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Commentary

GRP78 Is a Targetable Receptor on Cancer and Stromal Cells

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Glucose-Regulated Protein 78 (GRP78), also known as BiP (Binding Immunoglobulin Protein), is a member of the Hsp70 family of chaperone proteins that is essential for embryonic development. GRP78 is highly conserved across species, and is localized primarily in the endoplasmic reticulum, where it regulates protein folding and activates the unfolded protein response pathway (UPR) during stress conditions [6]. Under normal conditions, GRP78 binds to and inactivates the ER stress sensors ATF-6, PERK and IRE1. ER stress drives the accumulation of unfolded proteins to titrate GRP78 away from the stress sensors toward plasma membrane localization. GRP78 is highly expressed on the cell surface of a variety of cancer types owing to their inherently elevated ER stress levels, and weakly expressed on normal cells [8]. Global profiling of cell surface GRP78 is highly correlated with pathological states making it a relevant target for therapy.

Several natural ligands can elicit a diverse signaling response upon binding to cell surface GRP78. On tumor cells, GRP78 can bind to α2-macroglobulin and Cripto, leading to cell survival and proliferation, or to Kringle 5 and Par-4, which drives apoptosis [3]. On endothelial cells, GRP78 interacts with T-cadherin, which promotes cell survival [8]. Moreover, GRP78 antibodies, designed against the carboxyl-terminus, induced apoptosis of cancer cells and inhibited the growth of tumor xenografts [7]. As cell surface GRP78 agonists can trigger an apoptotic response, a considerable amount of effort has been invested in generating small molecules that can bind to cell surface GRP78 and activate the apoptotic pathways as an effective mode of anticancer therapy. Arap et al. [1] developed two targeted phage peptides containing predicted binding motifs for GRP78 wherein a dose of 300 mg/mouse was administered weekly via tail vein for four weeks. The peptides were tested in nude mice bearing DU145 prostate subcutaneous tumor xenografts and in immunocompetent *Balb/c* mice bearing EF43-fgf4 derived tumors. In both mouse models, the peptides were able to specifically bind GRP78, undergo cell internalization, and suppress tumor growth. Bone metastasis targeting peptidomimetic-78 (BMTP-78), a prototype drug developed by Arap et al. [1], consisting of a GRP78 binding peptide fused to a death-inducing domain, was tested in several human leukemia and lymphoma cell lines and toxicological studies were performed in rodents and nonhuman primates [9]. Remarkably, BMTP-78 reduced cell viability in 12 cell lines as well as in primary human AML cells. However, toxicology data in Sprague Dawley rats showed dose-dependent toxicity, with kidneys being the most affected organ. Moreover, BMTP-78 was found to be markedly toxic to non-human primates, causing fatal cardiac dysfunction. Although

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GRP78 is an attractive therapeutic target, these findings underscored the need for developing safer molecules.

The study by Kao and collaborators [5] published in this issue, describes a peptide that not only specifically targets GRP78 and suppresses tumor growth, but also accumulates in the tumor microenvironment for up to 72 h post-injection, with no apparent liver or kidney toxicity in mice. The group had previously identified isthmin (ISM), an antiangiogenic and proapoptotic protein that was able to bind both GRP78 and αvβ5 integrin on the plasma [4]. ISM is internalized in cancer cells, where it co-localizes with GRP78, but not αvβ5 integrin, and leads to apoptosis in B16F10 and 4 T1 tumor cells. ISM contains two domains: TSR (thrombospondin type I repeat) and C-terminal AMOP (adhesion-associated domain in MUC and other proteins), of which AMOP seems to be more critical for the function of ISM as mutation of critical residues in AMOP ($KD_{316–317}$) results in ablation of its activity. Kao et al. used the AMOP domain, known to induce apoptosis of HUVECs cells, to design cyclic peptides that target GRP78. All 16 synthetic peptides harbored the RKD core domain of AMOP and displayed proapoptotic activity. BC71 peptide exhibited the highest apoptotic activity toward HUVEC cells and decreased cell viability. Using neutralizing antibodies to N-terminal GRP78, C-terminal GRP78 and to αvβ5 integrin, the authors demonstrated that BC71 induced apoptosis by interacting with the N-terminal portion of GRP78 since only N-terminal GRP78 antibody was able to reduce apoptosis triggered by BC71. In accordance with observations from different groups on extracellular ligands of GRP78, BC71 leads to apoptosis via caspase-8 and p53 activation, as chemical inhibition of these targets decreased BC71-induced apoptosis by more than 50%. Tracking in vivo distribution following intravenous injection of the peptide into mice, Kao and colleagues demonstrated that Cy7-labelled BC71 accumulated in the area surrounding the 4 T1 mammary tumors in mice for at least 72 h post-injection. Reduced angiogenesis and extensive apoptosis, as indicated by TUNEL positivity, was observed upon treatment. Although preliminary toxicity results are promising, it needs further investigation as the peptide was tested in a single model. However, the anti-tumor efficacy of targeting cell surface GRP78 is consistent with previous studies and this article sheds new light on the anti-angiogenic effects of the treatment. Another interesting application is the use of the peptides as imaging agents or drug carriers since exploiting their ability to target tumor cells as-well-as endothelial cells surrounding the tumor.

It is noteworthy that, although targeting of GRP78 is promising and was shown to be efficient in suppressing tumor growth, this effect is limited to the extent of GRP78 expression on the cell surface. Regulation of NF-κB has been shown to induce ER stress and GRP78 translocation to the cell surface [2, 10]. However, it also causes immunosuppression and tumor cell immune evasion. Development of new strategies that combine agents that increase GRP78 translocation to the cell surface with peptides or small molecules targeting GRP78 could significantly improve the efficacy of cancer treatment.

Disclosure

The authors declare no conflicts of interest.

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