Cancer cells adapt to chronic stress in the tumor microenvironment by inducing the expression of GRP78/BiP, a major endoplasmic reticulum chaperone with Ca\textsuperscript{2+}-binding and antiapoptotic properties. GRP78 promotes tumor proliferation, survival, metastasis, and resistance to a wide variety of therapies. Thus, GRP78 expression may serve as a biomarker for tumor behavior and treatment response. Combination therapy suppressing GRP78 expression may represent a novel approach toward eradication of residual tumors. Furthermore, the recent discovery of GRP78 on the cell surface of cancer cells but not in normal tissues suggests that targeted therapy against cancer via surface GRP78 may be feasible.

Endoplasmic Reticulum Stress and Cancer

The endoplasmic reticulum (ER) is an essential perinuclear organelle for the synthesis and folding of secretory and membrane proteins, which accounts for about one third of the cell’s proteins. When the protein load exceeds the folding capacity of the ER, the cells trigger the unfolded protein response (UPR), which activates the PERK, IRE1/X-box binding protein-1 (XBP-1), and activating transcription factor-6 (ATF6) signaling pathways as protective measures, resulting in general translational attenuation, up-regulation of chaperones and folding enzymes, and enhanced ER-associated degradation of malformed proteins (1, 2). Depending on the severity of ER stress, the UPR can result in cell death through the activation of apoptotic pathways mediated specifically by the ER, as well as coupling with the mitochondrial pathways (2, 3). ER stress also induces autophagy, a cellular degradation process implicated in both cell death and survival (4).

Cancer cells are subject to ER stress because of both intrinsic and extrinsic factors (5). Cancer cells exhibit elevated glucose metabolism with increased glycolytic activity, and solid tumors often grow faster than their blood supply. The latter creates a tumor microenvironment characterized by glucose deprivation, acidosis, and severe hypoxia. These combined factors leads to the accumulation of underglycosylated and misfolded proteins in the ER, triggering the UPR (Fig. 1). In xenograft models, XBP-1 is required for survival under hypoxic conditions and tumor growth, whereas PERK confers advantage for tumor growth (6, 7). Another major UPR adaptive survival response is the induction of ER chaperone GRP78 in the tumor microenvironment (Fig. 1), which is the focus of this review.

**GRP78 Is a Key Survival Factor in Development and Cancer**

The glucose-regulated protein GRP78, also referred to as BiP (immunoglobulin heavy-chain binding protein), was discovered in the late 1970s together with GRP94 and GRP58 as cellular proteins induced by glucose starvation (1). Residing primarily in the ER, GRP78 belongs to the HSP70 protein family, which plays critical roles in the stress of oncogenesis. In addition to facilitating proper protein folding, preventing intermediates from aggregating, and targeting misfolded protein for proteasome degradation, GRP78 also binds Ca\textsuperscript{2+} and serves as an ER stress signaling regulator (1, 8). In nonstressed cells, GRP78 binds to ER transmembrane sensor proteins PERK, IRE1, and ATF6 and maintains them in an inactive form. When unfolded proteins pull GRP78 away from them, these pathways are activated, sending signals to the nucleus to trigger the UPR.

GRP78 is induced by physiologic stress that perturbs ER function and homeostasis, protecting against tissue or organ damage under pathologic conditions such as neurotoxic stress, myocardial infarction, and arteriosclerosis (1). During mouse development, homozgyous disruption of the Grp78 allele results in early embryonic lethality (9). Transcription of Grp78 is detectable as early as the two-cell stage and is required for both proliferation and survival for the embryonic inner cell mass, which are precursors of the pluripotent stem cells. Through synergistic interaction with cardiac specific transcription factor, Grp78 transcription is strongly induced in early embryonic heart, which uses glucose as the major energy source (10).

Although GRP78 expression is maintained at low basal level in major adult organs such as the brain, lung, and heart, it is strongly induced in tumors (3, 11). In support of the notion that GRP78 is more critically needed for the survival of stressed cells such as cancer, heterozygous GRP78 mice with half of wild-type (WT) GRP78 level are comparable to WT siblings in growth and development. However, tumor progression was significantly impeded in these mice as exemplified by a longer latency period, reduced tumor size, and increased tumor apoptosis.\(^1\) This is consistent with earlier studies that GRP78 conferred lysis resistance to cytotoxic T cells and tumor necrosis factor \(\alpha\), and that reduction of GRP78 in xenografts inhibited tumor formation and growth. GRP78 may also be (12) important for tumor metastasis because it is elevated in metastatic cancer cell lines, lymph node metastasis, and knockdown of GRP78 inhibits tumor cell invasion \textit{in vitro} and growth and metastasis in xenograft models (13, 14). The mechanism whereby GRP78 promotes growth and metastasis is just emerging. In addition to stress tolerance mediated by ER lumen GRP78, it has been reported that

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\(^1\) A. S. Lee, manuscript in preparation.
GRP78 is detected on the surface of highly metastatic prostate cancer cells and may mediate signal transduction pathways that induce proliferation and invasion (15).

Therapeutic Implications of GRP78 Induction in Cancer

Although therapeutic agents that target the tumor vasculature starve the tumors of vital nutrients, it has the unintended consequence of GRP78 induction. In xenograft models treated with antivascular and antiangiogenesis agents, GRP78 induction, which is hypoxia-inducible factor independent, is most intense in the viable tumor cells bordering necrotic regions created by the therapy (16). These cells are highly chemoresistant, correlating with GRP78 overexpression and inhibition of apoptosis. How might GRP78 suppress apoptosis? As a molecular chaperone, it prevents the formation of malfolded proteins. As a Ca$^{2+}$-binding protein, it preserves ER calcium homeostasis. Interestingly, whereas the majority of GRP78 resides in the ER lumen, a fraction of GRP78 exists as an ER transmembrane protein, with its N-amino portion in the cytosol (17). This could provide an explanation why GRP78 can be either directly inhibit the activity of proapoptotic effectors localized to the ER. For example, GRP78 binds and inhibits the activation of caspase-7, an executor caspase activated by both ER stress and genotoxic drugs (5, 17–19). GRP78 also binds and suppresses the activation of the BH-3 only proapoptotic protein BIK, its downstream target BAX, and prevents cytochrome c release from the mitochondria (20, 21). Furthermore, GRP78 in complex with other ER transmembrane proteins may also indirectly modulate the activity of these and other proapoptotic components. Thus, in multiple tumor types including lung, bladder, stomach, breast, gastric, and epidermoid carcinoma, GRP78 overexpression confers resistance to a wide variety of chemotherapeutic agents, and knockdown of GRP78 sensitizes the tumor cells to drug treatment (5). In malignant glioma, which represents a most malignant and resistant form of cancer, GRP78 is highly elevated, and knockdown of GRP78 enhances the efficacy of Temozolomide, the current standard of care for such cancer.2

GRP78-mediated drug resistance is not limited to proliferating tumor cells. Knockdown of GRP78 induces strong killing of growth-arrested, dormant cancer cells treated with Adriamycin/doxorubicin, a topoisomerase inhibitor, suggesting that dormant cells rely on GRP78, rather than cell cycle arrest, a commonly held concept, for drug resistance (20). Similarly, quiescent tumor-associated endothelial cells also depend on GRP78 for resistance because knockdown of GRP78 greatly enhances its drug sensitivity.3 These new findings imply that drugs against GRP78 will be particularly potent to eradicate residual tumor because they can overcome drug resistance not only in proliferating cancer cells, but also in dormant cancer cells, as well as in nontumor cells supporting tumor growth (Fig. 1).

GRP78 as a Therapeutic Target and Mediator of Cancer-Specific Therapy

Given the importance of GRP78 in cancer cell survival, it represents a prime target for anticancer agents. Interestingly, several naturally occurring compounds with putative anticancer activity inhibit either GRP78 expression or its activity at pharmacologic concentrations (Fig. 1). This includes genistein, an active ingredient of soy, (−)-epigallocatechin gallate (EGCG), a green tea component, and salicylic acid from plants (18, 22, 23). Nonetheless, these compounds act on many cellular targets and pathways in addition to GRP78. In contrast, the highly lethal bacterial ADP ribosylate cytoxin specifically cleaves GRP78 at a single amino acid, raising the interesting idea of exploiting this for anticancer therapy (24). Furthermore, GRP78 is an intracellular target for the melanoma differentiation–associated gene-7/interleukin 24 (MDA7/IL-24) produced by immune cells, which induces cancer-selective growth suppression and apoptosis in a wide range of human cancers (25).

Screening of compounds from microbes that specifically suppress the activation of the GRP78 promoter identified versipelostatin, which has no effect on basal GRP78 expression but inhibits transcriptional activation of the Grp78 gene by glucose starvation (26). This macrocyclic compound, which also disrupts some components of the UPR, selectively kills glucose-deprived cancer cells and acts synergistically with cisplatin in inhibiting tumor growth in xenografts. These observations, although preliminary and require more work on drug development, provide the proof of principle that inhibitors of GRP78 can be used in combination with standard therapeutic agents to enhance drug efficacy and possibly eliminate residual resistant tumor.

The recent discovery that GRP78 can be found on the cell surface of tumors but not in normal organs opens up an exciting opportunity of targeting cell surface GRP78 function as well as using it as a cancer-targeting marker (Fig. 1). How GRP78 escapes GRP78-mediated drug resistance is not limited to proliferating tumor cells. Knockdown of GRP78 induces strong killing of growth-arrested, dormant cancer cells treated with Adriamycin/doxorubicin, a topoisomerase inhibitor, suggesting that dormant cells rely on GRP78, rather than cell cycle arrest, a commonly held concept, for drug resistance (20). Similarly, quiescent tumor-associated endothelial cells also depend on GRP78 for resistance because knockdown of GRP78 greatly enhances its drug sensitivity.3 These new findings imply that drugs against GRP78 will be particularly potent to eradicate residual tumor because they can overcome drug resistance not only in proliferating cancer cells, but also in dormant cancer cells, as well as in nontumor cells supporting tumor growth (Fig. 1).

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**GRP78 as a Novel Biomarker for Tumor Behavior and Responsiveness to Therapy**

In human cancers, elevated GRP78 level generally correlates with higher pathologic grade, recurrence, and poor patient survival in breast, liver, prostate, colon, and gastric cancers; however, there are differing reports on lung cancer and an apparent exception for...
neuroblastoma that requires further investigation (13, 14). Auto-
antibodies against GRP78 at high levels in prostate cancer patients
have also been reported, associating with aggressive tumor
behavior (29, 30). Furthermore, expression of GRP78 was recently
examined retrospectively in prostate cancer patients during the
development of castration resistance (31). GRP78 is strongly up-
regulated during the transition from localized prostate cancer to
metastatic castration and may serve as a novel prognostic indicator
of recurrence in untreated patients with localized tumor.

In the management of breast cancer patients, there are at
present only two biomarkers that are used to predict potential
benefits of adjuvant therapy for the disease, hormone receptor
status, and Her2/neu status. The utility of these biomarkers to
eliminate ineffective treatment cannot be underestimated. For
women with hormone receptor-negative tumors, adjuvant hor-
monal therapy will not reduce the risk of recurrence. Therefore,
such women may be spared the toxicities of such agents. Similarly,
patients whose tumors do not overexpress Her2/neu may be
spared treatment with Herceptin. Unfortunately, similar tests that
would predict benefit from adjuvant systemic chemotherapy
agents do not exist. Such tests would be useful in avoiding the
toxicity associated with chemotherapy in patients who would not
benefit from treatment with these agents. Based on preclinical
studies strongly suggesting that GRP78-positive tumors may be
resistant to topoisomerase inhibitors (5, 16–20), a retrospective
study was conducted to evaluate the value of GRP78 as a
biomarker for treatment response. The study revealed that two-
thirds of breast cancer patients show moderate to high levels of
GRP78 in biopsies before treatment, and that in patients who
received adjuvant systemic chemotherapy with Adriamycin-based
regimens, GRP78 positivity indicated a higher risk of recurrence
(32). Thus, upon validation, GRP78 positivity might identify
patients who could be spared the toxicities of Adriamycin-based
adjuvant chemotherapy. Another observation that warrants addi-
tional investigation is whether GRP78 positivity might also identify
patients who are more likely to benefit from treatment with
Adriamycin followed by taxanes (32). In preclinical studies with
estrogen-positive human breast cancer cells, GRP78 confers
resistance to estrogen starvation–induced apoptosis through
suppressing the activity of BIK (21). Thus, GRP78 level may also

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**Figure 1.** ER stress induction of GRP78 in the tumor microenvironment. Both intrinsic and extrinsic factors lead to the up-regulation of GRP78 (also referred to as BiP) and its cell surface expression in tumor cells. Through inhibition of apoptosis, GRP78 facilitates tumor progression, immune resistance, metastasis, and drug resistance. Dormant tumor cells, as well as quiescent tumor endothelial cells, also rely on GRP78 to escape chemotherapy. Anticancer compounds that either inhibit the stress induction of GRP78 or suppress its catalytic function have been identified from multiple sources. When used in combination therapy, they should enhance drug efficacy, lower resistance and assist in eradicating residual tumor. GRP78 has also been identified as a cell surface receptor for Kringle 5 of human plasminogen and the activated form of the α2-macroglobulin. Because cell surface GRP78 is not detected in normal organs, it can serve as a conduit for cancer-specific delivery of cytotoxic agents via GRP78 binding peptides. Autoantibody levels against GRP78 in patient serum, as well as expression levels of GRP78 in biopsies, may represent novel biomarkers in stratifying patients for tumor behavior and responsiveness to treatment. Abbreviations: N, nucleus; ER, endoplasmic reticulum; C, cytoplasm.
provide guidance on selection of patients who will benefit from antiestrogen and aromatase inhibitor therapy. Although it should be emphasized that most human cancers are relatively heterogenous diseases and many factors need to be considered when building models to predict outcome, if our findings on GRP78 are confirmed, the integration of testing GRP78 expression level into the management strategies of breast cancer patients would substantially enhance treatment by reducing exposure to agents that are not likely to be beneficial to the patient (Fig. 1). This approach should also be applicable to other forms of cancer.

Concluding Thoughts and Future Directions

Elimination of residual tumor remains a major challenge for cancer therapy. The recent explosion in new knowledge on GRP78 suggests that it may represent a unifying mechanism for tumor resistance and a novel predictive biomarker to guide patient treatment, and this warrants vigorous testing in preclinical and clinical settings. The exciting recent discoveries also raise new important questions. How does GRP78 regulate tumor growth as well as invasion and metastasis? Does GRP78 also play important roles in the survival and function of nontumor cells supporting cancer growth and recurrence? Are there specific adaptive mechanisms for GRP78 cell surface expression in certain cancer cells? Finally, to test the therapeutic value of GRP78, it is critical to identify, through targeted screen or rational design, small-molecule inhibitors that are potent and specific modulators of GRP78 expression and/or activity. One approach is to target the catalytic activity of GRP78, which is required for its function. The recent establishment of conditional knock-out models of GRP78 will also provide further insight into the regulation and function of GRP78 in various types of cancers and other human diseases.

Acknowledgments

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19. Davidson DJ, Haskell C, Majest S, et al. Kringle 5 of human plasminogen induces apoptosis of endothelial cells? Finally, to test the therapeutic value of GRP78, it is critical to identify, through targeted screen or rational design, small-molecule inhibitors that are potent and specific modulators of GRP78 expression and/or activity. One approach is to target the catalytic activity of GRP78, which is required for its function. The recent establishment of conditional knock-out models of GRP78 will also provide further insight into the regulation and function of GRP78 in various types of cancers and other human diseases.

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