The unfolded protein response in glioblastomas: passing the stress test

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One may think of tumors as existentially stressed; their rapid, uncontrolled proliferation in the midst of unstable blood supplies results in a hypoxic, nutrient-deprived environment, and the threat of immune system attack is almost constant (if ineffective). Tumors are also subject to our treatment schemes for them, including radiation and chemotherapeutics. To cope with these stresses, tumors upregulate expression of chaperone proteins/heat shock proteins to facilitate protein folding and stability issues, and to reduce the impact of apoptotic drivers. Brain tumors, such as high-grade gliomas/glioblastomas (GBMs), are no exception [1], and these tumors often show high levels of chaperone proteins – some displayed on the surfaces of brain tumor cells – particularly compared with normal brain [2,3]. Whether these stress proteins represent useful immunotherapy moieties [3,4] or drug targets [5] remains to be seen. However, it seems likely that the stresses imposed on tumors, rather than simply inflicting damage, may actually drive processes that allow tumors to survive and even thrive amidst the stresses.

Cellular responses to stress take on a variety of forms, and sometimes those

responses manifest in different organelles. Subcellularly, the accumulation of unfolded proteins in the endoplasmic reticulum (ER) triggers a multipronged ‘stress management profile’ known as the unfolded protein response (UPR; for excellent reviews see [6,7]). This stress response initiates when sensors within the ER, such as the HSP 70 family member GRP78 (also known as BiP/HSPA5), detect unfolded or misfolded proteins in the ER lumen. Similar events occur in cells with high secretory outputs, where the cells experience large amounts of protein translation into the ER, such as in activated plasma cells that produce large quantities of antibodies. During ‘ordinary’ cellular stasis, one of GRP78’s roles is to bind to the ER luminal portions of three transmembrane molecules: PERK, IRE1 and ATF6. The interaction of GRP78 with these three ER membrane proteins maintains them in a monomeric state; when GRP78 releases them in order to perform chaperone duties for the unfolded proteins present in the ER, these three proteins now act as transducers of the UPR (it is likely that IRE1 has its own unfolded protein-sensing domain [8] and may not require GRP78’s

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tethering services). PERK and IRE1 dimerize or form higher-order oligomers with autokinase activities, while ATF6 is released into the Golgi for proteolytic processing.

PERK autophosphorylation induces its kinase activity, which leads to the phosphorylation of eIF2 α (or EIF2A). Phospho-eIF2 α inhibits translational initiation, thus bringing protein translation to a halt, which reduces the burden of unfolded proteins amassing in the ER. IRE1 *trans*-autophosphorylates to activate its kinase domain, which activates its unique endoribonuclease function. The endonuclease domain excises a 26-base intron from the XBP-1 mRNA (referred to as the ‘unspliced’ form, XBP-1u), and allows splicing to generate a longer (‘spliced’ XBP-1s) mRNA with a frame shift that now codes for a longer, more stable and active protein transcription factor. ATF6 migrates to the Golgi where it is cleaved by proteases S1P and S2P, releasing the cytosolic, N-terminal portion of ATF6 that is now an active transcription factor [9,10].

Both XBP-1 and ATF6 drive transcription of target genes that include those encoding AFT4 and more XBP-1, but also GRP94, GRP78 and calreticulin (to provide more chaperone capacity in the ER). XBP-1 transcriptional targets include members of the protein disulfide isomerase chaperone family, the chaperone FK506, and the large chaperone GRP170, as well as even more XBP-1. ATF4 target genes include the chaperone *HERPUD* or *HERP*, but also *CHOP* (also known as *GADD153*). The latter is a transcription factor regulating expression of apoptosis pathways. However, ATF4 also transactivates a promoter site in the *GADD34* gene with associated transcription and translation. GADD34 protein interacts with the type 1 protein serine/threonine phosphatase (PPI), which dephosphorylates eIF2 α , releasing the translational block instigated by PERK, thus allowing protein synthesis in the cytosol to recommence [11].

These fascinating complex pathways and interactions serve to essentially hedge bets on cell survival – the cell makes valiant attempts to promote protein folding and relieve the stress on the ER. However, if that fails, the cell is ready to set off an apoptotic cascade, presumably for the preservation of the tissue as a whole. On the other hand, the cell also sets up to return to normal by restarting the stalled cytosolic translational machinery. The fine balance in these

pathways is not understood, but perturbations in control of the pathways could have implications for cell proliferation and survival.

GBMs have active cell surface and extracellular environments that require autocrine and paracrine signaling, membrane remodeling as the cells proliferate and the ability to change shape during migration/invasion, for angiogenic activities, and for secretion/release of extracellular materials for local and systemic impacts. The UPR could clearly enhance these actions by providing a large and active chaperone cohort for folding, stabilization and packaging of surface- or extracellularly destined client proteins, as well as some of the lipid species required for the intracellular vesicular traffic in the secretory pathway. Thus, while initial stress may have instigated the UPR in GBM cells, the benefits may lead to ‘fixing’ of the UPR as a permanent feature in the tumor. Recent work in this area demonstrated other effects of the UPR on gliomas, such as an override of the protein translation inhibition – showing that GBM cells can continue normal protein synthesis activities through the stress, and possibly utilizing the extended folding capacity of the ER driven by the UPR [12]. One outcome of the continued protein synthesis was exacerbated cell proliferation, accompanied by an almost complete resistance to temozolomide upon UPR induction in glioma cells. Metabolic features such as glycolysis and lipogenesis were enhanced, and those metabolites were consistent with rapidly proliferating and drug-resistant tumor cells. Recurrent tumors from patients with GBMs showed elevated levels of nearly every category of lipid compared with primary tumors, along with activation in the ATF6 pathway (with higher levels of spliced XBP-1) compared with primary GBMs. Some of these features are similar to other findings, in particular the high expression of GRP78 and its role in chemoresistance [13] and genetic evidence for the utility of the UPR in other brain tumor types [14].

While there are relatively few publications regarding gliomas and the UPR, some themes are emerging in that literature. One would be the implication of IRE1 as an important player in glioma UPR biology [15–17]. In a study by Eppe *et al.*, the presence of XBP-1s mRNA and protein, particularly in the recurrent tumor setting, suggests an active IRE1/XBP-1 axis of the UPR (and IRE1 was overexpressed in xenograft GBMs) [12]. XBP-1 is known to play a role in

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lipogenesis via upregulated fatty acid synthase (FASN; which was also upregulated in the recurrent tumors compared with primary tumors) [18]. As recurrent tumors are, by definition, therapeutically resistant, these findings suggest an important role for the UPR in terms of lipid metabolism as it relates to drug resistance. Ongoing projects in our laboratory show that UPR induction in tumor cells leads to 3–5-fold increases in the production of exosomes, virus-sized extracellularly released membrane-enclosed vesicles (EPPLE *ET AL.*, EXOSOMES FROM GLIOMAS PASSAGE THE UNFOLDED PROTEIN RESPONSE TO UNSTRESSED CELLS: MECHANISMS OF RESISTANCE AND TUMOR PROGRESSION, MANUSCRIPT IN PREPARATION). Passage of these vesicles onto recipient (unstressed ‘naive’) GBM cells drives proliferation and profound chemoresistance, suggesting potential ties between the UPR, metabolic outputs and the resistant phenotypes seen in recurrent GBMs.

From a therapeutic perspective, most of the attention focuses on a UPR-related process, that of ER-associated degradation. This is the proteolytic arm of the UPR, where unrecoverable proteins are transported out of the ER and degraded by the 26S proteasome. Bortezomib (Velcade®; Millenium Pharmaceuticals, Cambridge MA, USA) is a US FDA-approved specific inhibitor of catalytic activity of the 26S proteasome, used in the treatment of multiple myeloma. By stifling one of the clearance mechanisms for an overloaded ER, certain UPR-related mRNAs and proteins are upregulated, but the apoptotic axes are activated while the cytoprotective ones

are suppressed [19]. Bortezomib has been used alone or in combination with other drugs in ten clinical trials for brain and CNS cancers [101], but only one is currently recruiting. Other avenues attempt to target major chaperones in the UPR, such as GRP78 [13,20], but none of these are at the clinical trial stage.

The UPR appears to be a formidable defense in the stressful world of brain tumors. It is unclear at what point the stresses applied to GBMs become overwhelming, resulting in cell death, or if these stresses simply augment tumor survivability. We may need to think in terms of targeting processes, such as the UPR or associated metabolic pathways, rather than targeting individual players. For instance, the IRE1/XBP-1 axis that leads to FASN-driven lipogenesis may be attractive, given its activation in recurrent GBMs. Targeting IRE1’s kinase or endonuclease activity coupled with FASN inhibition could be a reasonable combination with multi-tiered effects. May be then we can get stressed-out tumors to finally crack.

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■ Website

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