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Roles of GRP78 in Physiology and Cancer

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ABSTRACT

As one member of 70 kDa heat shock proteins, glucose-regulated protein 78 (GRP78) participates in protein folding, transportation and degradation. This sort of capacity can be enhanced by stresses under which GRP78 is induced rapidly. Unlike its homologues, GRP78 presents multifaceted subcellular position: When ER retention, it serves as the switch of unfolded protein response; When mitochondrial binding, it directly interacts with apoptotic executors; When cell surface residing, it recognizes extracellular ligands, transducing proliferative signals, especially in certain tumors. The close correlation between GRP78 and neoplasm provides us further insight into the event of carcinogenesis and cancer cell chemoresistance, indicating its prognostic predicting significance and validating potential therapeutics for clinical usage, especially because its small molecular inhibitors are emerging quickly these years. What's more, GRP78-related signaling may be helpful for clearer understanding of its biological mechanisms. J. Cell. Biochem. 110: 1299–1305, 2010.

KEY WORDS: GRP78; CANCER; UNFOLDED PROTEIN RESPONSE; CELL SURFACE; SIGNAL TRANSDUCTION; HEAT SHOCK PROTEINS

Heat shock proteins (HSPs) are molecular chaperones that facilitate proper protein folding. The dramatic induction of HSPs is one critical unifying component of adaptive physiological responses, which can be provoked by environmental stresses. With similar structures, HSPs exist in virtually all living organisms, helping to survive unfavorable environments. However, in some moods, neoplasm shares this character [Jolly and Morimoto, 2000].

One particular member of HSPs that show close correlation with tumor is glucose-regulated protein 78 (GRP78), which belongs to the HSP70 family. It is also referred to as BiP (immunoglobulin heavy chain-binding protein). In the late 1970s, upon rapid depletion of glucose from culture medium of chick embryo fibroblasts, the amount of one protein with molecular weight 78 kDa was significantly elevated, hence the name came out.

The primary functions of GRP78 are related to binding to hydrophobic patches of nascent polypeptides in the endoplasmic reticulum (ER) and availing proteins disaggregation if their misfolding is irreversible. If polypeptide production exceeds a certain threshold, unfolded protein response (UPR) is initiated [Schroder and Kaufman, 2005]. The result is a decrease in biosynthetic burden of the ER, and an increase in the folding capacity, like, by means of GRP78 expression elevation [Lee, 2005].

Besides, GRP78 sustains cytosolic calcium homeostasis and forms complex with pro-apoptotic moleculars, such as caspase family members, thus curtailing programmed cell death. In certain kinds of tumor, GRP78 acts as a receptor on the plasma membrane, transducing signals associated with cancer cell survival, proliferation, metastasis, etc. Encouragingly, targeting cell surface GRP78 has already achieved success at experimental level.

UNFOLDED PROTEIN RESPONSE AND HSPs

In eukaryotic cells, protein synthesis and modification take place in the ER. When peptide production exceeds the folding capacity of the ER, the accumulated misfolded protein elicits UPR.

UPR is orchestrated through activation of protein kinase-like ER kinase (PERK), inositol requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6), all three of which are bound and sequestered by GRP78 in normal conditions. When unfolded protein load triggers the dissociation of GRP78 from these three sensors, UPR signaling is initiated. PERK phosphorylates eukaryotic initiation factor 2α (eIF2α), preventing the influx of additional nascent polypeptides into the ER; dimerization of IRE1 turns on its endoribonuclease activity and splices mRNA of XBP1, a basic

Abbreviations used: ER, endoplasmic reticulum; UPR, unfolded protein response; ERSE, ER stress response element; EGCG, (−)-epigallocatechin gallate.

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leucine zipper family transcription factor; released ATF6 is transported to the Golgi apparatus, where it is cleaved to a functional fragment [Schindler and Schekman, 2009]. These damage control pathways synergistically render eukaryotic cells resist to ER stresses by means of dampening global protein syntheses except essential ones [Dudek et al., 2009].

Since the induction of GRP78 is carried out through ER stress response element (ERSE) bound by several transcription factors [Gal-Yam et al., 2006], including nuclear form ATF6 and ATF4, undoubtedly, UPR can elevate the level of GRP78 expression. In turn, the overexpressed GRP78 copes with the unfolded protein load and relieves the biosynthetic burden in stressed cells, thus maintaining their integrity and survival.

Be that as it may, depending on prolonged and severe stress crack, UPR elicits pro-apoptotic pathways in the form of CHOP/GADD153 (CCAAT/enhancer-binding protein-homologous protein/growth arrest- and DNA damage-inducible gene) to forge a link with growth suppression and cell apoptosis [Sauane et al., 2008]. This opposite pathway makes the survival equilibrium list to the reverse side in order to protect the whole organism.

The expression of GRP78 can be enhanced by various drugs that are capable of generating unfolded protein load, by means of interruption of glucose metabolism, disruption of protein glycosylation and trafficking. For example, under glucose starvation with the treatment of 2-deoxyglucose or glucosamine, the expression of GRP78 increases in a dose-dependent manner. Besides, GRP78 is induced by protein glycosylation blocker tunicamycin, protein biosynthesis inhibitor cycloheximide and protein trafficking inhibitor brefeldin A, which specifically get in the way of vesicle transportation from ER to Golgi apparatus, and even render resorption of Golgi membrane to the ER. When GRP78 is overexpressed, it regulates the transportation of secreted and membrane proteins. Deletion of KAR2, a yeast karyogamy gene homologous to mammalian GRP78, presents a recessive lethal mutation due to a rapid block of protein secretion.

One word of caution is required here. However important GRP78 shows to be, it is not an exclusive player in the stage of UPR, if not for a group of companions. Comparison of the promoters of GRP78 and another ER protein GRP94 reveals the common regulatory domain which is recognized by the same trans-acting factors. Transfection with targeting GRP78 siRNA results in upregulation of GRP94 in HeLa cells, suggesting the compensational feature of GRP94 in the absence of GRP78. Through creation of targeting allele models, GRP78 is found to be obligatory for early embryonic development via controlling secretion of neuronal factors for neural maturation. The GRP78 heterozygous mice, which express half of wild-type level of GRP78 protein, have no effect on organ development but significantly impede tumor angiogenesis [Dong et al., 2008]. In this case, another glucose-regulated protein GRP94, is upregulated in the heterozygous GRP78 cells. Similarly, strong sequence homology exists among HSP70 family members, and down-regulation of HSP70 causes elevation of GRP78 in an interferential dose-dependent manner [Li et al., 2009]. The close relationship of HSPs allows us to observe GRP78-related biological phenomenon less biased and less obscure.

CYTOSOLIC INTERACTION PARTNERS

Although some functions of GRP78 can be compensated by its homologues, it cannot be replaced, thanks to its unique pattern of subcellular positioning. As a highly hydrophilic protein with several short hydrophobic domains, GRP78 not only resides in the ER lumen but also localizes in the extra-ER regions.

Under normal conditions, GRP78 is concentrated in the perinuclear ER with the help of its C-terminal retention signal. As a member of HSP70 family, it participates in proper polypeptide folding, facilitates degradation of misfolded products, and affects the transportation of membrane or secretary proteins. With the calcium (Ca^{2+})-binding activity, GRP78 preserves intracellular Ca^{2+} homeostasis by holding back the depletion of ER intracisternal Ca^{2+}, which can be released by calcium–ATPase pump inhibitor thapsigargin, calcium ionophore A23187, and ionomycin. Besides, GRP78 controls efflux of Ca^{2+} from the ER by regulating the IP_3R channel. So the Ca^{2+} flux from ER to mitochondria is under control, promoting bioenergetics and sustaining cellular survival. GRP78 can also be redistributed to mitochondria to be a potent anti-apoptosis-binding element [Sun et al., 2006], and forms complex with BIK, a pro-apoptotic BH3-only protein, decreasing apoptosis of human breast cancer cells induced by such ER stress as estrogen deprivation. Besides, it binds to caspase-7 and caspase-12, thus blocking the main cytosolic apoptotic executors, in spite of no direct combination with caspase-3.

The substrate-binding affinity of HSP70 proteins is modulated by their nucleotide bound state. The ATP-bound state of GRP78 has a low affinity while ADP-bound state has a high one. HSP40s, its co-chaperones, stimulate the ATPase activity of GRP78, that is, they favor its substrate binding. Additionally, some HSP40s have the ability to bind substrate polypeptides and deliver them to GRP78 [Ushioda et al., 2008]. On the other hand, nucleotide exchange factors mediate the exchange of ADP for ATP, thus inducing substrate release. This principle applies in the case that GRP78 binds newly synthesized polypeptides for correct protein folding. Whether it still works when GRP78 binds the pro-apoptotic molecules mentioned above needs to be confirmed. If it's so, and low ATP is characteristic of glucose deprivation, sustained binding to these molecules is seen as apoptosis suppression.

How can GRP78 detect when it is necessary to act the anti-apoptosis binding, especially in normal cell survival adaptation? Reactive oxygen species (ROS) is generally regarded to be one of its cytoplasmic induction provokers. The proof of principle is that the rapid induction of GRP78 can be caused by redox potential changer homocysteine [Zulli and Hare, 2009], and attenuated by antioxidant addition, and ROS-dependent factors are found to be confined with the ERSE region of GRP78 promoter. Once GRP78 expression is elicited, cells are protected against apoptosis via suppression of oxidative stress. Because of this, hypoxic or ischemic preconditioning, which brings about ROS in the process of reperfusion, confers to upregulation of GRP78 expression so that cardiomyocyte injury due to subsequent lethal ischemia is relieved. Preconditioning-induced lethal resistance reduces in non-GRP78 inducing mutant cells or in GRP78 knockdown cells. However, the level of GRP78 remains unchanged in rat brains with and without ischemic tolerance [Garcia

et al., 2004], suggesting complicated influencing factors in vivo experiments and warranting additional investigation.

CELL SURFACE GRP78

What makes GRP78 more special is that it can position on plasma membrane. Under certain circumstances, the overall quantity of GRP78 can be induced by ER stress to such extent that a fraction shows on tumor cell surface and even releases into the culture medium, whereby autoantibodies against GRP78 are produced and can be isolated from cancer patient sera. The antibodies with binding site located in the NH_2-terminal domain of GRP78 show agonist properties, and are pro-proliferative and anti-apoptotic. In contrast, the ones binding the COOH-terminal domain inhibit cell proliferation and trigger apoptosis [Misra and Pizzo, 2010]. Apart form autoantibodies, several extracellular molecules with the property of GRP78 binding regulate cellular biology too.

The plasma proteinase inhibitor α2-macroglobulin binds to its NH_2-terminal domain with high affinity and activates PI3K/Akt and MAPKs pathways [Shu et al., 2008; Kern et al., 2009], which promote proliferation and survival in a variety of tumors [Misra et al., 2009b]. In addition to α2-macroglobulin, teratocarcinoma-derived growth factor I (Cripto) [Shani et al., 2008] and truncated cadherin (T-cadherin) [Philippova et al., 2008], are able to bind plasmic membrane GRP78 and elicit similar signaling events [Kelber et al., 2009]. As we know, signal pathways have cross-talks, which can help us to deduce possible findings. For instance, Akt negatively regulates the function or expression of several BH3-only proteins, including BAD and mdm2, which down-regulate tumor suppressor p53 [Kern et al., 2009]. Akt also phosphorylates human procaspase-9 and this phosphorylation correlates with a decrease in the protease activity of caspase-9. Under certain conditions, PI3K/Akt pathway activates NFκB survival signaling or inhibit JNK/p38 apoptotic signaling. However, disruption of GRP78 binding to these ligands using RNAi knockdown or antibody immunoneutralization precluded activation of Akt and MAPKs pathways in different types of cells [McFarland et al., 2009], including normal cells (such as renal epithelia and vascular endothelia) [Philippova et al., 2008] and cancer cells (such as melanoma and prostate cancer cells) [Misra et al., 2009a].

On the other hand, angiogenesis inhibitor plasminogen kringle 5 forms complex with cell surface GRP78 by binding a segment in the COOH-terminal domain, and induces apoptotic effect [McFarland et al., 2009]. Kringle 5 can also inhibit cancer cell proliferation and promotes procaspase-7 cleavage by binding to cell surface voltage-dependent anion channel. However, kringle 5 is not the only target of GRP78 for regulating coagulation. By physical interaction with the extracellular domain of tissue factor on endothelium cell surface, GRP78 attenuates procoagulant activity. Again, a word of caution is required here. Curiously, interaction with NH_2-terminal domain may cause apoptosis of cells, like prostate apoptosis response-4 (Par-4) binding to cell surface GRP78 [Burikhanov et al., 2009]. The exact mechanism still needs to be uncovered. So far, cell surface GRP78 has been identified in human prostate cancer [Gonzalez-Gronow et al., 2006], ovarian cancer [Chinni et al., 1997], gastric cancer [Rauschert et al., 2008], and melanoma [Papalas et al., 2010]. On infection diseases, GRP78 is identified as a coreceptor of MHC-I molecule for coxsackievirus internalization [Triantafilou et al., 2002] and thus enhances the magnitude of virus infection.

How is GRP78 redistributed from the ER to the cell surface? A plausible scenario is that, upon overproduction, excess GRP78 may leave the ER by vesicular transport and reside on the plasma membrane. This process requires several elements, among which MTJ-1, a DnaJ-like transmembrane protein, receives most attention. Its essential role was confirmed by MTJ-1 silencing, because of which the cell surface localization of GRP78 is abolished [Misra et al., 2005].

Not only does GRP78 regulate downstream signaling as cell surface receptor, but also can it be induced by activation of signal transduction, like MAPKs pathways. For instance, inhibition of MEK/ERK cascade using specific inhibitor U0126 or RNAi suppresses the expression of GRP78 in some kinds of cancer cell lines [Jiang et al., 2007]. And the p38-specific inhibitor, SB203580, abrogates the upregulation of GRP78 induced by sustained hemodynamic shear stress in human endothelial cells. Okadaic acid, a specific inhibitor of endogenous serine/threonine protein phosphatase activity, stimulates GRP78 transcription, whereas genistein, a general tyrosine kinase inhibitor derived from soy, eliminates its transcriptional activation. Whether okadaic acid and genistein influence the induction of GRP78 only through MAPKs pathways, or have something to do with other signal pathways, is still uncovered. Anyway, the induction of GRP78 appears to be a downstream event herein. We would like to summarize GRP78-related signaling as a loop feedback (Fig. 1).

CANCER CORRELATION WITH GRP78

Recently, a great part of findings about GRP78 have been focused on carcinogenesis and tumor progression. Taken full-scale analyze on the discoveries, the correlation between GRP78 and tumor malignancy appears a positive correlation as well as a negative correlation, whereas the predominance lies at the former.

Overexpression of GRP78 presents in a variety of tumors, including urinary, digestive, mammary, cerebral, and respiratory system tumors. In urinary system tumors, although GRP78 exists in benign prostatic tissue, prostate cancer, and lymphonode metastasis specimens, the immunoreactivity intensity presents significantly higher in primary tumor compared to benign epithelia [Daneshmand et al., 2007]. Likewise, the level of GRP78 in human renal carcinoma is markedly higher than adjacent non-tumor tissue [Fu et al., 2010]. For prostate cancer patients with old age, castration is often employed to control cancer progression. But overexpressed GRP78 are correlated with castration-resistance, leading to higher risk for clinical recurrence and worse overall survival [Daneshmand et al., 2007].

Induction of GRP78 also shows in gastric cancer specimens compared with adjacent tumor-free mucosa and is directly correlated with increased lymphonode metastasis. The increased metastasis may be mediated by the activation of cell motility mechanism p21-activated kinases (PAKs), which can be elevated

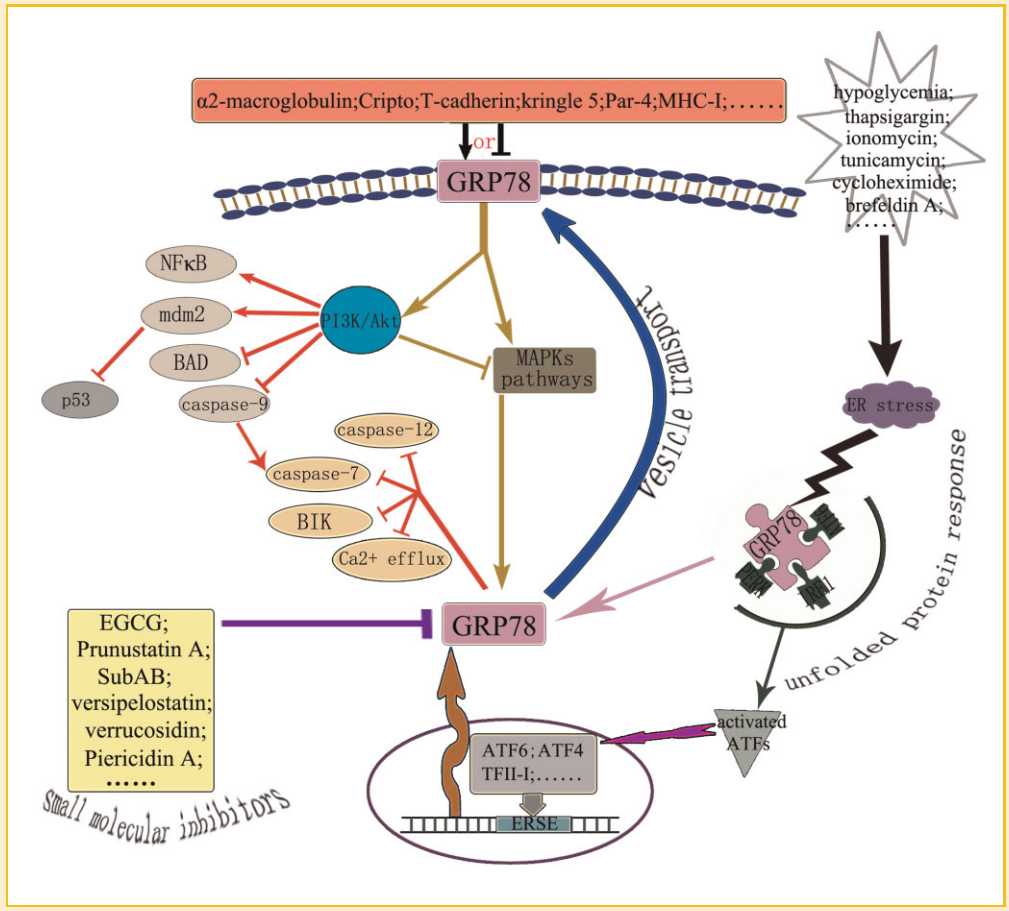

Fig. 1. GRP78-related signal transduction presents in the form of a loop feedback. A variety of insults can cause ER stress, resulting in unfolded protein load. The unfolded protein triggers dissociation of GRP78 from UPR sensors, including PERK, IRE1, and ATF6. These sensors elicit damage control pathways synergistically, part of which are activated ATFs. Nuclear form ATFs act on ERSE, elevating the expression of GRP78. Overexpressed GRP78 may be redistributed to cell surface by means of vesicle transport. Binding to the NH_2-terminal domain of GRP78 is pro-proferative and anti-apoptotic, while binding to the COOH-terminal domain inhibits cell proliferation and triggers apoptosis. The biological effect is found to be associated with the activation of PI3K/Akt and MAPKs pathways. In turn, interestingly, the activation of MAPKs pathways favors the rapid induction of GRP78. Several small molecular inhibitors of GRP78, such as EGCG, may be utilized for clinical usage of cancer treatment, since close correlation exists between GRP78 and various tumors.

several fold upon binding of GRP78 with its ligand α2-macroglobulin. In hepatocellular carcinoma, upregulation of GRP78 is linked with the capability of tumor venous infiltration, while HSP70 shows no significant association with any pathologic feature. And the activation of focal adhesion kinase (FAK) was found to play a critical role in the invasion of hepatocellular carcinoma [Su et al., 2010]. Importantly, the postoperative overall survival rate of patients with positive GRP78 expression is lower than that of those spared [Zheng et al., 2008]. For mammary tumor, the overexpression occurs in most of the more aggressive human breast cancer cells, which present negative estrogen receptor and resistance to regular cytotoxic drug regimens, such as adriamycin, etoposide, taxol, and vinblastine. One retrospective cohort study of 127 breast cancer patients with the treatment of adriamycin-based chemotherapy revealed an association between GRP78 positivity and shorter time to recurrence [Lee et al., 2006].

As regard to cerebral tumors, the level of GRP78 expression is low in normal adult brain tissues, but significantly higher in malignant glioma specimens and cell lines. It is positively correlated with proliferation rate and inversely correlated with median survival period of patients. For the non-surgical management of glioma patients, regular therapeutics consists of camptothecine, etoposide, temozolomide, and γ-radiation. But GRP78 curtails their effectiveness [Pyrko et al., 2007]. Moreover, endothelial cells derived from blood vessels of malignant glioma tissues constitutively overexpress GRP78, indicating that vasculature may concomitantly participate in chemotherapeutic agent resistance [Virrey et al., 2008]. In addition, the expression of GRP78 is stronger in poorly differentiated human lung cancer than in well or moderately differentiated one. Its expression corresponds well with resistance to etoposide-induced apoptosis. By employing immunohistochemistry, GRP78 level shows positively correlated with increased melanoma thickness and mitotic index, further making GRP78 eligible as a tumor biomarker [Zhuang et al., 2009].

Importantly, treatment with GRP78 inhibitor or knockdown of GRP78 potentiates chemotherapy-induced apoptosis in most of tumors mentioned above [Pyrko et al., 2007; Virrey et al., 2008].

Why is GRP78 overexpressed in tumors compared with normal tissues? Possibly due to the fact that tumor cells produce much more mutant proteins which require GRP78 to form complex with [Sorgjerd et al., 2006], while GRP78 associates to a far lesser degree with wild-type products. Since tumor biosynthesis runs at a high speed, even the pool of wild-type protein exceeds the threshold that normal ER capacity can endure, like progressive virus infection [Joyce et al., 2009]. Tumor therapy prior to biopsy, in the form of irradiation or heavy-metal compounds, also results in the induction of GRP78, which is then detected at labs. More likely, GRP78 induction is caused by relative shortage of nutrient and oxygen [Hardy et al., 2008], for outpaced blood supply of solid tumor tissues usually cannot meet their greedy requirement.

Nevertheless, opposite sound comes along. Several studies point out that GRP78 is inversely correlated with neoplastic microvessel density in human lung tumors and patients with positive GRP78 expression tend to have better prognosis than those with negative expression. These researchers regard positive GRP78 expression as a significant factor for predicting favorable outcome. The implication is testified by multivariate analysis of clinicopathologic characteristics. Upon validation that highly differentiated human esophagus adenocarcinoma show higher GRP78 level compared to low differentiated tumors, patients with strong expression of GRP78 get a survival benefit [Langer et al., 2008]. Similarly, overexpression of GRP78 was also found to be correlated with early clinical stages and better survival in neuroblastoma patients [Weinreb et al., 2009].

These sorts of prognostic predicting paradox may be influenced by individual variation or by different regimens, because GRP78 mediates resistance to some drugs while hypersensitivity to some others. Cells that overexpress GRP78 are resistant to topoisomerase II inhibitors, including etoposide [Virrey et al., 2008], amsacrine, and doxorubicin, but hypersensitive to DNA cross-linking agents, including melphalan, cisplatin, and carmustinum, owing to the correlation between upregulation of GRP78 and impairment of DNA cross-link repair.

TARGETING GRP78 THERAPEUTICS

Now that the majority of tumors exhibit positive correlation with GRP78, the targeting regimens are worthy of consideration. Several drugs seem to be qualified for clinical utilization. For instance, (−)-epigallocatechin gallate (EGCG), one of the main green tea components, can block tumor promoting function of GRP78 by targeting its ATP-binding domain [Wang et al., 2009]. Nevertheless, it acts on other cellular targets besides GRP78. So is salicyclic acid from plants [Grivennikov et al., 2010]. The bacterial AB_5 subtilase cytotoxin specially cleaves GRP78 [Byres et al., 2008], making it plausible of exploiting this for anti-cancer therapy. Prunustatin A, which is isolated from *Streptomyces violaceoniger*, inhibits GRP78 expression induced by 2-deoxyglucose. Additionally, membrane-permeant calcium chelator suppresses thapsigargin-induced GRP78 expression in a concentration-dependent manner. There are other small molecular inhibitors too, such as versipelostatin, verrucosidin, and Piericidin A, which might be eligible for the potential usage of tumor treatment.

Pep42, a cyclic oligopeptide that specifically binds to cell surface GRP78, can be internalized into cancer cells [Liu et al., 2007]. Therefore, it presents an effective prodrug-conjugated vehicle for a large number of cytotoxic drugs in order to accurately target neoplasm [Yoneda et al., 2008]. For instance, by means of cellular uptake and intracellular trafficking, its conjugate with taxol promotes apoptosis of tumor cells, especially the highly metastatic ones [Arap et al., 2004]. As proof of principle that the promoter of GRP78 contains a strong enhancer and can be further induced by stress, which characterizes microenvironment of solid tumors, the promoter can be utilized as a potent approach for gene delivery system for targeting oncogenesis transcription. Actually, GRP78 promoter-driven expression of suicide gene is strong to eradicate refractory human tumors [Dong et al., 2004].

PROSPECT THOUGHTS

The unsettled conclusion is whether GRP78 serves just as a biomarker in cancer, or otherwise, much more likely, an important aggravating prone factor. If some sorts of tumors could be reversed or at least controlled by targeting GRP78, how can we make it applicable for effective clinical utilization with unbearable side-effects spared, in the face of a great many drugs that show significant inhibiting potency? Since GRP78 is extremely important in physiological conditions and shows negative correlation with several kinds of neoplasm, prescreening is necessary before carrying out clinical cancer interference. Meaningfully, the signal transduction pathways facilitate us to elucidate many findings more clearly and fundamentally, but some potential mediators may take important action during the process as well as already uncovered ones, provoking further researches to dig them out.

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