The unfolded protein response in glioblastoma stem cells: towards new targets for therapy

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Chapter 2

The endoplasmic reticulum stress/ unfolded protein response in gliomagenesis, tumor progression and as a therapeutic target in Glioblastoma

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Abstract

Endoplasmic reticulum (ER) stress disrupts amongst others protein homeostasis in cells leading to the activation of the unfolded protein response (UPR) that is crucial for restoring this balance and cell survival. Hypoxia, reactive oxygen species and nutrient deprivation, conditions commonly present in the tumor microenvironment, are well-known triggers of the UPR. Apart from being an adaptive response, recently the UPR has been implicated in oncogenesis. Here we review the current understanding of the UPR in the most life threatening brain tumor in adults, glioblastoma multiforme (GBM). The UPR is controlled by BiP/GRP78 and three different sensors, PERK, IRE1 and ATF6. In orthotopic GBM mouse models IRE1 was reported to control angiogenesis, invasion and mesenchymal differentiation. Furthermore, PERK also was found to stimulate GBM growth. However, a direct role of the UPR in gliomagenesis remains to be demonstrated. Patient samples display chronic activation of the UPR and *in vitro* standard chemo- and radiotherapy partially act by aggravating ER stress leading to cell death. The UPR has been linked to enhanced sensitivity for apoptosis-inducing agents such as TRAIL and MDA-7. A number of agents such as proteasome inhibitors and several natural products were reported to exert cytotoxicity by enhancing ER stress in GBM cells, and some demonstrated activity in clinical studies. Finally, ER stress was suggested to be implicated in the maintenance of homeostasis in GBM stem cells. Taken together, the UPR appears to play an important role in GBM tumor progression and is a promising target for developing novel therapeutic interventions.

1. Introduction

Glioblastoma multiforme (GBM) is an extremely aggressive brain tumor and clinically difficult to treat cancer [1]. Current standard treatment consists of surgery combined with radiotherapy and chemotherapy [2]. However, the inability to surgically remove all tumor cells together with resistance to therapy, including novel targeted agents, results inevitable in recurrent disease leading to a poor median survival of patients of 12-15 months [3]. To improve prognosis of GBM patients a better understanding of the molecular mechanisms underlying the development of GBM or that cause resistance to current therapies is warranted. This will lead to the design and development of novel therapies that will improve prognoses. In this review we will explore the role of endoplasmic reticulum (ER) stress/ unfolded protein response (UPR) in gliomagenesis, tumor progression and as a potential novel therapeutic target in GBM.

The ER is an intracellular organelle that plays a central role in the synthesis of proteins and lipids. For example, transmembrane proteins and secreted proteins are produced by ribosomes on the ER membrane and enter the ER lumen where they are correctly folded with the help of chaperone proteins. Subsequently, proteins obtain posttranslational modifications, particular glycosylation, and when required are transported to other cellular organelles, the plasma membrane or destined for secretion. Another key function of the ER is storage of calcium ions (Ca^{2+}) that are important for cellular signal transduction. Moreover, several ER resident proteins involved in the correct assembly of proteins are dependent on Ca^{2+}.

The functioning of the ER can be disrupted by various physiological and pathological stimuli, including nutrient/ glucose deprivation, Ca^{2+} depletion, hypoxia, oxidative stress and viral infections [4]. These events alter or imbalance the protein folding capacity of the ER causing ‘ER stress’ and affect protein homeostasis leading to the production of misfolded proteins that are detrimental for proper cell functioning. avoid this and restore homeostasis, protein production in the ER is closely monitored by quality control mechanisms that are linked to adaptive stress responses, named the UPR. The accumulation of misfolded proteins triggers the UPR leading to activation of biochemical mechanisms that alleviate ER stress, or when homeostasis cannot be restored the activation of (apoptotic) cell death [4-6].

Interestingly, it is increasingly recognized that the UPR plays an important role in tumorigenesis. Cancer cells are often exposed to severe microenvironmental conditions such as hypoxia, hypoglycemia and low pH that will induce ER stress and activate the UPR adaptive system [6,7]. These cell extrinsic stressors affect intracellular protein production. For example, hypoxia affects the disulphide forming process leading to aberrant protein folding and low glucose will reduce ATP production that is required for the protein folding machinery [8,9]. Thus, cancer cells use the UPR to survive harsh conditions rather than undergoing apoptosis. Furthermore, the UPR has been found to confer increased resistance to chemotherapeutics in tumor cells attributed to cytoplasmic cytotoxicity of these compounds resulting in interference with proper protein production [10]. On the other hand, cellular malignant transformation is accompanied by enhanced growth requiring an increased production of membranes and secreted proteins. This demands a higher protein production capacity providing elevated intrinsic stress and activation of the UPR. In addition, recent research has demonstrated the involvement of the UPR in reprogramming tumor cells during oncogenesis and in determining a range of tumorigenic hallmarks such as cellular transformation, metastatic potential, genomic stability, angiogenesis, immunogenic tolerance and metabolic status of cells [8,11]. The chronic ER stress experienced by most cancer cells together with novel insights obtained in the importance of the UPR in tumor progression has stimulated the exploration for agents that modulate the UPR as a possible novel therapeutic approach for treating cancer.

Here, we will focus on the current understanding of the role of the UPR in GBM and its exploitation as a possible therapeutic target in this deadly disease.

2. The UPR

The UPR consists of three different parallel signaling routes or branches that are triggered by different sensory ER transmembrane proteins, protein kinase R (PKR)-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1) and activating transcription factor 6 (ATF6). The chaperone BiP/GRP78 that is located in the ER lumen, and involved in proper protein assembly plays a central role in the activation of these three branches. In the absence of ER stress, BiP/GRP78 is associated with all three

sensors PERK, IRE1 and ATF6 preventing their activation. Upon stress BiP/GRP78 is sequestered by misfolded proteins leading to activation of the three transmembrane proteins, via homodimerization, autophosphorylation and cleavage processes, and subsequent activation of the corresponding UPR branches in order to restore ER homeostasis [12-15]. This is achieved amongst others by inhibition of global protein synthesis, stimulation of ER-associated protein degradation (ERAD) by proteasomes in the cytoplasm and upregulation of the expression of chaperones and foldases. When cells cannot cope with the level of ER stress, apoptosis is activated in which UPR-dependent activation of the transcription factor C/EBP-homologous protein (CHOP) plays an important role by modulating anti- and pro-apoptotic proteins, such as Bcl-2 family members that control mitochondrial apoptosis [5,16]. The pathways triggered in the three different UPR branches are briefly described below (see also Figure 1).

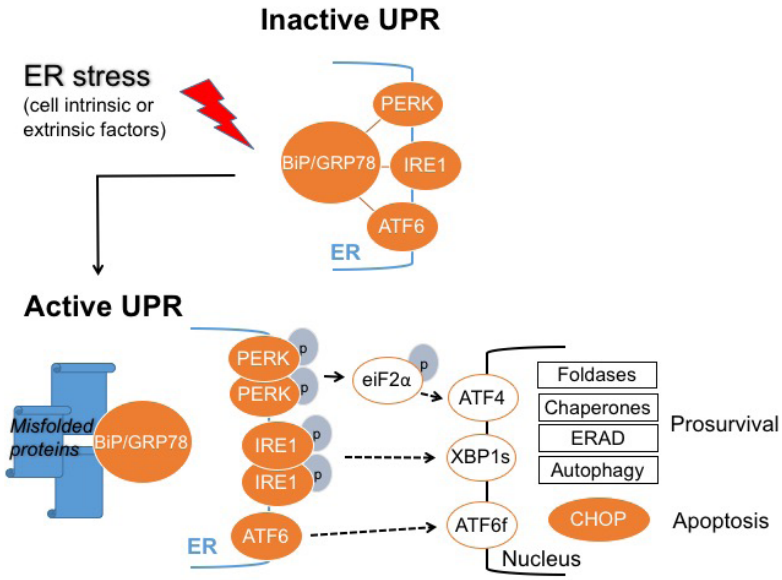

Figure 1: Simplified representation of ER stress/UPR signaling. *ER stress induced by either cell intrinsic or extrinsic factors trigger the UPR controlled by three key ER transmembrane proteins PERK, IRE1 and ATF6. In the absence of stress these transmembrane proteins are kept inactive by binding to BiP/GRP78, which dissociates to bind misfolded proteins produced as result of ER stress. PERK and IRE1 are activated by homodimerization and autophosphorylation and ATF6 is activated by protease cleavage in the Golgi. Signaling cascades lead to translocation of specific transcription factors, ATF4, XBP1s and ATF6f,*

to the nucleus where they regulate transcription adaptive, prosurvival responses to restore homeostasis or apoptosis in case of overwhelming damage (See for more details text and references).

PERK dimerization results in autophosphorylation and subsequent phosphorylation of the eukaryotic translation initiator factor 2α (eIF2α), leading to inhibition of global protein synthesis and thus preventing a further accumulation of misfolded proteins in the ER. Several mRNAs escape translational suppression by eIF2α and are part of the UPR such as the activating transcription factor 4 (ATF4). TF4 controls the expression of adaptive genes involved in amongst others ERAD, protein folding, amino acid biosynthesis as well pro-apoptotic CHOP [15,17]. PERK was also found to directly phosphorylate NRF2 that controls the antioxidant response pathway [18].

IRE1 comprises a serine/threonine protein kinase domain and an endoribonuclease (RNase) domain and is activated upon dimerization and autophosphorylation. It catalyzes the removal of an intron in the mRNA encoding the X-box binding protein (XBP1) yielding spliced *XBP1* mRNA that produces functional XBP1 protein. XBP1 is a transcription factor that regulates the activation of genes active in ERAD, protein folding and ER membrane expansion. The RNAase activity of IRE1 is also involved in the degradation of mRNAs, rRNAs and microRNAs through a process called regulated IRE1-dependent decay (RIDD) [19]. IRE1 has also been linked to the activation of JUN N-terminal kinase (JNK) through recruitment of tumor necrosis factor (TNF) receptor-associated factor 2 (TRAF2). JNK can phosphorylate and inhibit the anti-apoptotic activity of Bcl-2 and Bcl-xL or activate the pro-apoptotic function of BIM and Bid [5,16]. ATF6 is an ER transmembrane transcription factor that is activated by transport to the Golgi apparatus where it is cleaved by proteases in two fragments to generate a cytosolic fragment, ATF6f. ATF6f translocates to the nucleus where it promotes the transcription of ERAD genes and XBP1 [20].

Depending on the stimulus the three UPR branches activate overlapping as well as distinct sets of genes via mechanisms that are currently not well defined. For example, the transcription factors XBP1s and ATF6f can activate distinct genes as well as cooperatively induce genes by forming functional heterodimers [21]. Furthermore, although the PERK/ATF4 pathway is best known for activating the pro-death transcription factor CHOP also ATF6 and XBP1 have this ability [16].

The UPR is also tightly linked to autophagy, a major cellular catabolic process that sequesters large protein aggregates and damaged organelles for degradation in autophagosomes [22]. Autophagy serves as an alternative ERAD mechanism and is interlinked with all three UPR branches, that are able to modulate essential regulators of autophagy such as the mammalian target of rapamycin complex 1 (mTORC1), Beclin1 and the expression levels of autophagy regulatory genes [23]. A more detailed outline of the UPR and mechanisms of action are out of the focus of this review and have been addressed in several recent reviews [8,11,17,23].

3. GBM and the UPR

In cancer, initially the UPR was believed to function primarily as an adaptive system to support the survival of tumor cells. However, more recently it was recognized that the UPR, particularly the UPR transcription factors ATF4, XBP1 and ATF6, are important for reprogramming gene expression during tumor development [11]. We will continue by providing an overview of the current knowledge on the role of the UPR in different aspects of GBM biology, tumor behavior and its possible clinical value as therapeutic target.

3.1. UPR and gliomagenesis

The direct involvement of the UPR in gliomagenesis has been hardly explored. Thus far, only somatic mutations in the IRE1 encoding gene *ERN1* were reported that may represent a driver mutation in tumors, including in GBM [24,25]. However, mutations in *ERN1* are very rare in GBM (frequency < 1%) and likely play no important role in gliomagenesis. Moreover, the functional involvement of IRE1 in gliomagenesis has not yet been demonstrated.

In other studies, changes in the expression of ER stress-related genes have been associated with GBM development. For example, analysis of the genome of GBM patient samples for gene copy number variations revealed strong amplification at chromosome 7p11.2 that could be pinpointed to the *SEC61γ* and *EGFR* genes

[26]. Whereas EGFR amplification is a well-known genomic event in GBM, *SEC61γ* amplification was novel and observed in 77% of the clinical samples inspected. *SEC61γ* is one of the subunits of the SEC61 complex that forms a transmembrane pore for the translocation of proteins into the ER lumen and integration of transmembrane proteins into the ER membrane. In GBM cells in *vitro* induction of ER stress by tunicamycin, an inhibitor of N-linked glycosylation, enhanced SEC61γ levels amongst the known UPR proteins. Knockdown of SEC61γ expression in GBM cells resulted in reduced colony formation that was associated with inhibition of EGFR dependent Akt survival pathway stimulation and activation of apoptosis [26]. Thus, enhanced SEC61γ levels may provide a survival mechanism of GBM cells and as such contribute to gliomagenesis, although this remains to be proven.

A link between ER stress and self-renewal properties of GBM stem cells (GSCs) has been reported. GBM is believed to originate from GSCs, and GSCs also have been associated with the aggressive characteristics of GBM such as high tumor vascularization, invasive behavior, chemo- and radio-resistance and relapse of disease after surgery [1]. GSCs possess self-renewal ability and high tumor-initiating potential and are able to differentiate into bulk tumor cells that are commonly believed to lack or have reduced tumor forming ability [1,27]. Using an *in vivo* RNAi screening approach in a murine GBM model, the epigenetic modifying polycomb-group protein BMI1, known to stimulate self-renewal properties of GSCs and tumor aggressiveness, was found to exert its functions amongst others via affecting TGF-β/ BMP - and ER stress pathways [28]. Both pathways could at least in part be linked to reduced expression and altered functioning of various genes, including the stress inducible transcription factor ATF3. ATF3, a downstream target of p53, has been implicated in UPR signaling, particularly in the PERK branch [29]. ATF3 expression in the mouse GBM model was suppressed by BMI1 and was linked to enhanced stemness and in GBM patient samples low ATF3 expression correlated with poor prognoses [28]. Based on these data a role for ATF3 as a tumor suppressor gene in GBM was proposed. However, the relationship between ATF3 and the UPR in GBM, as well as its more direct role in progression from a normal (stem) cells to GSCs (gliomagenesis) has not been explored yet.

3.2. The UPR and GBM tumor progression

The contribution of the UPR in GBM tumor progression has been better explored. Drogat et al. were the first to demonstrate that overexpression of a dominant negative (dn) IRE1 in the U87 GBM cell line resulted in inhibition of hypoxia or glucose deprivation induced VEGFA expression [30]. Of note, in parallel to the well known hypoxia-inducible transcription factors (HIFs) hypoxia was reported to induce the UPR. Particularly, ATF4/XBP1 and HIF1/XBP1 heterodimers have been found to transcriptionally activate the expression of pro-angiogenic genes, including VEGF [31,32]. The assessment of tumor growth of U87 control or IRE1 deficient cells upon orthotopic intracranial implantation in mice revealed much smaller tumors in IRE1dn cells, which was accompanied by reduced vascularization, further confirming the importance of VEGFA in IRE1 adaptive signaling. In a follow-up study Auf and coworkers showed that IRE1 is indeed a key regulator of angiogenesis as well as invasion in the U87 model [33]. Using a similar approach IRE1 signaling was impaired by overexpression of IRE1dn in U87 cells and after intracranial implantation reduced tumor growth together with enhanced invasion was observed when compared to U87 control tumors. In addition, a strong decrease in tumor vascularization was detected in U87dn tumors, which also could be confirmed in a chicken egg angiogenesis assay. Comparison of transcriptional profiles revealed a strong decrease in IL-1b, IL-6, IL-8 and VEGFA in U87dn cells; overexpression of IL-6 in these cells rescued blood vessel formation [33]. Furthermore, in IRE1-impaired U87 cells the upregulation of an important modulator of astrocytoma cell migration, SPARC, was reported together with increased mesenchymal marker expression. The same group extended on studying the role of SPARC in IRE1 signaling and found that ER stress in U87 leads to a decrease in *SPARC* mRNA levels as a result of IRE1-dependent *SPARC* mRNA cleavage [34]. SPARC is a secretory matrix protein that regulates the interaction with the extracellular matrix thereby stimulating the migratory ability of cells. The authors propose that inactive IRE1 enhances SPARC leading to enhanced stress fiber formation and focal adhesions in a Rho-dependent way thus stimulating tumor invasion.

In another study, unexpectedly the circadian clock regulating PER1 mRNA was identified as a substrate for IRE1-dependent cleavage and to affect tumor formation in the U87 model [35]. Obstruction of IRE1 functioning in U87 cells resulted in sustained

PER1 levels that was associated with reduced tumorigenesis *in vivo*. The tumor growth inhibitory effect of PER1, which functions as a transcriptional suppressor, was found to involve inhibition of the production of the proangiogenic chemokine CXCL3. Furthermore, in GBM patient material with low PER1 and high XBP1 levels were associated with lower survival rates [35]. More recently, the contribution of the kinase and RNase domains present in IRE1 were more closely examined for contribution to either angiogenesis or invasion. IRE1 kinase or RNase deficient mutants were generated by amino acid substitutions and stably transfected under control of a doxycycline-inducible promoter in U87 cells [36]. It should be noted that in these models endogenous IRE1 is still expressed and may have some residual activity. The induced expression of all IRE1 mutants resulted in reduced tumor growth that was at least partially linked to differences in proliferation whereas apoptosis was not involved. Double mutant IRE1 resulted in a strong reduction of tumor vascularization and enhanced infiltration. Interestingly, intracranial tumors with disrupted IRE1 RNase activity were associated with enhanced tumor invasion whereas vascularization was comparable to control conditions. Disruption of the kinase domain resulted in tumors with less pronounced differences, with intermediate levels of invasion and wild-type vascularization patterns [36]. Moreover, the RNase defective mutants also resulted in enhanced expression of mesenchymal markers, consistent with a mesenchymal phenotype in GBM being associated with a more invasive phenotype as also shown by others [37,38]. It was concluded that both the kinase and RNase domains of IRE1 have proangiogenic activity and that particularly the RNase domain, when invalidated, stimulates a mesenchymal shift and enhanced invasion [36]. The underlying molecular mechanisms have not been elucidated as yet, although IRE1-TRAF2 dependent JNK and NF-κβ signaling may be involved in blood vessel formation. However, apart from cell autonomous mechanisms also changes in tumor cell-microenvironment interactions will be involved. From a therapeutic standpoint the inhibition of IRE1 RNAse activity is not a valid strategy as it will enhance tumor invasion. Targeting the kinase activity seems a more promising approach that will reduce tumor growth and may have antiangiogenic activity. Overall, these data indicate that at least the IRE1 branch of the UPR is involved in GBM progression and aggressiveness.

3.3. Enhanced UPR activity in GBM

In line with the proposed protective function of the UPR against a hostile tumor microenvironment, elevated UPR activity in GBM has been reported in several studies. RNA expression analyses of different *in vitro* cultured GBM cell lines and normal brain tissue revealed elevated levels of oxidative- and ER stress pathways in GBM, including ER chaperones and ATF4 [39]. Similarly, BiP/GRP78 was significantly enhanced in GBM cell lines and patient tumor specimens [40]. Moreover, the level of expression could be positively correlated with proliferation rates. Forced overexpression of BiP/GRP78 in C6 rat glioma cells was shown to provide resistance to ER- and oxidative stressors [41,42].

Epple and coworkers also reported elevated levels of BiP/GRP78 and the UPR transcription factors in GBM patient samples and in U87-derived mouse xenografts [43]. In normal brain tissue and in cultured U87 cells no elevated UPR activity was found, however, in U87 cells this could be strongly induced upon stress. Although only studied in one cell line, this was taken as evidence for the notion that the harsh tumor microenvironment largely contributes to elevated UPR activity in GBM. The authors also reported that the level of BiP/GRP78 segregated GBM (grade IV) from grade III gliomas and, moreover, was associated with poor prognosis in GBM. Furthermore, metabolic flux analysis using ^{13}C-glucose showed that ER stress enhanced the uptake of glucose and the glycolysis flux, accompanied by increases in several amino acids, acetate and glutathione, indicative of effects on protein synthesis, lipid synthesis and oxidative defense, respectively [43]. More recently, evidence for elevated activation of the PERK branch was provided in glioma grade III and particularly in GBM samples when compared with normal brain tissue [44]. Further, PERK silencing suppressed cell viability of U87 and U251 cells *in vitro* and a functional PERK branch was found to be important for cell survival under low glucose conditions. PERK-dependent Akt phosphorylation and translocation of the glycolysis regulating Hexokinase 2 (HK2) to the mitochondrial outer membrane was responsible for cell survival, since EGF that was also able to activate Akt and HK2 in the GBM cells could rescue cell survival under low glucose. Finally, the authors showed that PERK silencing in U87 cells resulted in significant decreased tumor formation capacity upon subcutaneous implantation in mice, suggesting a possible important role for PERK in glioma progression [44]. This

in addition to the IRE1 branch that was more extensively studied in this context as discussed above in 3.2..

Together these findings indicate that UPR activity in GBM is significantly elevated when compared to normal tissue and contributes to GBM development. This provides a therapeutic window for designing novel UPR targeting strategies and the development of novel treatments for this deadly disease.

4. The UPR as a therapeutic target in GBM

4.1. Conventional treatments and ER stress

GBM is known to be highly resistant to traditional chemotherapy and radiotherapy. Interestingly, the cytotoxic effects of these standard treatments were found to depend at least in part on the UPR response. For example, the frequently used DNA alkylating drug temozolomide (TMZ) appeared to induce BiP/GRP78 and CHOP, and siRNA mediated knockdown of BiP/GRP78 expression in GBM cell lines *in vitro* enhanced CHOP activation and sensitized for TMZ [40]. Furthermore, treatment with epigallocatechin 3-gallate (EGCG), a green tea derived inhibitor of BiP/GRP78 that targets its ATP-binding domain, sensitized glioma cells *in vitro* to TMZ. In a follow up study employing U87 and U251 intracranial mouse models, EGCG alone did not affect tumor growth but enhanced the therapeutic effect of TMZ [45]. Another ER stress regulated protein, Prolyl 4-hydroxylase, beta polypeptide (P4HB), also was demonstrated to affect sensitivity to TMZ. P4HB is a multifunctional protein acting as an ER stress inducible molecular chaperone with disulphide isomerase activity. P4HB expression has been associated with TMZ resistance and recurrent GBM, and ectopic overexpression or siRNA silencing was demonstrated to results in resistance or sensitivity to TMZ, respectively, in GBM cell lines [46].

Radiotherapy-induced cell death in GBM cells was shown to be in part mediated by ER stress involving the PERK and IRE1 branches [47]. Furthermore, enhancing ER stress by hypoxia and celecoxib, a COX-2 inhibitor also known to activate ER stress, enhanced sensitivity for radiotherapy. In GBM xenograft mouse models concomitant treatment with tunicamycin also sensitized for radiotherapy [48]. Moreover, in the

same study tunicamycin-dependent inhibition of N-linked glycosylation interfered with trafficking of EGFR and MET receptors to the cell membrane, which was earlier linked to reduced receptor signaling through the Akt survival route, resulting in enhanced radiosensitivity [49].

4.2. Apoptosis targeted therapy and ER stress

The efficacy of a number of apoptosis targeting agents in GBM was also reported to involve the UPR. The activity of TRAIL was synergistically enhanced by Amiodarone, a Ca^{2+} ion channel inhibitor, in various glioma cells [50]. Examination of the underlying mechanism revealed that augmented intracellular Ca^{2+} levels by Amiodarone resulted in CHOP accumulation and subsequent enhancement of TRAIL-receptor 2 (TRAIL-R2) expression and apoptosis. The protease inhibitor Nelfinavir and the polyether ionophore antibiotic Monensin in a similar way could increase TRAIL-R2 expression and sensitize for TRAIL-induced apoptosis [51,52]. In addition, Monensin also enhanced the proteasome-dependent degradation of c-FLIP, a well-known inhibitor of the TRAIL/TRAIL receptor, death-inducing signaling complex (DISC). Nefinavir, developed for treating HIV patients, triggers apoptosis via CHOP and the ER stress-associated caspase-4 in GBM cells [53]. The ER stress inducing agent 2,5-dimethyl-celecoxib (DMC) reduced viability of GBM cells and also sensitized for TRAIL-dependent cell death, however not by enhancing TRAIL-R2 expression but by enhancing caspase-8 activation and downregulating anti-apoptotic Survivin [54].

MDA-7/IL-24 is able to selectively induce apoptosis in cancer cells via multiple mechanisms including the activation of ER stress and, moreover, was found to directly interact with BiP [55]. In primary glioma cells, recombinant MDA-7 triggered apoptosis amongst others via PERK-dependent JNK1-3 phosphorylation and subsequent activation of BAX and the induction of mitochondrial apoptosis [56]. Moreover, MDA-7/IL24 was able to induce PERK-dependent autophagy that contributed to cell death. This was corroborated by another study showing that an autophagy-inducing drug enhanced the ability of MDA-7/IL-24 to kill primary GBM cells [57].

4.3. Other UPR targeting strategies

Several other agents, mostly natural products, were reported to potently activate the UPR and cause cytotoxicity in GBM models. Below some examples are provided.

The active component from marijuana, delta(9)-tetrahydrocannabinol (THC), was reported to induce cell death in GBM cells through stimulation of autophagy [58]. THC induced ceramide accumulation and subsequent activation of the UPR via eIF2α phosphorylation that promoted autophagy via tribbles homolog 3-dependent (TRB3-dependent) inhibition of mTORC1. Autophagy in this context activated apoptosis and was required for THC cytotoxicity *in vitro* and in a U87 intracranial model.

Piperlongumine, a natural plant alkaloid, showed preferential killing of human and mouse glioma cell cultures that was associated with enhanced ROS levels [59]. The cytotoxic effect of piperlongumine could be linked with ER stress induction via oxidative inactivation of peroxiredoxin 4 (PRDX4) an enzyme involved in ROS reduction that is overexpressed in high grade gliomas and mediates protein folding in the ER.

Perillyl alcohol (POH), a naturally occurring monoterpene, has cytotoxic effects in GBM cells that was at least in part due to the activation of ER stress since siRNA-mediated knockdown of CHOP significantly reduced apoptosis [60]. Notably, POH only enhanced TMZ-induced apoptosis in TMZ sensitive GBM cell lines. When combined with other ER stress–inducing cytotoxic drugs, celecoxib and nelfinavir, apoptosis was further enhanced in GBM cells independent of TMZ sensitivity. Intranasal administration of POH in a U251 intracranial mouse model resulted in significant survival benefit. In a follow up study the same group reported on the generation of a TMZ-POH conjugate, named NEO212, which was more potent than TMZ or combined POH/TMZ treatment in killing both TMZ sensitive and resistant GBM cells [61]. Recently, NEO212 was shown to target the GSC compartment 10-fold more effective than TMZ in newly generated patient-derived GBM cell lines.

The combined use of ER stress activators was demonstrated to enhance antitumor effects. For example, the proteasome inhibitor Bortezomib, which apoptosis-inducing activity can be partially linked to accumulation of damaged proteins and UPR activation, combined with Celecoxib or DMC resulted in enhanced UPR activation

and apoptosis in GBM cells [62]. This was mediated by elevated induction of CHOP and JNK1/caspase-4 activation upon combined treatment. This suggests that the combined use of agents that induce ER stress via different mechanism may potentiate each other.

Valproic acid (VPA), is a histone deacetylase (HDAC) inhibitor able to reactivate epigenetically silenced genes and is a promising therapeutic agent for treating cancer. A novel antitumor function of VPA involves the acetylation of BiP and in glioma cells VPA induced expression of BiP and CHOP together with the ER-embedded adaptor SEL1L that controls ERAD and has been linked with cell survival and cell fate decisions [63]. Knockdown of SEL1L together with VPA enhanced ER stress and apoptosis, and inhibited growth of neurosphere GBM cultures. A role for SEL1L as an adoptive mechanism implicated in the maintenance of stemness homeostasis in GSC was proposed.

Although, investigations in preclinical studies, as illustrated above, aggravators of ER stress currently have been not well explored in the clinic. A phase I study of nelfinavir with concurrent TMZ and radiotherapy has been conducted in GBM patients [64]. Although well tolerated, the possible clinical benefit remains to be demonstrated in follow up studies. The addition of amongst others celecoxib to conventional TMZ treatment of GBM patients in a phase II study was feasible but did not lead to detectable benefits [65]. A phase II study in which the HDAC inhibitor vorinostat was combined with bortezomib for treating recurrent GBM provided no clear benefit [66]. On the other hand, intranasal administration of POH in patients with recurrent GBM survived significantly longer than the untreated group [67]. Furthermore, the addition of VPA to common chemo-radiotherapy in newly diagnosed GBM patients appeared to have therapeutic benefit as well [68]. It should be noted that in the clinical studies mentioned above the agents were not administered with the purpose to elevate ER stress as a potential therapeutic strategy in GBM, but merely focused on other activities ascribed to these agents. Nonetheless, the findings provided hints of clinical activity of ER stress aggravators in GBM.

5. The UPR as a therapeutic target in GBM

Although a direct involvement of the UPR in gliomagenesis has not been demonstrated yet, the evidence reviewed here clearly indicates an important role for the UPR in GBM growth and progression (see also Figure 2).

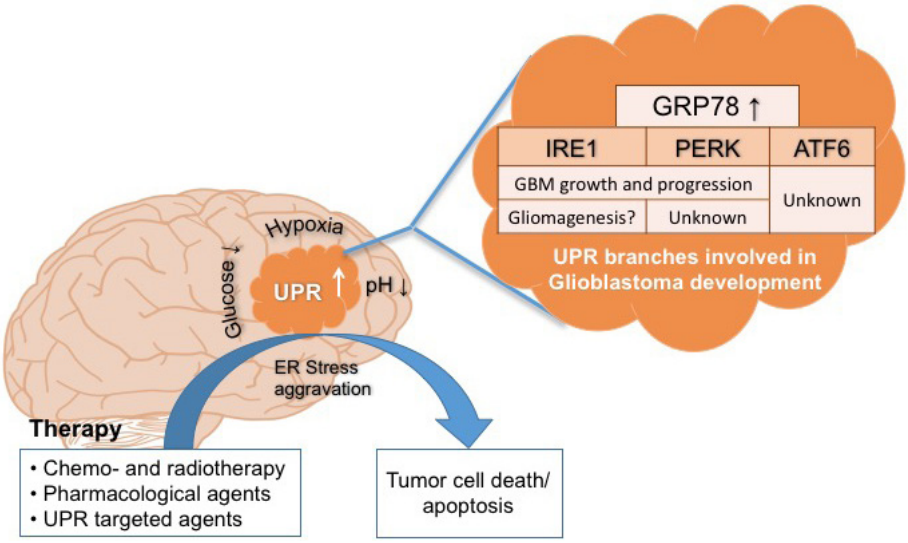

Figure 2: Schematic representation of the role of ER stress/ UPR in GBM. *The UPR, particularly the IRE1 and PERK branches have been implicated in GBM growth and progression, including angiogenesis, mesenchymal differentiation and invasion. Currently the possible involvement of the UPR in oncogenic transformation (gliomagenesis) is unknown. In GBM the activity of the UPR is elevated compared to normal brain tissue. This as a consequence of the harsh conditions in the tumor microenvironment (for example hypoxia, low glucose and low pH) leading to ER stress and UPR activation. The enhanced activity of the UPR provides a therapeutic window for therapies that aggravate ER stress and UPR activation. Conventional chemo-radiotherapy acts partially by enhancing UPR-dependent cell death. Several pharmacological agents and natural products are known to inflict ER stress and cytotoxicity in GBM cells. Novel designed agents that target specific the UPR (branches) may also be effective therapeutics. The involvement of the UPR in gliomagenesis and GBM progression has been studied only to a limited extent (See text for more details).*

Specifically, the IRE1 arm has been associated with angiogenesis and infiltrative growth in orthotopic GBM mouse models. However, overall the number of studies investigating the contribution of the UPR and the individual branches in GBM aggressiveness are

limited and have been mainly conducted in a few GBM cell line models. Studies in patient-derived cell culture models are required to support these findings. Additionally, more extensive and detailed studies are necessary to examine the involvement of the UPR branches and downstream effectors in GBM development and progression. The elevated levels of UPR activity in GBM samples of patients substantiate the view that GBM cells use this adaptive pathway to withstand the harsh conditions of the tumor microenvironment. Aggravation of ER stress by standard therapies and other pharmacological agents contributes to the induction of cell death. However, the clinical benefit of treatment with ER stress inducing agents remains to be better studied. Rather than using natural products with pleiotropic effects, including aggravation of ER stress, the development and testing of more specific UPR arm targeting molecules, such as small molecules targeting IRE1 or PERK [69], is warranted to examine the potential of the UPR as a promising targeted for therapy in GBM.

Conflict of interest

The authors declare that they have no conflict of interest.

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