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Mini Review Article

Therapeutic Potential of Glucose Regulated Protein 78 in Cancer

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Abstract

Endoplasmic reticulum has a critical role in the synthesis and folding of secretory and membrane proteins. High accumulation of proteins in ER activates the unfolded protein response and glucose regulated protein 78 or GPR78 plays an essential role in this pathway. Unfolded protein response is activated in cancerous cells due to their adverse condition to survive and it has been shown that GRP78 can be expressed in tumor cell membrane. Overexpresion and localization of GRP78 makes it a suitable target for the treatment of cancer. This review describes cellular localization, biological function, and role of GRP78 in cancer induction. Methods for tumor inhibition via GRP78 are also discussed.

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Introduction

The endoplasmic reticulum (ER) has an essential role in the synthesis and folding of secretory and membrane proteins. Due to accumulation of unfold proteins over the capacity of the ER, the unfolded protein response (UPR), as a protective process is activated leading to translational attenuation, up-regulation of chaperones and folding enzymes and enhanced ER-associated degradation of misfolded proteins. Several mediators such as PKR-like ER-associated kinase (PERK), IRE1/X-box binding protein-1 (XBP-1), and activating transcription factor-6 (ATF6) are inVolved in this process [1-4]. Severity of ER stresss pecifies the UPR which can cause cell death by activating the ER mediated apoptotic pathways, as well as coupling to the mitochondrial pathways [4, 5]. ER stress can also induce autophagy, a cellular degradation process implicated in both cell death and survival [6].

GRP78 is a 78 kDa molecular chaperone with the binding ability to hydrophobic patches on nascent polypeptides in the ER to prevent their aggregation ,thereby promoting folding of entire polypeptides in to proper native con formations [7]. Due to this function , GRP78 is expressed in all mammalian cells as one of the initial components in the signaling cascade of the UPR to inhibit aggregation of unfolded proteins [7].

The available pool of GRP78 in the ER induces secondary signaling mediators that directly act to prevent the accumulation of unfolded proteins in the cell or across the nuclear membrane [8]. GRP78 shows response to Ca^{2+}, glucose and energy depletion, accumulation of unfolded proteins in the ER. These events are regulated by GRP78 interactions with some of the secondary signaling proteins such as inositol requiring kinase 1 (IRE1), PKR-like ER-associated kinase (PERK), and activating transcription factor 6 (ATF6) [2, 9].

Localization of GRP78 in the cell

The presence of the KDEL retention motif on C-terminus of GRP78 makes this protein as an ER lumen-localized chaperone [10]. However, that the presence of GRP78 in nucleus and mitochondria has also been reported [11, 12]. Recently, an isoform of GRP78 in the cytosol generated by alternative splicing of the same gene is also reported [13].

GRP78 is contributing in many functions in different parts of the cell including the plasma membrane. [5, 14-23]. Berger et al reported the cell surface localization of GRP78 for the first time in 1997 [24].

Cancer and ER stress

The metabolic environment of tumors is often acidic, hypoxia, with lack of nutrient and low amounts of both amino acids and glucose [5]. These phenomena occur due to both poor vascularization and rapid growth of tumor cells, and the intrinsic property of cancer cells with high glucose metabolism and higher glycosylation rates. The microenvironment of tumor cells resembles physiologically to ER under stress condition where it causes the UPR is to be activated to help the cells survival [25, 26]. In the case of cancer, the proapoptotic pathways inactivated due to mutations resulting from tumorigenesis, thereby suppressing will be eliminated in the cancerous cells. This, coupled with the activation of the prosurvival UPR pathways, offers an advantage for cancer progression. [27, 28]

Mechanism for GRP78 in promoting cancer progression GRP78 binds and inhibits the activation of caspase-7, an executor caspase activated by both ER stress and genotoxic drugs. GRP78 also binds and suppresses the activation

of the proapoptotic protein BIK, its downstream target BAX, and preventing release of cytochrome C from the mitochondria. The results obtained from the mouse model showed that Grp78 heterozygosity extremely prolongs the latency and delays the progression of the oncogene-induced mammary tumors in the well-established model without affecting mouse growth, organ development, and antibody production [29].

Based on results reported by Zhou et al. in the MCF-7/BUS-10, a human breast cancer cell line, overexpression of GRP78 suppresses apoptosis induced by BIK and NOXA, alone or in combination. They farther reported that BCL-2 and GRP78 form independent complex with BIK where the increased expression of GRP78 results in decrease BIK binding to BCL-2 [30]. Overexpression of GRP78 also inhibits estrogen starvation-induced BAX activation, mitochondrial permeability transition, and consequent apoptosis in this cell line [31].

Developmental oncoprotein Cripto binds to cell surface GRP78 and promotes prosurvival signaling via MAPK/PI3K and Smad2/3 pathways. Cripto-dependent increase in cellular proliferation is prevented by immuno-neutralization of the Cripto-GRP78 complex at the cell surface [32]. Down regulation of MHC-I expression on the cell surface due to overexpression of GRP78 is another mechanism for GRP78 function. As a result of this regulation the capacity of the immune system to control of tumor progression is limited [33]. Interaction of activated α2M (α2M*) with GRP78 induces activation of PAK2, ERK1/2, p38 MAPK and P13K thereby promoting cell proliferation [34, 35]. Activation of Akt and NF-ƙB lead to cell survival in prostat cancer. α2M* leads to positive regulation, synthesis and secretion of PSA and a complex between α2M* and PSA serves as a ligand for GRP78. The interaction between GRP78 and this complex further increases the above signaling cascade. Three major mechanisms mediated by GRP78 for cancer progression were suggested by researchers studying on genetic model of breast cancer in the GRP78 heterozygous mice: protection against apoptosis, enhancement of tumor cell proliferation, and promotion of tumor angiogenesis [36-38]. Induction of tumor angiogenesis was approved by another research group. The GRP78 (+/-) mice exhibited a high reduction in the microvessel density (MVD) of the endogenous mammary tumors, where as no effect was obsereved on the MVD of normal organs. These observations suggest that the host vasculature is regulated by GRP78 function within the tumor microenvironment [36]. Knockdown of GRP78 significantly suppresses the VEGF-induced activation of ERK1/2, PLC-γ and VEGF receptor-2 (VEGFR-2).

Consequently the regulatory role of GRP78 in VEGF-induced endothelial cell proliferation through VEGFR-2-mediated signalingis also reported [39, 40]. GRP78 has been shown directly to interact with apoptotic pathway intermediates, to block caspase activation, eventually leading to apoptosis inhibition and increased cell survival [31, 41].

Overexpression in cancer

Due to its antiapoptotic properties, GRP78 induction has been reported to be as a prosurvival factor in cells undergoing ER stress. It has been illustrated that GRP78 level is highly elevated in various cancer cell lines, solid associated human cancer biopsies, and has association with malignancy and metastasis [31, 42, 43].

The GRP78 transcription is elevated under various stress conditions suggesting the inVolvement of GRP78 in cell survival enhancement. GRP78 seems to be directly connected to apoptotic pathway intermediates, to block caspase activation which eventually leads to increase cell survival due to apoptosis inhibition [35, 44]. Since tumor progression requires cell proliferation as well as inhibition of tumor cell death, therefore the inherent antiapoptotic properties of GRP78 could play a potential role in cancer progression. This is further supported with the expression level of GRP78, which is markedly higher in primary tumors compared with that of benign tissues. This has been documented in various cancers, including breast, hepatocellular carcinoma [45], lung cancer [46], and prostate cancer [47, 48]. In two different studies Xing and colleagues showed that GRP78 was up- regulated in colon cancer tissue but not in normal tissue. Their results reviled significant increase in GRP78 expression of colorectal carcinoma at the protein level, but there was no difference in the relative mRNA expression levels [49, 50].

Expression of GRP78 in mRNA and protein level was compared between normal primary and esophageal adenocarcinoma tissues. Increased expression of GRP78 in cancer tissue at two levels was related to tumor stage progress [51]. Over expression of GRP78 in glioma was shown by Lee and colleagues. They showed that decreasing of caspase 7 activation and resistance to etoposide and cisplatin-induced apoptosis, is related to the enhancement of GRP78 expression. Using western blot , approximatly 3-fold increase in GRP78 protein level in lung cancer tissue was observed, compared to normal tissue [52].

Induction of chemoresistance

In most of the cases, chemotherapy is the initial strategy for cancer treatment. However, after sometimes cancer grows back without showing response to the initial therapy.GRP78/BiP has been found to be overexpressed, both at the gene and protein levelat this stage. Increased expression of GRP78/BiP in the surviving cells has been found during the treatment of human breast cancer cells MDA-MB-435 with anti-angiogenic factor Combretastatin A4P supporting the correlation of higher GRP78 levels with higher resistance [53]. In addition GRP78 increased expression has been observed in a panel of MCF-7 human breast cancer cell line refractory to several treatments compared to the parental line [54]. Intriguingly, it has been shown that high level of GRP78 expression induced resistance to doxorubicin in breast cancer patients [55, 56]. These observations indicate that GRP78 could be a specific marker to predict doxorubicin resistance in breast cancer.

In addition, Virrey *et al*., showed that GRP78 induces chemoresistance development in brain endothelial cells and it is believed that the incidence of tumor vascularization and metastatic spread is attributed to this phenomenon [57]. GRP78 inhibition resensitize acute lymphoblastic leukemia cells refractory to vincristine [58]. GRP78 also plays an important role to contribute to castration resistant

prostate cancer (CRPC) development [59]. Immunohistochemical analysis revealed GRP78 overexpression in CRPC and its positive correlation with poor survival recurrence [47].

GRP78 expression has straight relation with the resistance of glioma cells to temozolomide. It has been shown that GRP78 knockdown lowers resistance of cells to temozolomide, where as cells with overexpression of GRP78, confers the higher resistance. Knockdown of GRP78 also sensitizes glioma cells to 5-fluorouracil and CPT-11[60].

Yidan Lin et al. had proposed a role of GRP78 in lung cancer. GRP78 is one of the factors which can regulate phosphorylation of Akt and it is essential to the ER stress-tolerant ERST-associated Cisplatin resistance in lung cancer cells [61].

Tumor treatment with GRP78

GRP78 is highly expressed in various situations such as glucose deprivation, chronic anoxia, and acidic pH, all persisting in poorly vascularized solid tumors. There are reports indicating the importance of GRP78 at different stages of tumorigensis, and variety of reports implicate the potential of GRP78 as a cancer therapeutic target or prognostic factor. In several studies it showed that GRP78 is the target of anticancer agents [62-64].

Either GRP78 expression or its activity is inhibited by pharmacological concentrations of several naturally occurring compounds with putative anticancer activity such as genistein, an active ingredient of soy, (-)–epigallocatechin gallate (EGCG), a green tea component, and salicyclic acid from plants [65-67]. To describe one of these components, EGCG directly interact with GRP78 at its ATP-binding site and regulates its function by competing with ATP binding [65, 68]. On the other hand, some synthetic drugs such as Pyrviniumpamoate, an old anthelmintic medicine, can suppress glucose deprivation-induced GRP78 transcription, and its combination with doxorubicin enhances its antitumor activity [69].

It has been shown that the HKH40A, the 8-methoxy analog of WMC79, promotes it's antitumor activity by reduction of GRP78 protein in cancer cell line[70]. Using GRP78 targeting peptides, when linked to Taxol, induced apoptosis in the targeted cancer cells [71].

Recentluy GRP78 is reported to be present on the surface of tumor cells but not in normal organs which opens up an exciting opportunity of targeting cell surface GRP78 as well as using it as a cancer-targeting marker [72]. Cellular growth suppression and apoptosis induction is occurred by ligation of surface GRP78 with antibody against the C-terminal domain of GRP78 which is exposed exteracellulary. Antibody binding led to reduction of GRP78 expression,up regulation of P53 activity, apoptosis promotion and inhibition of cellular proliferation in melanoma and prostate cell lines [73].

Misra *et al*., demonstrated that incubation of 1-LN prostate cancer cell with antibody directed towards C-terminal domain of GRP78 led to molecular changes in Ras/MAPK and PI 3-kinase/AKT signaling pathways, reduction of anti appototic Bcl-2 and up regulation of pro-apoptotic BaD, BaX and BaK [74]. Cell death by SAM-6, an anti GRP78 monoclonal antibody, was reported by Brandlein *et al*., This IgM antibody binds to glycosylated form of GRP78 leading to apoptosis viainduction of an intrinsic-like form of apoptosis and overaccumulation of large non-physiologic intracellular lipid [75]. Since the targeting of C-terminal domain of GRP78 (CGRP) with antibody is reported as a viable approach for tumor suppression, we evaluated the structure and antigenicity of this domain in previous study [76]. Our results showed that CGRP can play an important role as a target in cancer studies.

The GRP78 promoter-driven transgene is largely quiescent in major adult organs, but highly active in cancer cells and cancer-associated macrophages, which can diffuse to tumor necrotic sites devoid of vascular supply and facilitate cell-based therapy. Thus, controlling the transcription process by using the GRP78 promoter suggests multiple novel approaches for human cancer gene therapy, such as suicide genes, immunosuppressors and tumor suppressors. For example when the GRP78 promoter was used to drive the expression of Herpes simplex virus-thymidine kinase (HSV-tk) suicide gene in a retroviral system, complete eradication of sizable tumor mass, with no recurrence of tumor growth was occurred [77-79].

Conclusion

Together, studies suggest that over expression of GRP78 in cancer cell induces cancer progression and resistance to chemotherapeutic agents. Cell surface expression of GRP78 exclusively on cancer cells is an opportunity for tumor targeting via this protein. For more reliable application of GRP78, some key issues such as the basic mechanism for localization of GRP78 on the cell surface, downstream signaling after ligand binding and how to use this protein as a biological marker in cancer detection must be farther investigated.

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