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A systematic review on the potential of modulating the IRE arm of the UPR in U87 and U251 glioblastoma cells for improved therapeutic efficacy

Mehdi Ghavamizadeh[1,2,†], Roozbeh Kiani[1,2,†], Fatemeh Shams[2,3], Parmis Taghizadeh[1,2], Ali Honari-Jahromi[1,2], Seyed Mohammad Hossein Afzali-Joibari[4,5], Mozhdeh Zamani[6,*], Sanaz Dastghaib[3,*], Pooneh Mokarram[1,*]

1) Autophagy Research Center, Department of Biochemistry, Shiraz University of Medical Sciences, Shiraz, Iran
2) Student Research Committee, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3) Endocrinology and Metabolism Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
4) Nanomedicine and Nanobiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
5) Department of Medical Physics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
6) Autophagy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

ABSTRACT

Glioblastoma multiforme (GBM) remains the most aggressive primary brain tumor with poor prognosis and limited response to current therapies. Recent studies suggest that the unfolded protein response (UPR), particularly the inositol-requiring enzyme 1 (IRE1) signaling arm, plays a pivotal role in GBM pathophysiology by mediating cellular adaptation to endoplasmic reticulum stress. This systematic review evaluates the role of IRE1 in GBM cell lines (U87, U251) and investigates whether its activation or inhibition affects migration, proliferation, apoptosis, and cell death. A comprehensive search was conducted on PubMed/ Medline, Scopus, Web of Science, and Embase using various keywords up to October 14, 2025, following the PRISMA guidelines. The search aimed to identify original English-language studies that specifically examined and analyzed the IRE1 arm of the UPR pathway in glioblastoma cells. Out of 466 records, 26 studies met the inclusion criteria. Twenty studies explored IRE1 activation, while six investigated its inhibition. IRE1 activation yielded dual effects—promoting apoptosis via JNK or XBP1 pathways in some contexts, while supporting tumor survival and angiogenesis through XBP1-mediated transcription and RIDD suppression in others. Dual role of IRE1 could sensitize GBM cells to chemotherapy agents, reduced migration and proliferation, and induced apoptosis. IRE1 acts as a context-dependent regulator in GBM, showing both pro-survival and pro-death roles. Most studies report that IRE1 activation promotes glioblastoma cell death, while fewer address its inhibition. Thus, both activation and inhibition may offer therapeutic potential depending on cellular context and downstream signaling.

Keywords: Glioblastoma multiforme; Unfolded protein response; Inositol-requiring enzyme-1; XBP-1 protein; Therapeutics

†These authors have equally contributed to this work.
*Corresponding Authors: Autophagy Research Center, Department of Biochemistry, Shiraz university of Medical Sciences, Shiraz, Iran; Tel/Fax:+98 711 3230 3029; Email: Mozhdeh.zamani63@gmail.com, dastghaib@sums.ac.ir, AND mokaramp@sums.ac.ir

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INTRODUCTION

Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor, characterized by rapid progression and poor prognosis, with a median survival of 15 months despite advances in surgery, temozolomide (TMZ) chemotherapy, and radiotherapy. GBM frequently recurs due to its adaptability to microenvironmental stimuli and complex survival mechanisms. Multiple signaling pathways—including Wnt/β-Catenin, NF-κB, PI3K/AKT/mTOR, EGFR, TGF-β, and endoplasmic reticulum (ER) stress responses, unfolded protein response (UPR), and autophagy—contribute to resistance to cell death [1-3].

GBM cells use the UPR pathway to adapt to the ER stress. UPR-associated transcription factors regulate genes involved in angiogenesis, immune evasion, inflammation, autophagy, and apoptosis. Targeting ER stress and its sensory arms has shown therapeutic promise. The UPR, triggered by ER stress, involves three main sensors: Inositol-requiring enzyme 1 (IRE1), Protein Kinase R-like ER Kinase (PERK), and Activating transcription factor 6 (ATF6) [4]. IRE1, the most evolutionarily conserved, plays a central role in tumor biology. Upon UPR activation, IRE1 exerts RNase and kinase functions. Its RNase domain splices X-box binding protein 1 (XBP1) mRNA to generate spliced XBP1s, a transcription factor regulating UPR-related genes including; ER Chaperones, lipid biosynthesis, endoplasmic reticulum-associated degradation (ERAD) components, angiogenesis, and tumor progression [5, 6]. Under persistent ER stress, IRE1 shifts toward regulated IRE1-dependent decay (RIDD), which initially reduces misfolded protein burden by degrading ER-localized mRNAs. As stress intensifies, RIDD becomes pro-apoptotic by targeting mRNAs encoding survival proteins. Additionally, IRE1 activates the ASK1/JNK pathway via TRAF2 interaction, linking ER stress to apoptosis. RIDD counters XBP1-driven effects by degrading mRNAs involved in angiogenesis and immune cell recruitment. Elevated XBP1 promotes vascularization and immune infiltration, contributing to GBM aggressiveness, whereas strong RIDD activity correlates with reduced vascular and invasion markers (Fig. 1) [7, 8].

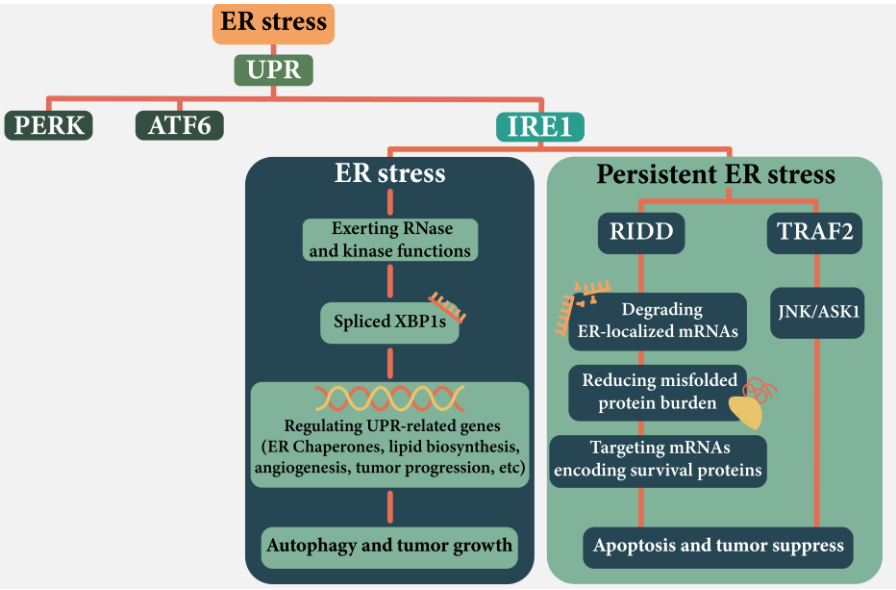

Figure 1: Schematic of IRE1 signaling under ER stress. IRE1 exhibits a dual role: under moderate ER stress, it promotes adaptive responses via XBP1 splicing, enhancing UPR-related gene expression, autophagy, and tumor growth. Under persistent or severe ER stress, IRE1 activates RIDD and TRAF2 pathways, leading to degradation of ER-localized mRNAs, reduction of misfolded protein burden, and induction of apoptosis and tumor suppression.

In summary, IRE1 functions as a molecular switch that determines whether GBM cells adapt to ER stress through XBP1s-mediated survival signaling or undergo apoptosis via RIDD activation or XBP1-induced CHOP expression under sustained stress. Overexpression of UPR components correlates with aggressive gliomas and increased sensitivity to ER stress-inducing

treatments. IRE1 acts as a pro-survival mediator in cancer, with tumor cells exploiting UPR hyperactivation to resist stress. Preclinical studies in cancers such as multiple myeloma, prostate, leukemia, and triple-negative breast cancer support IRE1 inhibition as a promising treatment approach alone or with standard therapies [9, 10]. It has been shown that IRE1 in particular plays a decisive role in tumorigenesis and tumor aggressiveness, as well as post-therapy response in, for example, cancers of the breast, pancreas, prostate, and brain [11].

In vitro studies utilizing GBM cell lines have been instrumental in dissecting the role of IRE1 [12]. The U87 and U251 cell lines are among the most commonly used models in GBM research. U87 cells, originally derived from a female patient in 1966, exhibit epithelial morphology and possess genetic aberrations such as PTEN loss and EGFR amplification [13], leading to large, well-demarcated tumors [14]. However, it is important to note that the commonly used U87MG cell line (from ATCC) was later found to be non-identical to its original patient source. U251 cells, on the other hand, harbor *p53* mutations and *PDGFRA* amplification, resulting in highly invasive tumor behavior [15]. These distinct genetic backgrounds and phenotypic characteristics make U87 and U251 valuable models for studying the diverse mechanisms of IRE1α signaling in GBM [16, 17]. Therefore, these two cell lines were selected to represent different molecular and phenotypic aspects of GBM—U87 as a model of less invasive but well-differentiated tumors, and U251 as a highly aggressive and invasive counterpart—allowing a more comprehensive evaluation of IRE1-related pathways.

According to the available published data, IRE1 signaling plays a dual role in GBM, balancing between tumor-promoting through XBP1 activity and tumor-suppressive by the RIDD pathway. This systematic review examines the effects of chemicals or genetic alterations on IRE1 activity and its consequences on cell survival, invasion, apoptosis, and other characteristics in GBM pathophysiology. Through investigations on U87 and U251 cell lines, we intend to explore how inhibiting the IRE1 branch of the UPR affects GBM progression and therapy resistance.

METHODS

Focused Question: "What is the specific function of the IRE1 arm of the UPR pathway in glioblastoma cells? Should this branch be activated or suppressed to inhibit cancer cell growth? What insights does targeting the IRE1 arm provide for cancer treatment? The reviewers followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist [18] (Supplementary File 1). It was registered in PROSPERO, the international database for prospectively registered systematic reviews in health and social care. (Registration ID: CRD420251041595)

Search Strategy: A systematic search without any time and language restriction was conducted on September 26, 2024, in electronic databases including PubMed/Medline, Scopus, WOS, and Embase to determine studies measuring the IRE1 arm in glioblastoma cells under various interventions. For our search, we employed a combination of MeSH and non-MeSH keywords, which included ("Glioma" OR "Glioblastoma" OR "GBM" OR "Glioblastomas") AND ("IRE1" OR "IRE" OR "ire1alpha" OR "inositol requiring enzyme 1 alpha"). Also, to verify a comprehensive literature search, we checked the reference list of available systematic reviews and meta-analyses and did a manual search in Google Scholar. Furthermore, an updated search was performed on October 14, 2025, to include any pertinent research released since the first search.

Eligibility Criteria: The inclusion criteria comprised original studies published in English that specifically assessed and investigated the IRE1 protein or RNA expression in U87 and U251 glioblastoma cell lines after various interventions and their outcomes. Studies evaluating the effects of upstream pathway manipulation via genetic modification or chemical compounds,

including molecules specifically designed to target IRE1 directly, were included. Review articles, unpublished manuscripts, gray literature, and studies on other cancer types or cell lines were excluded from evaluation. Studies that only assessed downstream markers of IRE1 activity, such as XBP1 splicing, without directly evaluating IRE1 activation or inhibition, were excluded. This was done to ensure that included studies specifically addressed modulators acting on IRE1 itself, rather than reporting indirect downstream effects**.** All bioinformatic studies that used RNAseq data from previous papers were excluded. All papers that only evaluated two simultaneous upstream manipulations (e.g., hypoxia and various gene expressions) in IRE1 positive and knockdown cells were excluded. Based on these criteria, titles and abstracts were screened by two reviewers (FS, and SD) independently. In the event of a disagreement between the two reviewers over an article's eligibility, a third expert reviewer (RK, MG, and PT) adjudicated its inclusion.

Data extraction and risk of bias assessment: Two reviewers (FS, and AHJ) extracted the following data from each study: author/year, compound/method, cell line/model, effect on IRE1 pathway, outcome, and analysis technique. The methodological quality and risk of bias of the included studies were assessed separately according to the type of experimental model used. For in vitro studies (using GBM cell lines), we used the Quality Assessment Tool for In Vitro Studies (QUIN) [19]. This tool looks at study design, use of controls, replicates, reporting quality, and statistical analysis. Since the QUIN tool is primarily intended for laboratory-based research, we introduced minor modifications to better align it with glioblastoma cell line studies, such as verifying cell line authentication and confirming the inclusion of both positive and negative controls. For animal studies, we applied the SYRCLE Risk of Bias (RoB) tool [20]. This is an adaptation of the Cochrane RoB framework specifically for animal experiments, and it covers several areas, including selection bias, performance bias, detection bias, attrition bias, and reporting bias. If a study had both in vitro and in vivo components, we evaluated each part separately with the appropriate tool. For both tools, a qualitative approach was used. Each study was evaluated across the relevant domains of the respective tool, and the overall judgment of bias was categorized as low, moderate, or high risk, depending on the number and significance of methodological limitations observed. Two independent reviewers (MZ, and PM) carried out all assessments in duplicate. Any disagreements were resolved through discussion until consensus was reached.

RESULTS

A total of 466 records were initially identified (PubMed: 91, Scopus: 149, WOS: 95, Embase: 131). After removing duplicates, 185 records (39.7%) persisted and were screened. Among them, 129 records had irrelevant titles and abstracts, resulting in 56 articles for further investigation. After a full-text review of the remaining articles, 30 papers were excluded for not meeting the inclusion criteria (e.g., studies that evaluated the pathway in cell lines other than U87 and U251). In summary, 26 papers (5.9%) met the criteria for inclusion in this systematic review. The detailed process of study selection is shown in Figure 2.

Within the pertinent records, 20 papers (77%) addressed the activation of the IRE1 pathway in GBM, whereas 6 studies (23%) investigated the outcomes and diverse methods of IRE1 pathway inhibition. The articles were published between 2010 and 2024. Eighteen studies utilized a single glioma cell line, while eight incorporated both types of cell lines and xenografted mouse models.

In order to account for in vitro and animal studies, we considered the methodological assessment criteria differently and evaluated the quality of in vitro studies based on a specific QUIN (Quality In Vitro) tool, and the quality of animal in vivo studies based on the SYRCLE RoB tool. In the total of 37 components that we considered, 26 in vitro and 11 in vivo animal components, the overall risk of bias was 24 works (65%) at low risk, 12 works (32%) at

moderate risk, and 1 work (3%) at high risk. Most of the studies in QUIN that were assessed (in vitro) also received a low-risk rating (88%), indicating the clarity with which all the experimental conditions, controls, replicates, and statistical analyses were reported. On the contrary, almost all of the SYRCLE rated studies (in vivo) received a moderate risk rating (82%) because of poor reporting of randomization, allocation concealment, and blinding of outcome assessment, and in some instances poor reporting of sample sizes or attrition. The deficiencies such as random allocation of animals are evident in both in vivo and in vitro studies and merit the introduction of appropriate precautions. The other frequently unreported issue is the lack of reporting on blinding, which is of concern, especially in in vivo studies. Incomplete items related to risk report are considered to weaken the animal experiments. The below summary Table (Table 1) indicates the risk of bias assessment results and the detailed checklist for item-specific risk assessments of all included studies is provided in Supplementary file 2.

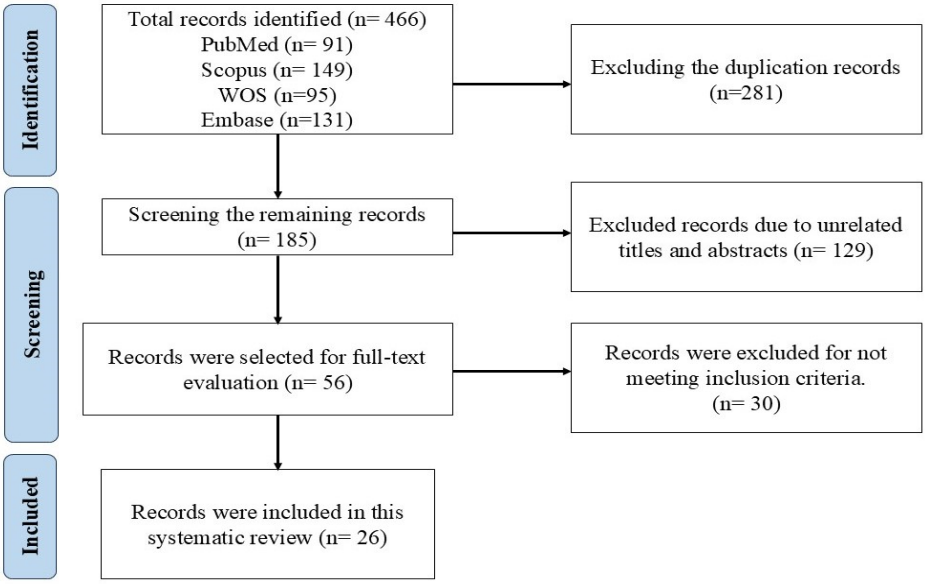

Figure 2: PRISMA flowchart.

Table 1: Summary results of risk of bias assessment of the included studies

Tool	Number	Low risk	Moderate risk	High risk
QUIN (in vitro)	26	23 (88%)	3 (12%)	0 (0%)
SYRCLE (animal)	11	1 (9%)	9 (82%)	1 (9%)
Overall	37	24 (65%)	12 (32%)	1 (3%)

Overall, inhibition of IRE1 via pharmacological or genetic approaches consistently enhanced apoptosis and reduced GBM cell survival, highlighting the therapeutic potential of targeting this pathway. Six studies showed that *IRE'* axis can be sensitized to apoptosis through their pharmacological or genetic inhibition, proving to be highly therapeutic (Table 2).

Güler et al. (2023) indicated that flavopiridol inhibited proliferation, colony formation, and migration in *IDH1*-wild-type and mutant U87 GBM cells. Mechanistically, flavopiridol inhibited the oncogenic transcription factor FOXM12. This repression of FOXM1 inhibited major stress regulators such as NF-κB), PARP, and major mediators of the unfolded protein response (UPR). Of these UPR mediators, flavopiridol markedly decreased IRE1 expression in a dose- and time-dependent manner. Notably, the study confirmed that IRE1 is downstream of FOXM1 signaling, as targeted siRNA knockdown of FOXM1 was also sufficient to suppress expression of IRE1. This generalized repression of FOXM1-dependent pathways (GRP78 and PERK) eventually enhanced apoptosis [21].

Table 2: Summary of inhibition studies detailing IRE effects, pathways, and outcomes in GBM models

Author / Year	Compound / Method	Cell Line / Model	Effect on IRE	Downstream Pathway	Outcome	Analysis Technique
Güler et al., 2023	Flavopiridol (24h (300 nM), 48h (100 and 200 nM) and 72h (50 and 100 nM)	U87 (IDH Wild-Type and IDH-Mutant)	↓ Expression	Inhibits FOXM1, NF-kB, UPR (IRE1, PERK, GRP78)	↓Proliferation, ↑Apoptosis, ↓Migration	Western blot, MTS assay, wound healing, Annexin V, Hoechst, colony formation, siRNA knockdown
Kavitha et al., 2015	Asiatic Acid	U87/nude mice both ectopic and orthotopic xenograft	↓ Expression	↓IRE1/Calnexin, ↑GRP78/Calpai, ER stress	↑Apoptosis, ↓Viability (better than TMZ)	Western blot, calcium assay, Annexin V, EM, xenograft, imaging
Doultsinos et al., 2021	MTX, Folinic Acid, Cefoperazone, Fludarabine	U87	↓phosphorylation through inhibition of kinase and RNase	↓P-IRE1, Inhibit XBP1 splicing (UPR)	↑TMZ sensitivity, ↑Apoptosis, ↓survival	Western blot, RNA cleavage assay, WST1, MST binding, qRT-PCR, docking/screening
Pelizzari-Raymundo et al., 2023	Z4, Z4P	Both / orthologous tumor of U87	↓Phosphorylation through inhibition of kinase	↓IRE1-P, ↓XBP1 splicing, ↓RIDD, preserve SPARC	↑TMZ efficacy, ↑Apoptosis, Full tumor regression, ↓ Cell Viability, ↓Invasion / Migration	Western blot, RNA cleavage, MST, qRT-PCR, migration, bioluminescence, kinome profiling
Bi et al., 2017	PRRT2 Overexpression, miR-30a-5p modulation	U87	Downregulation (mRNA/protein)	↓IRE1/XBP1, ↓PERK/ATF4, ↓ATF6/GRP78, ↓UPR	↑Apoptosis, ↓Viability, tumor suppression	Western blot, qRT-PCR, luciferase assay, CCK-8, caspase 3/7
Li et al., 2018	M1 virus+ IRE1 inhibitor (STF083010, KD)	U87, U251	Inhibition of IRE activity	↓ Autophagy, ↓ XBP1 splicing (UPR)	↑ Viral protein accumulation, ↑ apoptosis, ↑ oncolysis, ↑ survival	Western blot, flow cytometry, autophagy inhibition, immunohistochemistry, xenograft model, viral titer

Asiatic acid, a plant-derived triterpenoid, induces ER stress-mediated apoptosis in U87MG, LN18, and U118MG cells by inhibiting IRE1 and Calnexin and activating GRP78, Calpain, and intracellular calcium. This synergy disrupts ER homeostasis, shifts Bcl2 family protein dynamics towards pro-apoptotic signaling, and triggers mitochondrial dysfunction and caspase activation. Asiatic acid penetrated the blood-brain barrier in vivo and outperformed Temozolomide in orthotopic GBM models with enhanced tumor suppression [22].

In a drug repurposing approach, Doultsinos et al. (2021) indicated four FDA-approved drugs—Methotrexate, Folinic Acid, Cefoperazone, and Fludarabine Phosphate—to be IRE1 inhibitors that suppress both kinase and RNase activities in U87MG and patient-derived RADH87 cells. Inhibition of IRE1by these molecules suppressed *XBP1* splicing and impaired pro-survival UPR signaling. In combination with Temozolomide, they reduced its effective IC50 and increased apoptosis, especially under ER stress [11].

By direct inhibition of *IRE1*, Pelizzari-Raymundo et al. (2023) identified Z4 and blood-brain barrier-permeable Z4P as novel IRE1 kinase inhibitors in U87MG, U251, and primary GBM cells. Drugs reduced IRE1 phosphorylation, *XBP1* splicing, and RIDD activity. Combination therapy of Z4P with Temozolomide caused complete tumor regression and relapse prevention in orthotopic models, with cell migration also severely repressed [23].

The *PrRt2* (Proline-Rich Transmembrane Protein 2) is a tumor suppressor in U87MG and A172 glioblastoma cells by inhibiting UPR. When *PRRT2* is overexpressed, all three arms (*IRE1–XBP1*: down-expression *IRE1, XBP1; PERK–ATF4*: down-expression *PERK, ATF4; ATF6–GRP78*: down-expression *ATF6, GRP78*) of the UPR pathway are suppressed, leading to compromised cell survival (CCK-8 assay) and forced caspase activation (3/7 activation). *PRRT2* is inversely correlated with UPR effectors in glioblastoma specimens. Furthermore, the

oncogenic action of miR-30a-5p is mediated via direct inhibition of *PRRT2* (luciferase assay), which contributed to tumor growth [3].

ER stress caused by M1 virus in glioblastoma initiates the IRE1-XBP1 pathway, promoting autophagy and limiting oncolytic activity. Inhibition of *IRE1* by STF083010 or gene knock-down suppressed autophagy, enhanced apoptosis, and significantly promoted viral antitumor effects in vitro and in vivo. The results demonstrate the potential of *IRE1-XBP1*-autophagy pathway inhibition to enhance virotherapy for glioblastoma [24]. Collectively, these studies indicate that IRE1 inhibition converges on XBP1 and JNK pathways, leading to enhanced apoptosis in both U87 and U251 cells. Of the included articles, 20 studies specifically focused on the activation or upregulation of IRE1, employing a variety of interventions such as small molecules, genetic manipulations, and environmental stressors (Table 3). The findings, detailed below, highlight the diverse cellular outcomes of IRE1 activation in GBM models and its potential implications for the tumor microenvironment.

Table 3: Summary of activation studies detailing IRE effects, pathways, and outcomes in GBM models

Author/Year	Compound / Method	Cell Line / Model	Effect on IRE	Downstream Pathway	Outcome	Analysis Technique
Chang/2020	Aspirin (500μM)	U87MG	↑Phosphorylation	Noxa, Bax/Bak	Apoptosis	Western blot, DCFH-DA
Chang/2021	Gefitinib (10μM)	U87MG, H4	↑Phosphorylation	ROS/JNK/Noxa	Apoptosis	Western blot, MTT
Adamczyk/2023	Doxorubicin + DNMT2KO	U251MG	↑Phosphorylation	Caspase-3	Apoptosis	Western blot, MTT
Yoo/2014	Bortezomib (100nM)	U251T3, LN229	↑Expression	HSP90	HSV-1replication	Western blot, Plaque assay
Zhang/2022	HA15 + TAK-243	U87, LN229	↑Activity	XBP1s, TRAF2-JNK	Apoptosis	RT-qPCR, Flowcytometry
Kim/2010	SNAP (500μM)	U87MG, CRT-MG	↑Expression	XBP1s, CREB	Apoptosis	Western blot, TUNEL
Jung/2021	Miconazole (20μM)	U251MG, U87MG	↑Activity	ROS, LC3-II	Autophagic cell death	Western blot, TEM
Hwang/2010	Glucosamine (5mM)	U87MG	↑Expression	LC3-II, Beclin-1	Autophagic cell death	Western blot, MTT
Cui/2023	S4 (10μM)	U87MG, LN229	↑Activity	XBP1s, CRT, HSP70	Immunogenic cell death	Flowcytometry, ELISA
Zhang/2015	Sulphureuine B (20μM)	U87MG	↑Activity	XBP1s, Caspase-12	Apoptosis	Western blot, Trypan blue
Li/2024	SelK knockdown	U87MG, U251	↑Phosphorylation	Caspase-3	Apoptosis	Western blot, BrdU
Lu/2012	BFP (10μM)	U251, U87	↑Expression	ROS	Apoptosis	Western blot, Annexin V/PI
Oztatlici/2024	4-HC (50μM)	U87MG, T98G	↑Phosphorylation	Caspase-3/9	Apoptosis	Western blot, MTT
Dastghaib/2020	Simvastatin + TMZ	U87MG, U251	↑Activity	XBP1s, GRP78	Apoptosis	RT-qPCR, Trypan blue
Shen/2014	Ursolic Acid (25μM)	U87MG	↑Activity	JNK, AMPK-mTOR	Autophagic cell death	Western blot, IF
Lo/2020	Betulinic Acid (20μM)	U87MG, A172	↑Expression	XBP1s, CHOP	Apoptosis	Flowcytometry, Western blot
Wang/2024	Nano-hydroxyapatite	U87MG	↑Expression	CHOP, CRT, HSP70	Apoptosis, ICD	Western blot, JC-1
Xiu/2022	Curcumin + Radiation	U251MG, GL261	↑Activity	XBP1s, PERK-eIF2α	Apoptosis, ICD	Western blot, IF
Feng/2020	PLP2 knockdown	U87MG, U251	↑Expression	XBP1s, CHOP, LC3-II	Apoptosis, Autophagy	Western blot, TEM
Bobak/2016	Arginine deprivation	U251	↑Activity	XBP1s, LC3-II, p62	Autophagic cell death	Western blot, blue

Fourteen studies reported that IRE1 activation induced apoptosis in GBM cells. Chang et al. (2020) demonstrated that Aspirin upregulated IRE1 phosphorylation in U87MG cells, triggering mitochondrial apoptosis through the activation of Noxa and Bax/Bak [25]. Similarly, Chang et al. (2021) found that Gefitinib enhanced IRE1 activity in U87MG and H4 cells, promoting apoptosis via the ROS/IRE/JNK/Noxa axis [26]. Doxorubicin combined with DNA methyltransferase 2 (DNMT2) knockout in U251MG cells increased IRE1 phosphorylation, leading to apoptosis under endoplasmic reticulum (ER) stress conditions [27]. Zhang et al. (2022) showed that combining HA15, a GRP78 inhibitor, with TAK-243, a ubiquitin-activating enzyme 1 (UBA1) inhibitor, enhances ER stress in U87 and LN229 glioblastoma cells. HA15

disrupts GRP78-mediated protein folding, while TAK-243 blocks UBA1, increasing misfolded protein accumulation. This synergy activates the IRE1 pathway, inducing apoptosis via XBP1 splicing and tumor necrosis factor receptor-associated factor 2 (TRAF2)-c-Jun N-terminal kinase (JNK) signaling [28]. In another study, SNAP upregulated IRE1 in U87MG and CRT-MG cells, resulting in apoptosis mediated by XBP1 and CREB phosphorylation [29]. Sulphureuine B activated IRE1 in U87MG cells, promoting apoptosis via XBP1 and caspase-12 [30]. SelK knockdown in U87MG and U251 cells enhanced IRE1 activity, leading to apoptosis and reduced proliferation under ER stress [5]. Lu et al. (2012) showed that BFP upregulated IRE1 in U251 and U87 cells, inducing apoptosis through ROS-mediated ER stress [31]. Cyclophosphamide (4-HC) increased IRE1 phosphorylation in U87MG and T98G cells, driving apoptosis via caspase-3 activation [32]. Simvastatin combined with TMZ activated IRE1 in U87MG and U251 cells, resulting in cell death through XBP1 splicing [7]. Betulinic Acid in U87MG and A172 cells upregulated IRE1, promoting apoptosis via XBP1 and CHOP [11]. Nano-hydroxyapatite enhanced IRE1 expression in U87MG cells, inducing apoptosis through ER stress and mitochondrial disruption [33]. Curcumin combined with ionizing radiation activated IRE1 in U251MG and GL261 cells, leading to apoptosis alongside immunogenic cell death [34]. Lastly, PLP2 knockdown in U87MG and U251 cells upregulated IRE1, triggering both apoptosis and autophagy via XBP1 and CHOP [35]. In summary, IRE1 activation predominantly induces apoptosis in GBM cells via XBP1, JNK, and CHOP-mediated pathways, confirming a conserved pro-apoptotic role across multiple studies

Five studies identified IRE1 activation as a driver of autophagic cell death in GBM. Jung et al. (2021) reported that Miconazole activated IRE1 in U251MG and U87MG cells, inducing autophagic cell death through ROS and ER stress, as evidenced by increased LC3-II levels [26]. Glucosamine upregulated IRE1 in U87MG cells, promoting autophagic cell death via ER stress [36]. Shen et al. (2014) found that Ursolic Acid activated IRE1 in U87MG cells, leading to autophagic cell death through the IRE/JNK and CaMKK-AMPK-mTOR pathways [37]. PLP2 knockdown, as noted earlier, also induced autophagy alongside apoptosis in U87MG and U251 cells via IRE1 activation [35]. Additionally, Bobak et al. (2016) demonstrated that arginine deprivation in U251 cells activated the IRE1-XBP1 axis, resulting in ER stress and autophagic responses that reduced cell survival [38]. Overall, IRE1 activation can also trigger autophagic cell death through ER stress-mediated pathways, including JNK and AMPK-mTOR signaling, highlighting a secondary cell death mechanism.

The induction of immunogenic cell death by IRE1 involves coordinated mechanisms through both canonical UPR pathways and immune-related signaling cascades [39, 40]. IRE1 activation triggers XBP1 mRNA splicing, generating the transcriptionally active XBP1s isoform that facilitates calreticulin translocation from the ER lumen to the cell surface, a critical "eat-me" signal for dendritic cell recognition [41, 42]. Concurrently, IRE1 kinase activity recruits TRAF2, activating the JNK pathway that promotes mitochondrial dysfunction and release of damage-associated molecular patterns including ATP and high mobility group box 1 **(**HMGB1) [43, 44]. Importantly, crosstalk between IRE1 and the PERK-eIF2α axis amplifies immunogenic signaling, as eIF2α phosphorylation is essential for sustained calreticulin exposure during ICD [45, 46]. This integrated response, combining XBP1s-mediated transcriptional programs with PERK-dependent translational control, creates a cellular environment permissive for DAMP emission and immune activation (Fig. 3) [47, 48].

Three studies linked IRE1 activation to immunogenic cell death (ICD) in GBM. Cui et al. (2023) showed that S4 activated IRE1 in U87MG and LN229 cells, inducing ICD characterized by increased calreticulin (CRT) and HSP70 expression [49]. Xiu et al. (2022), as mentioned above, reported that Curcumin combined with ionizing radiation in U251MG and GL261 cells activated IRE1, promoting ICD through XBP1 splicing and PERK-eIF2α signaling, enhancing anti-tumor immune responses [34]. Wang et al. (2024) also noted that Nano-hydroxyapatite in U87MG cells upregulated IRE1, leading to ICD alongside apoptosis, with elevated CRT and HSP70 levels [33]. Collectively, these studies demonstrate that IRE1 activation promotes

immunogenic cell death (ICD) via XBP1s, CRT exposure, and PERK-eIF2α crosstalk, enhancing anti-tumor immune responses.

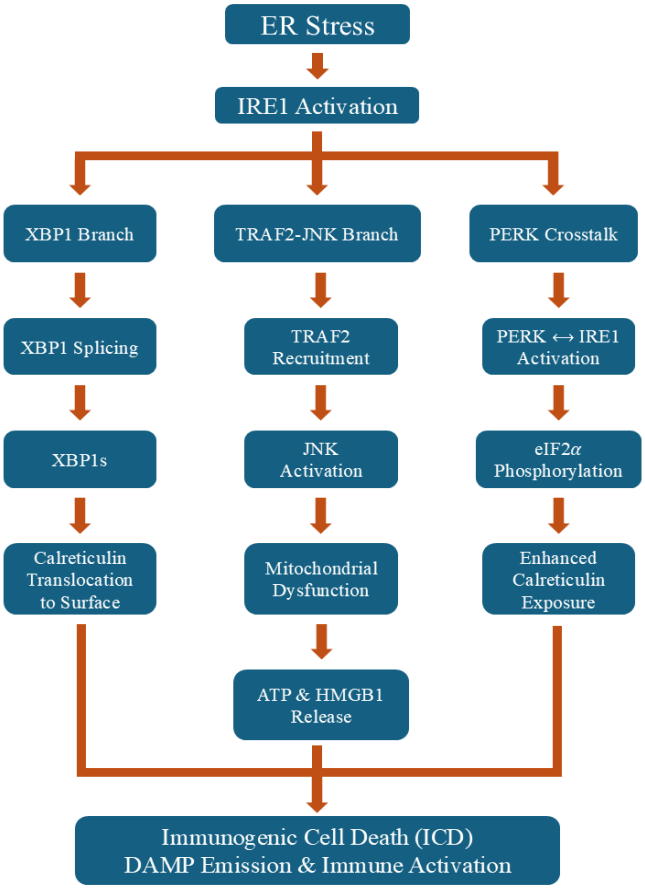

Figure 3: Schematic representation of IRE1-mediated pathways leading to immunogenic cell death (ICD). ER stress activates IRE1, triggering XBP1, TRAF2–JNK, and PERK crosstalk pathways. These events promote calreticulin exposure, ATP/HMGB1 release, and DAMP emission, culminating in immune activation and ICD.

Besides canonical cell death pathways, IRE1 activation can mediate unique outcomes, such as oncolytic virus enhancement, expanding the functional spectrum of this pathway. One study reported a unique outcome of IRE1 activation. Yoo et al. (2014) found that Bortezomib upregulated IRE1 in U251T3 and LN229 cells, enhancing HSV-1 replication and improving anti-tumor effects through an oncolytic mechanism involving HSP90 [50].

Table 4 presents the number of studies in which activation of the IRE1 signaling pathway, through various mechanisms, leads to different types of cell death, including apoptosis, autophagic cell death, immunogenic cell death, and specific effects observed in HSV-1 infection. It is noteworthy that some articles reported the involvement of more than one cell death pathway simultaneously.

Table 4: Categorization of cellular outcomes from IRE Activation studies in GBM

Outcome	Number of Studies	Compounds / Methods	Pathways	Models Used
Apoptosis	14	Aspirin, Gefitinib, HA15 + TAK-243	XBP1s, JNK, CHOP, ROS	U87MG, U251, LN229
Autophagic Cell Death	5	Miconazole, Ursolic Acid, Glucosamine	LC3-II, JNK, AMPK-mTOR	U87MG, U251
Immunogenic Cell Death	3	S4, Curcumin+Radiation, Nano-HA	XBP1s, CRT, HSP70	U87MG, LN229, GL261
Specific Effects (HSV-1)	1	Bortezomib	HSP90	U251T3, LN229

DISCUSSION

This review focuses on the IRE1 branch of the UPR and how it affects the fate of U87 and U251 glioblastoma cells. It does so by looking at the available research (Tables 2-4). Evidence shows that both activating and inhibiting IRE1 can make cells less viable and cause more cell death in various GBM models. This suggests that IRE1 has a complicated and context-dependent role in tumor biology [5, 11, 21, 24-27, 35].

Most of the studies that have been published recently have found that different inducers can activate the IRE1 pathway, which leads U87 and U251 glioblastoma cells to die. This could be a possible strategy to treat GBM [11, 30, 33, 38]. In this regard, IRE1 activation led to the death of GBM cells by chemicals such Aspirin, Gefitinib, Miconazole, Glucosamine, S4, BFP, Ursolic Acid, Simvastatin+TMZ, and Curcumin, which are also shown in Table 3 [7, 25-27, 31, 34, 36, 37, 49].

A possible mechanism underlying these effects could be that IRE1 activation is known to initiate a canonical adaptive response through its endoribonuclease (RNase) activity, which mediates the unconventional splicing of XBP1 mRNA [51]. The spliced form, XBP1s, drives the transcription of genes involved in protein folding, secretion, and ER-associated degradation (ERAD), thereby promoting cell survival under ER stress [52]. However, prolonged or excessive activation of IRE1, especially in the context of unresolved ER stress induced by the aforementioned pharmacological agents, may alter its function to promote cell death. This change is generally caused by regulated IRE1-dependent decay (RIDD) of certain mRNAs and microRNAs, which breaks down transcripts that are necessary for cell survival and growth [53]. Additionally, IRE1 can activate the c-Jun N-terminal kinase (JNK) pathway through its association with TRAF2, initiating apoptotic signaling. So, if IRE1 is active for too long or too much, it could push the cells past the adaptive threshold and start intrinsic apoptotic pathways [54].

Conversely, fewer studies highlighted that inhibition of IRE1, either pharmacologically or genetically, has also been reported to induce cell death in U251 and U87 cells [3, 11, 21-24]. This may be attributed to the loss of its pro-survival signaling, particularly the abrogation of XBP1s-mediated transcriptional programs that are crucial for maintaining ER homeostasis and metabolic adaptation in the tumor microenvironment. In this regard, Flavopiridol, Asiatic acid, MTX, Folinic Acid, Cefoperazone, Fludarabine and, Z4, Z4P are such compounds, which mentioned earlier (Table 2) and led to GBM cell death through IRE1 inhibition [11, 21-23].

The possible reason for these opposite effects could be that the IRE1-XBP1 axis is important for keeping tumors growing in GBM cells, which commonly live in low-oxygen and low-nutrient environments. Blocking this route can cause unresolved ER stress, a buildup of misfolded proteins, and more oxidative stress, which can eventually kill cells by apoptosis or other controlled cell death pathways [5]. This aligns with prior research indicating that the regulation of UPR pathways through various molecular mechanisms may present effective anticancer therapies by impeding tumor growth and progression [55].

The duality role of the IRE1's manipulation may reflect differences in temporal dynamics, ER stress levels, or the interplay with other UPR arms such as PERK and ATF6. For example, transient or mild ER stress might favor the adaptive, pro-survival functions of IRE1, while chronic or acute stress could enhance its cytotoxic outputs [56].

Additionally, the cell type–specific context, including genetic background, mutational status (e.g., p53, PTEN) [57], and metabolic dependencies of U251 versus U87 GBM cells, may further modulate the cellular response to IRE1 signaling [58]. Method of IRE1 modulation is also important, since some pharmacological agents might have off-target effects or affect various domains of IRE1 differently (e.g., kinase inhibitors vs. RNase inhibitors) [59, 60].

Another possible explanation lies in the concept of noncanonical functions of IRE1. Beyond its classical roles in XBP1 splicing and RIDD, IRE1 has been implicated in various signaling networks that influence tumor progression, immune evasion, and inflammation [61]. These

pleiotropic roles suggest that the cellular outcome of IRE1 modulation may depend not only on its intrinsic enzymatic activity but also on how it's signaling interfaces with oncogenic or tumor-suppressive pathways.

IRE1 signaling is very important for shaping the TME since it controls angiogenesis, immunological evasion, and inflammation. IRE1 activation is an important part of the UPR that helps tumor cells deal with stress and changes the TME to help the tumor grow. In terms of angiogenesis, IRE1 signaling boosts the growth of blood vessels by increasing the levels of pro-angiogenic molecules like vascular endothelial growth factor (VEGF). This encourages neovascularization, which gives tumors the nutrients and oxygen they need to develop and stay alive. IRE1 signaling helps make an immunosuppressive TME, which helps the immune system avoid detection. It changes the levels of immunological checkpoint molecules and affects how immune cells like tumor-associated macrophages (TAMs) are recruited and polarized. This favors phenotypes that weaken effective anti-tumor immunity and helps tumors evade immune surveillance. In terms of inflammation, IRE1 activation controls the generation of cytokines and chemokines that cause long-term inflammation in the TME. This inflammatory environment promotes tumor development and progression by creating a favorable habitat for cancer cells and inhibiting anti-tumor immune responses [62].

So, IRE1's pro-survival and pro-tumoral functions are dominated by crosstalk with inflammatory and growth factor signaling. For instance, IRE1 promotes the NF-κB pathway by interacting with TRAF2, which recruits IKB kinases (IKK) and promotes nuclear translocation of NF-κB [63]. It is also activated via an IRE1/UBE2D3/MIB1 signaling axis recently demonstrated to control NF-κB-dependent production of inflammatory chemokines that result in recruitment of myeloid cells into the tumor [64]. A unique pro-survival response also takes place with the interaction of IRE1 and EGFR signaling; the IRE1-induced JNK pathway can stabilize the EGFR ligand epiregulin (EREG) and result in an autocrine activation loop of EGFR and tumor growth [14]. IRE1 signaling is also connected to PI3K/AKT/mTOR signaling since GRP78, which regulates IRE1, affects PI3K/Akt/mTOR signaling to support radiation resistance in GBM [65].

IRE1's interactions with its own downstream targets and other parts of the UPR also control this balance between life and death in the cell. On the side that causes death, IRE1 can work with TRAF2 to turn on JNK, which makes ER stress a direct cause of apoptosis [66]. JNK activation could be converted into autophagic cell death by the AMPK-mTOR pathway [37]. The interaction with the other two UPR sensors, PERK and ATF6, is also very important. Inhibition of all three branches of the UPR simultaneously has been demonstrated to impede glioma cell proliferation; however, the inhibition of the IRE1 branch alone may result in the compensatory activation of alternative survival pathways such as PERK or ATF6 [67-70].

Taken together, these findings underscore the context-dependent nature of IRE1 signaling in GBM and suggest that both its hyper activation and inhibition can compromise tumor cell survival, albeit by distinct mechanisms. This dual role presents both a challenge and an opportunity in designing therapeutic strategies targeting the IRE1 arm of the UPR. Further studies dissecting the downstream effectors of IRE1 and the cellular conditions that determine its pro-survival versus pro-death outputs will be critical for the rational development of IRE1-targeted therapies in glioblastoma. So, IRE1 signaling orchestrates a multifaceted influence on the tumor microenvironment, fostering angiogenesis, enabling immune evasion, and sustaining inflammatory conditions that drive tumor aggressiveness.

More study is needed to understand what IRE1 does in GBM biology and to find biomarkers that can help doctors better classify patients and improve treatment outcomes. Significantly, evidence highlights IRE1 activation as a principal initiator of several types of cell death in GBM cells, especially in the context of ER stress. To implement these findings in clinical practice, comprehensive evaluation via clinical trials is needed to determine the safety and efficacy of IRE1-targeted medicines among various patient populations.

A primary obstacle in translating systemic therapies into effective treatments for GBM is the formidable blood-brain barrier (BBB), which restricts the entry of many promising

compounds into the central nervous system. The BBB permeability varies among several compounds reviewed here. Asiatic acid, Z4, Z4P and Flavopiridol demonstrates effective brain penetration [21-23]. In contrast, Methotrexate, Folinic acid, and Cefoperazone exhibit poor BBB penetration without specialized delivery [71-73]. Beyond the BBB, the GBM's adaptive microenvironment including immune infiltration and angiogenesis, is additional challenges.

Furthermore, the therapeutic challenge is compounded by the significant tumoral heterogeneity characteristic of GBM [74]. Even within a single tumor, different cell populations may exhibit varying dependencies on the UPR and the IRE1 pathway. This genetic and phenotypic diversity suggests that a subpopulation of cancer cells might be intrinsically resistant to IRE1 inhibition, potentially leading to treatment failure and tumor recurrence. Therefore, patient stratification based on biomarkers of IRE1 dependency could be essential for the success of such targeted therapies. Another key concern is the potential for on-target, off-tumor toxicity. The IRE1 pathway is not cancer cell-specific; it is a fundamental homeostatic mechanism in normal tissues [75]. Systemic inhibition of such a fundamental pathway has the potential to lead to undesirable side effects, and a balance between anti-tumor activity and systemic toxicity will be a key challenge in clinical development. Notably, most of the evidence presented in this review is based on preclinical studies employing in vitro cell cultures and animal models. While such models are valuable for understanding mechanisms, they fail to fully capture the heterogeneity of human GBM. Translating these promising preclinical results into true clinical reality requires rigorous confirmation by well-executed human clinical trials. It is significant that, owing to the bifunctional characteristics of IRE1 and the various downstream signaling pathways that may be initiated upon its activation, both the activation and inhibition of IRE1 could serve as viable therapeutic approaches in glioblastoma cells. Nonetheless, additional research is necessary to elucidate these pathways.

A significant focus for future research is the development of more selective IRE1 modulators. Creating compounds that only bind to the kinase or RNase domain of IRE1 could lead to a more targeted treatment plan. Additionally, the development of predictive biomarkers is essential for the clinical applicability of IRE1-targeted therapeutics. Looking at genomic, transcriptomic, or proteomic signatures (like the initial expression levels of important UPR elements like GRP78, IRE1 phosphorylation, or the ratio of spliced to unspliced XBP1) can help find the patients who are most likely to benefit from these treatments. This is a step toward a personalized era in GBM. Finally, it is most important to break through the long-standing barrier of the blood-brain barrier. New ways to distribute drugs need to be looked into in future studies. This will be an important part of the process for getting the right amount of drugs to the tumor microenvironment and getting the desired impact.

This systematic review highlights the therapeutic potential of targeting the IRE1 arm of the UPR in GBM. Both inhibition and activation of IRE1 have demonstrated anti-tumor effects through distinct mechanisms, including apoptosis, autophagic cell death, and immunogenic cell death. Based on the currently available literature, most studies have reported that activation of the IRE1 pathway by various inducers promotes glioblastoma cell death, while fewer data exist regarding its inhibition. These findings suggest that the divergent outcomes cannot be solely attributed to the genetic background of GBM cell lines but are also influenced by experimental conditions, tumor microenvironment, and crosstalk with other pathways such as TRAF2–JNK, PERK–eIF2α, and XBP1. Overall, IRE1 functions as a context-dependent regulator whose dual roles may offer complementary therapeutic opportunities. Nevertheless, most of the current evidence remains preclinical, and further studies are needed to clarify these mechanisms and evaluate their translational potential in vivo.

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Authors' Contribution: SD, MZ, and PM designed the study. FS, SD accessed pertinent literature sources. RK, MG, and PT screened the retrieved literatures. FS, and AHJ extracted data from included studies. MG, PT, SMHA-J and AHJ drafted the manuscript. MZ, SD and PM revised the manuscript. All authors read and approved the final manuscript.

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