Glucose-regulated proteins in cancer: molecular mechanisms and therapeutic potential

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Abstract | The glucose-regulated proteins (GRPs) are stress-inducible chaperones that mostly reside in the endoplasmic reticulum or the mitochondria. Recent advances show that the GRPs have functions that are distinct from those of the related heat shock proteins, and they can be actively translocated to other cellular locations and assume novel functions that control signalling, proliferation, invasion, apoptosis, inflammation and immunity. Mouse models further identified their specific roles in development, tumorigenesis, metastasis and angiogenesis. This Review describes their discovery and regulation, as well as their biological functions in cancer. Promising agents that use or target the GRPs are being developed, and their efficacy as anticancer therapeutics is also discussed.

The glucose-regulated proteins (GRPs) GRP78 (also known as BiP and HSPA5), GRP94 (also known as GP96 and HSP90B1), GRP170 (also known as ORP150 and HYOU1) and GRP75 (also known as mortalin and HSPA9) are stress-inducible molecular chaperones that belong to the heat shock protein (HSP) family (BOX 1). Unlike most of the HSPs, which reside mainly in the cytosol and the nucleus, these GRPs are found in the endoplasmic reticulum (ER) or the mitochondria, which are important organelles for the regulation of protein quality control and metabolic balance[1–4]. In their traditional chaperone roles, these GRPs facilitate protein folding and assembly, as well as the export of misfolded proteins for degradation. Coupled with their Ca^{2+} binding functions, they maintain the integrity and homeostasis of the ER and the mitochondria under physiological and pathological conditions.

GRP overexpression is widely reported in cancer cell lines and is associated with aggressive growth and invasive properties[5,6] (see Supplementary information S1 (table)). During the past decade, exciting discoveries have been made in identifying common and distinctive functions of these GRPs in cancer. GRP78 regulates the balance between cancer cell viability and apoptosis by sustaining ER protein folding capacity and by maintaining ER stress sensors and ER-associated pro-apoptotic machineries in their inactive state[7]. GRP94 is essential for the processing of proteins that have been implicated in tumorigenesis, such as insulin-like growth factor 1 (IGF1), Toll-like receptors (TLRs) and integrins[4]. GRP170, which has an ADP–ATP exchange function, is both a co-chaperone for GRP78 and an independent chaperone, and it is crucial for vascular endothelial growth factor A (VEGFA) processing and maturation[2,8,9]. GRP75 interacts with the tumour suppressor p53, thereby inactivating the capacity of p53 to function as a transcription factor and inducing apoptosis[10]. Furthermore, these GRPs, which are traditionally thought to exclusively reside in the ER lumen, can be actively translocated to other cellular locations and can be secreted, and they have additional functions that control signalling, proliferation, invasion, apoptosis, inflammation and immunity[11–14]. ER stress, as well as the development of therapeutic resistance, actively promotes the cell surface expression of GRP78, which functions as an upstream regulator of the PI3K–AKT oncogenic signalling pathway[15–17]. GRP78 is also a downstream target of AKT activation[18,19]. At the cell surface, GRP94 and GRP170 function in antigen presentation, and their secreted forms have the ability to elicit innate and adaptive immune responses, which could be useful in the development of cancer vaccines[1,2,20].

Through the use of cancer cell lines, xenografts and conditional knockout mouse models, the important roles of these GRPs in cancer are being established[5,20,21]. Promising therapeutics that are specifically directed against the GRPs, including conjugated peptides and toxins, antibodies, small molecules and microRNAs, are being developed[5,20,22]. Thus, these GRPs represent novel

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doi:10.1038/nrc3701

Key points

- The glucose-regulated proteins (GRPs) GRP78, GRP94, GRP170 and GRP75, are members of the heat shock protein family. They primarily reside in the endoplasmic reticulum (ER) or the mitochondria, and they are induced at the transcriptional level upon ER stress.
- As molecular chaperones, the GRPs regulate protein quality control and degradation, with GRP78 additionally functioning as a pivotal regulator of the unfolded protein response and the apoptotic machinery that is associated with the ER.
- The GRPs can be actively translocated to other cellular locations and secreted, and they can assume additional functions that control cellular signalling, proliferation, invasion, apoptosis, inflammation and immunity, which have major implications in cancer progression and therapeutic resistance.
- Specific roles of GRPs in development, tumorigenesis, metastasis and angiogenesis have been identified *in vitro* and are supported by genetically engineered mouse models.
- GRP overexpression is widely reported in many human cancers and is associated with aggressive properties, which suggests that GRPs have potential prognostic value. Interfering with the production or activities of GRPs in tumours that overexpress these proteins might provide new approaches for cancer treatment.
- The discovery that cell surface GRP78 is preferably expressed in cancer cells and stressed endothelial cells has led to the development of therapeutic agents that specifically target cell surface GRP78. These agents can induce cancer cell apoptosis and suppress tumorigenesis in mouse models with minimal toxicity.
- Although the GRPs are attractive targets for drug development, they can also function as mediators for cancer-specific drug delivery, transcriptional targeting of cancer and vaccine development.
- GRP170 can present full-length antigens to induce an immune response, which suggests that GRP170 could function as part of a new vaccine platform to augment antitumour immune responses.

prognostic markers and targets[5], as well as mediators or vaccines, that warrant vigorous investigation for anticancer therapy[2,23].

GRPs in the stress response

The GRPs are ubiquitous chaperones that are constitutively expressed at basal level and that sustain organ homeostasis through different mechanisms (see Supplementary information S2 (table)). The induction of the GRPs is widely used as an indicator for the onset of ER stress, and studies into the transcriptional activation mechanism of the GRPs (BOX 2) have facilitated the discovery of novel intracellular signalling pathways, whereby stress from the ER can be communicated to the nucleus to initiate the transcription of the unfolded protein response (UPR)-associated genes[20,24,25]. Cancer cells are subjected to ER stress that is triggered by both intrinsic and extrinsic factors, such as altered cell metabolism, hyperproliferation, hypoglycaemia, hypoxia, acidosis, viral infection and genetic lesions that lead to the production of mutated proteins that misfold[20,26]. These adverse conditions impinge on proper protein folding in the ER, thereby creating ER stress. GRP78 regulates the UPR by binding to and inactivating all three ER stress transducers — PRKR-like ER kinase (PERK; also known as eukaryotic translation initiation factor 2α kinase 3), inositol-requiring enzyme 1 (IRE1; also known as ERN1) and activating transcription factor 6 (ATF6)[7,20] — under non-stressed conditions. When misfolded proteins accumulate in the ER, GRP78 binds to them, thereby releasing the UPR sensors and leading to the activation of the UPR pathways[27–31] (FIG. 1). Conversely, when GRP78 is depleted or inactivated, the UPR can be spontaneously triggered, with diverse physiological consequences[32–35] (see Supplementary information S2 (table)). Nonetheless, GRP induction is not limited to ER stress. For example, autophagy-defective tumour cells upregulate ER chaperones in response to metabolic stress[36], and histone deacetylase inhibitors activate *GRP78* transcription without concomitantly inducing stress response in general[37] (BOX 2).

Biological functions of the GRPs in cancer

As summarized in this section, the GRPs, in both UPR-dependent and UPR-independent functions, have important roles in regulating various processes at multiple cellular locations that are essential for tumorigenesis (FIG. 2).

Proliferation. GRP78 expression levels correlated with proliferative rates of human glioma cell lines, and knockdown of GRP78 by small interfering RNA suppressed their growth[38]. In a mouse mammary tumour virus–polyoma middle T mammary tumour model, *Grp78* heterozygosity was sufficient to prolong the latency period and impede cancer growth, in part through the suppression of tumour cell proliferation[39] (TABLE 1). How might GRP78 facilitate proliferation? As an ER chaperone, GRP78 controls processing and maturation of a wide variety of cell surface receptors and secretory proteins that are crucial for the ability of cancer cells to respond to extrinsic proliferative signals[20]. GRP78 is also a regulator of WNT–β-catenin proliferative signalling, through its stabilization of WNT in the ER. When GRP78 was dissociated from WNT under hypoxic conditions, WNT was not properly processed, which led to its proteasomal degradation and to reduced WNT secretion[40].

GRP78 might also promote cell proliferation from the cell surface. ER stress or ectopic expression of GRP78 leads to localization of a subfraction of GRP78 on the cell surface[15]. Specific proteins have been reported to transport GRP78 to the cell surface in different cell types, such as the carrier protein MTJ1 (also known as DNAJC1) in macrophages and the tumour suppressor prostate apoptosis response 4 (PAR4; also known as PAWR) in the prostate cancer cell line PC-3 (REFS 41,42).

Cell surface GRP78 acts as a multifunctional receptor that affects both cell proliferation and viability[11–14]. For example, in prostate cancer cells, cell surface GRP78 functions as a receptor for the activated form of the plasma proteinase inhibitor α2-macroglobulin[43] (α2M*), thereby triggering ERK and AKT activation, as well as increased DNA and protein synthesis[44]. AKT signalling, which promotes proliferation and inhibits apoptosis, is also triggered by autoantibodies against the amino terminus of GRP78 that are found in cancer patients[45]. How might GRP78 regulate AKT activation? Cell surface GRP78 co-localizes with PI3K, which is an activator of AKT, and co-immunoprecipitates with PI3K subunits[16,17]. Furthermore, in cell culture model systems, an overexpression of GRP78 leads to increased production of phosphatidylinositol-(3,4,5)-trisphosphate

(PIP3; a signalling molecule downstream of PI3K), and mutation of the N-terminal region of GRP78 reduced both the binding of cell surface GRP78 to PI3K and PIP3 production[16]. A requirement for GRP78 during a serum-stimulated increase in PIP3 production has also been reported in human leukaemic cells[34].

PTEN, which encodes a plasma membrane lipid phosphatase that antagonizes the PI3K signalling pathway, is a major tumour suppressor gene in human cancer[46]. A biallelic conditional knockout mouse model of *Grp78* and *Pten* in the prostate epithelium or bone marrow showed that GRP78 deficiency reduces PI3K–AKT activation, which normally occurs as a result of PTEN loss in these cells and potently inhibits prostate tumorigenesis[47] and leukaemogenesis[48] (TABLE 1). Although cell surface GRP78 has been shown to regulate PI3K signalling, further studies are required to determine whether GRP78 in the ER or other cellular locations might also regulate PI3K–AKT signalling. Recent studies showed that GRP78 is a downstream target of the IGF1 receptor (IGF1R)–PI3K signalling pathway in mouse embryo fibroblasts, as well as in cancer cell lines[18,19], and this could represent a feedback regulatory mechanism that balances GRP78 expression and cancer cell proliferation.

Another pro-proliferative mechanism of GRP78 is the interaction of cell surface GRP78 with Cripto (also known as teratocarcinoma-derived growth factor 1), which is a glycosylphosphatidylinositol (GPI)-anchored, developmentally regulated oncoprotein[49]. Disruption of the cell surface GRP78–Cripto complex blocked Cripto activation of MAPK and PI3K pathways, and blocked Cripto modulation of activin A, activin B, nodal and transforming growth factor-β1 (TGFβ1) signalling[50]. Thus, cell surface GRP78 is a necessary mediator of Cripto proliferative signalling in human cancer.

GRP94 controls the maturation and secretion of IGFs, which are important mitogenic factors[51], and binding of IGF1 or IGF2 to IGF1R leads to PI3K–AKT activation. GRP94 regulates the processing of the low-density lipoprotein receptor-related protein 6 (LRP6), which is a WNT co-receptor[52]. Without GRP94, LRP6 is not exported from the ER to the cell surface, and this leads to the attenuation of the pro-proliferative and pro-survival WNT–β-catenin signalling pathway. This is the proposed mechanism for the attenuation of multiple myeloma and inflammatory colorectal cancer in mouse models where *Grp94* is deleted in B cells[53] and macrophages[54], respectively (TABLE 1). In breast cancer cells that are able to proliferate under chronic exposure to reactive oxygen species (ROS) *in vitro*, the expression of GRP94, but not of HSP90 or GRP78, is increased[55]. ROS are counteracted by the production of antioxidants and the formation of disulphide bonds in proteins in the ER, and this is promoted by GRP94.

Box 1 | **Discovery of the GRPs**

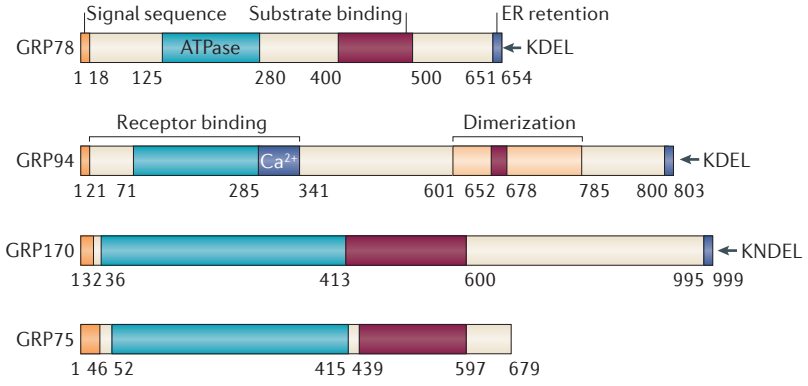
The glucose-regulated proteins (GRPs) were discovered in the mid-1970s as constitutively expressed cellular proteins that were induced by glucose starvation or by a block in protein glycosylation; hence, they were named GRPs[169–171]. GRP78, which is encoded in humans by heat shock 70 kDa protein 5 (*HSPA5*), shares 60% amino acid homology with HSP70, including the ATP-binding domain, which is required for their ATPase catalytic activity (see the figure), and GRP78 is an HSP70 analogue in the endoplasmic reticulum (ER). GRP78 is identical to BiP, which was originally discovered as an immunoglobulin heavy chain-binding protein[172–174]. This led to the designation of GRP78 as an ER molecular chaperone, and it is now established as a ubiquitous protein that is essential for processing a wide range of client proteins and maintaining the structural integrity of the ER[1,32,175].

Following the discovery of hamster GRP94 in 1984 (REF. 176), GRP94 has been identified as endoplasmin (discovered as a Ca^{2+}-binding protein[177]), ERP99 (discovered as a major ER glycoprotein[178]) and as the tumour rejection antigen GP96 (REF. 179). GRP94, which is encoded in humans by *HSP90B1*, shares 50% amino acid homology with HSP90 and is one of four HSP90 isoforms[180]. As well as being an ER chaperone, GRP94 is also a regulator of innate and adaptive immunity[1,4,181].

GRP75, which is encoded in humans by *HSPA9*, was first identified as a 66 kDa protein ($p66^{mot-1}$) that was linked to mortality and had anti-proliferative properties[182]. cDNA cloning and a homology search showed 80% homology to yeast mitochondrial HSP and 70% homology with mouse HSP70 (encoded by *Hspa1a*). Although GRP75 can localize to multiple subcellular sites, its primary location is in the mitochondria, as directed by its amino-terminal leader sequence[3] (see the figure).

Studying proteins that were induced by glucose starvation led to the discovery of a 150 kDa protein, GRP170 (REF. 183). GRP170, which is encoded in humans by hypoxia up-regulated 1 (*HYOU1*), is a large HSP70-like and HSP110-like protein in the ER[184] that is induced by hypoxia[185,186] (see the figure).

Therefore, all of the GRPs can function as chaperones that can be induced during cellular stress.



Overexpression of GRP75 in mouse fibroblasts leads to anchorage-independent growth, as well as formation of tumours, when transplanted into nude mice[56]. Contributing factors might include the role of GRP75 as a mitochondrial protein importer and its ability to retain p53 in the cytoplasm, thereby leading to the downregulation of p53 target genes such as cyclin-dependent kinase inhibitor 1A (*Cdkn1a*) and *Mdm2*. This effect on p53 has been shown in a subset of neuroblastomas[57]. Another client protein of GRP75 is fibroblast growth factor 1 (FGF1), which has broad mitogenic activities and which functions as a modifier of endothelial cell migration and proliferation, and is therefore pro-angiogenic[58].

Apoptosis. In general, the GRPs are suppressors of apoptosis[20]. Caspase 7, which is an executioner caspase that is associated with the ER, can be activated by the chemotherapeutic agent etoposide; and GRP78, in a manner that is dependent on its ATP-binding activity, forms a complex with caspase 7 (FIG. 1) and protects cells from apoptosis that is induced by etoposide[59,60]. A functional relationship was recently shown at the outer surface of the ER between GRP78, the pro-apoptotic protein BIK and the anti-apoptotic protein BCL-2 (REFS 61,62). GRP78 and BCL-2 form separate complexes with different domains of BIK. BIK sequestration of BCL-2 reduces the interaction of BCL-2 with the ER, leading to ER Ca^{2+} release, translocation of the pro-apoptotic protein BAX to the mitochondria and the release of cytochrome *c* to the cytosol, which initiates apoptosis. However, high levels of GRP78 sequester BIK, which releases the inhibition of BCL-2, thereby suppressing apoptosis[62].

These observations, however, raise the important question of how GRP78 — as an ER lumen protein — can interact with cytosolic proteins that associate with the outer ER membrane. Intriguingly, two independent studies showed that a subpopulation of GRP78 from isolated microsomes was resistant to sodium carbonate extraction and existed as a partially protease-resistant (presumably transmembrane) protein[59,60]. However, despite the presence of some weak hydrophobic motifs that support this possibility, the primary amino acid sequence of GRP78 does not predict a traditional transmembrane configuration under normal physiological conditions. Thus, the interaction between GRP78 and cytosolic proteins must be mediated either by an unconventional form of GRP78 that spans the ER membrane or by luminal GRP78 in a complex with other ER transmembrane proteins; this issue remains to be resolved.

ER stress induces alternative splicing of the *GRP78* transcript, and this generates a cytosolic isoform of GRP78 (GRP78va) that regulates PERK signalling and increases leukaemia cell survival[63]. ER stress also promotes the localization of GRP78 to the mitochondria, which are physically and functionally interconnected with the ER (BOX 3). Mitochondria-associated GRP78 can bind to RAF1, and this interaction is involved in the maintenance of mitochondrial permeability and is therefore protective against ER-stress-induced apoptosis[64]. In support of the anti-apoptotic functions of GRP78, knockout of *Grp78* in various tissues led to caspase activation and tissue atrophy[33,34,65,66] (see Supplementary information S2 (table)). In breast, prostate and leukaemic cancer models, heterozygous and/or homozygous knockout of *Grp78* increased tumour apoptosis and impeded tumour progression[39,47,48] (TABLE 1).

Through an interaction with α2M*, cell surface GRP78 promotes 1-LN prostate cancer cell survival by activating the AKT and nuclear factor-κB (NF-κB) signalling cascades[67]. In hypoxic HT1080 fibrosarcoma cells, cell surface GRP78 functions as a receptor for Kringle 5, which is a human plasminogen factor that, upon internalization, competes with procaspase 7 binding to the ATP-binding domain of GRP78 in the ER, and this leads to caspase 7 activation and tumour cell apoptosis[68]. However, one study indicates that cell surface GRP78, along with PAR4, has a pro-apoptotic function through mediating TNF-related apoptosis-inducing ligand (TRAIL; also known as TNFSF10) activation, thereby triggering the extrinsic apoptotic pathway in PC3, HeLa and H460 cells[42]. Nonetheless, in MCF-10A and MDA-MB-231 cells, GRP78 prevents TRAIL-induced apoptosis and is therefore a pro-survival factor[69]. Thus, the effect of GRP78 on TRAIL-induced apoptosis might be context dependent. Besides modulating apoptosis, GRP78 has been implicated in protective autophagy through maintenance of ER structural integrity[32] and modulation of mTOR signalling in oestrogen-resistant breast cancer cells[70].

GRP94 maintains ER Ca^{2+} homeostasis and protects cancer cells from apoptosis[71]. GRP94 deficiency in human multiple myeloma cells resulted in apoptosis through inhibition of the WNT–survivin pathway[53]. In an SkBr3 breast cancer cell line that abundantly expressed cell surface HER2 (also known as ERBB2), pharmacological inactivation of GRP94 destabilized HER2 and inhibited RAF1–MAPK survival signalling at the cell membrane[72]. In cancer cells, GRP170 is upregulated by

Box 2 | **Transcriptional activation of GRP promoters**

The promoter of the 78kDa glucose-regulated protein (*GRP78*) contains three endoplasmic reticulum (ER) stress response elements (ERSEs), which are located upstream of the TATA element[187,188]. In non-stressed cells, nuclear transcription factor Y (NFY), SP1 and histone deacetylase 1 (HDAC1) bind to the ERSEs and maintain *GRP78* at a low basal transcriptional level[189]. Upon ER stress, activating transcription factor 6 (ATF6) dissociates from GRP78 in the ER and translocates to the Golgi, where it is cleaved to generate a form of ATF6 that can enter the nucleus, ATF6(N) (see FIG. 1). ATF6(N), which binds to the ERSE through binding to NFY, also associates with YY1 and increases the binding of YY1 to the *GRP78* promoter[189]. YY1-associating protein arginine *N*-methyltransferase (PRMT) and the histone transacetylase p300 are also recruited to the *GRP78* promoter, concurrent with histone 4 acetylation and arginine 3 methylation that are known to activate transcription[189]. Together, these transcription factors and chromatin modifiers form an ERSE-binding complex, which can include the transcription factor TFII-I, which functions as a scaffold protein[190]. Although inositol-requiring enzyme 1 (IRE1)–X-box-binding protein 1 (XBP1) is also an important branch of the response to ER stress, mouse embryonic fibroblasts that were devoid of XBP1 showed only a modest effect on ER stress induction of *Grp78* and no effect on *Grp94* (REF. 191). Thus, XBP1 might contribute to GRP transcription, but it is not obligatory. ER stress induction of *GRP78* might also be partly attributed to ERSE-independent pathways, which are mediated by ATF4 (a basic leucine zipper transcription factor) in a complex with cyclic AMP-responsive element-binding protein 1 (CREB1)[30]. Promoters of *GRP94*, *GRP75* and *GRP170* contain the ERSE consensus sequence and are similarly regulated[4,25,125,192].

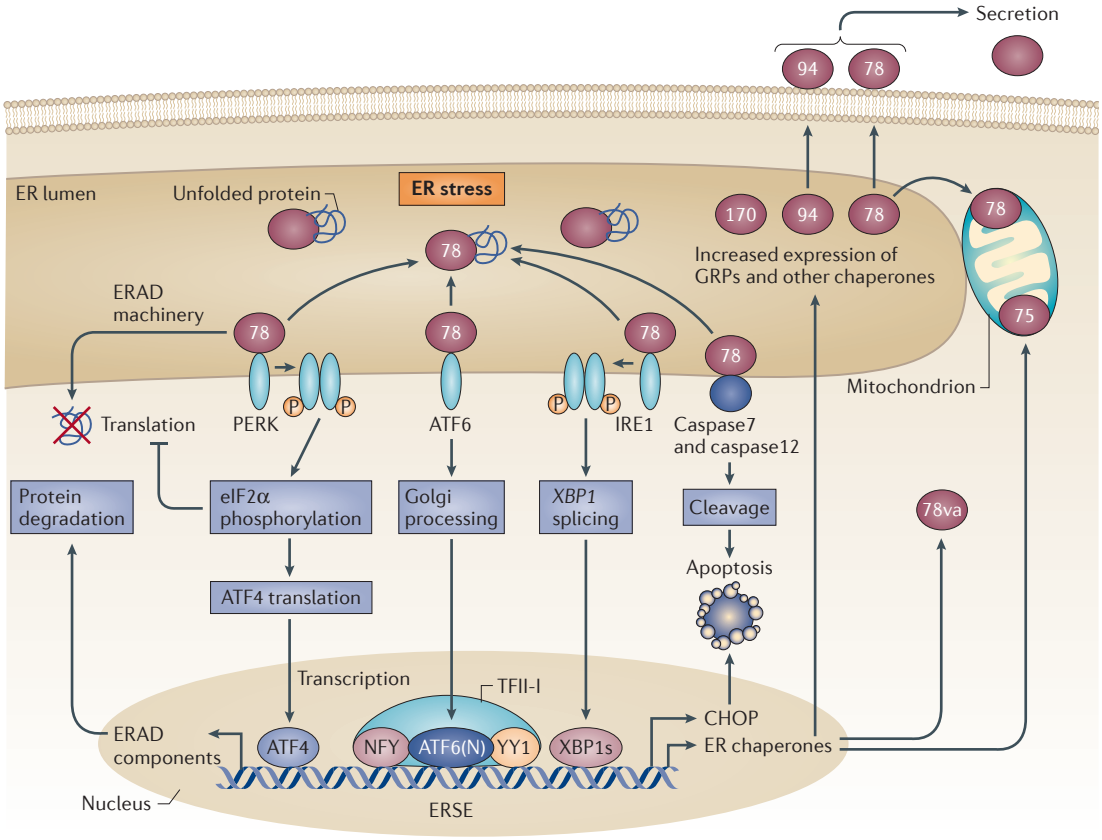


Figure 1 | **GRPs in the unfolded protein response and the stress response.** Endoplasmic reticulum (ER) luminal glucose-regulated protein 78 (GRP78) functions as a unfolded protein response (UPR) signalling regulator by binding to and maintaining the ER stress sensors (PRKR-like ER kinase (PERK), activating transcription factor 6 (ATF6) and inositol-requiring enzyme 1 (IRE1)) in inactive forms. It also binds to ER-associated caspase 7 and caspase 12 and suppresses their activation. Upon ER stress, GRP78 is titrated away through binding to misfolded proteins. This triggers the UPR, as exemplified by the dimerization of PERK and IRE1, and triggers the activation of their downstream signalling pathways, which leads to arrest of translation and ER-associated protein degradation (ERAD). The UPR also generates the active nuclear form of ATF6 (ATF6(N)), as well as ATF4 and the spliced form of X box-binding protein 1 (XBP1s), which function together with other transcriptional factors, including YY1, nuclear transcription factor Y (NFY), TFII-I and chromatin modifiers, to activate the ER stress response element (ERSE) present in the promoters of ER stress-responsive genes. A major UPR response is to induce the transcription of ER folding proteins, such as the GRPs, to increase the ER protein folding capacity, and to induce the transcription of the mitochondrial chaperone GRP75. Stressed cells actively promote the relocalization of GRP78 and GRP94 to the plasma membrane and, in some instances, their secretion; these cells also generate a cytosolic isoform of GRP78 (GRP78va) through alternative splicing. Nonetheless, UPR can also induce transcription of the pro-apoptotic transcription factor *CHOP*; and following release from GRP78, caspase 7 and caspase 12 are activated, thereby triggering apoptosis. Thus, the UPR regulates the balance between survival and cell death in stressed cells, and the upregulation of the GRPs represents a major adaptive, protective action that occurs through the maintenance of cellular homeostasis. eIF2α, eukaryotic translation initiation factor 2α; P, phosphorylation.

hypoxia and by drugs such as celecoxib (a non-steroidal anti-inflammatory drug) and proteasome inhibitors, and knockdown of GRP170 activated the expression of the UPR pro-apoptotic factor CHOP and stimulated apoptosis[73,74]. GRP170 might also protect cancer cells against cell death by blocking ER Ca^{2+} release or delaying the onset of the UPR by binding to the ER stress sensors[75,76].

Angiogenesis. Eliminating the tumour vasculature, which supplies nutrients and oxygen within the tumour, is a key strategy for anticancer therapy. Tumour-associated endothelial cells are physiologically and functionally different from endothelial cells that are derived from normal tissues, and they express high levels of GRP78 compared with normal organs[38,77,78]. *Grp78*$^{+/-}$ mice, as well as mice with conditional heterozygous knockout of *Grp78* in the host endothelial cells, showed a marked reduction in tumour microvessel density (MVD) but did not show any difference in the MVD of normal organs[39] (TABLE 1). *Grp78* knockdown impairs immortalized endothelial cell proliferation, survival and migration *in vitro*, and this is consistent with its requirement for neoangiogenesis in primary tumour and metastatic growth[39].

GRP78 is expressed on the surface of proliferating endothelial cells[68,79]. Cell surface GRP78 associates with the GPI-anchored truncated cadherin (T cadherin) and mediates T cadherin-dependent endothelial cell survival[80]. The plasminogen Kringle 5 is reported to bind

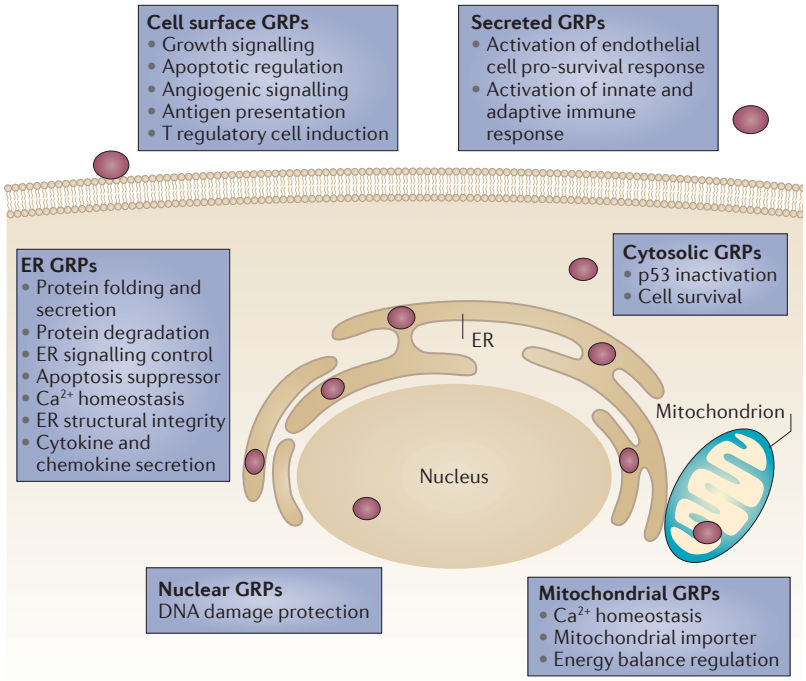


Figure 2 | **GRPs in survival and immunity.** Most of the glucose-regulated protein (GRP) molecules GRP78, GRP94 and GRP170 are located in the endoplasmic reticulum (ER) lumen and function as ER folding proteins, and GRP75 is primarily a mitochondrial chaperone. Under ER stress or pathological stress conditions, a subfraction of GRP78 and GRP94 translocate to the cell surface and their secreted forms can be detected. Cell surface GRPs control crucial growth and apoptotic signalling functions, as well as immune functions — notably, antigen presentation. ER stress also induces GRP78 translocation into the nucleus and the mitochondria, and it induces alternative splicing of the *GRP78* transcript, which leads to the generation of a cytosolic isoform of GRP78. As is evident in the purple boxes, GRPs can have both pro-survival and immune functions in various subcellular locations.

to GRP78 in glioma endothelial cells and induce apoptosis[68] that can be increased by radiation, as this results in the internalization of cell surface GRP78 by LRP1 and in the activation of p38 MAPK[78]. Other studies, however, suggest that the apoptotic effect of Kringle 5 in proliferating endothelial cells and in 1-LN prostate cancer cells was mediated by the cell surface voltage-dependent anion channel, which co-localizes with GRP78 and is regulated by GRP78 (REF. 81). VEGF can induce cell surface expression of GRP78 in endothelial cells, and knockdown of GRP78 suppressed VEGF-mediated MAPK signalling and endothelial cell proliferation[82]. However, although knockdown of GRP170 had no effect on the expression of GRP78 and GRP94, it resulted in retention of VEGF in the ER and blocked its secretion[83]. Collectively, these studies suggest that targeting GRP78 and GRP170 could achieve a dual effect in suppressing tumour growth as well as in tumour angiogenesis.

Invasion and metastasis. Tumour metastasis is a multistep process that involves the degradation of the extracellular matrix (ECM), tumour cell migration and invasion, the induction of angiogenesis and tumour cell survival in new tissues. The level of intracellular GRP78, as well as cell surface GRP78, is increased in metastatic cancer cell lines, lymph node metastases and human metastatic lesions[6,84–86]. Knockdown of GRP78 suppresses tumour cell invasion *in vitro* and suppresses metastatic growth in xenograft and syngeneic tumour models[87–89]. In addition to protecting metastatic tumour cells from the adverse host environment and promoting angiogenesis, GRP78 has been shown to promote cell motility. One mechanism for this is through cell surface GRP78 functioning as a co-receptor for ligands that signal the activation of kinases known to enhance migration, such as AKT, focal adhesion kinase (FAK) and p21-activated kinase 2 (PAK2)[88,90]. It has also been proposed that cell surface GRP78 functions as a bridge protein for the urokinase-type plasminogen activator (uPA)–urokinase plasminogen activator surface receptor (uPAR) protease system, which can mediate degradation of the ECM and promote invasion[88]. Like GRP78, GRP94 overexpression is associated with lymph node metastasis and carcinoma recurrence, and silencing of GRP94 inhibits migration and proliferation of MDA-MB-231 breast cancer cells *in vitro*[55,91]. GRP94 client proteins include cell interaction and cell matrix components, such as integrins, and this might explain the influence of GRP94 on cell invasion. It was recently shown that a cell-permeable peptide that competitively inhibited the interaction between GRP94 and integrins blocked cell invasion[92]. GRP75 overexpression is associated with liver cancer metastasis[93], and GRP170 upregulation is observed in invasive breast cancer[94] (see Supplementary information S1 (table)). Thus, the GRPs are novel protein targets for the inhibition of cancer metastasis.

Inflammation and immunity. ER stress can drive a pro-inflammatory programme in tumour cells and macrophages that facilitates tumour progression. Additionally, stressed tumour cells secrete mediators that stimulate macrophages to produce pro-inflammatory cytokines, thereby further amplifying the pro-inflammatory response of tumour cells[20,95]. However, cancer cell survival requires resistance against host immune defences. GRP78 regulates inflammation and immunity through multiple mechanisms[20,96]. As a major ER chaperone, GRP78 facilitates the processing and secretion of cytokines and chemokines[96,97]. Acute ablation of GRP78 in adult mice results in alteration of their chemokine and cytokine profile[34] (see Supplementary information S2 (table)). In terms of immune evasion, GRP78 protects fibrosarcoma cells from lysis by cytotoxic T lymphocytes (CTLs) and tumour necrosis factor *in vitro,* and when fibrosarcoma cells that were incapable of inducing GRP78 were injected into mice, tumours were either not formed or rapidly regressed with evidence of a cytotoxic T cell response[98].

GRP78 is an obligatory binding partner for cell surface major histocompatibility complex (MHC) class I molecules[99]. Functioning as the α2M* cell surface signalling receptor, GRP78 regulates the G_s-mediated cyclic AMP (cAMP) production and the pro-inflammatory

Table 1 | **Roles of GRP78 and GRP94 in mouse models of cancer**

Cancer type	Mutant mouse model	Heterozygous or homozygous	Key phenotypes	Refs
Breast carcinoma	*Grp78*$^{+/-}$; *MMTV–PyT*	+/–	Prolongs latency period and impedes tumour growth by reducing tumour cell proliferation and angiogenesis, and by increasing apoptosis	39
Prostate adenocarcinoma	*Grp78*$^{f/+}$; *Pten*$^{f/f}$; *probasin* Cre or *Grp78*$^{f/f}$; *Pten*$^{f/f}$; *probasin* Cre	+/– or –/–	• Suppresses tumorigenesis • AKT activation is decreased	47
Leukaemia	*Grp78*$^{f/+}$; *Pten*$^{f/f}$; *Mx1*–Cre	+/–	• Suppresses leukaemic blast cell expansion • PI3K–AKT signalling decreased • Normal haematopoietic phenotype	48
Liver cancer	*Grp78*$^{f/f}$; *Pten*$^{f/f}$; *Alb*–Cre	–/–	• Exacerbates steatosis and liver injury • Activation of liver progenitor cells and accelerates tumorigenesis • Strong GRP78 re-expression in cancer lesions	111
	Grp94$^{f/f}$; *Pten*$^{f/f}$; *Alb*–Cre	–/–	• Minor liver injury and disrupts cell adhesion protein organization • Liver progenitor cell proliferation increased • ERK activation evident and accelerates tumorigenesis	113
Multiple myeloma	*Hsp90b1*$^{f/f}$; *Cd19*-Cre; *Xbp1*s transgene*	–/–	• Suppresses tumour growth • Inhibits WNT–β-catenin signalling	53
Colorectal cancer	*Hsp90b1*$^{f/f}$; *LysM*-Cre*	–/–	• Reduction in number and size of colitis-associated colon cancer • Colonic epithelial β-catenin mutation decreased • WNT signalling decreased	54

Alb, albumin; GRP, glucose-regulated protein; *Hsp*, heat shock protein; LysM, lysozyme M; *MMTV*, mouse mammary tumour virus; Mx1, myxovirus resistance 1; *PyT*, polyoma middle T; Xbp1s, spliced form of X-box-binding protein 1. **Hsp90b1* is also known as *Grp94*.

cyclooxygenase 2 (COX2)–prostaglandin E (PGE)–cAMP signalling cascade[100,101]. Regulatory T cells (T_{Reg} cells) are a subpopulation of T cells that drive immune suppression. In some cancers, increased numbers of T_{Reg} cells promote cancer progression by active suppression of the immune responses against the tumour. Cell surface GRP78 in T cells forms a complex with and confers stabilization to cell surface TGFβ, which is an immune regulator and an inducer of T_{Reg} cells[102]. Some cancer cells secrete GRP78, which modulates the differentiation of human monocytes into mature dendritic cells, and subsequent recruitment of T cells leads to the generation of T_{Reg} cells[103].

GRP94 has an important role in immunity because it facilitates MHC class I molecule-mediated antigen presentation by inducing the maturation and activation of various cells that are involved in innate and adaptive immune responses, as well as through the secretion of pro-inflammatory cytokines[20]. GRP94 is the unique and obligatory chaperone of TLRs — it facilitates their maturation and translocation to the cell surface. Macrophage-specific knockout of *Grp94* resulted in a lack of response to TLR ligands and a loss of innate immune function[104] (see Supplementary information S2 (table)), as well as reduced colitis and inflammation-associated colon tumorigenesis[54]. Thus, GRPs regulate inflammation and immunity both in tumour cells and through interactions with the tumour microenvironment (FIG. 2).

Stem cell regulation. The notion that cancers are perpetuated by a small population of tumour-initiating cells (TICs) that show stem cell-like properties suggests a link between deregulated stem cell activation and cancer development. Initially identified in leukaemia, TICs have also been implicated in solid tumours. Haematopoietic stem cells (HSCs) must maintain a balance between quiescence and activation to respond to demands for hematopoiesis and the need for long-term stem cell maintenance. Consistent with the pro-survival properties of GRP78, acute inducible ablation of GRP78 in the adult haematopoietic system resulted in an intrinsic reduction of the HSC pool through increased apoptosis[34] (see Supplementary information S2 (table)). Inactivation of PTEN in bone marrow HSCs led to activation of the PI3K–AKT pathway, expansion of the HSC population, development of a myeloproliferative disorder and eventually leukaemia[105]. Strikingly, heterozygous knockdown of *Grp78* in *Pten*-null mice was sufficient to inhibit PI3K–AKT activation, restore the HSC population to a normal level and suppress leukaemic blast expansion[48] (TABLE 1). This effect is mediated at least in part by GRP78 at the cell surface, as treatment of the *Pten*-null mice with a GRP78-targeted antibody also suppressed AKT activation and leukaemic blast formation[17]. Despite the similarity of GRP78 and GRP94 as ER chaperones, acute loss of GRP94 in the bone marrow led to AKT activation and expansion of HSCs, and this corresponded with a loss of surface β4 integrin expression

and HSC niche attachment[106,107] (see Supplementary information S2 (table)). These findings provide the first evidence that GRPs regulate HSC homeostasis through distinct pathways with different outcomes.

In head and neck TICs, the expression of GRP78 at the cell surface is associated with self-renewal, suppression of differentiation and radioresistance[108,109]. RNA interference (RNAi)-mediated silencing of GRP78 suppressed the growth of head and neck TICs in a mouse xenograft model, which suggests that cell surface GRP78 is a novel biomarker of TICs and a potential therapeutic target[108,109]. PTEN inactivation, which occurs in about one-half of all cases of human liver cancer, results in steatosis, liver injury and inflammation, which lead to liver progenitor cell (LPC) proliferation and the development of liver cancer[110]. Reduction of the expression levels of GRP78 to less than 25% of wild type in the mouse liver resulted in steatosis but did not trigger LPC activation or malignancies[111,112] (see Supplementary information S2 (table)). However, a similar reduction in GRP78 expression in the *Pten*-null liver model increased steatosis and liver injury, and it accelerated hepatocellular carcinoma (HCC) and cholangiocarcinoma formation[111] (TABLE 1). Strikingly, intense GRP78 re-expression was observed in the cancer lesions, and GRP78 expression in the surrounding liver tissue also reverted back to the wild-type level[111], which suggests repopulation of the liver by GRP78-positive, unrecombined cells that confer a survival advantage, as reported for other tissues[35]. Knocking out *Grp94* in the liver caused only mild injury, but in the *Pten*-null mice, loss of *Grp94* perturbed cell adhesion, stimulated LPC proliferation and accelerated HCC and cholangiocarcinoma progression[113] (TABLE 1). In human liver cancer, as well as in the *Pten*-null mouse model, the levels of GRPs are upregulated and correlate with poor prognosis (see Supplementary information S1 (table)). So, how can these observations be reconciled? One plausible explanation is that in organs where loss of the GRPs leads to progenitor cell activation, tumorigenesis might be accelerated when coupled with other carcinogenic events. However, there is generally a gain (rather than a loss) of GRP function in cancer, owing to stress-induced expression of GRPs. Under these conditions, the GRPs, with their pro-proliferation and anti-apoptotic functions, protect tumour cells from the host defence systems and promote tumour progression and resistance.

GRPs in therapeutic resistance

The expression of GRPs, in both tumour cells and the stromal cells, as an adaptive response to stress that is induced by cancer treatments, could represent a major obstacle to therapeutic efficacy[5,26,77,114]. GRP78 has been extensively documented to confer resistance against a wide range of therapies, including chemotoxic drugs, anti-hormonal agents, DNA-damaging agents, anti-angiogenesis drugs and chromatin-modifying drugs, as well as radiation therapy[20,21,115–117]. The effects are observed in proliferating and dormant cancer cells, TICs, and in tumour-associated endothelial cells, and they involve not only the ER form of GRP78 but also the stress-induced cytosolic isoform[63], the secreted form[118] and the cell surface form of GRP78 (REF. 108). Although less well studied, GRP94 and GRP170 have been linked with chemoresistance in various tumours[71,84], and GRP75 has been linked with resistance to cisplatin in ovarian cancer[119].

Targeting GRPs

As the GRPs are crucial factors in the multiple steps of tumorigenesis and are often induced in tumours that have developed resistance against conventional therapy, they are attractive targets for drug and vaccine development to combat cancer progression and recurrence. GRP inhibitors that function at multiple levels have recently been identified (FIG. 3). Importantly, the cell surface expression of GRPs — primarily in malignant cells, but not in normal cells, *in vivo* — offers the opportunity

Box 3 | **GRP interconnectivity in the endoplasmic reticulum and mitochondria**

In addition to protein folding and secretion, the endoplasmic reticulum (ER) is central to Ca^{2+} homeostasis and the regulation of apoptosis. As low-affinity, high-capacity Ca^{2+}-binding proteins, the glucose-regulated proteins (GRPs) GRP78 and GRP94 help to maintain the balance of Ca^{2+} in the ER[1]. Mitochondria are the site of oxidative phosphorylation-dependent ATP generation, which is crucial for maintaining energy homeostasis, and they also integrate and transduce apoptotic signals, and participate in the regulation of intracellular Ca^{2+}. Structural and functional analyses show zones of close contact between the ER and the mitochondria, and these zones are referred to as mitochondria-associated membranes[193] (MAMs). MAMs enable the efficient transmission of Ca^{2+} from the ER to the mitochondria, and molecular chaperones, such as GRP75, calnexin, calreticulin, ERp44, ERp57 and the σ1 receptor co-occur at MAMs[194]. Signalling from the ER to the mitochondria can be crucial in the induction of mitochondrial-dependent cell death pathways[61,195,196]. The unfolded protein response promotes GRP78 localization to the mitochondria[64,197], slows the increase of Ca^{2+} levels in mitochondria after stress and reduces free radical generation, and it is thereby associated with protection against ischaemic injury[196]. For cancer cells, the overexpression of GRP78 might protect them against damage that results from potentially carcinogenic free radicals that are generated from an endogenous or exogenous source. GRP78 might also regulate the energy balance of mitochondria through the modulation of GRP75 and peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α) expression[198]. In specific tissues, GRP78 haploinsufficiency leads to compensatory upregulation of GRP75, GRP94 and other ER chaperones[33,198]; conversely, GRP94 deficiency triggers GRP78 upregulation[199].

Collectively, these findings demonstrate the interconnectivity of the GRPs and adaptive responses in cells, which maintains functional Ca^{2+} and ATP homeostasis in the ER and the mitochondria. These mechanisms might result in a survival advantage to cancer cells in the face of the various metabolic and environmental perturbations that occur in growing tumours.

for cancer-specific therapy and drug delivery without harming the normal organs. Additionally, because GRPs are upregulated in the cancer microenvironment, their promoters could be of use in the development of gene therapies for the treatment of cancer.

Inhibitors of the GRPs. In principle, agents that inhibit the synthesis, stability or activity of the GRPs can simultaneously suppress their function at various cellular locations. The challenge is to minimize the toxicity to normal organs. Various heterozygous knockout mouse models have shown that a 50% decrease in GRP78 expression has no effect on normal organs but significantly impedes tumour growth and angiogenesis[89] (TABLE 1). This implies that agents that selectively block the stress induction of GRP78 will affect tumours that require a high level of GRP78 and will spare normal organs. Natural compounds with anticancer properties that suppress GRP78 induction have been reported; however, they exert pleiotropic effects[5,120]. Specific cancers also express microRNAs that can cooperatively function to suppress *GRP78* translation (FIG. 3) and reverse chemoresistance[121]. However, specific inhibitors of *GRP78* stress induction remain to be identified.

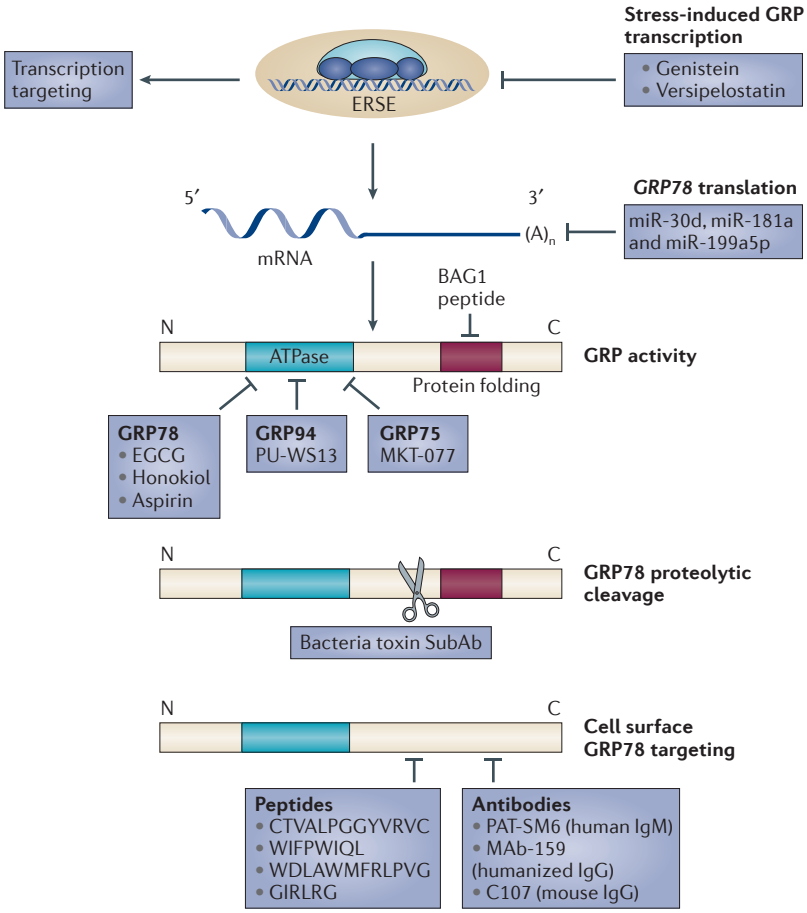


Figure 3 | **Summary of agents that target the GRPs.** Stress-induced glucose-regulated protein (GRP) expression can be suppressed at the transcriptional level by inhibiting transcription factors that are required for the stress induction of the GRP promoter. Several microRNAs (miRNAs) have been identified that suppress the translation of *GRP78* mRNA in cancer cells. As the chaperone function of the GRPs depends on the ATPase catalytic activity, compounds or peptides that bind to their ATP-binding domains or that alter their ATPase activity are effective in suppressing GRP function. A BAG1 peptide binds to the GRP78 substrate-binding domain and inhibits its protein refolding activity. GRP78 can be specifically cleaved by the bacterial toxin SubAb, which renders it non-functional. Cell surface GRP78 can be effectively targeted by specific peptides in conjugated or non-conjugated forms, as well as by the human plasminogen factor Kringle 5 (K5). Antibodies against cell surface GRP78 are able to suppress GRP78-mediated oncogenic signalling and induce cancer cell death by multiple mechanisms. In addition, the inducible GRP promoter that contains the ER stress response elements (ERSEs) can be used to direct cytotoxic gene expression in cancer cells. The GRP inhibitors listed have shown a wide range of anticancer effects *in vitro* and *in vivo*. ATF, activating transcription factor; Ig, immunoglobulin; NFY, nuclear transcription factor Y; XBP1s, spliced form of X-box-binding protein 1.

Alternatively, as the *GRP78* promoter is highly active in aggressive solid tumours, this offers the opportunity to use the *GRP78* promoter to direct the expression of suicide genes, immunosuppressors and tumour suppressors in anticancer therapy. As a proof-of-principle, the *Grp78* promoter was used to drive the expression of the herpes simplex kinase suicide gene in a retroviral system, and this resulted in the eradication of sizable tumours[122,123]. The recent systemic administration of a dual tumour-targeted phage that contained the RGD tumour homing ligand and the *Grp78* promoter showed persistent transgene expression *in vitro* and significant killing of therapy-resistant tumours *in vivo*[124]. Likewise, cancer-inducible transgene expression can be directed by the *Grp94* promoter in tumours of various origins and cancer-associated macrophages[125].

Selective destruction of GRP78 at the protein level might be possible owing to the discovery of a bacterial toxin SubAb, which cleaves GRP78 at a single site (L416–L417) in the hinge region that connects the ATPase and the substrate-binding domain of the molecule, thereby inactivating it[126]. In two studies, the systemic delivery of an engineered fusion protein that combined epidermal growth factor (EGF) and SubAb was toxic to EGF receptor (EGFR)-expressing cancer cells *in vitro* and caused a delay in the growth of human breast, prostate and glioblastoma xenografts in mice[127,128]. One of these studies[128] also showed that modest cleavage of GRP78 in normal mouse liver cells owing to EGFR expression did not lead to weight loss, and this is consistent with the findings in genetic models that normal organs, including the liver, can tolerate a partial decrease in GRP78 levels[39].

As the ATPase catalytic activity of GRP78 is necessary for its anti-apoptotic function[60], targeting its ATP-binding domain can effectively inactivate GRP78 in cancer. Several plant compounds, including (-)epigallocatechin gallate (EGCG), honokiol and aspirin (also known as salicylate), directly bind to this domain and inhibit the ATPase activity (FIG. 3), and this is associated with the sensitization of cancer cells to chemotoxic agents[129–131]. Furthermore, an unconjugated peptide that is derived from the co-chaperone BAG1 binds to the substrate-binding domain of GRP78 and inhibits its protein refolding activity, and prostate cancer cells that stably expressed this peptide showed reduced growth and apoptosis in xenograft models in a manner that was

dependent on binding to GRP78 (REF. 132). In glioblastoma cells, GRP78 can also be inactivated via acetylation by vorinostat, which is a deacetylase inhibitor with antitumour activity[133].

Specific inhibitors against GRP94 function have recently been identified on the basis of the unique secondary nucleotide-binding pocket of this GRP[72,134]. One of the compounds, PU-WS13, has been shown to reduce the viability of breast cancer cells that express high levels of cell surface HER2 (REF. 72) and the viability of human multiple myeloma cells[53] *in vitro*. Interestingly, honokiol induces calpain-mediated cleavage of GRP94 in human gastric cancer cells, and this is associated with apoptosis and a reduction in tumour growth[135]. GPM1, which is a chemical that can bind to GRP94, suppresses its surface presentation through increased retention in the ER. This chemical was shown to compromise the immune functions of GRP94 *in vivo*; however, its efficacy in cancer is not known[136]. MKT-077, which is a cationic rhodacyanine dye, binds to the nucleotide-binding domain of GRP75, abrogates its interaction with p53 and reactivates the transcriptional and pro-apoptotic activities of p53 in cancer cells but not in normal cells *in vitro*[137–139]. Additionally, virtual screening of a drug database has revealed several small-molecule inhibitors that are able to interrupt the p53–GRP75 complex[140].

Collectively, these proof-of-principle studies show that GRP inhibitors can selectively confer toxicity to cancer cells *in vitro* and *in vivo*, and this warrants further development and validation.

Cytotoxic agents that target cell surface GRP. Preferential expression of GRP78 on the surface of tumour cells *in vivo* enables specific tumour targeting with minimal harmful effects on normal cells[11,13,141]. As cell surface GRP78 expression is further detected in some TICs and increased in metastatic and drug-resistant tumours[6,16,117], as well as in hypoxic endothelial cells that support tumour growth[68,78], cytotoxic agents against cell surface GRP78 have the potential to target these cells in addition to the primary tumour.

Several synthetic peptides composed of GRP78-binding motifs that are fused to cell-death-inducing peptides or to cytotoxic drugs are able to promote apoptosis in cancer cells *in vitro*, including human prostate cancer cells, human breast cancer cells, human melanoma, chemotherapy-resistant B-lineage acute lymphoblastic leukaemia cells and multidrug-resistant gastric cells[6,142–148] (FIG. 3). Furthermore, xenograft and isogenic mouse models were used to validate the efficacy of the peptides in suppressing the growth of prostate cancer, breast cancer, melanoma and bone metastasis with no apparent toxicity[6,142,145]. The GRP78-binding peptides have been conjugated to nanoparticles or liposomes for more efficient drug delivery[145,149], and such agents are able to home to endothelial cells in tumours, thereby suppressing tumour growth in colon carcinoma-bearing mice and prolonging their survival[82]. Furthermore, a reconstructed protein that contains a GRP78-binding peptide and mung bean trypsin inhibitor shows targeted anticancer effects, both *in vitro* and *in vivo* in colorectal cancer[150].

MAb159, a high affinity GRP78-specific mouse monoclonal immunoglobulin G (IgG) antibody was recently identified that triggers endocytosis and the degradation of cell surface GRP78, and that activates both intrinsic and extrinsic apoptosis[17]. MAb159 causes cancer cell death and suppresses the growth of colon and lung xenografts, the metastatic growth of human breast and human melanoma xenografts and the growth of prostate cancer and leukaemia in genetically engineered mouse models, at least in part through inhibition of the PI3K signalling pathway[17]. MAb159 also functions in synergy with irinotecan, which is a topoisomerase I inhibitor, to suppress human colon cancer xenograft growth. A humanized MAb159 retains antitumour activity with no toxicity in mice and shows favourable pharmacokinetics[17]. In principle, this antibody can also be used as an *in vivo* imaging agent for the selection of patients who express cell surface GRP78 and to determine whether that predicts disease progression and response to therapy. Another screen yielded a mouse monoclonal IgG antibody that targets the carboxy-terminal domain of GRP78, C107, and that can induce apoptosis in melanoma cells *in vitro* and slow their growth as xenografts in mice[151]. A human monoclonal IgM antibody, PAT-SM6, which was isolated from a patient with gastric cancer and which can simultaneously bind to low-density lipoproteins and multiple GRP78 molecules on the surface of tumour cells, induces lipid accumulation and apoptosis in human multiple myeloma cells[152,153] and suppresses human melanoma growth, both *in vitro* and in xenografts[154]. Based on favourable safety profiles in Phase I studies, the efficacy of PAT-SM6 is being tested in clinical trials[154]. It has also been reported that autoantibodies against GRP78 from patients with ovarian cancer promote apoptosis and decrease the invasiveness of ovarian cancer cells[155]. In another study, autoantibodies against GRP78 from patients with prostate cancer triggered ER Ca^{2+} release in human bladder carcinoma cells and increased tissue factor procoagulant activity, which implies that blocking cell surface GRP78 signalling could potentially reduce the risk of cancer-related thrombotic events[156]. As a proof-of-principle that cell surface GRP75 may also be amenable to therapy, it was shown that intra-tumoural and intra-peritoneal injections of an antibody against GRP75 resulted in the suppression of tumour growth[22].

Vaccination strategies. Molecular chaperone preparations from tumours that carry tumour antigens offer a personalized, polyvalent vaccine therapy[2,23,157]. Although vaccination of lethally irradiated cancer cells that expressed autologous secretory GRP94 fusion proteins protected mice from primary tumour growth and metastasis[158], vitespen, which is a GRP94–peptide complex that is purified *ex vivo* from the tumour cells of individual patients, showed variable immunogenicity and overall limited efficacy in clinical trials, with clinical responses only in certain patient subsets[159–161]. Recent studies showed that immunization with a low dose of GRP94 activated CTLs with some tumour suppression in mice, whereas a high dose induced T_{Reg} cell proliferation and immune suppression, in a manner that was

dependent on TLR-mediated NF-κB activation[162]. The use of GRP94 fusion proteins with tumour antigens, the depletion of T_{Reg} cells and pooled GRP94 vaccines have all been proposed to enhance the antitumour activity of GRP94 immunization[163]; however, challenges remain with these approaches.

GRP170 can form complexes with full-length protein antigens, such as GP100 (also known as PMEL), and can increase their presentation to immune cells, thereby augmenting multivalent T cell-mediated antitumour immune responses[164]. Genetic modification of various poorly immunogenic melanomas to express a secretable form of GRP170 significantly suppressed tumour growth *in vivo*, and this was associated with increased tumour-infiltrating $CD8^+$ T cells and stimulation of dendritic cells[165]. The use of GRP170-secreting tumour cells as a cell-based vaccine is effective in treating established mouse prostate tumours[166]. Incorporation of a pathogen-associated molecule such as the NF-κB-activating domain of bacterial flagellin into GRP170 (FlaGRP170) produced a chimeric protein that maintains highly efficient antigen-processing and activation of dendritic cells, thereby mounting a superior antitumour immune response against metastatic tumours in mice[167]. The tumour-derived, secreted form of GRP78 is also able to elicit an antitumour immune response in mouse models, as a result of activation of cytotoxic T cells[168].

Conclusions and perspectives

The GRPs have functions that are distinct from the HSPs, and they affect both the tumour cells and the tumour microenvironment. As the stress induction of GRPs could be a major contributor to tumorigenesis and therapeutic resistance, their specific inhibitors and targeting agents hold great therapeutic promise. Their clinical efficacies, as well as large GRPs as vaccines, warrant vigorous testing in the clinical setting. However, answers to key issues on basic mechanisms, such as how the stress-induced relocalization of the GRPs from the ER to the cell surface and other organelles occurs, what their interactive partners are and the mechanisms of signalling, as well as the usefulness of GRPs as prognostic markers and companion imaging agents for precision cancer care, will greatly advance the understanding of GRP biology and the applications of GRPs in cancer.

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Acknowledgements
This work is supported by grants to the author from the US National Institutes of Health (NIH) grants CA027607 and AG034906, and to the Norris Comprehensive Cancer Center, Los Angeles, California (P30 CA014089). The author thanks L. Hightower, Z. Li, J. Subjeck, X.-Y. Wang and J. Luk for helpful discussions and the Lee Laboratory members, particularly J. Shen and Y. Zhang for their help in preparing the references and figures. The author also thanks E. Olivo and S. Olivo for their assistance in preparation of the manuscript. The author apologizes to those whose work is not cited owing to space limitations.

Competing interests statement
The author declares no competing interests.

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