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REVIEW

The role of heat shock proteins and glucose regulated proteins in cancer

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Abstract

Heat shock proteins (HSPs) are a family of evolutionary conserved proteins that work as molecular chaperones for cellular proteins essential for cell viability and growth as well as having numerous cyto-protective roles. They are sub-categorised based on their molecular weights; amongst which some of the most extensively studied are the HSP90 and HSP70 families. Important members of these two families; Heat shock proteins 70 and heat shock proteins 90 (Hsp70/90), are the glucose regulated proteins (GRP). These stress-inducible chaperones possess distinct roles from that of the other HSPs, residing mostly in the endoplasmic reticulum and mitochondria, but they can also be translocated to other cellular locations. Their ability in adapting to stress conditions in the tumour microenvironment suggests novel functions in cancer. GRPs have been implicated in many crucial steps of carcinogenesis to include stabilization of oncogenic proteins, induction of tumour angiogenesis, inhibition of apoptosis and replicative senescence, and promotion of invasion and metastasis.

Key words: Glucose regulated protein, cancer

INTRODUCTION

Heat shock proteins (HSPs) are highly conserved proteins, which are expressed ubiquitously in all organisms studied to date. They were first identified as a set of proteins whose expression was increased immediately after a sudden rise in temperature.[1] Further investigations have revealed that the expression of these proteins are also elevated following a variety of metabolic and environmental insults such as hypoxia, ischaemia, anoxia, exposure to UV light and chemicals (including anti-cancer chemotherapy), nicotine, surgical stress, nutritional deficiencies (e.g. glucose deprivation), emotional and mechanical stress, mechanical injuries and viral agents or other stresses such as exposure to toxic radicals and carcinogens.[2-4] They are further subcategorised into 6 different groups based on their molecular weights, namely the small HSPs (15-30 kDa), HSP40, HSP60, HSP70, HSP90 and the large HSP100. HSPs of high molecular weight function in an ATP dependent manner whereas the smaller ones are ATP-independent.[5]

The role of HSP as molecular chaperones make them an essential part of cell survival. Protein synthesis *in vivo* is largely aided by molecular chaperones in facilitating proper folding of nascent polypeptides, avoiding aggregation of proteins as a result of non-specific interaction and directing the translocation of proteins to their correct intracellular localization. During conditions of stress, when a protein is damaged, molecular chaperones restore the balance by facilitating their re-folding or, in the case of irreparably damaged proteins, their removal by protein degradation.[6]

Heat shock proteins are either expressed continuously or increased under conditions of stress and present at different subcellular compartments. This is exemplified by HSP90 which is expressed abundantly at all times in the cells whereas HSP70 expression is induced by stresses such as anticancer drugs and oxidative stress and is at a very low level under normal conditions.[1] Exposure of cells to various stress conditions result in an imbalance of protein metabolism. This phenomenon imposes a threat on the cells' ability to respond rapidly and precisely to the detrimental effect of stress on homeostasis. To counteract this problem, the heat shock proteins are produced. This coordinated activation of HSP expression is known as the heat

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shock response.[7] Inducible expression of heat shock proteins in response to stress conditions is pivotal in protecting cells from severe stress that would cause lethal molecular damage.[8] The HSPs are of considerable interest due to their involvement in many diseases. A large body of evidence supports the role of molecular chaperones in maintaining cancer phenotypes. Constitutive overexpression of HSPs is a fundamental requirement for the survival of cancer cells, with these observed at high levels in various tumours and are closely associated with poor prognosis as well as resistance to treatment.[9,10]

Important members of the HSP protein family are the glucose regulated proteins (GRPs), initially discovered in 1977 when chicken embryo fibroblasts were cultured in glucose-starved conditions. These GRP genes are induced following disturbance of endoplasmic reticulum (ER) functions, especially agents that disrupt calcium stores and inhibit glycosylation.[11] Unlike other HSPs that reside mostly in the cytoplasm of the cell, GRPs reside in the endoplasmic reticulum. GRPs are also different from other HSPs as they are not readily responsive to hyperthermia and oxidative stress, but to reducing conditions (e.g. anoxia) and conditions that interrupt normal functioning of the endoplasmic reticulum.[12] As cell stress generally results in accumulation of misfolded proteins, GRP expression has been used as a marker for the unfolded protein response (UPR). The UPR is a conserved, adaptive cellular program activated in response to the disruption of ER homeostasis by increasing the expression of molecular chaperones and elimination of misfolded proteins while attenuating the intake of nascent polypeptides into the ER.[13] GRPs function as molecular chaperones, facilitating protein folding and assembly as well as exportation of misfolded proteins for degradation. GRPs also play an essential role in maintaining the integrity and homeostasis of the ER and mitochondria under physiological and pathological conditions due to their Ca^{2+} binding ability.[14] Two of the most extensively researched GRPs are GRP78 from the HSP70 family and GRP94 from the HSP90 family with therapeutics specifically targeting these GRPs under development.

The GRP78 and GRP94 chaperone system

GRP78, more commonly known as BiP (immunoglobulin heavy chain binding protein), resides primarily in the ER. It has a conserved ATPase domain and a peptide-binding domain which enables binding of proteins with hydrophobic residues in the unfolded region.[11,15] Processing of unfolded protein substrates is carried out within a large multi-protein complex that is composed of GRP78 along with GRP94 and other proteins.[14] GRP94 comprises 4 domains: an N-terminal domain (NTD), an acidic linker domain (LD), a middle domain (MD) and a C-terminal domain (CTD).[16] GRP94 provides a platform for the oligomerization of the loaded protein cargo in a dimer form.[17]

Both of the chaperone proteins GRP78 and GRP94 are essential for triage decisions about protein fate. GRP78 has many functions but its best characterised role is in protein biosynthesis. As a chaperone protein, GRP78 plays an essential role in the assembling, folding and transportation of newly synthesised peptides. It has also been discovered to play a role in maintaining ER stress sensors and ER-associated pro-apoptotic machineries in their inactive state under normal physiological and pathological conditions.[18,19] GRP94 is also a molecular chaperone that instructs the folding and assembly of secreted and membrane proteins but it is very selective in its clients. Whilst the GRPs were initially thought to reside only in the endoplasmic reticulum, more recently it has been shown that they can be translocated to other cellular locations and can be secreted. In addition to their role as molecular chaperones, they also possess other functions that control proliferation, invasion, apoptosis, inflammation and immunity.[20-23]

Endoplasmic reticulum stress, the unfolded protein response and cancer

Various studies involving cancer cell lines, knockout mouse models and xenografts have established the important roles of GRPs in cancer. Reports from cell lines studies especially show an association of GRPs with aggressive growth and invasive properties.[15,24,25]

Cancer cells are highly susceptible to ER stress due to both intrinsic and extrinsic factors.[25] During tumourigenesis, cancer cells exhibit elevated glucose metabolism with increased glycolytic activity and the high proliferation rate of cancer cells requires increased ER activity in facilitating protein folding, assembly and transport. The rapid growth of cancer cells eventually outgrow their initial blood supply which then results in glucose deprivation,

acidosis and hypoxia which are hallmarks of the microenvironment of poorly vascularised solid tumours.[15,26] Other events like ER calcium depletion, DNA damage and energy fluctuations or perturbations can altogether interrupt the protein folding process and subsequently cause accumulation of unfolded and misfolded protein in the ER, which is collectively referred to as ER stress.[27-29] Tumour cells eventually accrue a protective mechanism, the UPR to overcome the chronic stresses and in order to thrive in niches where nutrient and gaseous exchange is compromised. The activation of the UPR has two primary functions: (1) to initially restore normal function of the cell by halting protein translation, at the same time as activating the signalling pathways that lead to increased production of molecular chaperones involved in protein folding;[30,31] (2) when the first objectives are not achieved or when the disruption is prolonged, apoptotic pathways are initiated to remove the stressed cells.[32,33]

Many cancer types ensure the correct folding of their key signalling pathway proteins as well as the ability to survive an hypoxic environment associated with tumour development by relying on the UPR.[34] Studies support the notion that the UPR is an important mechanism for cancer cells to maintain malignancy and therapy resistance.

GRP78, GRP94 and cancer

Accumulating evidence has shown that GRP78 expression is elevated in various cancer types, including oral, prostate, oesophageal, gastric, breast and lung cancer.[19,35-41] Expression of GRP78 was found to correlate with high levels of proliferation in glioma cells while knockdown decreased the cells' proliferative capacity.[42] Upregulation of GRP78 expression has been linked to protection of dormant tumour cells against drug toxicity.[43] Another study has also shown that downregulation of GRP78 inhibits cell growth and proliferation and at the same time alleviates chemoresistance.[44] By upregulating GRP78 expression cancer cells may simply be increasing the protein-folding capacity of the ER, thereby avoiding the induction of ER stress and cell death.[45] GRP94 overexpression is also associated with cancer aggressiveness and metastatic progression[46] and targeting GRP94 with a vaccination strategy involving the use of an adenovirus vector and a secretable form of GRP94, combined with radiation therapy was shown to be effective in preventing tumour metastasis in mouse models.[47] GRP94 expression has also shown to be associated with metastatic spread to the brain in patients with breast cancer[48] and is associated with poor prognosis in gastric carcinomas[38] and with chemo-resistance in lung cancer.[49] Whilst GRP78 resides in the endoplasmic reticulum under normal conditions, it can also be highly expressed on the surface of many tumour cells (Fig. 1). The cell surface GRP78 acts as a signalling receptor and regulates pathways involved in proliferation, apoptosis, invasion and metastasis.[20,50] This has been demonstrated in prostate cancer cell lines, where the cell surface GRP78 activates the p21-activated kinase-2 (PAK2) signalling pathway, which then binds with α2-macroglobulin to facilitate invasion and metastasis.[51]

One of the distinguishing features of cancer cells is their ability to develop resistance to chemotherapeutic agents. Patients who initially respond to therapy, experience relapse of their cancer, which becomes unresponsive to the initial therapy within a period that can vary from some months to years. GRP78 expression has been found elevated at this stage. The same finding was also found for GRP94 where immunostaining revealed higher expression in recurring breast tumours compared to that of the primary tumour.[46]

Immunohistochemical and genome wide analysis on patient samples of glioma, leukaemia, prostate and breast cancer have shown elevation of GRP78 in tumours refractory to therapy[42] (Figs 1, 2 and 3). The paradoxical upregulation of UPR activation in refractory tumours without inducing apoptosis is believed to be a result of an increased level of GRP78. The observation of cell surface GRP78 in malignant but not benign cells suggests that that GRP78 is implicated in cancer cell survival. Even though the underlying mechanisms of how GRP78 induces chemo-resistance remains enigmatic, two mechanisms of action are hypothesized: (1) the synergistic effect of both the UPR pro-survival branch and the receptor-mediated activation of the Akt/PI3K pathway; (2) activation of the Akt/PI3K pathway counteracts the pro-apoptotic action of UPR, resulting in cell survival. In support of this notion, experiments have been carried out by using antibodies against the N-terminal or C-terminal domain of GRP78. Using prostate cancer cell lines and melanoma cells it has been demonstrated that blocking the N-terminal significantly decreases cell proliferation through the Akt/PI3K signalling pathway,[52,53] whereas

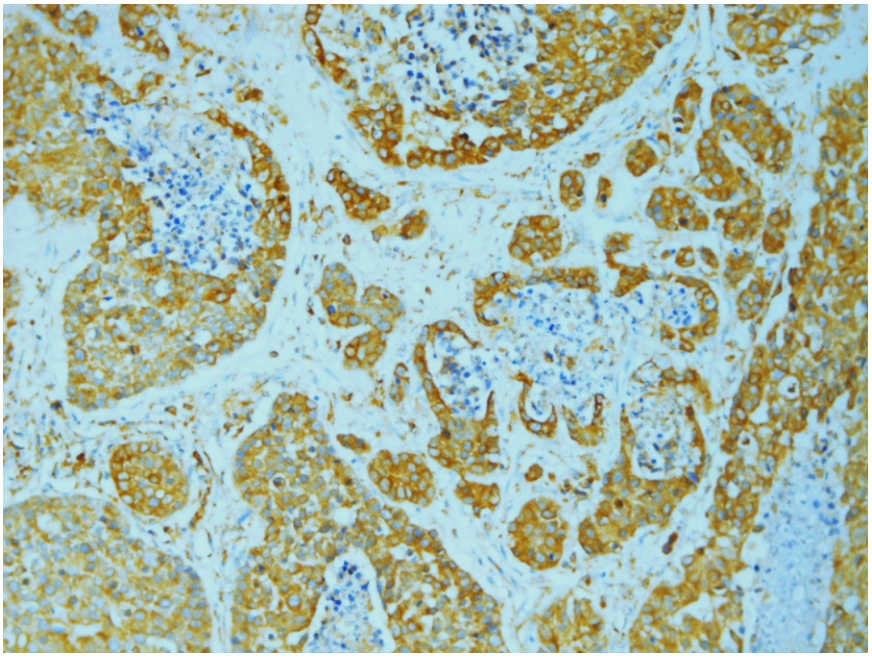

FIG. 1 Immunohistochemical staining of GRP78 in an infiltrating ductal carcinoma of the breast. GRP78 is localised to the cytoplasm and cell membrane of the tumour cells. Magnification x200.

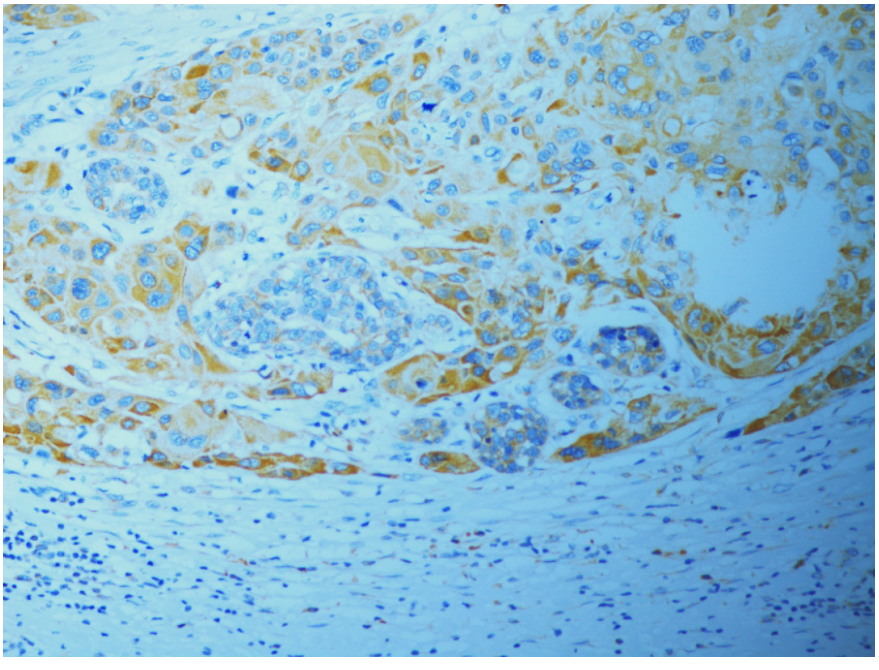

FIG. 2 Immunohistochemical staining of cytoplasmic GRP78 in large and bizarrely shaped tumour cells in a high grade infiltrating ductal carcinoma of the breast. Magnification x200.

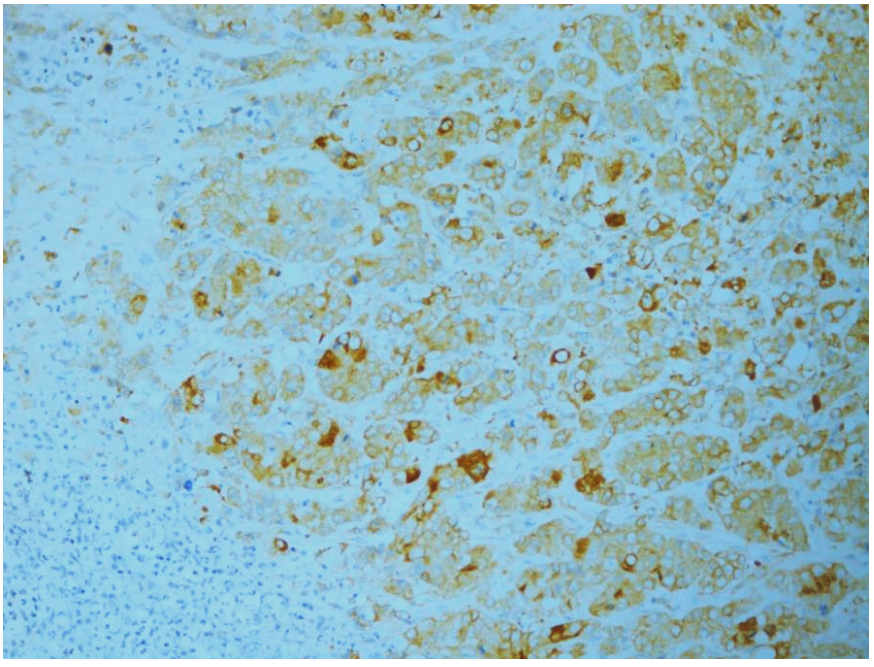

FIG. 3 Heterogenous expression of cytoplasmic GRP78 in the tumour cells of an infiltrating ductal carcinoma of the breast. Magnification x200.

targeting the C-terminal results in reduced cell proliferation and induced apoptosis through activation of p53 and suppression of the Akt/PI3Ksignalling pathways.[54,55] Patients who have developed chemo-resistance also exhibit high expression of GRP94.[46]

Another condition that contributes to the development of therapeutic resistance is hypoxia. Hypoxia prevails in the tumour bulk simultaneously with the development and progression of solid tumours. Rapid proliferation of tumour cells outgrows the blood supply, causing stresses including glucose starvation, hypoxia, and oxidative stress to the tumour cells. This condition evokes a series of intertwining intracellular signal cascades, resulting in cell cycle arrest, reduced drug delivery to the tumour and alteration in gene transcription activity, which contributes to tumour growth, angiogenesis and metastasis and hence, the enhanced the resistance of cancer cells to chemotherapeutic agents. Jiang *et al* (2009) showed that knockdown of GRP78 enhances the sensitivity to chemotherapeutic drugs in human melanoma cells.[56]

In addition to chemotherapeutic resistance, GRP78 overexpression is also associated with oestrogen antagonist sensitivity in breast cancer. This is demonstrated in a study where knocking down of GRP78 in oestrogen antagonist resistant breast cancer cell lines re-sensitizes them to anti-oestrogen treatment, followed by an observation of decreased proliferation and increased apoptosis.[57]

The role of GRPS in tumour cell invasion and metastasis

Treatment of malignant tumours remains a challenge to the oncologist. The majority of cancer patients are faced with mortality due to tumour metastasis. In a study carried out by Xia and colleagues (2014),[19] it was shown that 90% of oral squamous cell carcinoma patients with lymphatic metastasis were positive for GRP78 expression.

It is thought that GRP78 facilitates invasion and metastasis by using several different pathways. In prostate cancer, it is associated with activation of the p21-activated kinase 2 (PAK2) signalling pathway and binding to α2-macroglobulin by cell surface GRP78.[51] Meanwhile in colorectal cancer, activation of the uPA/uPAR protease system by the cell surface GRP78 facilitates invasion and metastasis.[58] In liver cancer, GRP78 has been associated with Matrix Metalloproteinase 2 (MMP2) expression, which along with MMP9 plays an important role in degradation of extracellular matrix and has long been associated with invasion and

metastasis. Experiments have demonstrated that knocking down cell surface GRP78 inhibits secretion of MMP2, and in contrast, forced expression of GRP78 promotes the secretion and activity of MMP2.[59]

GRP94 expression is particularly abundant in cancer metastases and in moderately and poorly differentiated cancers as well as late stage tumours.[37,60-62]

Reactive oxygen species (ROS) are abundantly produced in many types of cancer cells, where ROS is associated with increased cell growth and adaptation capacity. However, pro-oxidative-based chemotherapies could make tumour cells become sensitive to the treatment under such conditions. Targeting the altered redox state of cancer cells results in an increase of proteins of the ER machinery dedicated to protein folding, including GRP94 and members of the Protein Disulfide Isomerase Family (PDIs). These proteins are required by ROS-resistant cells to proliferate under a reducing basal condition, thereby allowing them to maintain a high proliferation rate even under conditions of heightened antioxidant capacity. GRP94 overexpression also leads, probably, through modulation of secreted proteins, to an acquisition of a high migratory capacity.[46]

In summary, the interactions between tumour cells and the tumour microenvironment are fundamental to cancer progression, and GRPs play a pivotal role in facilitating this. Although the accumulated evidence to date implicates GRPs with tumour progression, further studies are required to investigate the role of GRPs in prognosis and in therapeutic approaches that could potentially target GRPs to improve patient outcome.

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