GRP78 Induction in Cancer: Therapeutic and Prognostic Implications

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Abstract

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.

[\textit{Cancer Res} 2007;67(8):3496–9]

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 malfolded 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 lead to the accumulation of underglycosylated and misfolded proteins in the ER, triggering the UPR (Fig. 1). In xenograft models, XBP-1 is 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 allele results in early embryonic lethality (9). Transcription of \textit{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, \textit{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. 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

\(1\) A. S. Lee, manuscript in preparation.

\textit{doi:10.1158/0008-5472.CAN-07-0325

Cancer Res 2007; 67: (8). April 15, 2007 3496 www.aacrjournals.org Downloaded from cancerres.aacrjournals.org on April 14, 2017. © 2007 American Association for Cancer Research.
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 malformed 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 may be able to 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 genisten, 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 Ab, subtilase 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 suppression and apoptosis in a widerange of human cancers (25). As proof of principle that cell surface GRP78 can serve as a conduit for cancer-specific delivery of cytotoxic agents, systemic administration of synthetic chimeric peptides with GRP78 binding motifs fused to proapoptotic sequence–suppressed tumor growth without affecting normal tissues (27). Similarly, other GRP78 targeting peptides, when linked to taxol, induced apoptosis in the targeted cancer cells (28). Another major advance is that cell surface GRP78 is the receptor for the angiogenesis inhibitor Kringle 5 (K5) of human plasminogen (19). Following internalization, K5 blocked the antiapoptotic activity of GRP78 and induced cell death. Although the primary target for K5 is likely to be growth-stimulated endothelial cells supporting tumor progression, recombinant K5 also induced apoptosis in stressed fibrosarcoma cells where surface GRP78 was also detected (19). Therefore, in principle, this class of drugs, which are currently tested in clinical trials, should have dual antiangiogenic and antitumor activities while sparing normal organs and tissues.

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


\(^{4}\) F.M. Hofman, J. Virrey, D. Dong, et al. GRP78 confers chemoresistance to glioma-associated brain endothelial cells, submitted for publication.

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neuroblastoma that requires further investigation (13, 14). Autoantibodies 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 upregulated 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 hormonal 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 additional 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

![Figure 1. ER stress induction of GRP78 in the tumor microenvironment.](https://cancerres.aacrjournals.org/content/cr/67/8/3498/F1.large.jpg)
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 heterogeneous 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

Received 1/24/2007; revised 2/28/2007; accepted 3/2/2007.

Grant support: National Cancer Institute grants CA027607 and CA111700, the Susan G. Komen Foundation, and the L.K. Whittier Foundation. 1 thank Darcy Spicer, Susan Groshen, Peter Baumeister, Miao Wang, and members of the Lee laboratory for helpful discussions and assistance. Due to space limitations, I apologize that many important primary articles cannot be cited.

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