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Beyond the endoplasmic reticulum: atypical GRP78 in cell viability, signaling and therapeutic targeting

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SYNOPSIS

GRP78 is traditionally regarded as a major endoplasmic reticulum (ER) chaperone facilitating protein folding and assembly, protein quality control, Ca^{2+} binding and regulating ER stress signaling. It is a potent anti-apoptotic protein and plays a critical role in tumor cell survival, tumor progression and angiogenesis, metastasis and resistance to therapy. Recent evidence shows that GRP78 can also exist outside the ER. The finding that GRP78 is present on the surface of cancer but not normal cells *in vivo* represents a paradigm shift on how GRP78 controls cell homeostasis and provides an opportunity for cancer specific targeting. Cell surface GRP78 has emerged as an important regulator of tumor cell signaling and viability as it forms complexes with a rapidly expanding repertoire of cell surface protein partners, regulating proliferation, PI3K/AKT signaling and cell viability. Evidence is also emerging that GRP78 serves as a receptor for viral entry into host cells. Additionally, a novel cytosolic form of GRP78 is discovered prominently in leukemia cells. These, coupled with report of nuclear and mitochondria localized form in GRP78, point to the previously unanticipated role of GRP78 beyond the ER that may be critical for cell viability and therapeutic targeting.

Keywords

GRP78; cell surface; cytosol; nuclear; mitochondria; signaling

INTRODUCTION

Unlike other members of the Hsp70 gene family, GRP78 is encoded by a single copy gene in the eukaryotic genome and its induction is primarily regulated at the level of transcription [1,2]. Since its discovery in the 1980s, GRP78 is well established as an ER chaperone and widely used as a marker for ER stress. It is highly induced in a wide range of tumors through intrinsic factors such as altered glucose metabolism of cancer cells, compounded by extrinsic factors such as glucose deprivation, hypoxia and acidosis in the microenvironment of poorly-perfused solid tumor [3]. The induction of GRP78 by ER stress leads to an increase in GRP78 in the ER compartment, as well as promotion of GRP78 relocalization from the ER to the cell surface [4]. There GRP78 assumes a new function of co-receptor for cell surface signaling [5,6]. ER stress also enhances retention of intron 1 of the Grp78 transcript, leading to translation of a novel isoform of GRP78 (Grp78va) that lacks the ER signal peptide and is localized in the cytosol [7]. While GRP78va is in low abundance

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compared to the ER form of GRP78, it exhibits cytoprotective properties and has the potential to regulate UPR signaling from the cytosol. Additionally, GRP78 is reported to associate with the mitochondria and in mouse models [8], perturbation of GRP78 level in the ER elicits compensatory responses in the ER as well as in the mitochondria [9]. While least understood, GRP78 has been observed in the nucleus following overexpression [10]. Furthermore, a secreted form of GRP78 is linked to endothelial cell drug resistance [11]. In this review, we summarize the occurrence and putative function of GRP78 beyond the ER (Figure 1). These findings support the emerging notion of functional importance of minor subpopulations of various proteins generated by different means to influence fundamental biological processes.

CELL SURFACE GRP78

With the first report of cell surface localization of GRP78 in 1997 [12], evidence is rapidly accumulating that GRP78 exists on the cell surface of select cell types. However, there have been inconsistencies in literature on the expression of cell surface GRP78 in cultured cell lines, likely due to inherent diversity of cells maintained in culture, or technical issues. Nonetheless, whole organism targeting of cell surface GRP78 showed reactivity primarily observed with pathological tissues such as cancer and cell types under stress conditions such as hypoxia or glucose starvation. This suggests potential applications in targeted therapy. The key known functions of cell surface GRP78 is summarized in Figure 1 and 2.

GRP78 ON THE SURFACE OF TUMOR CELLS AND CONTROL OF ONCOGENIC SIGNALING

GRP78 was identified on the cell surface of malignant lymphocytes of lymphoma and leukemia from patients with acquired immunodeficiency syndrome [12]. KDEL motif containing proteins, including GRP78, were expressed on the surface of NG108-15, a neuroblastoma glioma hybrid cell line [13]. Established human cancer cell lines of neuroblastoma (SH-SY5Y), lung adenocarcinoma (A549), colon adenocarcinoma (LoVo), acute lymphoblastic leukemia-B cells (Sup-B15 ALL-B cell) and ovarian tumor cells freshly isolated from patient ascites fluid showed cell surface GRP78 expression as determined by global profiling of cell surface proteome of tumor cells [14]. Cell surface GRP78 was also found on melanoma cell line Me6652/4, osteosarcoma SJSA-1, hepatoma cell line HepG2 [15], breast cancer cells (Hs578T, MDA-MB-231, BrCa-MZ-01, MCF-7) [16] and gastric cancer 23132/87 and pancreatic cancer cells BXPC-3 [17].

Through formation of complex with other proteins on the cell surface, GRP78 is reported to mediate tumor cell signal transduction (Figure 2). For example, in highly metastatic and invasive 1-LN prostate cancers, cell surface GRP78 acts as a receptor for activated α_2-macroglobulin leading to activation of PAK-2, and together with LIMK and cofilin phosphorylation, increases motility for metastasis [18,19]. The interaction of α_2-macroglobulin with cell surface GRP78 is also reported to promote cell proliferation by activating ERK1/2, p38 MAPK and PI3K and cell survival by Akt and NF-kB signaling cascade [20]. Autoantibody from serum of prostate cancer patients against an amino segment of GRP78 (Leu 98-Leu115) induces cell proliferation, suggesting that it serves as an agonist of activated α_2-macroglobulin, which recognizes the same site of GRP78 [21]. Additionally, treatment of human bladder carcinoma cell line with the same autoantibody against GRP78 increases the tissue factor procoagulant activity [22]. Interestingly, the autoantibody caused the release of Ca^{2+} from the ER store, which may account for the increase of risk of venous thromboembolism. In another example, Cripto, a multifunctional cell surface protein which is key to vertebrate embryogenesis and human tumor progression, was bound to cell surface GRP78 [23]. The complex of Cripto and GRP78 can enhance

tumor growth via inhibition of TGF-β signaling. Furthermore, it was determined that blockade of Cripto binding to cell surface GRP78 by an antibody against N-terminus of GRP78 inhibits oncogenic Cripto signaling and this involves the MAPK/PI3K and Smad2/3 pathway [24].

ANTI-CANCER THERAPY BY TARGETING CELL SURFACE GRP78 ON TUMOR CELLS

By screening the circulating pool of antibodies from patient serum, GRP78 was identified as a tumor antigen highly expressed in bone marrow metastases of prostate cancer patients, whereas weakly expressed in normal prostate tissue [25]. This opens up the opportunity for cell surface GRP78 ligand-directed targeting cancer therapy, such that synthetic chimeric ligand peptides containing programmed cell death-inducing sequence when administrated to the mice suppressed tumor growth in xenograft and isogenic mouse models of prostate and breast cancer [26]. In other applications, Pep42, a cyclic oligopeptide that specifically bound to cell surface GRP78 and internalized into cells, enabled taxol-conjugated Pep42 to target and kill melanoma cells by recognizing GRP78 on their surface [27], Further study showed that the fusion of Pep42 conjugated to apoptosis-inducing peptide D-$(KLAKLAK)_2$ selectively killed human cancer cell lines *in vitro* by binding to cell surface GRP78, but with minimal toxicity to normal cells where no GRP78 was detected on the cell surface [15].

GRP78 modification variants may represent novel targets for cancer therapy. Phage display derived human monoclonal antibodies isolated by binding to primary breast cancer cells recognize a modified form of cell surface GRP78, involving a putative glycosylation site at the C-terminus of GRP78 [16]. Another report indicates the existence of an 82 kDa tumor specific variant of GRP78 [17]. The epitope is an O-linked carbohydrate moiety and is specific for malignant cells, which may account for escape of GRP78 from immune surveillance and immune response. Cancer patient serum auto-antibody against this form of GRP78 when added to malignant cells leads to lipid accumulation and cell death [28].

A commercial polyclonal antibody directed against C-terminus of GRP78 was reported to induce apoptosis in melanoma cells (A375) and prostate cancer cells (1-LN, DU145), but not in another prostate cancer cell line, PC-3, where GRP78 expression was undetectable on the surface [29]. The proposed mechanism is that this antibody leads to up-regulation of p53, inhibition of NF-kappa B1 and NF-kappa B2 activation, and suppression of Ras/MAPK and PI3K/Akt signaling [29–32]. In another study using prostate cancer PC-3 cells, apoptosis induced by extracellular Par-4 and TRAIL was observed to be dependent on the binding of Par-4 to cell surface GRP78, and resulted in activation of the extrinsic apoptosis pathways and this was enhanced by ER stress or TRAIL [33]. Par-4 was previously regarded as cytosolic and nuclear protein that promotes cell death, however, it was found that Par-4 can spontaneously secrete in normal and cancer cell culture and it was proposed that ER stress or TRAIL caused translocation of Par-4-GRP78 complex from ER to plasma membrane [34,35]. Nonetheless, how Par-4 enters the ER and the conflicting reports of whether GRP78 is expressed at significant level on the surface of PC-3 cells remain to be resolved since other studies showed no cell surface GRP78 expression in PC-3 cells compared to high level in more malignant and invasive1-LN cells [36,37].

GRP78 ON THE SURFACE OF PROLIFERATING ENDOTHELIAL CELLS

GRP78 is expressed on the cell surface of proliferating endothelial cells and monocytic cells [38,39] (Figure 2). GRP78 associates with major histocompatibility complex (MHC) class I on the surface of these cells and is required for MHC class I expression [40]. GPI-anchored T-cadherin is reported to associate with GRP78 on the surface of vascular endothelial cells,

and in this capacity, GRP78 influences endothelial cell survival as a cell surface signaling receptor [41].

As tumor progression typically requires angiogenesis for nutrient and oxygen supply, anti-angiogenic therapy exploits this requirement to block tumor growth. Kringle 5 of human plasminogen has been shown to be a binding partner of GRP78 on the surface of proliferation endothelial cells and stimulated tumor cells [39]. Recombinant Kringle 5 (rK5) induces apoptosis of proliferating endothelial cells and tumor cells through binding of surface expressed GRP78 and enhancing caspase-7 activity by disruption of GRP78-procaspase-7 complex [39]. Further study shows that prior irradiation significantly sensitizes the glioma microvessel endothelial cells to rK5-induced apoptosis, which required low-density lipoprotein receptor related protein 1 (LRP1) and GRP78 [42]. In addition, the expression of cell surface GRP78 is elevated in VEGF-activated HUVEC cells and required for endothelial cell proliferation [43]. The same study showed that cell surface GRP78 is a promising target for effective liposome drug delivery in cancer anti-neovascular therapy [43].

GRP78 was recently identified as the endothelial cell receptor required for Mucorales to penetrate and damage endothelial cells. Moreover, serum from mice vaccinated with recombinant GRP78 protected diabetic ketoacidosis mice from infection with mucormycosis, providing a novel approach for therapeutic intervention to lethal mucormycosis [44]. GRP78 also exists on the atherosclerosis plaque endothelial surface and negatively regulates tissue factor-mediated initiation of coagulation cascade [38]. In another study, a novel peptide, RoY, was demonstrated to alleviate mouse hind limb ischemia through binding surface expressed GRP78 on hypoxic endothelial cells [45]. Furthermore, another peptide derived from ADAM15, metalloprotease has also shown to activate GRP78 on endothelial cell membrane under hypoxic condition, inducing VEGF-independent angiogenesis, implying cell surface GRP78 can serve as angiogenic receptor for ischemia disease therapy [46].

CELL SURFACE GRP78 AS CO-RECEPTOR FOR VIRUS INTERNALIZATION

Evidence is emerging that GRP78 serves as a critical portal for viral entry into host cells (Figure 2). Previous studies on viral entry of Coxsackie virus A9 into host cells determined that it required major histocompatibility complex class I molecules. GRP78 was later found to act as co-receptor for virus internalization by associating with major histocompatibility complex class I molecules on the cell surface [47]. GRP78 expressed on liver cancer cell surface acts as receptor for dengue virus serotype 2 entry and antibodies directed against both the N and C-terminus of GRP78 majorly affected the binding of the virus to the cell surface as well as the virus infectivity [48]. Recently, on the study of Borna disease virus, which is characterized by highly neutropic and noncytopathic infection, GRP78 was also found on the surface of Borna disease virus targeted cells. The Borna disease virus entry was mediated by association of cell surface GRP78 with the N-terminus cleaved product of envelope glycoprotein of Borna disease virus, GP1 [49]. The antibody against N-terminus of GRP78 (N20) was shown to inhibit GP1 binding to cells expressing GRP78 on cell surface and reduce virus infection.

POTENTIAL MECHANISM FOR GRP78 TRANSLOCATING FROM ER TO CELL SURFACE

The mechanisms for GRP78 trafficking from ER to plasma membrane are just emerging. The C-terminal tetra-peptide, KDEL, has been shown to prevent GRP78 secretion and maintain it within the ER lumen [50]. Since the KDEL receptor expression was not

coordinately up-regulated with ER stress in HeLa cells [51], increase in intracellular level of GRP78 triggered by ER stress may exceed the retention capacity of KDEL retrieval system, resulting in escape from ER to cell surface. It is also possible that the activity of the various components of the KDEL system is being altered under ER stress or pathological conditions. Another possible mechanism for GRP78 transport to cell surface may involve masking of the KDEL motif by glycosylation or other modification to the protein sequence adjacent to KDEL. Glycosylated form of GRP78 has been detected and potential glycosylation sites exist at the C-terminus in close proximity to the KDEL motif [4,16,17]. Additionally, specific GRP78 interacting protein partners may facilitate its transport from ER to the cell surface and this can be cell type specific and/or acting in combination. For example, a DnaJ-like transmembrane protein, MTJ-1, binds GRP78 and silencing MTJ-1 expression apparently suppress cell surface GRP78 expression in macrophages [52]. However, in PC3 cells, Par-4 was reported to be required for translocation of GRP78 from the ER to the plasma membrane [33].

In cell cultures, ER stress agent, thapsigargin, actively promotes cell surface expression of GRP78, as the increase in cell surface GRP78 is several fold higher than the increase in intracellular GRP78 induced by thapsigargin [4]. Nonetheless, ER stress is not required for cell surface localization of GRP78. Thus, ectopic expression of GRP78 can induce the translocation in the absence of ER stress as indicated by the lack of CHOP induction [4]. Moreover, deletion of the ER retention motif KDEL alters GRP78 relocation in a dose-dependent manner, such that GRP78 devoid of the KDEL motif showed more surface localization at lower dosages. This suggests that the KDEL retrieval system plays a significant role in regulating the extent of GRP78 leaving the ER. The mutation analysis of putative O-linked glycosylation site threonine 648, which is two amino acids away from KDEL, shows no effect on modification and translocation of GRP78 to cell surface [4]. It is still possible that the modification only plays a minor role in the translocation progress thus it is below detection limit or the experiments were not performed in the correct cell types.

The extracellular domains of GRP78 exposed on the cell surface have been identified by flow cytometry assay using different epitopes at N-, C-terminus and a middle segment of GRP78. These studies show that the N-, C-terminus and middle portion of GRP78 are all exposed on cell surface [4,15,33,45]. Furthermore, the blockade of function of Cripto, GPI-anchored T-cadherin and Par-4 by antibody against N-terminus of GRP78 is in agreement with the existence of extracellular N-terminal region of cell surface GRP78 [24,33,41]. Similarly, antibody against the C-terminus of GRP78 also affects cellular signaling consistent with exposed C-terminus of GRP78 on the cell surface [26].

Since addition of extracellular recombinant GRP78 did not appear to bind to the surface of the recipient cells, cell surface GRP78 is unlikely due to adhesion of secreted GRP78 to the cell surface [4]. This raises the issue of whether GRP78 exists as a transmembrane protein. While GRP78 is a hydrophilic protein, TMpred program showed several potential transmembrane segments, with a predicted structural model of cell surface GRP78 presented as a transmembrane protein with C-terminus exposed extracellularly [4,10]. This is supported by biochemical analysis of microsomal fractions showing that a subpopulation of GRP78 is ER-transmembrane protein with N terminus protruding to cell cytosol, as proven by limited trypsin digestion and cell membrane separation by carbonate sodium extraction [10]. Future studies are required to fully dissect the domains of GRP78 that are extracellular.

GRP78 IN THE CYTOPLASM

Although unexpected, GRP78 has also been detected in the cytoplasm with functions including regulation of UPR signaling, assembly of viral protein, and amelioration of lead

neurotoxicity (Figure 1). A cytosolic GRP78 isoform (GRP78va), which is generated by alternative splicing, has recently been discovered [7] (Figure 3). GRP78va mRNA contains the intron 1 of *Grp78* and encodes a truncated form of GRP78 due to alternative translational initiation. The abundance of *Grp78va* is likely affected by nonsense-mediated RNA decay (NMD), given that the retention of intron 1 introduces a premature stop codon. However, ER stress-induced eIF2α phosphorylation leads to repression of NMD [53], which consequently stabilizes the *Grp78va* mRNAs. Therefore, GRP78va is upregulated by ER stress, due to elevated pre-mRNA level of *Grp78* and increased stability of *Grp78va* transcript. Notably, GRP78va is overexpressed in leukemic cells and leukemia patient samples. As this isoform is devoid of the signaling peptide and retains the major functional domains of GRP78, it is localized in the cytosol, where it can potentially interact with many client proteins. The first GRP78va associated protein reported is P58IPK, the inhibitor of PERK during UPR (Figure 3). While P58IPK primarily resides in the ER, inefficient translocation can lead to cytosolic localization [54]. When overexpressed, GRP78va interacts with P58IPK and antagonizes its inhibitory effect on PERK, resulting in activated PERK signaling and increased cell survival under ER stress. This study suggests that GRP78va has the potential to influence survival of cancer cells in adaptation to ER stress through modulating UPR signaling and other yet unknown processes in the cytosol.

GRP78 plays an important role in human cytomegalovirus (HCMV) assembly and egress [55]. During HCMV infection, the vigorous synthesis of viral proteins requires increased capacity of the protein maturation machinery and therefore GRP78 expression is induced [56]. GRP78 actively binds to the viral proteins US2 and US11, which facilitates the virus-mediated degradation of major histocompatibility complex class I and II. A recent study indicated that GRP78 is localized in the cytosolic assembly compartment possibly through the mask of its C-terminal KDEL signal by the protein complex in assembly [57].

GRP78 also plays a protective role in lead-induced oxidative stress in astroglia cells [58]. It has been shown that lead (Pb) directly targets GRP78 through direct binding and the GRP78 expression is elevated [59]. Further studies disclosed the compartmentalized cytosolic distribution of GRP78 in the astrocytoma cells exposed to Pb. The induction and redistribution of GRP78 protects astroglia against Pb neurotoxicity. However, the detailed molecular mechanism needs further investigation to interpret why and how GRP78 acts in the cytoplasm in astroglia cells under Pb insults.

GRP78 IN THE MITOCHONDRIA

The ER and the mitochondria are physically and functionally linked and there is increasing evidence of the GRPs influencing ER and mitochondrial crosstalk to maintain mitochondrial function (Figure 1). For example, the ER chaperone GRP94 interacts with the essential mitochondria chaperone, GRP75, to facilitate GRP75-mediated import of the mitochondrial proteins [60]. Interestingly, GRP78 is also implicated in regulation of mitochondria energy balance. Grp78 heterozygosity ameliorates high-fat diet induced obesity and type 2 diabetes, as a consequence of activated adaptive UPR and increased insulin sensitivity in white adipose tissue (WAT) [9]. The partial reduction of GRP78 in WAT leads to elevated expression of GRP75, suggesting elevated energy expenditure in the mitochondria likely as a compensatory measure. It is possible that GRP78 might physically interact with GRP75 in the mitochondria, since it has been reported that GRP78 is also localized into the mitochondria under ER stress [8]. Submitochondrial fractionation studies identified the GRP78 mitochondrial location mainly in the intermembrane space, inner membrane and mitochondria matrix, where GRP75 also locates. ER stress and UPR signaling induce the overexpression of GRP78, which results in the mitochondria localization of GRP78. Furthermore, Ca^{2+} transfer from ER to mitochondria at contact sites between the organelles

can induce mitochondrial dysfunction and programmed cell death after stress. Recently it was discovered that overexpressing GRP78 protects astrocytes against ischemic injury, reduces net flux of Ca^{2+} from ER to mitochondria, increases Ca^{2+} uptake capacity in isolated mitochondria, reduces free radical production, and preserves respiratory activity and mitochondrial membrane potential after stress [61]. Collectively, these findings imply that GRP78 can potentially regulate mitochondrial function, such as balancing energy expenditure and maintaining mitochondria homeostasis especially under ER stress.

GRP78 IN THE NUCLEUS

GRP78 has also been observed in the nucleus when it is ectopically overexpressed or induced by ER stress [10,62]. Gilvocarcin V (GV) is an antitumor antibiotic with a coumarin-based aromatic structure that promotes protein-DNA cross-linking when photoactivated by near-UV light. Interestingly, the mature form of GRP78 lacking the hydrophobic leader was selectively cross-linked to DNA in human fibroblasts by photoactivated GV [63]. A proteomics study to isolate proteins involved in irradiation-induced DNA protein crosslink in mammalian cells using γ-rays under either aerated and/or hypoxic conditions identified GRP78 as cross-linked to DNA [64].

Recently it was reported that capsaicin, a pungent ingredient of red pepper, induces apoptosis and also promotes cytoplasmic CHOP expression and nuclear translocation of GRP78 in human hepatoma HepG2 cells [65]. It was found that capsaicin-induced apoptosis is mediated through elevation of intracellular production, ROS, regulation of mitochondrial Bcl2 family and caspase-3. In another example, knockdown of GRP78 sensitizes cells to UVC-induced cell death, primarily due to the impaired DNA repair capacity [66]. Taken together, it is tempting to speculate that the nuclear form of GRP78 might play a role against DNA damage induced apoptosis through a distinct regulatory mechanism in the nucleus (Figure 1).

SECRETED GRP78

In the early 1990's, immunogold electron microscopy studies showed that in the exocrine pancreatic cells, the ER luminal proteins GRP78, PDI and GRP94 are exported from the ER to other intracellular organelles, such as nuclear envelope cisternae, the trans-Golgi cisternae, secretory granules, plasma membranes, and even secreted into extracellular space [67]. Although all of these chaperone proteins have the KDEL ER retention signal, it was speculated that the saturation of KDEL receptors or defects in the protein sorting system might cause the inability to retrieve these KDEL-bearing proteins to the ER lumen, especially in the cells with intensive requirements on protein synthesis and maturation. Following these observations, secretion of GRP78 was also detected in tumors. In the study of the inhibitory effects of Bortezomib's on tumor angiogenesis, it was discovered that a few tumor cell lines secreted a high amount of GRP78 into the tumor microenvironment [11]. The inhibition of ubiquitin-proteasome pathway by Bortezomib, a proteasome inhibitor, causes accumulation of misfolded proteins, leading to ER stress and UPR activation. In that study, it was proposed that induction of GRP78 under ER stress in tumor cells leads to increased secretion of GRP78, and by binding to cell surface receptors of endothelial cells, extracellular GRP78 activates ERK and AKT pathways and protects endothelial cells from the antiangiogenic effect of Bortezomib [11] (Figure 1). In a proteomic study of gastric cancer, GRP78 was identified in sera of 28% patients but not in healthy individuals [68]. Importantly, circulating GRP78 autoantibodies have been detected in gastric and prostate cancer patients and implicated in tumor cell proliferation [21,68]. Further studies would help to clarify whether the soluble GRP78 in patient sera is from tumor cells or only dead cells in peripheral blood. Interestingly, GRP78 was also detected in oviductal fluids from women in

the periovulatory period [69]. It was found to be secreted from human oviduct epithelial cells and has the ability to modulate sperm-zona pellucida binding during fertilization [69]. Therefore, secreted GRP78 can potentially regulate a multitude of biological processes in both pathological and physiological conditions.

CONCLUSIONS AND PERSPECTIVES

GRP78 localized in multiple intracellular organelles is correlated with facilitating cell adaptation to stress for survival (Figure 1). This also raises the following issues. How does ER stress promote GRP78 export from the ER, especially to the cell surface, cytosol and mitochondria? Is it possible that the protein transportation machinery is altered in cancer cells, or cells with high protein processing demands, such as pancreatic acinar cells, and those experiencing ER stress or oxidative stress? Since cell surface GRP78 plays a critical role for cell signaling and survival, blocking its expression on the cell surface may sensitize cancer cells and proliferating endothelial cells within the tumor microenvironment to chemotoxic agents. For the cytosolic isoform of GRP78, what are its interactive partners and what pathways are affected? Finally, GRP78 is only a paradigm of ER proteins. There might be similar regulation of redistribution on other ER chaperone proteins, such as GRP94 and PDI. It will be important to explore atypical forms of these proteins outside the ER as they may also have unexpected functions important for controlling cell viability and signaling.

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Abbreviations used

ER	endoplasmic reticulum
GV	Gilvocarcin V
HCMV	human cytomegalovirus
LRP1	lipoprotein receptor related protein 1
MHC	major histocompatibility complex
NMD	nonsense-mediated RNA decay
rK5	Kringle 5
WAT	white adipose tissue

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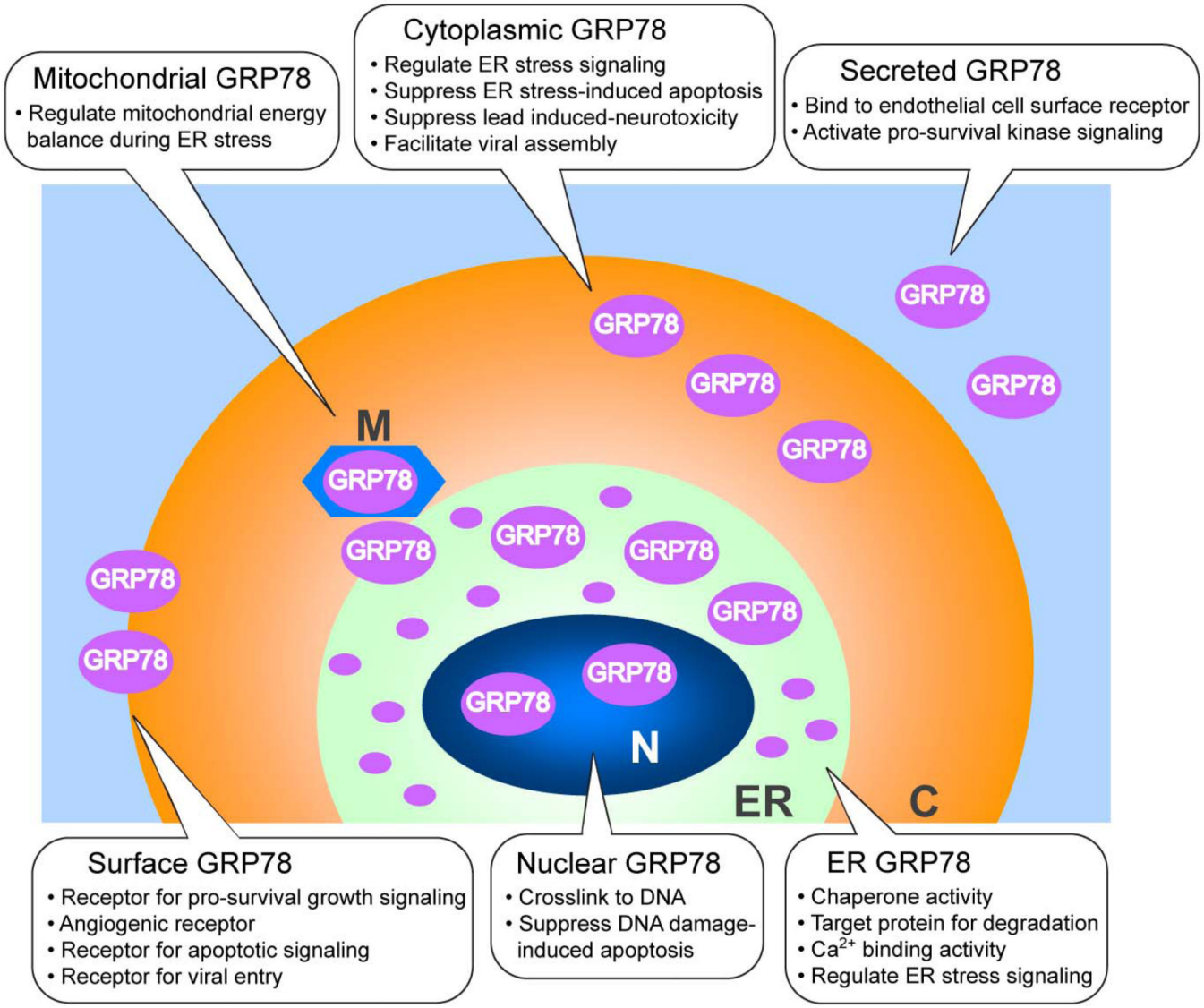

Figure 1. Summary of GRP78 functions in different subcellular compartments

GRP78 is traditionally recognized as a major ER chaperone facilitating protein maturation and degradation, Ca^{2+} binding and regulating ER stress signaling. In non-stressed cells, GRP78 is primarily located in the ER lumen with a subfraction detected as a transmembrane protein. GRP78 also exists outside the ER and acts in multifaceted cellular activities. For example, ER stress promotes cell surface expression of GRP78 and generation of a cytoplasmic isoform resulting from alternative splicing. Cell surface GRP78 emerges as an important receptor in cell signaling, viability and anti-cancer therapeutic targeting. The cytoplasmic GRP78 isoform is a newly identified regulator of the ER stress signaling pathway, in addition to the function of canonical GRP78 in the cytoplasm. Beyond the ER, the mitochondrial, nuclear and secreted forms of GRP78 have been linked to cellular homeostasis and therapeutic resistance. Abbreviations: N, nucleus; ER, endoplasmic reticulum; C, cytoplasm; M, mitochondria.

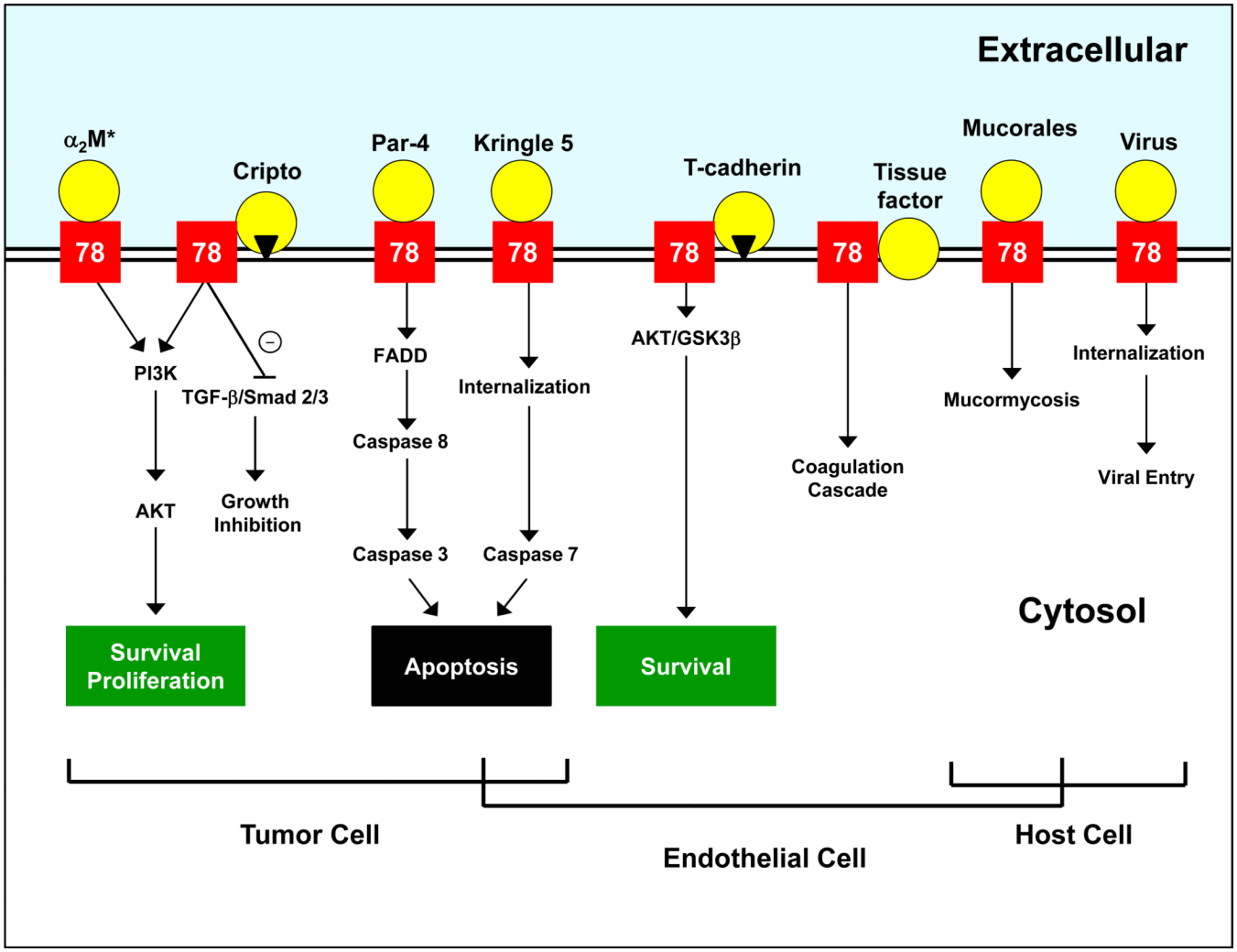

Figure 2. Examples of GRP78 on the cell surface serving as a receptor and regulator of cell signaling

Cell surface GRP78 forms complexes with a variety of extracellular ligands (e.g., activated α_2-macroglobulin, Kringle 5, Par-4) and cell surface anchored (▼) proteins (e.g., Cripto, T-cadherin) in tumor and endothelial cells leading to pro-survival or pro-apoptotic pathways. It also regulates the coagulation cascade through interaction with integral membrane protein (Tissue factor) and facilitates fungal (*R. oryzae*) and viral entries (e.g., Coxsackie virus A9, Borna disease virus and dengue virus serotype 2) in the respective host cells. 78: GRP78; α_2M*: activated α_2-macroglobulin.

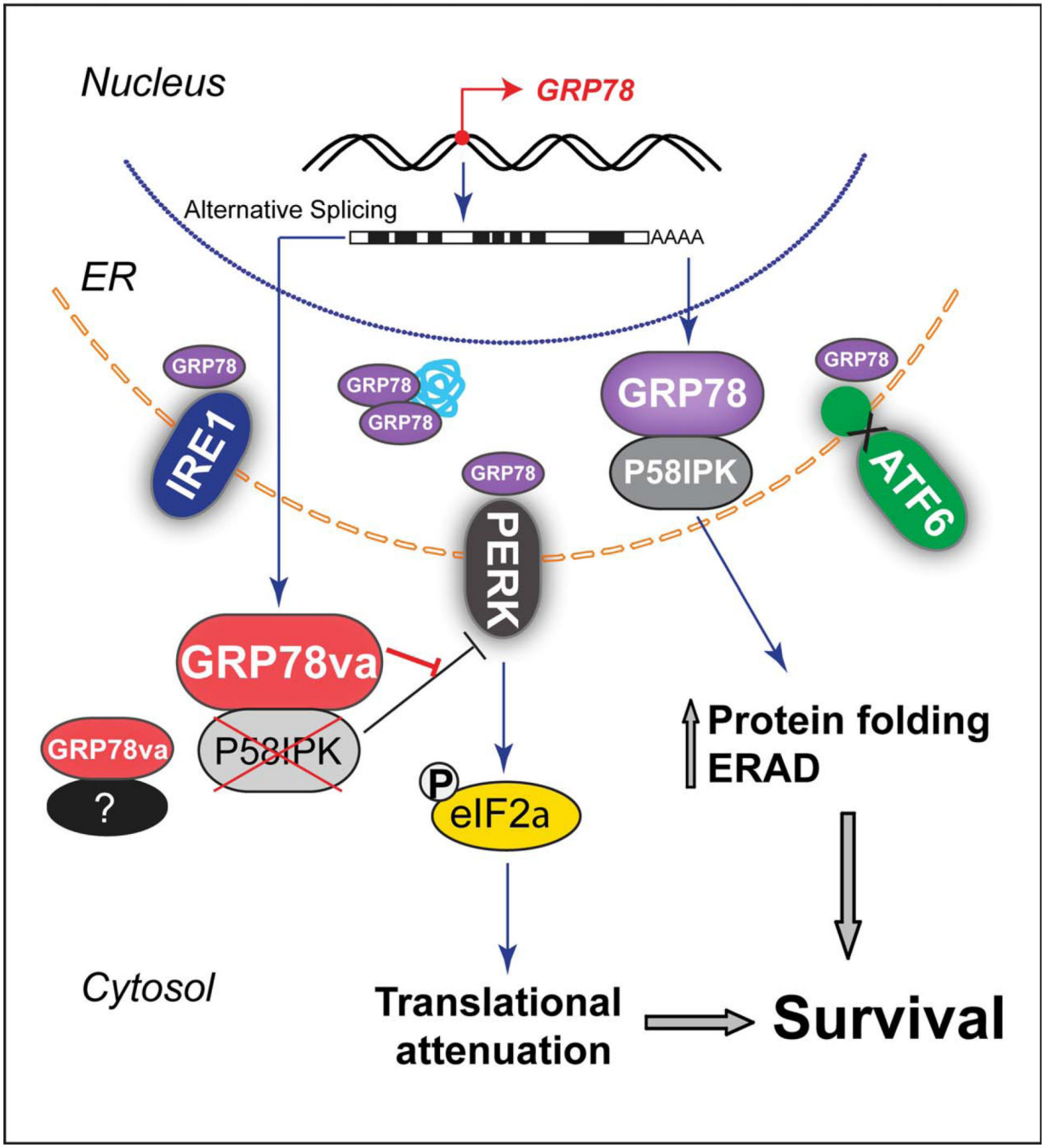

Figure 3. Role of GRP78 and its isoform GRP78va in regulating UPR signaling pathway and cell survival

ER stress induces *Grp78* transcription, resulting in upregulation of GRP78 and GRP78va, the latter is generated by alternative splicing. In the ER lumen, GRP78, in association with $P58^{IPK}$ and other co-chaperones, enhances protein folding and degradation of misfolded proteins (ERAD). Release of GRP78 from PERK, IRE1 and ATF6 induces the UPR signaling. PERK activation leads to eIF2α phosphorylation and translation attenuation, contributing to cell survival. In the cytosol, GRP78va enforces PERK signaling by inactivation of the cytosolic $P58^{IPK}$.