The glucose-regulated proteins: stress induction and clinical applications

Amy S. Lee

A protective mechanism used by cells to adapt to stress of the endoplasmic reticulum (ER) is the induction of members of the glucose-regulated protein (Grp) family. The induction of mammalian Grp proteins in response to ER stress involves a complex network of regulators and novel mechanisms. The elucidation of Grp function and regulation opens up new therapeutic approaches to diseases associated with ER stress and cancer.

The mammalian stress response is an evolutionarily conserved mechanism that allows cells to respond to adverse environmental or metabolic conditions. This response is represented at the molecular level by the induced synthesis of specific sets of cellular proteins with protective functions. For example, heat shock and inflammation strongly induce the expression of heat shock proteins (Hsps)[1]. This review focuses on the family of so-called glucose-regulated proteins (Grps), a term that encompasses a variety of endoplasmic reticulum (ER) chaperones that can be induced in cell culture under conditions of glucose starvation[2,3]. The *Grp* genes can also be induced by other perturbations of ER function such as agents that affect calcium stores or inhibit glycosylation. As these stress conditions generally lead to the accumulation of misfolded proteins in the ER, induction of *Grp* gene expression has been used extensively as a marker for the unfolded protein response (UPR). The UPR, an adaptive process conserved from yeast to human that acts in response to the disruption of ER homeostasis, also involves transient attenuation of new protein synthesis, degradation of misfolded proteins and onset of apoptosis[4]. Here, I address the characterization of mammalian Grp induction, regulation and function. The role of the Grps (Grp78 and Grp94) in cell survival during stress, and their clinical implications in cancer chemotherapy, adaptive immunity and gene therapy, will be discussed.

Unique inducers for the mammalian *Grp* genes

The Grps were discovered in 1977 by Ira Pastan through the observation that two proteins of molecular size 78 and 94 kDa were strongly induced in chicken embryo fibroblasts cultured in glucose-free medium[5]. These proteins were subsequently identified as Grp78 (also referred to as the immunoglobulin binding protein BiP) and Grp94 (alias gp96). Grp94 is the most abundant glycoprotein in the ER. In addition, whereas Grp78 is evolutionarily conserved from yeast to humans, Grp94 has only been identified in vertebrates, suggesting that Grp94 might serve unique functions that are restricted to higher eukaryotes.

Expression of misfolded proteins, underglycosylated proteins and treatment of cells with reducing agents, induce the expression of both yeast and mammalian *Grp* genes; however, other stress inducers are unique for the *Grp* genes of higher eukaryotes (Fig. 1). For example, thapsigargin, a non-phorbol ester tumor promoter that specifically inhibits the ER Ca^{2+}-ATPase, is a highly potent ER stress inducer. This, as well as the Ca^{2+} ionophore A23187, ionomycin and EGTA, activate *Grp* transcription through depletion of Ca^{2+} stores in the ER (Ref. 6). This type of Ca^{2+} stress is not observed in yeast. In addition, chronic ethanol exposure potentiates inducers of *Grp* transcription[7], and the mood-stabilizing drug sodium valproate, a branched-chain saturated fatty acid used in the treatment of bipolar disorder and epilepsy, induces *Grp78* gene expression in rat brain[8].

Most studies of *Grp* induction have been conducted with cells in culture; however, there is now new information on Grp expression *in vivo*. The Grps might be required for tissue-specific development. For example, Grp78 is elevated in embryonic mouse heart and is induced following hypoglycemic stress of the heart[9] and within the rat endometrium at the pre-implantation stage[10]. High levels of Grp78 and Grp94 expression were observed in the brains of immature rats compared to the levels found in the brains of adult animals. Their expression was also high in the rat adult brain following cerebral ischemia, head trauma or seizure, induced by the glutamate agonist kainate[11]. Pathological conditions such as tumor growth correlate with Grp78 and Grp94 overexpression[3]. This could be partly caused by the activation of *Grp* gene expression through glucose starvation, acidosis and hypoxia, which are hallmarks of the microenvironment of poorly vascularized solid tumors[12]. Grp78 induction is also associated with injury and dysfunction of human vascular endothelial cells as a result of hyperhomocysteinemia or genetic disorder[13]. Indeed,

Amy S. Lee
University of Southern California/Norris Comprehensive Cancer Center, Dept of Biochemistry and Molecular Biology, University of Southern California Keck School of Medicine, 1441 Eastlake Avenue, Los Angeles, CA 90089-9176, USA.
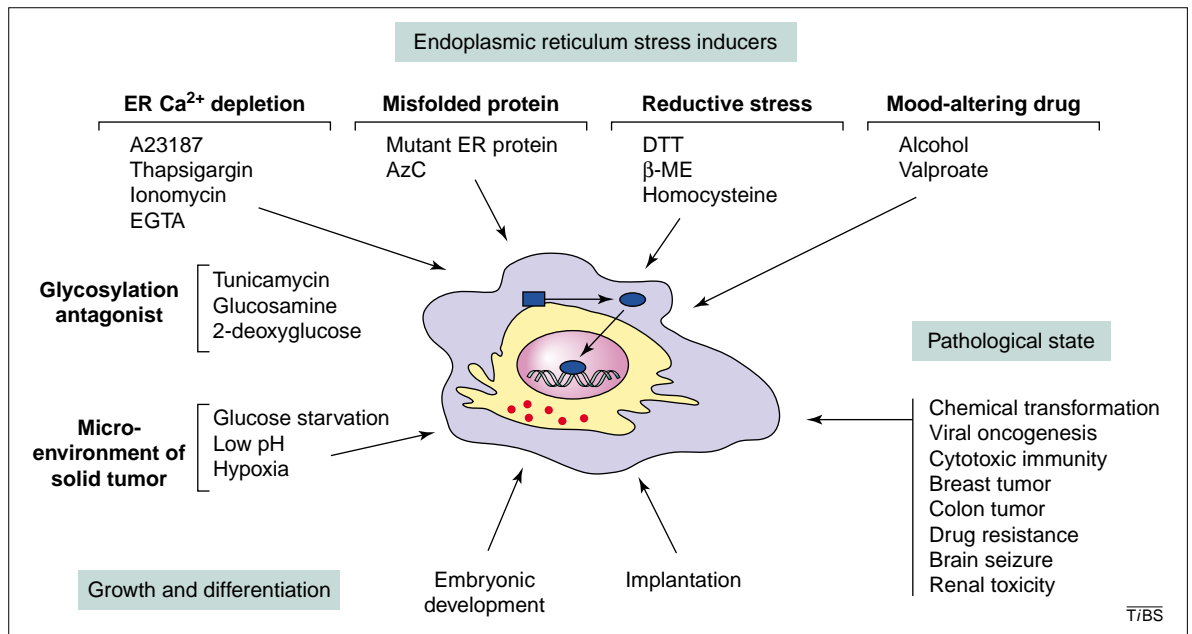e-mail: amylee@ hsc.usc.edu

Fig. 1. Summary of known induction conditions for the mammalian Grp protein family. Stress conditions affecting ER homeostasis trigger signaling pathways from the ER (yellow) to the nucleus (pink). This results in the activation of specific transcription factors (blue) that promote *Grp* gene transcription and accumulation of Grp (red) to high levels. Abbreviations: A23187 is a Ca^{2+} ionophor; AzC, azetidine-2-carboxylic acid; β-ME, β-mercaptoethanol; DTT, dithiothreitol; EGTA, ethyleneglycol-bis(β-aminoethyl ether); ER, endoplasmic reticulum.

the majority of pathologically toxic conditions show areas of inflammation, apoptosis and necrosis, which could represent physiological stresses for Grp induction *in vivo*.

Conservation and divergence of the induction mechanism of yeast and mammalian *Grp* genes

Stress induction of both yeast and mammalian *Grp* genes is primarily regulated at the level of transcription (Fig. 2). The promoter of *KAR2*, the yeast homolog of Grp78, contains one copy of the unfolded protein response element (UPRE). This element, CAGCGTG, serves as the binding site for an activator Hac1, which undergoes ER stress-induced mRNA splicing catalyzed by an ER membrane protein, Ire1p, using an unconventional mechanism[4]. The yeast UPR is negatively regulated by the phosphatase Ptc2p, which directly interacts with and dephosphorylates the phosphorylated form of Ire1p. In yeast, deletion of the gene encoding Ire1p, *IRE1*, abolishes the UPR and the associated induction of *Grp* genes. Some features of the yeast UPR are conserved in mammalian cells[14] (Fig. 2). For example, a subset of mammalian *Grp* promoters contain a UPRE-like sequence[15], to which yeast Hac1 can bind and activate transcription[14]. Mice and humans have two *IRE1* alleles. Support of an Ire1p-dependent pathway for mammalian UPR induction is based on the observation that overexpression of Ire1p activates the *Grp* promoter in a manner that is dependent on the endoribonuclease activity of Ire1p (Refs 4,16,17). This shows that the induction of Grp by Ire1p overexpression is mediated by its enzymatic activity and not merely as a consequence of creating ER stress through overexpressing an ER protein. However, the target for the endoribonuclease activity of Ire1p in mammalian cells has not yet been identified and the mammalian homolog of yeast Hac1 has not been isolated. Surprisingly, genetic disruption of both *IRE1* alleles in mice had no effect on the UPR (Ref. 17). Therefore, unless there are as yet undiscovered mammalian *IRE1* alleles, it appears that the putative Ire1p homolog is not obligatory for the mammalian UPR.

Evidence is accumulating suggesting that mammalian cells use Ire1p-independent signaling pathways to activate the *Grp* genes in response to ER stress. Different stress stimuli could also elicit distinct or overlapping pathways. WEIHI7.2 lymphoma cells are able to increase *Grp78* transcript levels in response to a block in protein glycosylation but not in response to depletion of the ER Ca^{2+} store; further investigation suggested that the proto-oncogene product c-fos was involved in *Grp78* induction following ER Ca^{2+} release[18]. Stress induction of Grp78 and Grp94 is suppressed by genistein, an inhibitor of protein tyrosine kinases that works by obstructing the ATP-binding site of the kinases[19]. ER-stress treatment is also known to induce JNK and p38 MAP kinase signaling[17]. However, the direct relationship between the targets of these and other stress-activated kinases, and their role in the induction of *Grp* genes upon ER stress, remain to be established.

Complex regulatory network for induction of *Grp* transcription

An unusual feature shared by mammalian *Grp* promoters is that they contain multiple copies of the
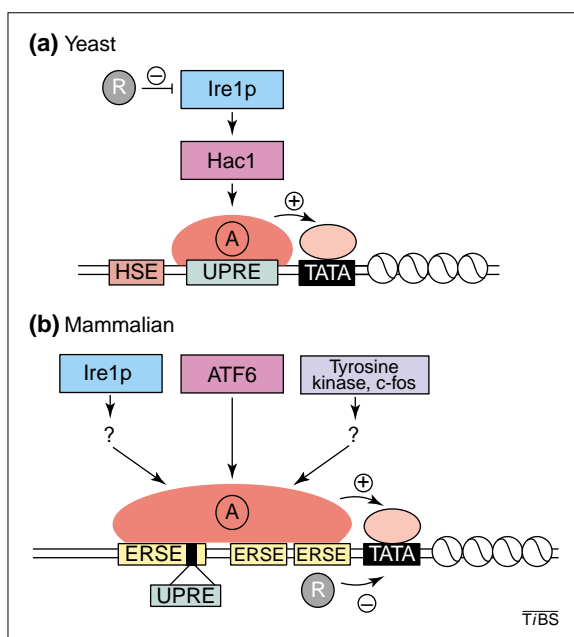
Fig. 2. Induction mechanisms of yeast and mammalian *Grp* genes. (a) In yeast cells, ER stress leads to activation of Ire1p, which induces mRNA splicing and translation of Hac1. The multimeric transcription activating complex (A) consists of Hac1, which binds directly to the UPRE, as well as ada proteins and Gcn5, which have histone acetyltransferase activity. The phosphatase Ptc2p acts as a negative regulator (R) through inactivation of Ire1p. The promoter of *KAR2*, the yeast homolog of *Grp78*, also contains a heat shock element (HSE). (b) In mammalian cells, the *Grp* promoters contain multiple endoplasmic reticulum stress elements (ERSEs) but no HSE. A subset of mammalian *Grp* promoters, including *Grp78*, also contains UPRE-like sequences. ER stress leads to activation of Ire1p; however, its downstream target is not known. ATF6, an ER transmembrane protein, undergoes proteolytic cleavage and other modifications upon ER stress. The nuclear form of ATF6 is a strong activator of the mammalian *Grp* genes. Other transcription factors such as NF-Y, YY1 and TFII-I, can also bind and activate the ERSE. Both NF-Y and YY1 are known to interact with proteins that modify histone acetylation and with cofactors that bind to the TATA element. Other activating pathways might involve tyrosine kinases and the proto-oncogene product c-fos; however, their downstream targets are not known. Members of the cold shock domain proteins, dpA and YB-1, have been shown to suppress ER stress induction of the *Grp78* promoter.

CCAAT element flanked by GC-rich sequences. These turn out to be repetitive units of the ER stress response element (ERSE)[20,21], which is an evolutionarily conserved tripartite structure CCAAT(N_9)CCACG (N represents a 9-bp region) (Fig. 2). This sequence is notably more complex than the UPRE. Recently, another sequence motif, ATTGG(N)CCACG, referred to as ERSE-II, has been identified in the promoter of the gene encoding Herp – a novel ER transmembrane protein. This motif can also confer the ER stress response[22]. The CCAAT-binding factor NF-Y (alias CBF) binds the CCAAT motif of the ERSE and is required for both basal and stress-induced transcription from the *Grp* promoter[19,21,23,24]. By contrast, transcription factor Ying Yang 1(YY1) binds to the CCACG motif in gel shift assays[25]. Overexpression of YY1 enhances *Grp78* promoter activity under ER stressed conditions, whereas overexpression of the cold shock

domain proteins dbpA and YB-1, which also show affinity for the CCACG motif, suppress *Grp78* promoter activity[26]. In genistein-treated cells, in which Grp78 induction is severely suppressed, interaction of both NF-Y and YY1 with the ERSE is inhibited[19]. Within a subset of vertebrate *Grp* promoters, the N_9 sequence is highly conserved and has a strikingly high GC content that binds an ER-stress-inducible complex[15,21,27]. Indeed, mutation of the GC-rich motif reduces ER stress induction[21,23]. Through affinity chromatography, the protein that binds to the GC-rich motif of the ERSE has been identified as the transcription factor TFII-I, known to facilitate protein–protein interactions[27]. Suppression of TFII-I expression by antisense approaches inhibited ER stress induction of the ERSE. Because the stimulatory activity of TFII-I is dependent on tyrosine phosphorylation, it can potentially provide a link between the tyrosine kinase signaling pathway and *Grp* transcription. The yeast UPR model predicts that chromatin modification by acetylation could be important for induction of ER stress response genes[4]. Both NF-Y and YY1 have been shown to interact with cofactors that can modify histone acetylation. Therefore, in addition to enhancing factor cooperativity, they might modify the chromatin structure of *Grp* genes in response to ER stress[14]. Furthermore, YY1 and TFII-I, through their ability to interact with components of the basal transcription machinery, such as TBP, TFIIB and TAFII55I-1, can also link the upstream regulatory and the basal transcription complexes.

Although not detectable as an ERSE-binding complex in gel shift assays using nuclear extracts, ATF6, a transmembrane protein with an ER and perinuclear location, is a potent activator of the *Grp* promoters[20]. There are two forms of ATF6, a 90-kDa form referred to as ATF6(α) and a 110-kDa form referred to as CREB-RP or ATF6(β). Both forms of ATF6 undergo ER-stress-induced cleavage, generating cytoplasmic fragments of 50–60 kDa that enter the nucleus and activate the ERSE through complex formation with NF-Y in a manner dependent on the CCACG motif[24]. In the presence of a proteasome inhibitor, ER-stress-induced cleavage of exogenous ATF6 expressed at a low level can be visualized and is mediated by the Site-I and Site-2 proteases (S1P and S2P, respectively)[28]. These novel findings also imply that ATF6 has to undergo ER stress-induced translocation to the Golgi apparatus to be processed by these specific proteases. Interestingly, Brefeldin A, a drug that causes redistribution of the Golgi components into the ER and blocks protein transport out of the mixed ER–Golgi system[29], induces *Grp78* transcription in hamster fibroblasts; this induction is mediated by the region of the Grp78 promoter containing the ERSE (Ref. 30). Future studies will be required to determine whether the impairment of Grp induction

Table 1. Proteins encoded by mammalian genes that are induced upon ER stress[a]

Protein	Localization	Function	Refs
Grp78/BiP	ER lumen	Protein chaperone, Ca^{2+}-binding protein, cytoprotection	2–4
Grp94/gp96	ER lumen, transmembrane, cell surface	Protein and peptide chaperone, Ca^{2+}-binding protein, cytoprotection	2–4,53
Grp170	ER lumen	Protein and peptide chaperone	54
ERp72	ER lumen	Protein chaperone, Ca^{2+}-binding protein	23,57
PDI	ER lumen	Disulfide bond exchange	57
Calreticulin	ER lumen	Protein chaperone, glycoprotein folding, Ca^{2+}-binding protein	58
Grp58/ERp57	ER lumen	Protein chaperone, glycoprotein folding	57,58
SERCA-2β	ER transmembrane	ER Ca^{2+}-ATPase pump	15
Herp	ER transmembrane	Contains ubiquitin-like domain	22
Oxidoreductin 1-Lβ	ER	ER redox homeostasis	33
Adapt78	Perinuclear, ER, cytoplasm	Cytoprotection	34
CHOP	Nucleus	Transcription factor, growth arrest, apoptosis	17
GLUT-1	Plasma membrane	Glucose transporter	35
Asparagine synthetase	Cytoplasm	Amino acid synthesis	36

[a]Abbreviations: BiP, immunoglobulin-binding protein; CHOP, a nuclear transcription activator associated with growth arrest and induction of apoptosis; ER, endoplasmic reticulum; GLUT, glucose transporter; Grp, glucose-regulated protein; PDI, protein disulfide isomerase; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase.

in S2P-deficient cells is absolute and whether there are other mechanisms for ATF6 activation. In cells depleted of ER Ca^{2+}, the level of the 90-kDa uncleaved ATF6(α) actually increases at the peak of *Grp78* stress induction concurrent with the appearance of other modified forms that can be recovered in soluble nuclear fractions[31]. It is possible that ATF6 is retained in the ER through interaction with ER chaperones. Following ER stress, a fraction of ATF6 could dissociate from the chaperone complex and, with changes in ER membrane fluidity, assume new cellular locations. Because ATF6 is a substrate of p38 MAP kinase[32], its activity can also be modulated by phosphorylation.

Multiple functions of the Grps
A list of proteins encoded by mammalian genes that exhibit induction profiles similar to *Grp78* and *Grp94* in response to ER stress is summarized in Table 1. The majority of the Grps [Grp78, Grp94, Grp170, ERp72, PDI, calreticulin and GRP58 (alias ERp57)] are ER molecular chaperones that assist in protein folding and assembly. Grp78, Grp94, ERp72 and calreticulin are also Ca^{2+}-binding proteins. Newly identified ER proteins that are inducible by ER stress include SERCA-2β, Herp, oxidoreductin 1-Lβ and adapt78, all with functions related to maintenance of ER homeostasis. SERCA-2β is an ER transmembrane protein that pumps Ca^{2+} from the cytosol into the ER lumen[15]. Blockage of SERCA-2β activity by thapsigargin results in ER Ca^{2+} depletion and subsequent Grp induction[6]. Herp contains ubiquitin-like domains at its N-terminus, and genes encoding both Grp78 and Herp are induced in human vascular endothelial cells exposed to homocysteine[13,22]. Oxidoreductin 1-Lβ is an N-glycoprotein required for maintaining a suitable redox environment in the ER (Ref. 33). Finally, Adapt78 is related to Grp78 and exhibits a cytoprotective function[34].

ER stress conditions can also induce genes encoding non-ER proteins, such as GLUT-1 (a glucose-transporter protein)[35], asparagine synthetase[36] and CHOP, a nuclear transcription activator associated with growth arrest and induction of apoptosis[17]. Although CHOP does not regulate *Grp* gene expression, the levels of two activators for the *Grp* promoters, ATF6(α) and TFII-I, increase upon ER Ca^{2+} release in some cell lines[27,31]. Autoregulation of these transcription factors in response to ER stress might represent novel feedback mechanisms that sustain the ER stress response.

Role of Grps in cell survival following ER stress
Overexpression, antisense and ribozyme approaches in tissue culture systems directly showed that Grp78, Grp94 and adapt78 can protect cells against cell death[3,37–43] (Fig. 3). Interestingly, two independent studies suggest that Grp protects more against stress incurred by depletion of ER Ca^{2+} than by a block in protein glycosylation[37,43]. Although the mechanism for the anti-apoptotic effect of the Grps is not understood, a Grp78 mutant that is defective in ATP hydrolysis, which is required for the protein's chaperone activity, loses its protective function[41]. Hsp70, which is related to Grp78 and is a major cytoplasmic chaperone, protects cells against apoptosis through multiple pathways[1]. One such mechanism involves preventing the formation of a functional apoptosome. Further experiments will be required to determine whether the Grps functionally interact with cell death components to mediate ER stress-induced apoptosis[4,17,44].

The protective function of the Grps suggests that their induction could be beneficial in situations
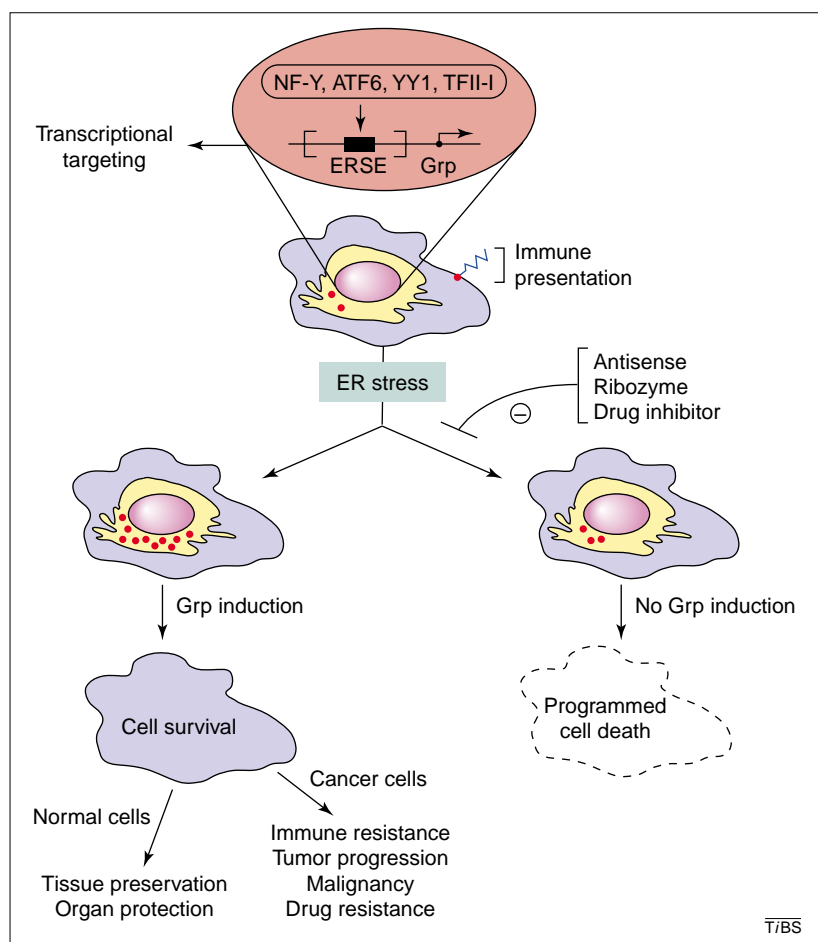
Fig. 3. Grp induction and protection. ER stress triggers transcriptional activation of the *Grp* genes mediated by the ERSE and subsequent increase in the levels of Grp proteins. Components of the transcription activating complex include NF-Y, ATF6, YY1 and TFII-I. The stress-inducible properties of the *Grp78* promoter can be used for transcriptional targeting in gene therapy. The induction of Grp can be blocked at the level of transcription by drug inhibitors such as genistein. The stability and translation of Grp can also be downregulated by ribozymes and antisense expression vectors. Suppression of Grp induction in ER stressed cells leads to higher cytotoxicity and programmed cell death. The induction of Grp confers a survival advantage. This can be beneficial to organ preservation, for example, by protecting against brain damage or endothelial cell injury. By contrast, induction of Grp in pre-neoplastic and neoplastic cells could lead to escape of the tumor cells from immune surveillance, resulting in tumor progression and malignancy. Induction of Grp in cancer cells can also lead to drug resistance. Grp94 derived from necrotic cancer cells acts to present antigenic peptides when taken up by scavenging antigen-presenting cells.

involving tissue or organ damage. The higher levels of Grp94 and Grp78 in the brains of immature rats when compared to those of adult animals might account for the higher resistance of immature rats to seizure[11]. In addition, specific induction of these Grps in the dentate gyrus region of the adult rat brain following seizure might contribute to a neuroprotective effect in that particular region of the brain. For early-onset familial Alzheimer's disease (FAD), overexpression of Grp78 in neuroblastoma cells expressing a mutant presenilin-1 (PS1) protein was reported to restore resistance to ER stress[45]. However, another study showed that the induction of Grp78 and CHOP by the UPR is independent of PS1 expression and that UPR is intact in individuals with sporadic Alzheimer's disease or presenilin-mediated FAD (Ref. 46). Thus,

whether clinical manipulation of Grp level will be therapeutic for FAD requires future resolution. Recently, expression of Grp78 was reported to prevent the aggregation and facilitate the proteasomal degradation of mutant prion proteins, which are implicated in neurodegenerative disorders such as prion diseases and transmissible spongiform encephalopathies[47]. However, when the proteasomal function is impaired, Grp78 might render the mutant prion protein even more pathogenic by promoting its refolding. Induction of Grp78 has also been observed in endothelial cells damaged by reductive stress that was caused by hyperhomocysteinemia which, with both genetic and environmental components, is a common risk factor for thrombotic vascular events such as premature arteriosclerosis, stroke, myocardial infarction and thrombosis[13,22]. Therefore, the induction of Grp could be an adaptive response evolved in mammals to protect endothelial cells against stress-induced cell death.

Grps as targets for cancer chemotherapy

Whereas Grp overexpression could limit damage in organs exposed to ER stress, the anti-apoptotic function of the Grps also predicts that their induction in neoplastic cells could lead to cancer progression and drug resistance (Fig. 3). In a variety of cancer cell lines, solid tumors and human cancer biopsies, the levels of Grp78 and Grp94 are elevated, correlating with malignancy[3,48]. In addition, induction of Grp78 has been shown to protect cancer cells from immune surveillance, whereas suppressing the stress-mediated induction of Grp78 enhanced apoptosis, inhibited tumor growth and increased the cytotoxicity of chronic hypoxic cells[3,38,39]. Therefore, targeted suppression of Grp expression or function in cancer cells could represent a novel approach to cancer chemotherapy. Genistein, which suppresses both the Grp and the heat shock responses, inhibits the growth of carcinogen-induced tumors in rats and in human leukemia cells transplanted into mice[19]. In another example, Grp94 has been shown to associate with and stabilize p185/erbB2 (also referred to HER-2/neu), which is commonly overexpressed in breast carcinomas and is associated with poor prognosis[49]. Treatment of breast cancer cells with geldanamycin, an antiproliferative agent, enables the degradation of p185 in the breast cancer cells by disrupting the Grp94–p185 complex.

With respect to drug resistance, preinduction of Grp in a variety of human cancer cell lines confers resistance to inhibitors of topoisomerase II (e.g. etoposide) but increases sensitivity to DNA cross-linking agents such as cisplatin[50,51]. Direct suppression of Grp94 levels by antisense knockdown strategies results in enhanced sensitivity to etoposide-induced cell death[42]. Interestingly, etoposide-induced cell death triggers proteolytic cleavage of Grp94 by calpain, which also cleaves

Bcl-xL during apoptosis, therefore turning an anti-apoptotic protein into a pro-apoptotic molecule[42,44]. Within the microenvironment of a solid tumor, unique stress conditions can lead to induction of Grp. Because the chaperone function of Grp is essential for its protective function, it is possible that this function requires retention of growth factor receptors, among other proteins, in the ER, leading to G1 arrest and, therefore, resistance to cytotoxic drugs targeting S-phase cells[50]. Other mechanisms for Grp-mediated protection could involve Grp interaction with effectors of apoptosis, leading to the blockage of cell death induced by drug treatment.

Role of Grp in immunotherapy

Grp94 has been shown to chaperone a broad array of peptides, including those derived from normal proteins as well as from foreign and altered proteins present in cancer or virus-infected cells[52]. Thus, tumor-derived Grp94 carries tumor antigenic peptides, and Grp94 preparations from virus-infected cells carry viral epitopes. Although Grp94 is normally intracellular, necrotic cells release Grp94–peptide complexes, which are taken up by scavenging antigen-presenting cells. Presentation of the peptides on the surface of these cells leads to stimulation of T lymphocytes and a pro-inflammatory response. Complexes of Grp94 with peptides, whether isolated from cells or reconstituted *in vitro*, have served as immunogens to elicit immune responses specific to the Grp94-chaperoned antigenic peptides[53]. Immunization using low concentrations of Grp94–peptide preparations in mice inhibited tumor growth. Autoimmunity has not been observed, suggesting that the immune response is targeted against the peptides and not against Grp94. In extrapolating the animal studies to human applications, cancer patients were immunized with autologous cancer-derived Grp94 preparations as a test for the feasibility of a patient-specific, chaperone-based vaccine for immunotherapy of human cancer[53]. Recently, another peptide-binding Grp, referred to as Grp170, has also been shown to be effective as a cancer vaccine[54]. If successful, this approach could also be applied as therapy for infectious diseases.

Use of the stress-inducible *Grp78* promoter in cancer gene therapy

Aggressive tumors often suffer from insufficient blood supply resulting in areas of acidosis, nutrient deprivation and hypoxia. Such adverse conditions specifically activate the *Grp78* promoter. The use of the stress-inducible *Grp78* promoter to drive therapeutic gene expression offers a novel approach for gene therapy of poorly perfused tumors and ischemic diseases. The *Grp78* promoter confers higher efficacy than viral promoters in eradication of sizable tumors in mouse models bearing fibrosarcoma and breast tumors[12,55]. An added advantage associated with use of the *Grp78* promoter is that it is also inducible by a form of laser therapy (photodynamic therapy), which promotes oxidative stress and ischemia *in vivo*[56], enabling the expression of the therapeutic gene to be further controlled temporally and spatially.

Conclusion

Since the discovery of Grp78 and Grp94 20 years ago, there has been an explosion of new knowledge regarding the function and regulation of Grp in cell culture. Future experiments addressing the physiological roles played by Grp *in vivo* will further enhance our understanding of the importance of these proteins in human development and in diseases associated with the disturbance of ER homeostasis.

References

1 Jolly, C. and Morimoto, R.I. (2000) Role of the heat shock response and molecular chaperones in oncogenesis and cell death. *J. Natl. Cancer Inst.* 92, 1564–1572

2 Lee, A.S. (1992) Mammalian stress response: induction of the glucose-regulated protein family. *Curr. Opin. Cell Biol.* 4, 267–273

3 Little, E. *et al.* (1994) The glucose-regulated proteins (GRP78 and GRP94): functions, gene regulation, and applications. *Crit. Rev. Eukaryotic Gene Expr.* 4, 1–18

4 Kaufman, R.J. (1999) Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. *Genes Dev.* 13, 1211–1233

5 Shiu, R.P. *et al.* (1977) Glucose depletion accounts for the induction of two transformation-sensitive membrane proteins in Rous Sarcoma virus-transformed chick embryo fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* 74, 3840–3844

6 Li, W.W. *et al.* (1993) Transactivation of the grp78 promoter by Ca^{2+} depletion. A comparative analysis with A23187 and the endoplasmic reticulum Ca^{2+}-ATPase inhibitor thapsigargin. *J. Biol. Chem.* 268, 12003–12009

7 Hsieh, K.P. *et al.* (1996) Interaction of ethanol with inducers of glucose-regulated stress proteins. Ethanol potentiates inducers of grp78 transcription. *J. Biol. Chem.* 271, 2709–2716

8 Wang, J.F. *et al.* (1999) Differential display PCR reveals novel targets for the mood-stabilizing drug valproate including the molecular chaperone GRP78. *Mol. Pharmacol.* 55, 521–527

9 Barnes, J.A. and Smoak, I.W. (2000) Glucose-regulated protein 78 (GRP78) is elevated in embryonic mouse heart and induced following hypoglycemic stress. *Anat. Embryol. (Berlin)* 202, 67–74

10 Simmons, D.G. and Kennedy, T.G. (2000) Induction of glucose-regulated protein 78 in rat uterine glandular epithelium during uterine sensitization for the decidual cell reaction. *Biol. Reprod.* 62, 1168–1176

11 Little, E. *et al.* (1996) Induction of glucose-regulated protein (glucose-regulated protein 78/BiP and glucose-regulated protein 94) and heat shock protein 70 transcripts in the immature rat brain following status epilepticus. *Neuroscience* 75, 209–219

12 Gazit, G. *et al.* (1999) Use of the glucose-starvation inducible grp78 promoter in suicide gene therapy of murine fibrosarcoma. *Cancer Res.* 59, 3100–3106

13 Outinen, P.A. *et al.* (1999) Homocysteine-induced endoplasmic reticulum stress and growth arrest leads to specific changes in gene expression in human vascular endothelial cells. *Blood* 94, 959–967

14 Foti, D.M. *et al.* (1999) Conservation and divergence of the yeast and mammalian unfolded protein response. Activation of specific mammalian endoplasmic reticulum stress element of the grp78/BiP promoter by yeast Hac1. *J. Biol. Chem.* 274, 30402–30409

15 Caspersen, C. *et al.* (2000) The sarco/endoplasmic reticulum calcium-ATPase 2β is an endoplasmic reticulum stress-inducible protein. *J. Biol. Chem.* 275, 22363–22372

16 Tirasophon, W. *et al.* (2000) The endoribonuclease activity of mammalian IRE1 autoregulates its mRNA and is required for the unfolded protein response. *Genes Dev.* 14, 2725–2736

17 Urano, F. *et al.* (2000) IRE1 and efferent signaling from the endoplasmic reticulum. *J. Cell Sci.* 113 Pt 21, 3697–3702

18 He, H. *et al.* (2000) Involvement of c-Fos in signaling *grp78* induction following ER calcium release. *Oncogene* 19, 5936–5943

19 Zhou, Y. and Lee, A.S. (1998) Mechanism for the suppression of the mammalian stress response by genistein, an anticancer phytoestrogen from soy. *J. Natl. Cancer Inst.* 90, 381–388

20 Yoshida, H. *et al.* (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, 33741–33749

21 Roy, B. and Lee, A.S. (1999) The mammalian endoplasmic reticulum stress response element consists of an evolutionarily conserved tripartite structure and interacts with a novel stress-inducible complex. *Nucleic Acids Res.* 27, 1437–1443

22 Kokame, K. *et al.* (2000) Herp, a new ubiquitin-like membrane protein induced by endoplasmic reticulum stress. *J. Biol. Chem.* 275, 32846–32853

23 Marcus, N. and Green, M. (1997) NF-Y, a CCAAT box-binding protein, is one of the *trans*-acting factors necessary for the response of the murine ERp72 gene to protein traffic. *DNA Cell Biol.* 16, 1123–1131

24 Yoshida, H. *et al.* (2001) Endoplasmic reticulum stress-induced formation of transcription factor complex ERSF including NF-Y (CBF) and activating transcription factors 6α and 6β that activates the mammalian unfolded protein response. *Mol. Cell. Biol.* 21, 1239–1248

25 Li, W.W. *et al.* (1997) Induction of the mammalian GRP78/BiP gene by Ca^{2+} depletion and formation of aberrant proteins: activation of the conserved stress-inducible *grp* core promoter element by the human nuclear factor YY1. *Mol. Cell. Biol.* 17, 54–60

26 Li, W.W. *et al.* (1997) Suppression of *grp78* core promoter element-mediated stress induction by the dbpA and dbpB (YB-1) cold shock domain proteins. *Mol. Cell. Biol.* 17, 61–68

27 Parker, R. *et al.* (2001) Identification of TFII-I as the endoplasmic reticulum stress response element binding factor ERSF: its autoregulation by stress and interaction with ATF6. *Mol. Cell. Biol.* 21, 3220–3233

28 Ye, J. *et al.* (2000) ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol. Cell.* 6, 1355–1364

29 Lippincott-Schwartz, J. *et al.* (1991) Brefeldin A's effects on endosomes, lysosomes, and the TGN suggest a general mechanism for regulating organelle structure and membrane traffic. *Cell* 67, 601–616

30 Liu, E.S. *et al.* (1992) Brefeldin A as a regulator of grp78 gene expression in mammalian cells. *J. Biol. Chem.* 267, 7128–7133

31 Li, M. *et al.* (2000) ATF6 as a transcription activator of the endoplasmic reticulum stress element: thapsigargin stress-induced changes and synergistic interactions with NF-Y and YY1. *Mol. Cell. Biol.* 20, 5096–5106

32 Thuerauf, D.J. *et al.* (1998) p38 mitogen-activated protein kinase mediates the transcriptional induction of the atrial natriuretic factor gene through a serum response element. A potential role for the transcription factor ATF6. *J. Biol. Chem.* 273, 20636–20643

33 Pagani, M. *et al.* (2000) Endoplasmic reticulum oxidoreductin 1-lβ (ERO1-Lβ), a human gene induced in the course of the unfolded protein response. *J. Biol. Chem.* 275, 23685–23692

34 Leahy, K.P. and Crawford, D.R. (2000) Adapt78 protects cells against stress damage and suppresses cell growth. *Arch. Biochem. Biophys.* 379, 221–228

35 Wertheimer, E. *et al.* (1991) The ubiquitous glucose transporter GLUT-1 belongs to the glucose-regulated protein family of stress-inducible proteins. *Proc. Natl. Acad. Sci. U. S. A.* 88, 2525–2529

36 Barbosa-Tessmann, I.P. *et al.* (1999) Activation of the unfolded protein response pathway induces human asparagine synthetase gene expression. *J. Biol. Chem.* 274, 31139–31144

37 Little, E. and Lee, A.S. (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, 9526–9534

38 Jamora, C. *et al.* (1996) Inhibition of tumor progression by suppression of stress protein GRP78/BiP induction in fibrosarcoma B/C10ME. *Proc. Natl. Acad. Sci. U. S. A.* 93, 7690–7694

39 Koong, A.C. *et al.* (1994) Increased cytotoxicity of chronic hypoxic cells by molecular inhibition of GRP78 induction. *Int. J. Radiat. Oncol. Biol. Phys.* 28, 661–666

40 Liu, H. *et al.* (1997) Endoplasmic reticulum chaperones GRP78 and calreticulin prevent oxidative stress, Ca^{2+} disturbances, and cell death in renal epithelial cells. *J. Biol. Chem.* 272, 21751–21759

41 Morris, J.A. *et al.* (1997) Immunoglobulin binding protein (BiP) function is required to protect cells from endoplasmic reticulum stress but is not required for the secretion of selective proteins. *J. Biol. Chem.* 272, 4327–4334

42 Reddy, R.K. *et al.* (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, 28476–28483

43 Miyake, H. *et al.* (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

44 Nakagawa, T. and Yuan, J. (2000) Cross-talk between two cysteine protease families. Activation of caspase-12 by calpain in apoptosis. *J. Cell Biol.* 150, 887–894

45 Katayama, T. *et al.* (1999) Presenilin-1 mutations downregulate the signalling pathway of the unfolded-protein response. *Nat. Cell Biol.* 1, 479–485

46 Sato, N. *et al.* (2000) Upregulation of BiP and CHOP by the unfolded-protein response is independent of presenilin expression. *Nat. Cell Biol.* 2, 863–870

47 Jin, T. *et al.* (2000) The chaperone protein BiP binds to a mutant prion protein and mediates its degradation by the proteasome. *J. Biol. Chem.* 275, 38699–38704

48 Fernandez, P.M. *et al.* (2000) Overexpression of the glucose-regulated stress gene GRP78 in malignant but not benign human breast lesions. *Breast Cancer Res. Treat.* 59, 15–26

49 Chavany, C. *et al.* (1996) p185erbB2 binds to GRP94 *in vivo*. Dissociation of the p185erbB2/GRP94 heterocomplex by benzoquinone ansamycins precedes depletion of p185erbB2. *J. Biol. Chem.* 271, 4974–4977

50 Tomida, A. and Tsuruo, T. (1999) Drug resistance mediated by cellular stress response to the microenvironment of solid tumors. *Anticancer Drug Des.* 14, 169–177

51 Belfi, C.A. *et al.* (1999) Increased sensitivity of human colon cancer cells to DNA cross-linking agents after GRP78 up-regulation. *Biochem. Biophys. Res. Commun.* 257, 361–368

52 Binder, R.J. *et al.* (2000) CD91: a receptor for heat shock protein gp96. *Nat. Immunol.* 1, 151–155

53 Janetzki, S. *et al.* (2000) Immunization of cancer patients with autologous cancer-derived heat shock protein gp96 preparations: a pilot study. *Int. J. Cancer* 88, 232–238

54 Wang, X-Y. *et al.* (2001) Characterization of heat shock protein 110 and glucose regulated protein 170 as cancer vaccines and the effect of fever-range hyperthermia on vaccine activity. *J. Immunol.* 166, 490–497

55 Chen, X. *et al.* (2000) Eradication of murine mammary adenocarcinoma through HSVtk expression directed by the glucose-starvation inducible grp78 promoter. *Breast Cancer Res. Treat.* 59, 81–90

56 Gomer, C.J. *et al.* (1991) Glucose regulated protein induction and cellular resistance to oxidative stress mediated by porphyrin photosensitization. *Cancer Res.* 51, 6574–6579

57 Ferrari, D.M. and Soling, H.D. (1999) The protein disulphide-isomerase family: unravelling a string of folds. *Biochem. J.* 339, 1–10

58 High, S. *et al.* (2000) Glycoprotein folding in the endoplasmic reticulum: a tale of three chaperones? *FEBS Lett.* 476, 38–41

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