.

Goldenberg-Cohen N, Raiter A, Gaydar V, Dratviman-Storobinsky O, Goldstein T, Weizman A, Hardy B (2012) Peptide-binding GRP78 protects neurons from hypoxia-induced apoptosis. Apoptosis Int J Program Cell Death 17: 278–88.

Gonzalez-Gronow M, Selim MA, Papalas J, Pizzo SV (2009) GR P78: a multifunctional receptor on the cell surface. Antioxid Redox Sign 11: 2299–306.

Gorbatyuk MS, Shabashvili A, Chen W, Meyers C, Sullivan LF, Salganik M, Lin JH, Lewin AS, Muzyczka N, Gorbatyuk OS (2012) Glucose regulated protein 78 diminishes alpha-synuclein neurotoxicity in a rat model of Parkinson disease. Mol Ther J Am Soc Gene Ther 20: 1327–37.

Gow A, Wrabetz L (2009) CHOP and the endoplasmic reticulum stress response in myelinating glia. Curr Opin Neurobiol 19: 505–10.

Greenamyre JT, Sherer TB, Betarbet R, Panov AV (2001) Complex I and Parkinson's disease. IUBMB Life 52: 135–41.

Guzel E, Basar M, Ocak N, Arici A, Kayisli UA (2011) Bidirectional interaction between unfolded-protein-response key protein HSPA5 and estrogen signaling in human endometrium. Biol Reprod 85: 121–7.

Hara H, Kamiya T, Adachi T (2011) Endoplasmic reticulum stress inducers provide protection against 6-hydroxydopamine-induced cytotoxicity. Neurochem Int 58: 35–43.

Hardy B, Raiter A (2010) Peptide-binding heat shock protein GRP78 protects cardiomyocytes from hypoxia-induced apoptosis. J Mol Med (Berl) 88: 1157–67.

Hetz C, Thielen P, Matus S, Nassif M, Court F, Kiffin R, Martinez G, Cuervo AM, Brown RH, Glimcher LH. (2009) XBP-1 deficiency in the nervous system protects against amyotrophic lateral sclerosis by increasing autophagy. Genes Dev 23: 2294–306.

Hidalgo C, Carrasco MA (2011) Redox control of brain calcium in health and disease. Antioxid Redox Sign 14: 1203–7.

Hirohata M, Ono K, Morinaga A, Ikeda T, Yamada M (2009) Anti-aggregation and fibril-destabilizing effects of sex hormones on alpha-synuclein fibrils in vitro. Exp Neurol 217: 434–9.

Huang SP, Chen JC, Wu CC, Chen CT, Tang NY, Ho YT, Lo C, Lin JP, Chung JG, Lin JG (2009) Capsaicin-induced apoptosis in human hepatoma HepG2 cells. Anticancer Res 29: 165–74.

Jaepel J, Blum R (2011) Capturing ER calcium dynamics. Eur J Cell Biol 90: 613–9.

Kadowaki H, Nishitoh H, Ichijo H (2004) Survival and apoptosis signals in ER stress: the role of protein kinases. J Chem Neuroanat 28: 93–100.

Kakiuchi C, Ishiwata M, Umekage T, Tochigi M, Kohda K, Sasaki T, Kato T (2004) Association of the XBP1-116C/G polymorphism with schizophrenia in the Japanese population. Psychiatry Clin Neurosci 58: 438–40.

Katayama T, Imaizumi K, Sato N, Miyoshi K, Kudo T, Hitomi J, Morihara T, Yoneda T, Gomi F, Mori Y, Nakano Y, Takeda J, Tsuda T, Itoyama Y, Murayama O, Takashima A, St George-Hyslop P, Takeda M, Tohyama M (1999) Presenilin-1 mutations downregulate the signalling pathway of the unfolded-protein response. Nat Cell Biol 1: 479–85.

Ke Z, Wang X, Liu Y, Fan Z, Chen G, Xu M, Bower KA, Frank JA, Li M, Fang S, Shi X, Luo J (2011) Ethanol induces endoplasmic reticulum stress in the developing brain. Alcohol Clin Exp Res 35: 1574–83.

Kim R, Emi M, Tanabe K, Murakami S (2006) Role of the unfolded protein response in cell death. Apoptosis Int J Program Cell Death 11: 5–13.

Kostyuk P, Verkhratsky A (1994) Calcium stores in neurons and glia. Neuroscience 63: 381–404.

Landowne D, Ritchie JM (1971) On the control of glycogenolysis in mammalian nervous tissue by calcium. J Physiol 212: 503–17.

Larner SF, Hayes RL, Wang KK (2006) Unfolded protein response after neurotrauma. J Neurotrauma 23: 807–29.

LeDoux SP, Druzhyna NM, Hollensworth SB, Harrison JF, Wilson GL (2007) Mitochondrial DNA repair: a critical player in the response of cells of the CNS to genotoxic insults. Neuroscience 145: 1249–59.

Lee AS (2001) The glucose-regulated proteins: stress induction and clinical applications. Trends Biochem Sci 26: 504–10.

Lee AS (2007) GRP78 induction in cancer: therapeutic and prognostic implications. Cancer Res 67: 3496–9.

Lee J, Giordano S, Zhang J (2012) Autophagy, mitochondria and oxidative stress: cross-talk and redox signalling. Biochem J 441: 523–40.

Lehotsky J, Racay P, Pavlikova M, Tatarkova Z, Urban P, Chomova M, Kovalska M, Kaplan P (2009) Cross-talk of intracellular calcium stores in the response to neuronal ischemia and ischemic tolerance. Gen Physiol Biophys 28: 104–14.

Li J, Ni M, Lee B, Barron E, Hinton DR, Lee AS (2008) The unfolded protein response regulator GRP78/BiP is required for endoplasmic reticulum integrity and stress-induced autophagy in mammalian cells. Cell Death Differ 15: 1460–71.

Li MH, Inoue K, Si HF, Xiong ZG (2011) Calcium-permeable ion channels involved in glutamate receptor-independent ischemic brain injury. Acta Pharmacol Sin 32: 734–40.

Lievremont JP, Rizzuto R, Hendershot L, Meldolesi J (1997) BiP, a major chaperone protein of the endoplasmic reticulum lumen, plays a direct and important role in the storage of the rapidly exchanging pool of Ca^{2+}. J Biol Chem 272: 30873–9.

Lin Y, Wang Z, Liu L, Chen L (2011) Akt is the downstream target of GRP78 in mediating cisplatin resistance in ER stress-tolerant human lung cancer cells. Lung Cancer 71: 291–7.

Little E, Lee AS (1995) Generation of a mammalian cell line deficient in glucose-regulated protein stress induction through targeted ribozyme driven by a stress-inducible promoter. J Biol Chem 270: 9526–34.

Liu H, Bowes RC, III, van de Water B, Sillence C, Nagelkerke JF, Stevens JL (1997) Endoplasmic reticulum chaperones GRP78 and calreticulin prevent oxidative stress, Ca^{2+} disturbances, and cell death in renal epithelial cells. J Biol Chem 272: 21751–9.

Liu M, Spellberg B, Phan QT, Fu Y, Lee AS, Edwards JE, Jr., Filler SG, Ibrahim AS (2010) The endothelial cell receptor GRP78 is required for mucormycosis pathogenesis in diabetic mice. J Clin Invest 120: 1914–24.

Lucero HA, Lebeche D, Kaminer B (1994) ER calcistorin/protein disulfide isomerase (PDI). Sequence determination and expression of a cDNA clone encoding a calcium storage protein with PDI activity from endoplasmic reticulum of the sea urchin egg. J Biol Chem 269: 23112–9.

Luo S, Mao C, Lee B, Lee AS (2006) GRP78/BiP is required for cell proliferation and protecting the inner cell mass from apoptosis during early mouse embryonic development. Mol Cell Biol 26: 5688–97.

Luvsandagva B, Nakamura K, Kitahara Y, Aoki H, Murata T, Ikeda S, Minegishi T (2012) GRP78 induced by estrogen plays a role in the chemosensitivity of endometrial cancer. Gynecol Oncol 126: 132–9.

Malhotra JD, Kaufman RJ (2007) Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? Antioxid Redox Sign 9: 2277–93.

Marambaud P, Dreses-Werringloer U, Vingtdeux V (2009) Calcium signaling in neurodegeneration. Mol Neurodegen 4: 20.

Mattson MP, LaFerla FM, Chan SL, Leissring MA, Shepel PN, Geiger JD (2000) Calcium signaling in the ER: its role in neuronal plasticity and neurodegenerative disorders. Trends Neurosci 23: 222–9.

McCormack JG, Halestrap AP, Denton RM (1990) Role of calcium ions in regulation of mammalian intramitochondrial metabolism. Physiol Rev 70: 391–425.

Michalak M, Parker JMR, Opas M (2002) Ca^{2+} signaling and calcium binding chaperones of the endoplasmic reticulum. Cell Calcium 32: 269–78.

Misra UK, Pizzo SV (2010a) Ligation of cell surface GRP78 with antibody directed against the COOH-terminal domain of GRP78 suppresses Ras/MAPK and PI 3-kinase/AKT signaling while promoting caspase activation in human prostate cancer cells. Cancer Biol Ther 9: 142–52.

Misra UK, Pizzo SV (2010b) Modulation of the unfolded protein response in prostate cancer cells by antibody-directed against the carboxyl-terminal domain of GRP78. Apoptosis Int J Program Cell Death 15: 173–82.

Misra UK, Gonzalez-Gronow M, Gawdi G, Wang F, Pizzo SV (2004) A novel receptor function for the heat shock protein Grp78: silencing of Grp78 gene expression attenuates alpha2M*-induced signalling. Cell Signal 16: 929–38.

Misra UK, Gonzalez-Gronow M, Gawdi G, Pizzo SV (2005) The role of MTJ-1 in cell surface translocation of GRP78, a receptor for alpha 2-macroglobulin-dependent signaling. J Immunol 174: 2092–7.

Misra UK, Deedwania R, Pizzo SV (2006) Activation and cross-talk between Akt, NF-kappaB, and unfolded protein response signaling in 1-LN prostate cancer cells consequent to ligation of cell surface-associated GRP78. J Biol Chem 281: 13694–707.

Misra UK, Mowery Y, Kaczowka S, Pizzo SV (2009) Ligation of cancer cell surface GRP78 with antibodies directed against its COOH-terminal domain up-regulates p53 activity and promotes apoptosis. Mol Cancer Ther 8: 1350–62.

Misra UK, Payne S, Pizzo SV (2011) Ligation of prostate cancer cell surface GRP78 activates a proproliferative and antiapoptotic feedback loop: a role for secreted prostate-specific antigen. J Biol Chem 286: 1248–59.

Miyake H, Hara I, Arakawa S, Kamidono S (2000) Stress protein GRP78 prevents apoptosis induced by calcium ionophore, ionomycin, but not by glycosylation inhibitor,

tunicamycin, in human prostate cancer cells. J Cell Biochem 77: 396–408.

Nagel F, Bahr M, Dietz GP (2009) Tyrosine hydroxylase-positive amacrine interneurons in the mouse retina are resistant against the application of various Parkinsonian toxins. Brain Res Bull 79: 303–9.

Naidoo N (2009) ER and aging-protein folding and the ER stress response. Ageing Res Rev 8: 150–9.

Naidoo N (2011) The unfolded protein response in mouse cerebral cortex. Methods Enzymol 489: 3–21.

Nakajima S, Hiramatsu N, Hayakawa K, Saito Y, Kato H, Huang T, Yao J, Paton AW, Paton JC, Kitamura M (2011) Selective abrogation of BiP/GRP78 blunts activation of NF-kappaB through the ATF6 branch of the UPR: involvement of C/EBPbeta and mTOR-dependent dephosphorylation of Akt. Mol Cell Biol 31: 1710–8.

Ni M, Zhang Y, Lee AS (2011) Beyond the endoplasmic reticulum: atypical GRP78 in cell viability, signalling and therapeutic targeting. Biochem J 434: 181–8.

Oida Y, Izuta H, Oyagi A, Shimazawa M, Kudo T, Imaizumi K, Hara H (2008) Induction of BiP, an ER-resident protein, prevents the neuronal death induced by transient forebrain ischemia in gerbil. Brain Res 1208: 217–24.

Orrenius S, Zhivotovsky B, Nicotera P (2003) Regulation of cell death: the calcium-apoptosis link. Nat Rev Mol Cell Biol 4: 552–65.

Ouyang YB, Giffard RG (2012) ER-mitochondria crosstalk during cerebral ischemia: molecular chaperones and ER-mitochondrial calcium transfer. Int J Cell Biology 2012: 493934.

Ouyang YB, Voloboueva LA, Xu LJ, Giffard RG (2007) Selective dysfunction of hippocampal CA1 astrocytes contributes to delayed neuronal damage after transient forebrain ischemia. J Neurosci 27: 4253–60.

Ouyang YB, Lu Y, Yue S, Xu LJ, Xiong XX, White RE, Sun X, Giffard RG (2011a) miR-181 regulates GRP78 and influences outcome from cerebral ischemia in vitro and in vivo. Neurobiol Dis 45: 555–63.

Ouyang YB, Xu LJ, Emery JF, Lee AS, Giffard RG (2011b) Overexpressing GRP78 influences Ca^{2+} handling and function of mitochondria in astrocytes after ischemia-like stress. Mitochondrion 11: 279–86.

Ouyang YB, Lu Y, Yue S, Xu LJ, Xiong XX, White RE, Sun X, Giffard RG (2012) miR-181 regulates GRP78 and influences outcome from cerebral ischemia in vitro and in vivo. Neurobiol Dis 45: 555–63.

Parmar T, Nimbkar-Joshi S, Katkam RR, Gadkar-Sable S, Chaudhari U, Manjramkar DD, Savardekar L, Jacob S, Puri CP, Sachdeva G (2009) Differential expression of calreticulin, a reticuloplasmin in primate endometrium. Hum Reprod 24: 2205–16.

Paschen W, Frandsen A (2001) Endoplasmic reticulum dysfunction—A common denominator for cell injury in acute and degenerative diseases of the brain? J Neurochem 79: 719–25.

Pfaffenbach KT, Lee AS (2011) The critical role of GRP78 in physiologic and pathologic stress. Curr Opinion Cell Biol 23: 150–6.

Pincus D, Chevalier MW, Aragon T, van Anken E, Vidal SE, El-Samad H, Walter P (2010) BiP binding to the ER-stress sensor Ire1 tunes the homeostatic behavior of the unfolded protein response. PLoS Biol 8: e1000415.

Pratt WB, Toft DO (1997) Steroid receptor interactions with heat shock protein and immunophilin chaperones. Endocr Rev 18: 306–60.

Puzianowska-Kuznicka M, Kuznicki J (2009) The ER and ageing II: calcium homeostasis. Ageing Res Rev 8: 160–72.

Pyrko P, Schonthal AH, Hofman FM, Chen TC, Lee AS (2007) The unfolded protein response regulator GRP78/BiP as a novel target for increasing chemosensitivity in malignant gliomas. Cancer Res 67: 9809–16.

Quinones QJ, de Ridder GG, Pizzo SV (2008) GR P78: a chaperone with diverse roles beyond the endoplasmic reticulum. Histol Histopathol 23: 1409–16.

Ramsay RG, Ciznadija D, Mantamadiotis T, Anderson R, Pearson R (2005) Expression of stress response protein glucose regulated protein-78 mediated by c-Myb. Int J Biochem Cell Biol 37: 1254–68.

Rao RV, Peel A, Logvinova A, del Rio G, Hermel E, Yokota T, Goldsmith PC, Ellerby LM, Ellerby HM, Bredesen DE (2002) Coupling endoplasmic reticulum stress to the cell death program: role of the ER chaperone GRP78. FEBS Lett 514: 122–8.

Reddy RK, Lu J, Lee AS (1999) The endoplasmic reticulum chaperone glycoprotein GRP94 with Ca(2+)-binding and antiapoptotic properties is a novel proteolytic target of calpain during etoposide-induced apoptosis. J Biol Chem 274: 28476–83.

Reddy RK, Mao C, Baumeister P, Austin RC, Kaufman RJ, Lee AS (2003) 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 278: 20915–24.

Saleh MC, Connell BJ, Saleh TM (2009) Estrogen may contribute to ischemic tolerance through modulation of cellular stress-related proteins. Neurosci Res 63: 273–9.

Sammels E, Parys JB, Missiaen L, De Smedt H, Bultynck G (2010) Intracellular Ca^{2+} storage in health and disease: a dynamic equilibrium. Cell Calcium 47: 297–314.

Schindler AJ, Schekman R (2009) In vitro reconstitution of ER-stress induced ATF6 transport in COPII vesicles. Proc Natl Acad Sci USA 106: 17775–80.

Schroder M (2006) The unfolded protein response. Mol Biotechnol 34: 279–90.

Sharma SV, Gajowniczek P, Way IP, Lee DY, Jiang J, Yuza Y, Classon M, Haber DA, Settleman J (2006) A common signaling cascade may underlie "addiction" to the Src, BCR-ABL, and EGF receptor oncogenes. Cancer Cell 10: 425–35.

Shields AM, Panayi GS, Corrigall VM (2012) A new-age for biologic therapies: long-term drug-free therapy with BiP? Front Immunol 3: 17.

Shu CW, Sun FC, Cho JH, Lin CC, Liu PF, Chen PY, Chang MD, Fu HW, Lai YK (2008) GRP78 and Raf-1 cooperatively confer

resistance to endoplasmic reticulum stress-induced apoptosis. J Cell Physiol 215: 627–35.

Simpkins JW, Perez E, Wang X, Yang S, Wen Y, Singh M (2009) The potential for estrogens in preventing Alzheimer's disease and vascular dementia. Ther Adv Neurol Disord 2: 31–49.

Sokka AL, Putkonen N, Mudo G, Pryazhnikov E, Reijonen S, Khiroug L, Belluardo N, Lindholm D, Korhonen L (2007) Endoplasmic reticulum stress inhibition protects against excitotoxic neuronal injury in the rat brain. J Neurosci 27: 901–8.

Stetler RA, Gan Y, Zhang W, Liou AK, Gao Y, Cao G, Chen J (2010) Heat shock proteins: cellular and molecular mechanisms in the central nervous system. Prog Neurobiol 92: 184–211.

Sudhof TC (2012) Calcium control of neurotransmitter release. Cold Spring Harbor Perspectives in Biology. 4: a11353.

Sun FC, Wei S, Li CW, Chang YS, Chao CC, Lai YK (2006) Localization of GRP78 to mitochondria under the unfolded protein response. Biochem J 396: 31–9.

Suyama K, Watanabe M, Sakabe K, Okada Y, Matsuyama D, Kuroiwa M, Mochida J (2011) Overexpression of GRP78 protects glial cells from endoplasmic reticulum stress. Neurosci Lett 504: 271–6.

Suzuki T, Lu J, Zahed M, Kita K, Suzuki N (2007) Reduction of GRP78 expression with siRNA activates unfolded protein response leading to apoptosis in HeLa cells. Arch Biochem Biophys 468: 1–14.

Ting J, Lee AS (1988) Human gene encoding the 78,000-dalton glucose-regulated protein and its pseudogene: structure, conservation, and regulation. DNA 7: 275–86.

Treiman M (2002) Regulation of the endoplasmic reticulum calcium storage during the unfolded protein response—significance in tissue ischemia? Trends Cardiovasc Med 12: 57–62.

Triantafilou K, Fradelizi D, Wilson K, Triantafilou M (2002) GRP78, a coreceptor for coxsackievirus A9, interacts with major histocompatibility complex class I molecules which mediate virus internalization. J Virol 76: 633–43.

Virrey JJ, Dong D, Stiles C, Patterson JB, Pen L, Ni M, Schonthal AH, Chen TC, Hofman FM, Lee AS (2008) Stress chaperone GRP78/BiP confers chemoresistance to tumor-associated endothelial cells. Mol Cancer Res 6: 1268–75.

Wang S, Kaufman RJ (2012) The impact of the unfolded protein response on human disease. J Cell Biol 197: 857–67.

Wang Q, He Z, Zhang J, Wang Y, Wang T, Tong S, Wang L, Wang S, Chen Y (2005) Overexpression of endoplasmic reticulum molecular chaperone GRP94 and GRP78 in human lung cancer tissues and its significance. Cancer Detect Prev 29: 544–51.

Wang M, Wey S, Zhang Y, Ye R, Lee AS (2009) Role of the unfolded protein response regulator GRP78/BiP in development, cancer, and neurological disorders. Antioxid Redox Sign 11: 2307–16.

Wang M, Ye R, Barron E, Baumeister P, Mao C, Luo S, Fu Y, Luo B, Dubeau L, Hinton DR, Lee AS (2010) Essential role of the unfolded protein response regulator GRP78/BiP in protection from neuronal apoptosis. Cell Death Differ 17: 488–98.

Wey S, Luo B, Tseng CC, Ni M, Zhou H, Fu Y, Bhojwani D, Carroll WL, Lee AS (2012) Inducible knockout of GRP78/BiP in the hematopoietic system suppresses Pten-null leukemogenesis and AKT oncogenic signaling. Blood 119: 817–25.

Wise PM, Dubal DB, Wilson ME, Rau SW, Liu Y (2001) Estrogens: trophic and protective factors in the adult brain. Front Neuroendocrinol 22: 33–66.

Wisniewska M, Karlberg T, Lehtio L, Johansson I, Kotenyova T, Moche M, Schuler H (2010) Crystal structures of the ATPase domains of four human Hsp70 isoforms: HSPA1L/Hsp70-hom, HSPA2/Hsp70-2, HSPA6/Hsp70B', and HSPA5/BiP/GRP78. PLoS ONE 5: e8625.

Wu J, Kaufman RJ (2006) From acute ER stress to physiological roles of the unfolded protein response. Cell Death Differ 13: 374–84.

Xu C, Bailly-Maitre B, Reed JC (2005) Endoplasmic reticulum stress: cell life and death decisions. J Clin Invest 115: 2656–64.

Xu L, Emery JF, Ouyang YB, Voloboueva LA, Giffard RG (2010) Astrocyte targeted overexpression of Hsp72 or SOD2 reduces neuronal vulnerability to forebrain ischemia. Glia 58: 1042–9.

Yang Y, Turner RS, Gaut JR (1998) The chaperone BiP/GRP78 binds to amyloid precursor protein and decreases Abeta40 and Abeta42 secretion. J Biol Chem 273: 25552–5.

Yoshida H, Haze K, Yanagi H, Yura T, Mori K (1998) Identification of the cis-acting endoplasmic reticulum stress response element responsible for transcriptional induction of mammalian glucose-regulated proteins. Involvement of basic leucine zipper transcription factors. J Biol Chem 273: 33741–9.

Yu Z, Luo H, Fu W, Mattson MP (1999) The endoplasmic reticulum stress-responsive protein GRP78 protects neurons against excitotoxicity and apoptosis: suppression of oxidative stress and stabilization of calcium homeostasis. Exp Neurol 155: 302–14.

Zhai L, Kita K, Wano C, Wu Y, Sugaya S, Suzuki N (2005) Decreased cell survival and DNA repair capacity after UVC irradiation in association with down-regulation of GRP78/BiP in human RSa cells. Exp Cell Res 305: 244–52.

Zhang LH, Zhang X (2010) Roles of GRP78 in physiology and cancer. J Cell Biochem 110: 1299–305.

Zhang Y, Sun LG, Ye LP, Wang B, Li Y (2008) Lead-induced stress response in endoplasmic reticulum of astrocytes in CNS. Toxicol Mech Methods 18: 751–7.

Zhang LH, Yang XL, Zhang X, Cheng JX, Zhang W (2011) Association of elevated GRP78 expression with increased astrocytoma malignancy via Akt and ERK pathways. Brain Res 1371: 23–31.

Zhengqi F (2012) The involvement of GRP78 on estrogen signaling in gastric cancer. In: Hao D, Ying C, Lijiang L, eds. *International Conference on Biomedical Engineering and Biotechnology*. CS Digital Library. pp. 1125–8.

Zu K, Bihani T, Lin A, Park YM, Mori K, Ip C (2006) Enhanced selenium effect on growth arrest by BiP/GRP78 knockdown in p53-null human prostate cancer cells. Oncogene 25: 546–54.

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