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Original Article

Ferroptosis-related gene regulation and miRNA/lncRNA Network on Temozolomide Resistance in Glioblastoma

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ABSTRACT

Objectives: This study aimed to elucidate ferroptosis-associated transcriptomic and post-transcriptional regulatory networks underlying temozolomide (TMZ) resistance in glioblastoma (GBM) by integrating RNA-seq data with curated ferroptosis, microRNA (miRNA), and long non-coding RNA (lncRNA) interaction databases.

Materials and methods: We performed an integrative transcriptomic analysis of parental and TMZ-resistant U251 and U87 GBMcells to characterize ferroptosis-associated regulatory networks. Differential expression and enrichment analyses revealed distinct, cell line-specific ferroptosis-related transcriptional programs linked to TMZ resistance. By integrating curated ferroptosis genes with experimentally validated miRNA and lncRNA interactions, we constructed a high-confidence ferroptosis-centred competing endogenous RNA network.

Results: Ferroptosis-related differentially expressed genes associated with TMZ response were identified. Then, the top miRNAs and lncRNAs were determined to have the most regulatory connections with these genes. Key regulatory hubs, including miR-16-5p, miR-155-5p, KCNQ1OT1, and NEAT1, were identified as potential modulators of ferroptosis and TMZ resistance. We visualised key messenger RNA-miRNA-lncRNA pathways, such as KCNQ1OT1–miR-16-5p-CCND1/CDK6.

Conclusion: These findings highlight post-transcriptional regulatory layers underlying chemoresistance in GBM. Overall, this study provides important insights into the effect of TMZ on GBM cells via the ferroptosis axis.

Keywords: Chemoresistance, ferroptosis, glioblastoma, small RNA, temozolomide.

Glioblastoma (GBM) is the most aggressive and malignant primary brain tumor. It presents major treatment challenges due to its invasive nature, intratumoral heterogeneity, and strong resistance to standard treatments, such as temozolomide (TMZ).[1] This resistance is a key barrier to improving patient outcomes. Elucidating the molecular mechanisms underlying therapeutic response and resistance remains a major challenge in GBM research. Ferroptosis is a regulated, iron-dependent form of cell death caused by uncontrolled lipid peroxidation. It has become a potential vulnerability in GBM and offers a new approach to overcoming chemoresistance.[2,3]

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Iron metabolism is closely linked to sensitivity to ferroptosis, acting as a double-edged sword in GBM biology. Glioblastoma cells often handle iron poorly, which promotes tumor growth and helps them evade ferroptosis. Recent studies show that modifying iron-regulatory pathways can restore sensitivity in resistant cells. For example, iron regulatory protein 1 (IRP1)-mediated ferroptosis can reverse TMZ resistance by regulating the lipocalin 2 (LCN2)/ferroportin 1 (FPN1) axis in an the nuclear factor κB subunit 2 (NFKB2) -dependent manner.[4] Additionally, loss of coatomer protein complex I subunit zeta 1 (COPZ1) leads to nuclear receptor coactivator 4 (NCOA4)-mediated ferritinophagy and ferroptotic cell death in glioma.[5] Furthermore, iron availability influences tumor cell migration and epithelial-mesenchymal transition (EMT), integrating ferroptosis into the broader metabolic network of GBM.[6-8] Despite its potential, GBM cells employ various strategies to resist ferroptosis, often elevating

lipid-peroxidation regulators and detoxification pathways.[2,3] Recognising these regulatory layers underscores the need for comprehensive approaches to overcoming ferroptosis resistance in GBM.

Recent evidence highlights the important roles of non-coding RNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), in modulating ferroptosis. These regulatory RNAs influence key processes such as iron metabolism, glutathione synthesis, and lipid peroxidation, thereby affecting ferroptotic sensitivity across diverse biological contexts.[9] In particular, ferroptosis-related lncRNAs have been shown to act as crucial regulators of ferroptosis, potentially impacting cancer progression and therapeutic resistance by interacting with miRNAs and other components of gene regulatory networks. Competing endogenous RNA (ceRNA) interactions enable lncRNAs to modulate gene expression by sequestering shared miRNAs, adding a significant layer of post-transcriptional regulation with potential relevance to ferroptosis sensitivity.[10,11] However, ferroptosis-centred ceRNA networks in GBM remain poorly understood, and their contribution to TMZ resistance has not been comprehensively mapped.

To address this gap, this study combines transcriptomic profiling with curated interaction databases to systematically map ferroptosis-related regulatory networks in GBM. We used the publicly available RNA-seq dataset GSE151680, which includes both parental and TMZ-resistant U251 and U87 GBM cells, to identify differentially expressed genes (DEGs) associated with TMZ response. We manually curated ferroptosis regulators from ferroptosis database (FerrDb) which contains ferroptosis related genes and diseases. By applying strict multi-layered filters, including FerrDb annotation, expression evidence, differential expression status, and miRNA connectivity, we constructed a high-confidence ferroptosis-associated ceRNA network. Ultimately, we identified the top miRNAs and lncRNAs with the most regulatory connections. We visualized key messenger RNA (mRNA)-miRNA-lncRNA pathways, such as KCNQ1OT1–miR-16-5p–CCND1/CDK6, to highlight crucial regulatory modules that may contribute to TMZ resistance.

MATERIALS AND METHODS

Glioblastoma dataset

To investigate ferroptosis-related regulatory networks in GBM cells treated with TMZ, we analyzed the publicly available RNA-seq dataset GSE151680 from the Gene Expression Omnibus. The dataset contains RNASeq data which is performed in Beijing Neurosurgical Institute and Beijing Tiantan Hospital in China. This dataset provides expression profiles from two human GBM cell lines, U251 and U87. The comparison groups were identified as malignant GBM (MG), referring to parental GBM cells for the control, and TMZ-resistant GBM cells (TR) for the experiment group. The U251 cells harbor a TP53 mutation, exhibit high proliferation, and are therapy-resistant, whereas U87 cells represent a distinct GBM model with different therapeutic responses. Each condition includes biological replicates, ensuring data robustness for differential expression and pathway analysis. All analyses were performed in R (version 4.3) using Bioconductor and CRAN packages, including DESeq2, clusterProfiler, ReactomePA, org.Hs.eg.db, msigdbr, multiMiR, and visualization tools such as ggplot2 and pheatmap. This study did not involve human participants or animal experiments and therefore did not require ethical approval.

Differential expression analysis

Separate analyses for U251 and U87 identified cell line-specific transcriptional responses to TMZ. Raw counts were processed and normalized using DESeq2, and differential expression between TMZ resistant (TR) and malignant GBM (MG) cells was assessed using a negative binomial model. Genes with an absolute log2 fold change ≥1 and an adjusted p-value (Benjamini–Hochberg) <0.05 were considered differentially expressed. To ensure data quality, a variance-stabilizing transformation was performed, confirming the consistency and robustness of the results.

Functional and pathway enrichment analyses

To elucidate biological processes impacted by TMZ, separate enrichment analyses were performed for up- and down-regulated DEGs

in each cell line. Using clusterProfiler, we conducted over-representation tests including Gene Ontology (GO) categories (biological process, molecular function, cellular component), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Reactome pathways. Terms with at least 10 genes and an adjusted p-value <0.05 were considered significant, providing a clear threshold for identifying relevant pathways. We generated dot plots and enrichment summary figures, and we exported all tables to ensure reproducibility. In addition, we performed Gene Set Enrichment Analysis (GSEA) using preranked lists derived from DESeq2 statistics, applying gseKEGG for KEGG pathways and Molecular Signatures Database (MSigDB) with ClusterProfiler in GSEA for Hallmark gene sets. Significant pathways (q-value or adjusted p-value <0.05) were further illustrated with enrichment curves and GSEA ridge/dot plots.

Integration of ferroptosis-related genes

To investigate ferroptosis-specific regulation, curated ferroptosis gene sets from FerrDb v2 (accessed November 2025) were integrated, including driver, suppressor, and marker genes. We combined these lists into a non-redundant reference set and intersected them with the expression matrix to identify ferroptosis-related transcripts. We then visualized ferroptosis-related DEGs in U251 and U87 using heatmaps. FerrDb annotations (driver/suppressor/marker) helped characterize the regulatory patterns of TMZ-responsive ferroptosis genes across both cell lines.

Identification of ferroptosis-associated miRNAs

To identify post-transcriptional regulators of ferroptosis-related DEGs, validated miRNA-mRNA interactions from multiMiR were integrated, including experimental support from miRTarBase, miRecords, and TarBase. Analyses were performed separately for U251 and U87 and then combined into a unified, ferroptosis-focused miRNA-gene interaction table. We retained only interactions involving FerrDb-annotated genes. Counting the number of targeted ferroptosis genes per miRNA enabled us to identify a core ferroptosis miRNA panel, highlighting key regulators that respond to TMZ in both cell lines.

Construction of a ferroptosis-related ceRNA Network

To explore how ferroptosis is regulated post-transcriptionally in GBM, we constructed a ceRNA network. We combined confirmed miRNA-mRNA and miRNA-lncRNA relationships from the miRNet database, which gathers experimental data from miRTarBase, miRecords, and TarBase. We standardized all interaction tables into one dataset that includes mature miRNA IDs, human gene symbols, and annotated lncRNAs. To ensure the final network accurately captured the regulation of ferroptosis during TMZ treatment, we used a stepwise filtering method. First, we kept only interactions involving genes listed in FerrDb as ferroptosis drivers, suppressors, or markers. Next, we included only lncRNAs that appeared in our RNA-seq data. Third, we limited the mRNAs to those that were differentially expressed and associated with ferroptosis in U251 or U87 cells. Finally, we identified a leading set of miRNAs linked to ferroptosis by ranking all miRNAs based on the number of ferroptosis-related DEGs they target, selecting those with the strongest associations. After applying these filters, we constructed an integrated ceRNA network linking ferroptosis-related mRNAs with their regulatory miRNAs and the lncRNAs that compete for the same miRNA-binding sites.

RESULTS

Distinct transcriptional programs underlie TMZ resistance in U251 and U87 GBM cells

To characterize transcriptomic alterations associated with acquired TMZ resistance, we compared MG and TR U251 and U87 GBM cells using RNA sequencing-based differential expression analysis. Volcano plots revealed extensive transcriptional remodeling upon TMZ resistance in both cell lines, with U251 cells exhibiting a markedly higher number of DEGs compared to U87 cells, as shown in Figure 1a, b. Comparative overlap analysis further revealed that only a subset of DEGs was shared between U251 and U87 cells, highlighting pronounced cell line-specific transcriptional adaptations associated with TMZ resistance, as shown in Figure 1c.

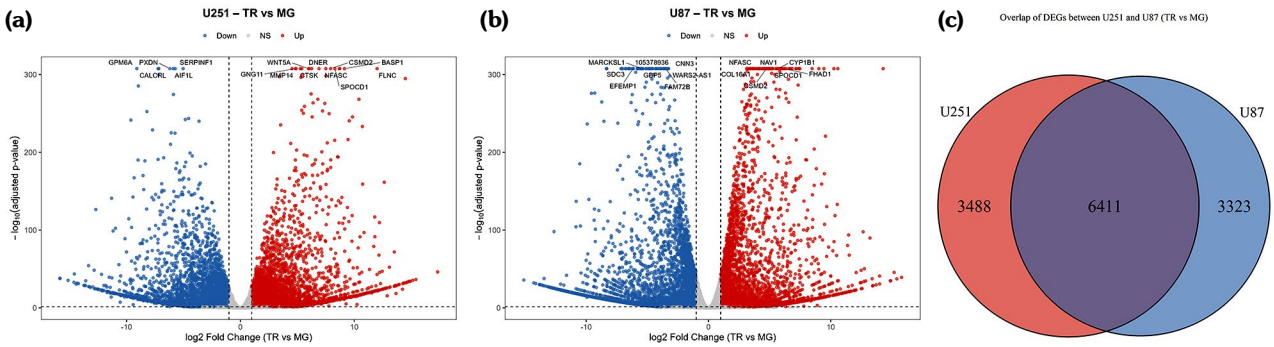

Figure 1. Transcriptomic alterations associated with temozolomide resistance in U251 and U87 GBM cells. **(a, b)** Volcano plots showing DEGs between temozolomide resistant (TR) and malignant (MG) GBM cells in U251 **(a)** and U87 **(b)** cell lines. Genes with an absolute $\log_2$ fold change ≥1 and an adjusted p-value (Benjamini-Hochberg correction) <0.05 were considered significantly differentially expressed and are shown in red (upregulated) and blue (downregulated), while non-significant genes are shown in gray. **(c)** Venn diagram depicting the overlap and cell line-specific distribution of DEGs between U251 and U87 GBM cells following TMZ resistance acquisition.

DEGs: Differentially expressed genes; GBM: Glioblastoma; TMZ: Temozolomide.

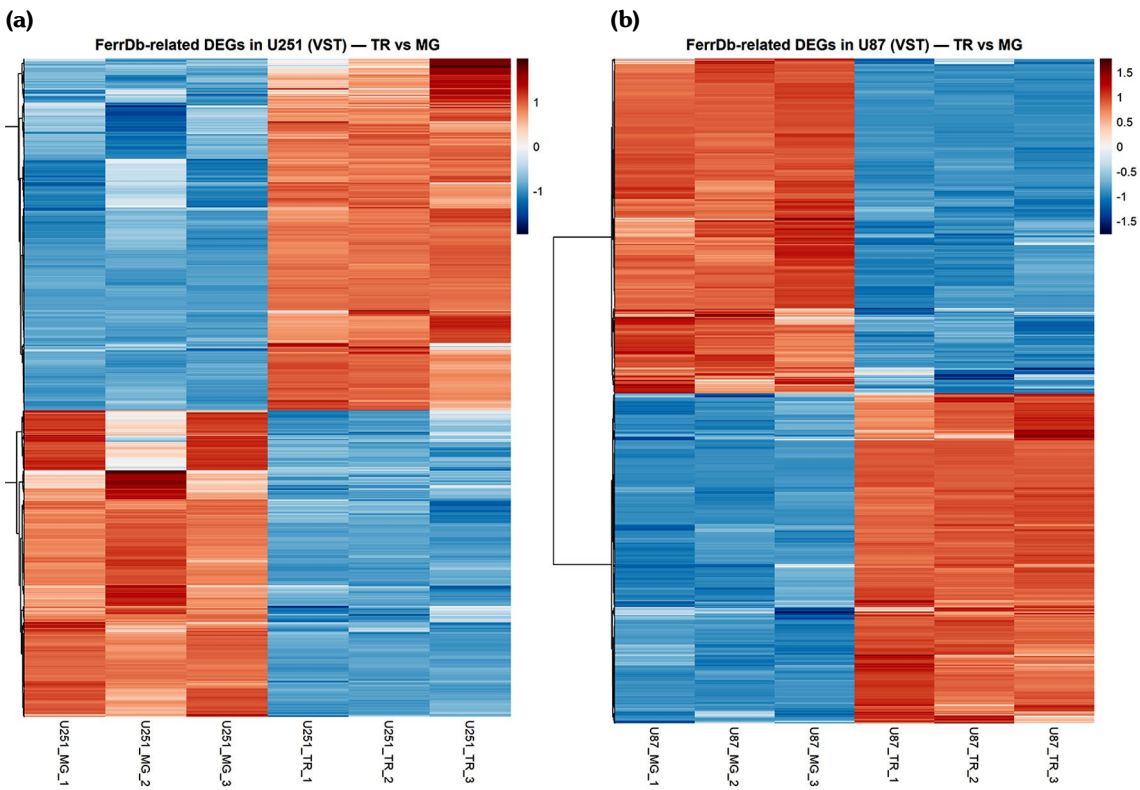

Figure 2. Ferroptosis-related gene expression changes in TMZ-resistant GBM cells. **(a, b)** Heatmaps of variance-stabilized expression values for ferroptosis-related differentially expressed genes (DEGs) derived from FerrDb (a manually curated database of ferroptosis regulators in U251 **(a)** and U87 **(b)** cells. Hierarchical clustering was performed using Euclidean distance and complete linkage. Data were row-scaled prior to clustering. Samples are grouped by TMZ resistance (TR) and parental malignant group (MG), highlighting distinct ferroptosis-associated transcriptional patterns associated with TMZ resistance. Rows represent genes and columns represent samples.

TMZ: Temozolomide; GBM: Glioblastoma; VST: Variance-stabilizing transformation; DEGs: Differentially expressed genes.

Ferroptosis-related transcriptional remodeling differs between TMZ-resistant U251 and U87 cells

To investigate whether TMZ resistance is accompanied by alterations in ferroptosis-related gene expression, resistance-associated DEGs were intersected with FerrDb annotations. Heatmap visualization of variance-stabilized expression profiles revealed coordinated but distinct ferroptosis-related transcriptional patterns in U251 and U87 cells, as shown in Figure 2a, b. Notably, TMZ-resistant U251 cells displayed a more pronounced ferroptosis-associated transcriptional shift compared to U87 cells.

Distinct functional reprogramming patterns underlie TMZ resistance

To elucidate the biological processes underlying TMZ resistance, GO enrichment analysis was performed separately for upregulated and downregulated DEGs in each cell line. In U251 cells, upregulated genes were predominantly enriched in biological processes related to inflammatory and immune responses, including cytokine-mediated signaling, regulation of inflammatory response, leukocyte migration and chemotaxis, as well as extracellular matrix organization, cell adhesion, angiogenesis, and tissue remodeling, as shown in Figure 3a. In contrast, downregulated genes were primarily associated with neurodevelopmental and differentiation-related programs, including axon development, gliogenesis, glial cell differentiation, neuron projection guidance, cell fate commitment, and regionalization, reflecting suppression of normal neural lineage specification and differentiation pathways in resistant U251 cells, as shown in Figure 3b.

Moreover, U87 cells displayed a distinct functional enrichment profile. Upregulated genes were predominantly associated with biological processes governing cell migration and invasion, including amoeboidal-type cell migration, leukocyte and mononuclear cell migration, chemotaxis, extracellular matrix organization, wound healing, angiogenesis, and cellular responses to hypoxia and oxygen availability, as shown in Figure 3c. Furthermore, downregulated genes were strongly enriched for neurodevelopmental and differentiation-related processes, including axon development, axon guidance, neuron projection guidance, pattern specification, regionalization, epithelial tube morphogenesis, sensory system development, and cell fate commitment, reflecting suppression of normal developmental programs in resistant cells, as shown in Figure 3d.

The KEGG pathway analysis further supported these findings. In U251 cells, upregulated genes were enriched in cytokine-cytokine receptor interaction, cytoskeleton in muscle cells, lipid and atherosclerosis, lysosome, and tumor necrosis factor (TNF) signaling pathways, as shown in Figure 3e. Downregulated genes were enriched in phosphatidylinositol-3-kinase and protein kinase B (PI3K-Akt), Wnt, and axon guidance pathways, as shown in Figure 3f. In U87 cells, upregulated KEGG pathways included immunoglobulin superfamily cell adhesion molecules (IgSF CAM) signaling, cytoskeleton in muscle cells, and cyclic adenosine monophosphate (cAMP) signaling, as shown in Figure 3g, whereas downregulated genes were enriched in PI3K-Akt, mitogen-activated protein kinase (MAPK), and calcium signaling pathways, as shown in Figure 3h.

GSEA reveals coordinated pathway-level alterations associated with TMZ resistance

To assess pathway-level transcriptional changes beyond individual DEGs, GSEA was performed using hallmark gene sets. In U251 cells, TMZ resistance was associated with enrichment of pathways related to EMT-, coagulation-, and inflammation-associated pathways, as shown in Figure 4a. On the other hand, U87 cells exhibited a distinct hallmark enrichment pattern, reflecting differential engagement of hypoxia-related signaling together with EMT and coagulation pathways, as shown in Figure 4b. These coordinated pathway-level alterations further support systematic transcriptional reprogramming during TMZ resistance.

Key miRNAs and lncRNAs form central regulatory hubs targeting ferroptosis-related genes

To investigate post-transcriptional regulation of ferroptosis-associated DEGs, experimentally validated miRNA-target interactions were

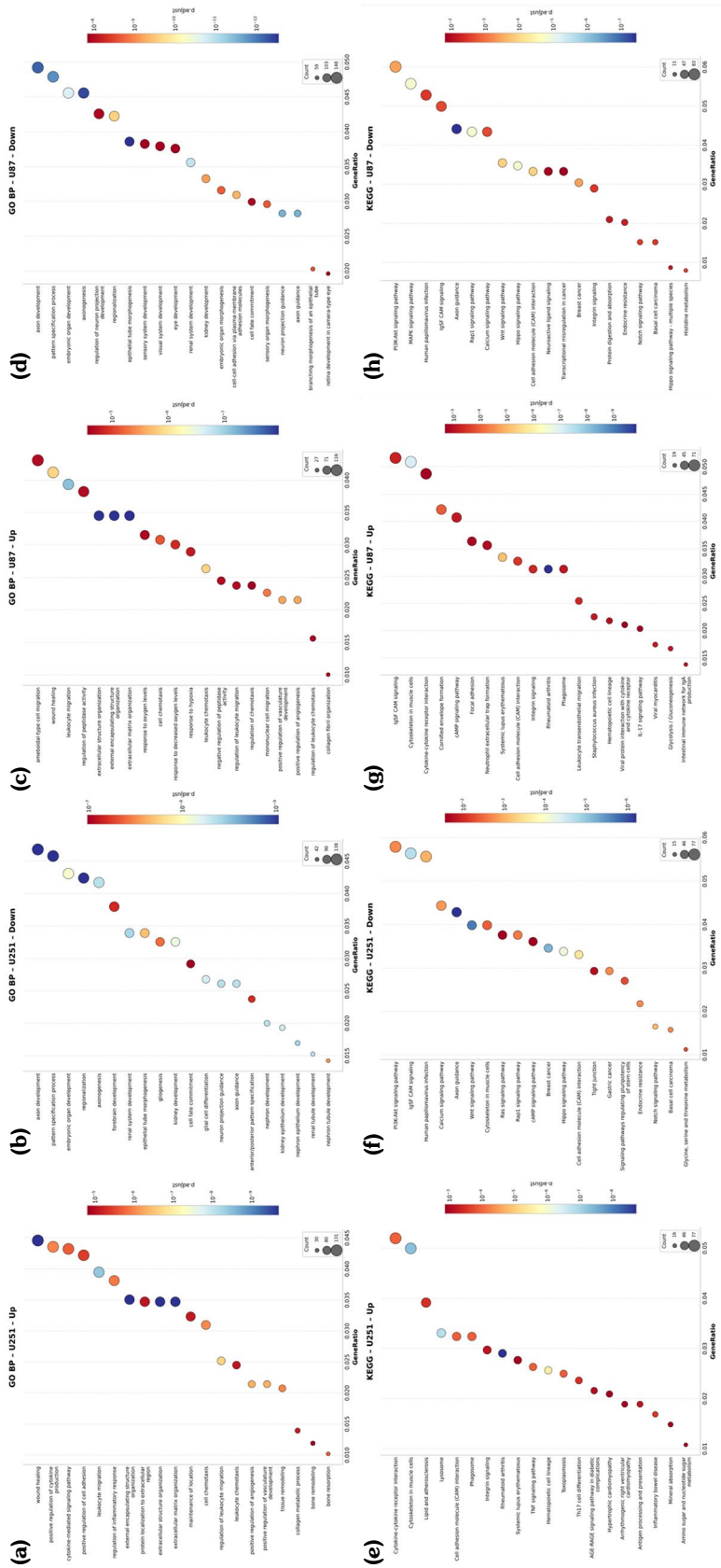

Figure 3. Functional enrichment analysis of resistance-associated DEGs. **(a, b)** GO biological process enrichment analysis for upregulated **(a)** and downregulated **(b)** DEGs in TMZ-resistant U251 cells. **(c, d)** GO biological process enrichment analysis for upregulated **(c)** and downregulated **(d)** DEGs in TMZ-resistant U87 cells. **(e, f)** KEGG pathway enrichment analysis for upregulated **(e)** and downregulated **(f)** DEGs in U251 cells. **(g, h)** KEGG pathway enrichment analysis for upregulated **(g)** and downregulated **(h)** DEGs in U87 cells. Dot size represents the number of genes associated with each term (GeneRatio), and color indicates the adjusted p-value (Benjamini-Hochberg correction).

DEGs: Differentially expressed genes; GO: Gene ontology; TMZ: Temozolomide; KEGG: Kyoto Encyclopedia of Genes and Genomes.

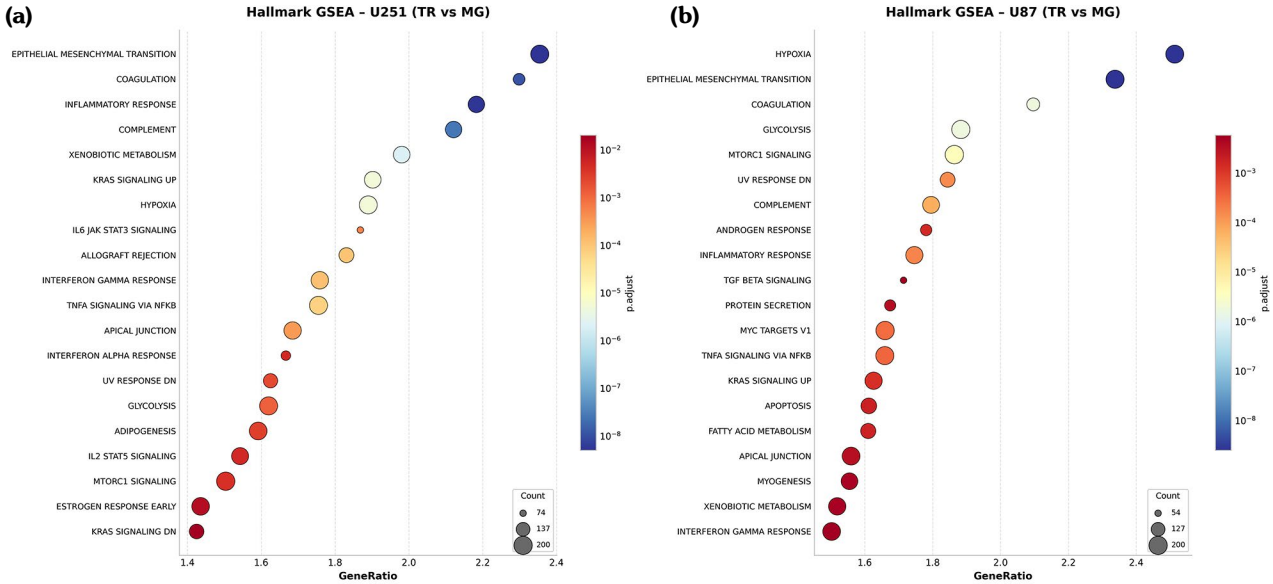

Figure 4. Hallmark GSEA reveals pathway-level transcriptional reprogramming. **(a)** Hallmark GSEA dot plot illustrating significantly enriched pathways in TMZ-resistant U251 cells compared to MG group. **(b)** Hallmark GSEA dot plot obtained depending on log2FC, showing enriched pathways associated with TMZ resistance in U87 cells compared to the MG cells. Analysis was performed using MSigDB v7.5.1. Dot size represents the number of genes associated with each pathway, and color indicates the adjusted p-value.

GSEA: Gene Set Enrichment Analysis; TMZ: Temozolomide; TR: Temozolomide resistant GBM cells; MG: Malignant glioblastoma cells.

obtained from the multiMiR database. Ranking miRNAs by the number of ferroptosis-related DEGs they target revealed hsa-mir-124-3p, hsa-mir-1-3p, hsa-mir-16-5p, hsa-mir-34a-5p, hsa-mir-155-5p as the most connected regulators, as shown in Figure 5a.

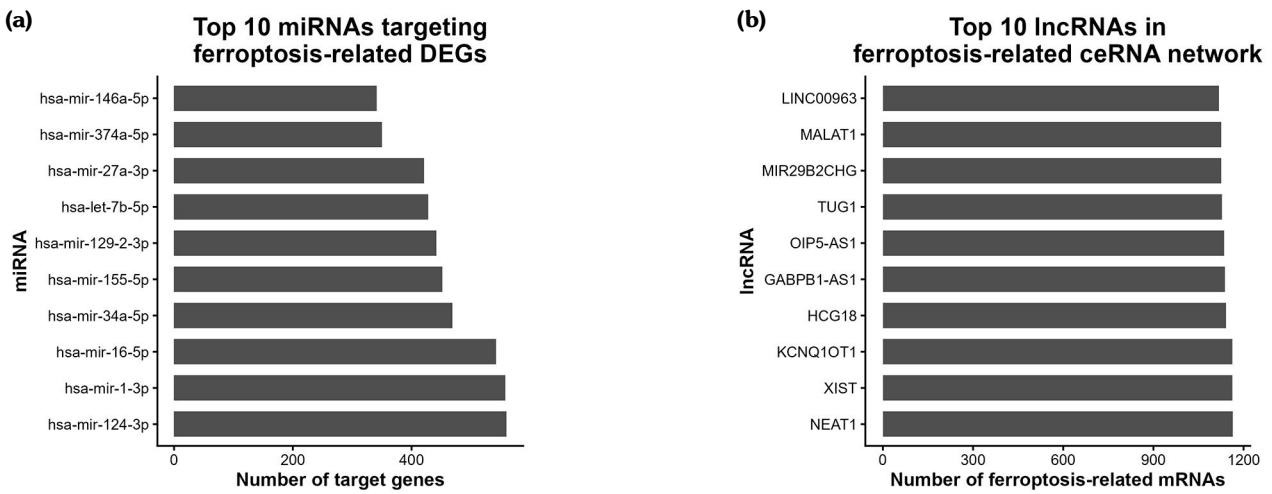

Figure 5. Identification of key post-transcriptional regulators targeting ferroptosis-related genes. **(a)** Bar plot showing the top 10 miRNAs ranked by the number of ferroptosis-related DEGs they target, based on experimentally validated interactions retrieved from multiMiR. **(b)** Bar plot depicting the top 10 long non-coding RNAs (lncRNAs) with the highest number of interactions with ferroptosis-associated microRNAs. Regulators are ranked based on the number of ferroptosis-related DEGs they target, irrespective of expression direction.

miRNAs: Micro ribonucleic acids; DEGs: Differentially expressed genes; lncRNAs: Long noncoding ribonucleic acids.

Parallel analysis of lncRNA-miRNA interactions identified NEAT1, XIST, KCNQ1OT1, HCG18, and GABPB1-AS1 as the most highly connected lncRNAs, each associated with over 1,000 ferroptosis-related mRNAs, as shown in Figure 5b.

Network topology analysis further identified two key regulatory modules: the KCNQ1OT1-miR-16-5p-CCND1/CDK6 axis and the NEAT1-miR-155-5p-BIRC3/CHAC1 axis, linking cell cycle regulation and inflammatory signaling to ferroptosis sensitivity.

DISCUSSION

Glioblastoma is the most common and lethal primary brain tumor in adults. Although aggressive treatment strategies are used, it has a poor prognosis. For the primary treatment strategy, surgical resection is followed by radiotherapy and concurrent/adjuvant TMZ chemotherapy. These strategies induce tumor cell death primarily through DNA alkylation and damage.[12] Temozolomide is an alkylating agent that adds a methyl group to O^6 position of guanine of DNA, which is leading to mismatches during replication, DNA strand breaks, and eventual apoptotic cell death when repair mechanisms are overwhelmed. However, intrinsic and acquired resistance mechanisms significantly limit TMZ efficacy, contributing to poor long-term outcomes and recurrence in GBM patients. Ferroptosis is a regulated, iron-dependent form of cell death. This feature can distinguish it from other forms of cell death, specifically apoptosis, by high levels of lipid peroxidation mediated by iron-catalyzed Fenton reactions, depletion of glutathione (GSH), and failure of glutathione peroxidase 4 (GPX4). The GPX4 is the key enzymatic defense that detoxifies lipid hydroperoxides, to prevent oxidative damage. Low antioxidant levels cause lethal lipid peroxide accumulation, leading to membrane damage and cell death. Unlike apoptosis, which depends on caspase activation and can be increased by p53 mutation, anti-apoptotic protein upregulation, or enhanced DNA repair, ferroptosis bypasses these traditional resistance pathways. Glioblastoma cells exhibit "ferroaddiction", meaning they have elevated intracellular iron and are metabolically predisposed to ferroptotic stress under conditions of impaired antioxidant defenses.[13] This makes ferroptosis an attractive alternative death pathway to exploit in therapy-resistant GBM.

Although TMZ's classical cytotoxicity operates via DNA damage, experimental data suggest that TMZ may also induce ferroptosis in GBM cells by modulating iron metabolism and disrupting antioxidant defences.[14] Elevated reactive oxygen species (ROS) and increased expression of iron importers like *DMT1 (divalent metal transporter 1)* have been reported in TMZ-treated GBM cells, accompanied by decreased *GPX4* expression, which are the hallmarks consistent with ferroptotic activation. Knockdown of *DMT1* attenuates this effect, emphasising the involvement of iron-dependent lipid peroxidation pathways in TMZ-induced death in some cellular contexts.[15]

To investigate ferroptosis-related genes in GBM cells treated with TMZ, we analyzed TR and MG groups in GBM cell lines U87 and U251. Temozolomide resistance has common features in different GBM cell lines. Volcano plot visualization demonstrated a clear separation between upregulated and downregulated genes in both cell lines. Several genes exhibited both high fold changes and strong statistical significance for up- and down-regulation in TMZ resistance groups (Figure 1a, b). As shown in our results (Figure 1c), two different TMZ-resistant GBM cell lines, U87 and U251, share more than 6000 DEGs compared to control cells. Temozolomide-resistant U251 cells displayed a more pronounced ferroptosis-associated transcriptional shift compared to U87 cells, suggesting differential engagement and regulation of ferroptotic pathways between the two GBM models.

The functional enrichment analysis suggests that TMZ resistance in GBM is accompanied by distinct, cell line-specific adaptive programs that converge on biological processes relevant to ferroptosis regulation. In U251 cells, upregulated genes relate to inflammatory and immune-related responses, including cytokine-mediated signaling, regulation of inflammation, leukocyte migration, and chemotaxis. Recent research has demonstrated that ferroptosis is closely linked to inflammation and leads to the production of certain substances that can aggressively

activate the immune system.[16] These substances are also responsible for regulating cellular inflammation, signal transduction, and cellular proliferation. The activation of different inflammation-mediated pathways may also lead to the development of ferroptosis.[17] In addition, pathways related to extracellular matrix organization, cell adhesion, angiogenesis, and tissue remodeling are involved. These enrichments suggest extensive remodeling of the tumor microenvironment accompanied by enhanced pro-tumorigenic signaling in TMZ-resistant cells. In U87, upregulated genes were predominantly associated with biological processes governing cell migration and invasion. These findings indicate extensive remodeling of the tumor microenvironment and enhanced invasive and stress-adaptive programs in TMZ-resistant U87 cells. Moreover, downregulated genes in both U251 and U87 were mainly enriched in neurodevelopmental and differentiation-associated processes, such as axon development, gliogenesis, glial cell differentiation, neuron projection guidance, cell fate commitment, and regionalization. This pattern indicates a suppression of normal neural lineage specification and differentiation programs in TMZ-resistant U251 and U87 cells.[18,19]

The KEGG analysis showed that, in U251 cells, upregulated genes were enriched in cytokine-cytokine receptor interaction, cytoskeleton in muscle cells, lipid and atherosclerosis, lysosome, and TNF signaling pathways. The activation of inflammation via the upregulation of several inflammation-related signaling pathways may induce the cell to undergo ferroptosis, controlled by main inflammatory pathways like the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING), NF-κB, which release proinflammatory cytokines (TNF), JAK-STAT (Janus kinase/signal transducers and activators of transcription), MAPK (mitogen-activated protein kinase) signaling pathways, and inflammasome.[20] Due to the primary features of atherosclerosis, which are oxidative stress, abnormal lipid metabolism, and inflammation, ferroptosis plays a role in the development and progression of atherosclerosis by regulating disease-related signaling pathways.[21] These findings indicate that our results support the relation between the TMZ-resistant mechanisms and ferroptosis in terms of related pathways. On the other hand, downregulated genes in U251 were enriched in PI3K-Akt, Wnt, and axon guidance pathways. Liu et al.[22] showed that in ovarian cancer, when a key proliferative and migration-related gene, TRIM46, was knocked down, it was connected to both ferroptosis and Wnt signaling pathways as same findings in our study. For the PI3K-Akt pathway, it is found that inhibition of the pathway induces the cell to become sensitive to ferroptosis, which leads the studies to overcome drug resistance.[23] Actin filaments are one of the cytoskeleton components and play a role in transferrin receptor (TFRC)-mediated iron absorption.[24] Therefore, it is related to the iron-dependent death mechanism, ferroptosis. It can support our findings on the upregulation of certain genes controlling the cytoskeleton in muscle cells. The downregulated genes support the hypothesis that the relation between ferroptosis and TMZ-resistance depends on the regulation of the significant cancer-related signaling pathways.

Gene set enrichment analysis revealed that TMZ-resistant U251 cells were significantly enriched for pathways associated with EMT, coagulation, and inflammatory response.[25-27] On the other hand, TMZ-resistant U87 cells exhibited a distinct hallmark enrichment profile characterized by hypoxia-related signaling together with EMT and coagulation pathways.[25,27,28] Collectively, these pathway-level alterations further support the concept of extensive transcriptional remodeling accompanying the acquisition of TMZ resistance.

MiRNAs control chemotherapy resistance via DNA repair, cell cycle, drug absorption and metabolism, and apoptosis. It has been shown that miRNAs regulate TMZ resistance in GBM cells by affecting signaling pathways and autophagy.[29] They also regulate chemotherapy resistance via ferroptosis mechanisms. For instance, treating GBM cells with the miR-147a mimic significantly induced ferroptosis, and the ferroptotic inhibitors inhibited the miR-147a mimic-mediated tumor suppression.[30] In our study, we have found miRNAs targeting ferroptosis-related DEGs and hsa-mir-124-3p,

hsa-mir-1-3p, hsa-mir-16-5p, hsa-mir-34a-5p, and hsa-mir-155-5p are the top-5 miRNAs ordered by the number of target genes. Hsa-miR-124-3p was found to be related to ferroptosis by regulating the cellular and oxidative stress response and inflammation via targeting stress response and transcription factor genes such as JUN, HIF1A, DUSP1, and ATF3.[31] In the same direction as our results, hsa-miR1-3p is related to endoplasmic reticulum stress in lung adenocarcinoma.[32] Fang *et al.*[33] showed that hsa-miR-16-5p regulates ferroptosis by upregulating ACSL4, a ferroptosis-inducing gene in diabetes. Hsa-mir-155-5p and hsa-mir-34a-5p were found to be related to ferroptosis via inflammation, oxidative stress, cell death, and targeting ferroptosis-related genes such as *cytochrome b beta (CYBB) and Acyl-CoA synthetase long chain family member 4 (ACSL4)*.[34]

In the lncRNA aspect, we found that NEAT1, XIST, KCNQ1OT1, HCG18, and GABPB1-AS1 are the most highly connected lncRNAs according to target genes. NEAT1 directly promotes ferroptosis by regulating lipid peroxidation and increasing ROS.[35] Similarly, XIST controls the ferroptosis resistance through the SLC7A11/GPX4 axis.[36] KCNQ1OT1 was also found to regulate ferroptosis through ACSL4-mediated lipid peroxidation.[37] Moreover, it is found that silencing HCG18 regulates GPX4-inhibited ferroptosis to prevent sorafenib resistance in hepatocellular carcinoma.[38] For GABPB1-AS1, it regulates oxidative stress during erastin-induced ferroptosis through mitochondrial ROS and antioxidant response mechanisms.[39] All these studies support our results, indicating the roles of miRNAs and lncRNAs in ferroptosis via target genes. We also identified two key regulatory modules, which are the KCNQ1OT1-miR-16-5p-CCND1/CDK6 axis and the NEAT1-miR-155-5p-BIRC3/CHAC1 axis, linking cell cycle regulation and inflammatory signaling to ferroptosis sensitivity. As indicated above, the activation of different inflammation-mediated pathways promotes cells to development of ferroptosis.[17] These findings highlight a select set of miRNAs and lncRNAs that function as central post-transcriptional regulatory hubs, potentially coordinating ferroptosis-related gene expression programs in TMZ-resistant GBM cells.

This study has potential limitations. First of all, the lack of a clinical validation step can limit the study. Two GBM cell lines can represent the general biological roles of the genes and ceRNAs, however, in every patient cohort the results may slightly change. Glioblastoma is characterized by high heterogeneity as well as a complex tumor microenvironment, including immune and stromal components, which are not captured by the U87 and U251 cell line models used in this study.[1] To give an entire biological background of ferroptosis in GBM, it is suggested that further analyses are required to characterize the systematic regulations and effects of these genes. Moreover, the performed *in silico* analyses strongly suggest an association between TMZ treatment and ferroptosis-related pathways; they do not provide direct causal evidence of ferroptotic cell death. Therefore, we also suggest *in vivo* validations of the results to evaluate tumor heterogeneity and the tumor microenvironment. Additionally, the ceRNA perspective was determined by target genes and their related pathways rather than evaluating them on transcriptomic sequencing data. As such, the proposed regulatory networks represent putative regulatory relationships that require further validation to confirm their biological relevance.

In conclusion, this study provides a systematic transcriptomic and *in silico* analysis of TMZ-treated U87 and U251 GBM cells, highlighting different common molecular responses associated with oxidative stress, lipid metabolism, and ferroptosis-related pathways. Therefore, it has a crucial impact on the effect of TMZ on GBM cells in terms of the ferroptosis axis. From a small RNA-centered perspective, the results suggest that ferroptosis-associated regulatory mechanisms may contribute to the cellular response to TMZ, contributing to understanding therapy-associated vulnerability and resistance in GBM. Since knowledge of the ferroptosis mechanism is still evolving in cancer research, our study can contribute to the field from a small RNA perspective. Moreover, these findings can be considered as preliminary results toward illuminating the molecular mechanisms underlying therapeutic response, which is the major challenge in GBM research.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Both authors contributed equally to the conception, analysis, and writing of this study.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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REFERENCES

1. Zhuo S, He G, Chen T, Li X, Liang Y, Wu W, et al. Emerging role of ferroptosis in glioblastoma: Therapeutic opportunities and challenges. Front Mol Biosci 2022;9:974156. doi: 10.3389/fmolb.2022.974156.
2. Wang X, Ren X, Lin X, Li Q, Zhang Y, Deng J, et al. Recent progress of ferroptosis in cancers and drug discovery. Asian J Pharm Sci 2024;19:100939. doi: 10.1016/j.ajps.2024.100939.
3. Ren Y, Mao X, Xu H, Dang Q, Weng S, Zhang Y, et al. Ferroptosis and EMT: Key targets for combating cancer progression and therapy resistance. Cell Mol Life Sci 2023;80:263. doi: 10.1007/s00018-023-04907-4.
4. Lan Y, Yang T, Yue Q, Wang Z, Zhong X, Luo X, et al. IRP1 mediated ferroptosis reverses temozolomide resistance in glioblastoma via affecting LCN2/FPN1 signaling axis depended on NFKB2. iScience 2023;26:107377. doi: 10.1016/j.isci.2023.107377.
5. Liu HJ, Hu HM, Li GZ, Zhang Y, Wu F, Liu X, et al. Ferroptosis-related gene signature predicts glioma cell death and glioma patient progression. Front Cell Dev Biol 2020;8:538. doi: 10.3389/fcell.2020.00538.
6. Guo W, Duan Z, Wu J, Zhou BP. Epithelial-mesenchymal transition promotes metabolic reprogramming to suppress ferroptosis. Semin Cancer Biol 2025;112:20-35. doi: 10.1016/j.semcancer.2025.02.013.
7. Ebrahimi N, Adelian S, Shakerian S, Afshinpour M, Chaleshtori SR, Rostami N, et al. Crosstalk between ferroptosis and the epithelial-mesenchymal transition: Implications for inflammation and cancer therapy. Cytokine Growth Factor Rev 2022;64:33-45. doi: 10.1016/j.cytogfr.2022.01.006.
8. Zhang H, Chen N, Ding C, Zhang H, Liu D, Liu S. Ferroptosis and EMT resistance in cancer: A comprehensive review of the interplay. Front Oncol 2024;14:1344290. doi: 10.3389/fonc.2024.1344290.
9. Chen X, Kang R, Kroemer G, Tang D. Broadening horizons: The role of ferroptosis in cancer. Nat Rev Clin Oncol 2021;18:280-96. doi: 10.1038/s41571-020-00462-0.
10. Balihodzic A, Prinz F, Dengler MA, Calin GA, Jost PJ, Pichler M. Non-coding RNAs and ferroptosis: Potential implications for cancer therapy. Cell Death Differ 2022;29:1094-106. doi: 10.1038/s41418-022-00998-x.
11. Zhou W, Thiery JP. Ferroptosis-related LncRNAs in diseases. BMC Biol 2025;23:158. doi: 10.1186/s12915-025-02268-x.
12. Rahman MA, Yadab MK, Ali MM. A modern approach to glioblastoma using temozolomide and nanoparticles carrier drug: A standard care of combination therapy and treatment. Biochem Pharmacol 2025;242:117276. doi: 10.1016/j.bcp.2025.117276.
13. Singh S, Mohapatra I, Barik D, Zheng H, Kim S, Sharma M, et al. Harnessing ferroptosis to transform glioblastoma therapy and surmount treatment resistance. Cell Death Discov 2025;11:448. doi: 10.1038/s41420-025-02744-x.
14. Li H, Wu Y, Chen Y, Lv J, Qu C, Mei T, et al. Overcoming temozolomide resistance in glioma: Recent advances and mechanistic insights. Acta Neuropathol Commun 2025;13:126. doi: 10.1186/s40478-025-02046-4.
15. Song Q, Peng S, Sun Z, Heng X, Zhu X. Temozolomide drives ferroptosis via a DMT1-dependent pathway in glioblastoma cells. Yonsei Med J 2021;62:843-9. doi: 10.3349/ymj.2021.62.9.843.
16. Li J, Li L, Zhang Z, Chen P, Shu H, Yang C, et al. Ferroptosis: An important player in the inflammatory response in diabetic nephropathy. Front Immunol 2023;14:1294317. doi: 10.3389/fimmu.2023.1294317.
17. Deng L, He S, Guo N, Tian W, Zhang W, Luo L. Molecular mechanisms of ferroptosis and relevance to inflammation. Inflamm Res 2023;72:281-99. doi: 10.1007/s00011-022-01672-1.
18. Benarroch E. What is the role of ferroptosis in neurodegeneration? Neurology 2023;101:312-9. doi: 10.1212/WNL.0000000000207730.
19. Jiang T, Ma W, Dong W, Zhou H, Mao X. Ferroptosis-associated transcriptional factors in neurological diseases: Molecular mechanisms and therapeutic prospects. Exp Mol Med 2025;57:2763-81. doi: 10.1038/s12276-025-01611-0.
20. Chen Y, Fang ZM, Yi X, Wei X, Jiang DS. The interaction between ferroptosis and inflammatory signaling pathways. Cell Death Dis 2023;14:205. doi: 10.1038/s41419-023-05716-0.
21. Wang Y, Zhao Y, Ye T, Yang L, Shen Y, Li H. Ferroptosis signaling and regulators in atherosclerosis. Front Cell Dev Biol 2021;9:809457. doi: 10.3389/fcell.2021.809457.
22. Liu S, Xiao C, Rong Y, Liu M, Yang K, Tang J, et al. Comprehensive analysis of ferroptosis-related genes indicates that TRIM46 is a novel biomarker and promotes the progression of ovarian cancer via modulating ferroptosis and Wnt signaling pathway. Am J Cancer Res 2024;14:4686-4707. doi: 10.62347/ONUY8904.

23. Su H, Peng C, Liu Y. Regulation of ferroptosis by PI3K/Akt signaling pathway: A promising therapeutic axis in cancer. Front Cell Dev Biol 2024;12:1372330. doi: 10.3389/fcell.2024.1372330.
24. Sun X, Ou Z, Xie M, Kang R, Fan Y, Niu X, et al. HSPB1 as a novel regulator of ferroptotic cancer cell death. Oncogene 2015;34:5617-25. doi: 10.1038/onc.2015.32.
25. Kapteijn MY, Zwaan S, Ter Linden E, Laghmani EH, van den Akker RFP, Rondon AMR, et al. Temozolomide and lomustine induce tissue factor expression and procoagulant activity in glioblastoma cells in vitro. Cancers (Basel) 2023;15:2347. doi: 10.3390/cancers15082347.
26. Nasir S, Nazir S, Hanif R, Javed A. Glioblastoma multiforme: Probing solutions to systemic toxicity towards high-dose chemotherapy and inflammatory influence in resistance against temozolomide. Pharmaceutics 2023;15:687. doi: 10.3390/pharmaceutics15020687.
27. Liu Q, Xue Y, Chen Q, Chen H, Zhang X, Wang L, et al. PomGnT1 enhances temozolomide resistance by activating epithelial-mesenchymal transition signaling in glioblastoma. Oncol Rep 2017;38:2911-8. doi: 10.3892/or.2017.5964.
28. Li G, Xiong Z, Li Y, Yan C, Cheng Y, Wang Y, et al. Hypoxic microenvironment-induced exosomes confer temozolomide resistance in glioma through transfer of pyruvate kinase M2. Discov Oncol 2024;15:110. doi: 10.1007/s12672-024-00963-9.
29. Palizkaran Yazdi M, Barjasteh A, Moghbeli M. MicroRNAs as the pivotal regulators of Temozolomide resistance in glioblastoma. Mol Brain 2024;17:42. doi: 10.1186/s13041-024-01113-6.
30. Xu P, Ge FH, Li WX, Xu Z, Wang XL, Shen JL, et al. MicroRNA-147a targets SLC40A1 to induce ferroptosis in human glioblastoma. Anal Cell Pathol (Amst) 2022;2022:2843990. doi: 10.1155/2022/2843990.
31. Lin Y, He J, Mou Z, Chen H, You W, Guan T, et al. Ferroptosis-related genes, a novel therapeutic target for focal segmental glomerulosclerosis. BMC Nephrol 2024;25:58. doi: 10.1186/s12882-024-03490-5.
32. Chen K, Dai P, Gu L. Building endoplasmic reticulum stress-related LncRNAs signatures of lung adenocarcinoma. J Gene Med 2024;26:e3731. doi: 10.1002/jgm.3731.
33. Fang X, Song J, Chen Y, Zhu S, Tu W, Ke B, et al. LncRNA SNHG1 knockdown inhibits hyperglycemia induced ferroptosis via miR-16-5p/ACSL4 axis to alleviate diabetic nephropathy. J Diabetes Investig 2023;14:1056-69. doi: 10.1111/jdi.14036.
34. Singh P, Rath SL. Neural Network-Enhanced Investigation of Ferroptosis and Druggability in Early-Onset Alzheimer's Disease. bioRxiv 2025:2025.06.21.660896. doi: 10.1101/2025.06.21.660896.
35. Zhang Y, Luo M, Cui X, O'Connell D, Yang Y. Long noncoding RNA NEAT1 promotes ferroptosis by modulating the miR-362-3p/MIOX axis as a ceRNA. Cell Death Differ 2022;29:1850-63. doi: 10.1038/s41418-022-00970-9.
36. Huang K, Yu L, Lu D, Zhu Z, Shu M, Ma Z. Long non-coding RNAs in ferroptosis, pyroptosis and necroptosis: From functions to clinical implications in cancer therapy. Front Oncol 2024;14:1437698. doi: 10.3389/fonc.2024.1437698.
37. Liu Y, Chen Q, Zhu Y, Wang T, Ye L, Han L, et al. Non-coding RNAs in necroptosis, pyroptosis and ferroptosis in cancer metastasis. Cell Death Discov 2021;7:210. doi: 10.1038/s41420-021-00596-9.
38. Li X, Li Y, Lian P, Lv Q, Liu F. Silencing lncRNA HCG18 regulates GPX4-inhibited ferroptosis by adsorbing miR-450b-5p to avert sorafenib resistance in hepatocellular carcinoma. Hum Exp Toxicol 2023;42:9603271221142818. doi: 10.1177/09603271221142818.
39. Qi W, Li Z, Xia L, Dai J, Zhang Q, Wu C, et al. LncRNA GABPB1-AS1 and GABPB1 regulate oxidative stress during erastin-induced ferroptosis in HepG2 hepatocellular carcinoma cells. Sci Rep 2019;9:16185. doi: 10.1038/s41598-019-52837-8.