



Article

The Natural Triterpenoid Alisol B Overcomes Temozolomide Resistance in Glioblastoma Through Multi-Target Mechanisms: Coordinated Epigenetic, Metabolic, and Cell-Cycle Reprogramming

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Abstract

Glioblastoma (GBM) is a highly aggressive and therapy-resistant brain tumor, necessitating innovative multi-target strategies. Natural compounds like the triterpenoid Alisol B from *Alisma orientale* hold promise due to their polypharmacological potential, yet their system-level mechanisms are unclear. Using an integrated multi-omics approach (transcriptomics, proteomics, lysine acetyl-proteomics) in resistant GBM cells and validating findings in vitro and in AB strain zebrafish (*Danio rerio*) xenografts, we found that Alisol B induces endoplasmic reticulum stress and G2/M arrest, initiated by extensive lysine acetylation reprogramming on histones and metabolic enzymes (e.g., FASN, FDFT1). This epigenetic rewiring leads to disrupted cholesterol biosynthesis, characterized by transcriptional activation of the mevalonate pathway alongside post-transcriptional suppression of terminal enzymes (DHCR7, CYP51A1), suggestive of toxic intermediate accumulation. Alisol B also downregulated the oncogenic axis (BIRC5-FOXM1-ITGA4) and SCD5. This study delineates Alisol B's novel multi-mechanistic action through concurrent epigenetic rewiring, metabolic dysfunction induction, and survival network dismantling. Our work elucidates the molecular pharmacology of a natural compound and provides a framework for developing polypharmacological therapies against resistant cancers, exemplifying natural products as tools to reveal new therapeutic paradigms.

Keywords: Alisol B; glioblastoma; natural products; multi-target therapy; epigenetic reprogramming; metabolic reprogramming; polypharmacology



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1. Introduction

Glioblastoma multiforme (GBM) is an adult primary brain tumor of the highest grade and aggressiveness. According to the WHO 2021 classification, isocitrate dehydrogenase-wildtype (IDH-wildtype) glioblastoma constitutes the most prevalent and therapy-resistant subtype, characterized by relentless proliferation, diffuse infiltration, and a median survival rarely exceeding 16 months with current standard care [1–3]. The pathophysiology of IDH-wildtype GBM is driven by a complex interplay of molecular alterations, including

aberrant activation of receptor tyrosine kinase (RTK)/phosphoinositide 3-kinase (PI3K) and rat sarcoma virus/mitogen-activated protein kinase (RAS/MAPK) signaling pathways, rampant cell-cycle dysregulation, and extensive metabolic reprogramming such as heightened aerobic glycolysis (the Warburg effect) to fuel rapid growth [4–6]. Beyond genetic mutations, epigenetic modifications, including alterations in DNA methylation and histone post-translational modifications, are increasingly recognized as critical regulators of tumor plasticity, heterogeneity, and therapeutic resistance [7,8]. Notably, metabolic and epigenetic pathways engage in dynamic crosstalk, driving glioma biology and revealing therapeutic vulnerabilities [9]. This complexity renders single-target therapies inadequate, necessitating strategies that co-target multiple pathological drivers [10]. Natural products, with their inherent structural complexity and polypharmacological potential, are thus promising candidates for disrupting the redundant signaling networks of resistant cancers [11–13]. Unraveling their molecular mechanisms is therefore critical to fully exploit their therapeutic potential against intractable malignancies like GBM.

Among various natural product scaffolds, triterpenoids have demonstrated potent anti-neoplastic activity across diverse cancers by modulating proliferation, apoptosis, and metabolism [14–16]. Alisol B, a bioactive tetracyclic triterpenoid isolated from the traditional medicinal plant *Alisma orientale* (Sam.) Juzep [17], exemplifies this promise. Previous investigations have established the capacity of Alisol B to provoke endoplasmic reticulum stress and induce cell cycle arrest in other malignancies [18,19]. While our preliminary work confirmed Alisol B's efficacy against temozolomide (TMZ)-resistant GBM cells [20], a systems-level understanding of its mechanism—particularly its impact on the proteome and the crucial regulatory layer of protein acetylation in IDH-wildtype GBM—remains completely unexplored. This gap impedes a holistic understanding of its polypharmacology and rational therapeutic development. To bridge this knowledge gap, the present study employs an integrated multi-omics approach to systematically elucidate the coordinated molecular mechanisms of Alisol B against therapy-resistant, IDH-wildtype glioblastoma.

Protein acetylation, a fundamental post-translational modification, regulates protein activity, stability, and interactions, thereby playing a central role in metabolism, epigenetics, and signaling [21–24]. In GBM, this regulatory layer intersects with profound metabolic reprogramming, characterized by hyperactive de novo synthesis of cholesterol and fatty acids to sustain proliferation, membrane dynamics, and oncogenic signaling [25,26]. Two key enzymes in these pathways are recognized as potential anti-cancer targets [27]: (i) Squalene synthase (FDFT1), the downstream rate-limiting enzyme in cholesterol biosynthesis that catalyzes squalene formation [28,29]; and (ii) Fatty acid synthase (FASN), which drives de novo fatty acid synthesis to produce essential lipid building blocks [30]. Given their central role in GBM metabolism and susceptibility to acetylation, FASN and FDFT1 represent critical nodes for investigating the acetyl-proteomic impact of Alisol B.

In this study, we employed an integrated experimental approach to elucidate the anti-GBM mechanisms of Alisol B. The zebrafish xenograft system, which offers unique advantages for studying tumor cell migration and drug responses in a vertebrate context, served as our primary in vivo platform [31–35]. We combined this in vivo model with advanced proteomic and lysine acetyl-proteomic analyses, together with functional validation in cellular models. This multi-omics strategy aimed to unbiasedly map the molecular landscape of Alisol B's action on GBM cells, with the goal of discovering its key targets and novel regulatory mechanisms. Our findings not only provide novel insights into the anti-glioma pharmacology of Alisol B but also help elucidate its efficacy in modulating cholesterol biosynthesis.

2. Results

2.1. Alisol B Dose-Dependently Inhibits T98G Glioblastoma Cell Proliferation

Alisol B (Figure 1A) dose-dependently inhibited the proliferation of T98G glioblastoma cells. Treatment with Alisol B (20, 30, 40, 50 μ M) for 24 h significantly reduced cell viability compared to the control ($p < 0.001$; Figure 1B). The half-maximal inhibitory concentration (IC_{50}) was determined to be 24.27 μ M.

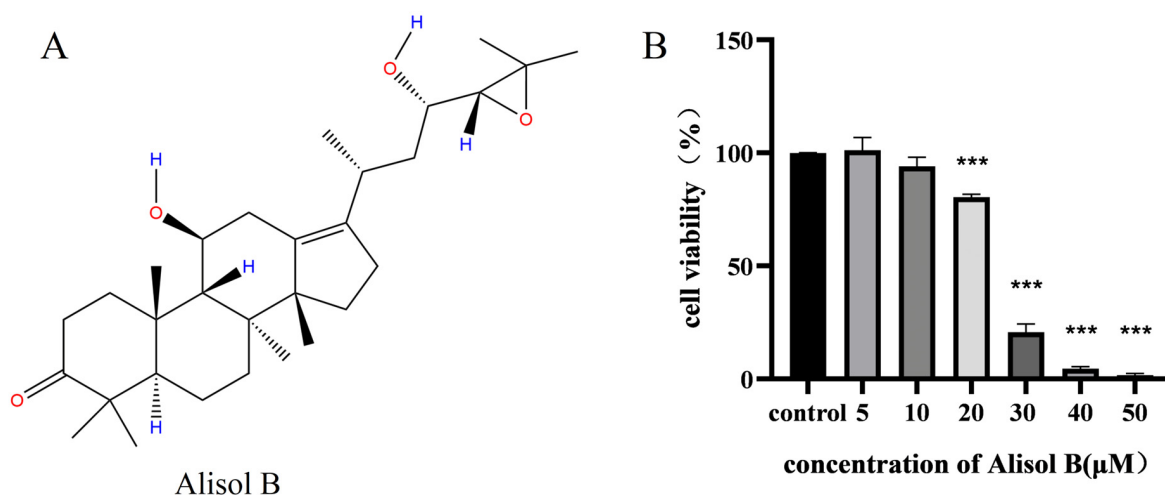


Figure 1. Alisol B inhibits the proliferation of T98G glioblastoma cells in a dose-dependent manner. (A) Chemical structure of Alisol B. Red atoms represent oxygen (O), and blue atoms represent hydrogen (H). (B) Cell viability of T98G cell after being treated by Alisol B ($n = 3$ independent biological replicates). The black bar represents the untreated control group (0 μ M), and gray bars represent groups treated with Alisol B at the concentrations indicated on the x-axis. *** $p < 0.001$, vs. Control.

2.2. Alisol B Induces Apoptosis in T98G Cells

Alisol B induced apoptosis in T98G cells in a concentration-dependent manner. Flow cytometry analysis after Annexin V-fluorescein isothiocyanate/propidium iodide (Annexin V-FITC/PI) staining showed that treatment with 30 μ M Alisol B for 24 h significantly increased the apoptosis rate compared to untreated cells ($p < 0.05$; Figure 2A,B), linking cell death to the activation of apoptotic pathways.

2.3. Alisol B Suppresses Lateral and Vertical Migration of T98G Cells

Alisol B inhibited the migratory capacity of T98G cells. In a wound healing assay, treatment with Alisol B (10, 15, 20 μ M) for 24 h significantly reduced wound closure in a dose-dependent manner (Figure 2C,D), consistent with the subsequent observation of ITGA4 downregulation (Section 2.9). Similarly, in a Transwell migration assay, Alisol B treatment caused a significant, dose-dependent reduction in the number of migrated cells ($p < 0.05$ vs. control; Figure 3A,B).

2.4. Alisol B Inhibits the Invasive Potential of T98G Cells

Alisol B potently inhibited the invasive potential of T98G cells. In a Matrigel-coated Transwell invasion assay, Alisol B treatment (10, 20, 30 μ M) resulted in a dose-dependent reduction in the number of invaded cells ($p < 0.05$ at all concentrations; Figure 3C,D). Maximal inhibition (72% decrease) was observed at 30 μ M ($p < 0.001$ vs. control).

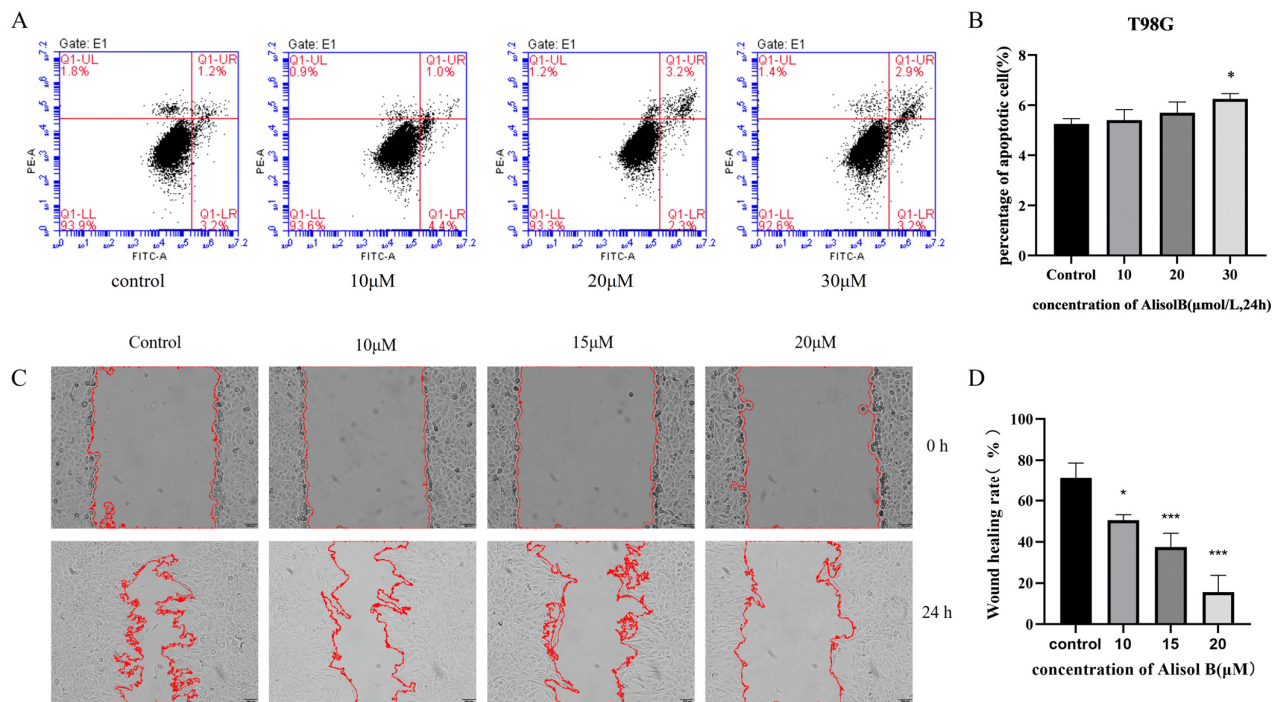


Figure 2. Alisol B induces apoptosis and inhibits the migration of T98G cells. ($n = 3$ independent biological replicates). (A) Flow cytometry plots of T98G cell apoptosis after Alisol B treatment. Q1-UL (Annexin V⁻/PI⁺): necrotic cells; Q1-UR (Annexin V⁺/PI⁺): late apoptotic cells; Q1-LL (Annexin V⁻/PI⁻): viable cells; Q1-LR (Annexin V⁺/PI⁻): early apoptotic cells. (B) Quantification of apoptotic cells. (C) Representative images of wound healing assay at 0 h and 24 h after treatment with Alisol B at indicated concentrations. Red lines indicate the initial wound edges at 0 h, superimposed on both 0 h and 24 h images to visualize cell migration. (D) Quantification of wound closure rate. The black bar represents the untreated control group (0 μM), and gray bars represent groups treated with Alisol B at the concentrations indicated on the x-axis. * $p < 0.05$, *** $p < 0.001$, vs. Control.

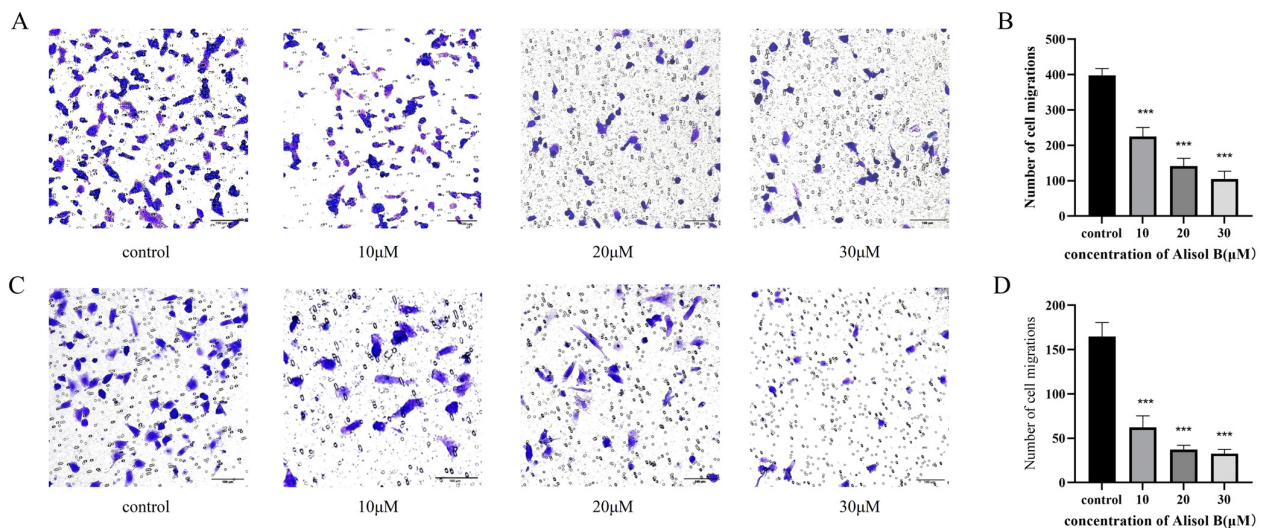


Figure 3. Alisol B inhibits transwell migration and invasion of T98G cells. ($n = 3$ independent biological replicates). (A) Representative images of migrated cells (B) Quantification of migrated cells. (C) Representative images of invaded cells. (D) Quantification of invaded cells. The black bar represents the untreated control group (0 μM), and gray bars represent groups treated with Alisol B at the concentrations indicated on the x-axis. *** $p < 0.001$, vs. Control.

2.5. Alisol B Inhibits Tumor Proliferation and Migration in a Zebrafish Xenograft Model

The zebrafish xenograft model, chosen for its suitability for real-time, in vivo imaging of tumor dynamics, was used to validate Alisol B's efficacy. First, the median lethal concentration (LC_{50}) of Alisol B in zebrafish was determined to be 35 μ M. Two subtoxic doses (1.75 μ M [1/20 LC_{50}] and 3.5 μ M [1/10 LC_{50}]) were selected for subsequent experiments.

T98G cells labeled with red fluorescent dye were microinjected into the yolk sac of 48 hpf zebrafish embryos. While fluorescence intensity was comparable across all groups at 2 h post-injection ($p > 0.05$), it was significantly reduced in Alisol B-treated groups at 48 h, indicating inhibition of tumor cell proliferation in vivo (Figure 4A,B).

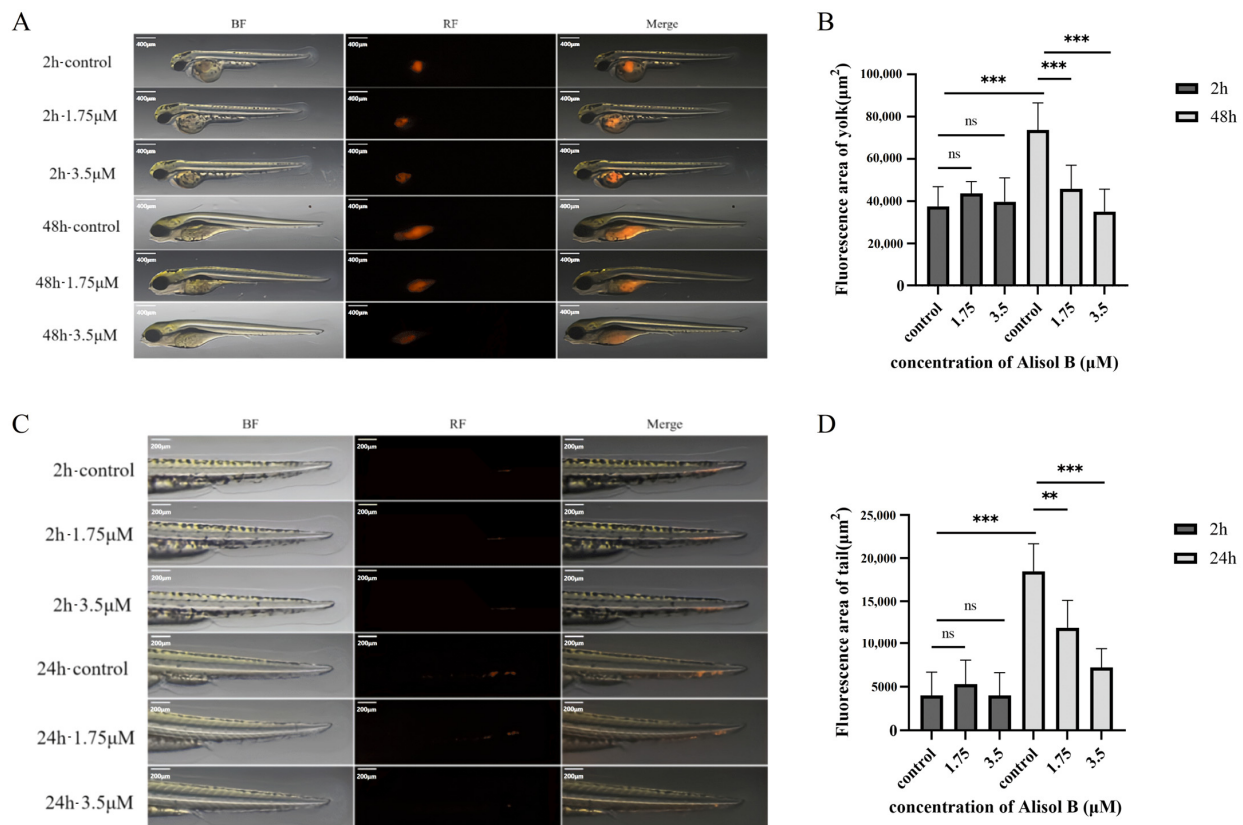


Figure 4. Alisol B inhibits the proliferation and migration of T98G glioma cells in a zebrafish xenograft model ($n \geq 10$ embryos per group). (A) Fluorescence imaging of yolk sac tumors at 2 and 48 h post-injection (hpi). BF: bright field; RF: red fluorescence (tumor cells); MERGE: overlay. Yellow indicates tumor cells. Scale bar: 400 μ m. (B) Quantification of yolk sac fluorescence area. (C) Fluorescence imaging of tumor cells in the tail region at 2 and 24 hpi. BF: bright field; RF: red fluorescence (tumor cells); MERGE: overlay. Yellow indicates tumor cells. Scale bar: 200 μ m. (D) Quantification of tail region fluorescence area. ** $p < 0.01$, *** $p < 0.001$, vs. Control, ns, not significant.

To assess anti-migratory effects, tumor cell dissemination to the tail region was quantified. At 24 h post-injection, both Alisol B treatment groups showed significant reductions in caudal tumor fluorescence area compared to the control (1.75 μ M: $p < 0.01$; 3.5 μ M: $p < 0.001$; Figure 4C,D), demonstrating inhibition of tumor cell migration in vivo.

2.6. Proteomic Profiling Reveals Alisol B-Induced Protein Expression Changes

Integrated proteomic analysis of T98G cells treated with 20 μ M Alisol B identified 6469 proteins. Functional annotation of all identified proteins is summarized in Figure 5A. Differential expression analysis (fold change >1.5 or $<1/1.5$, $p < 0.05$) revealed 124 significantly altered proteins (46 upregulated, 78 downregulated; Figure 5B).

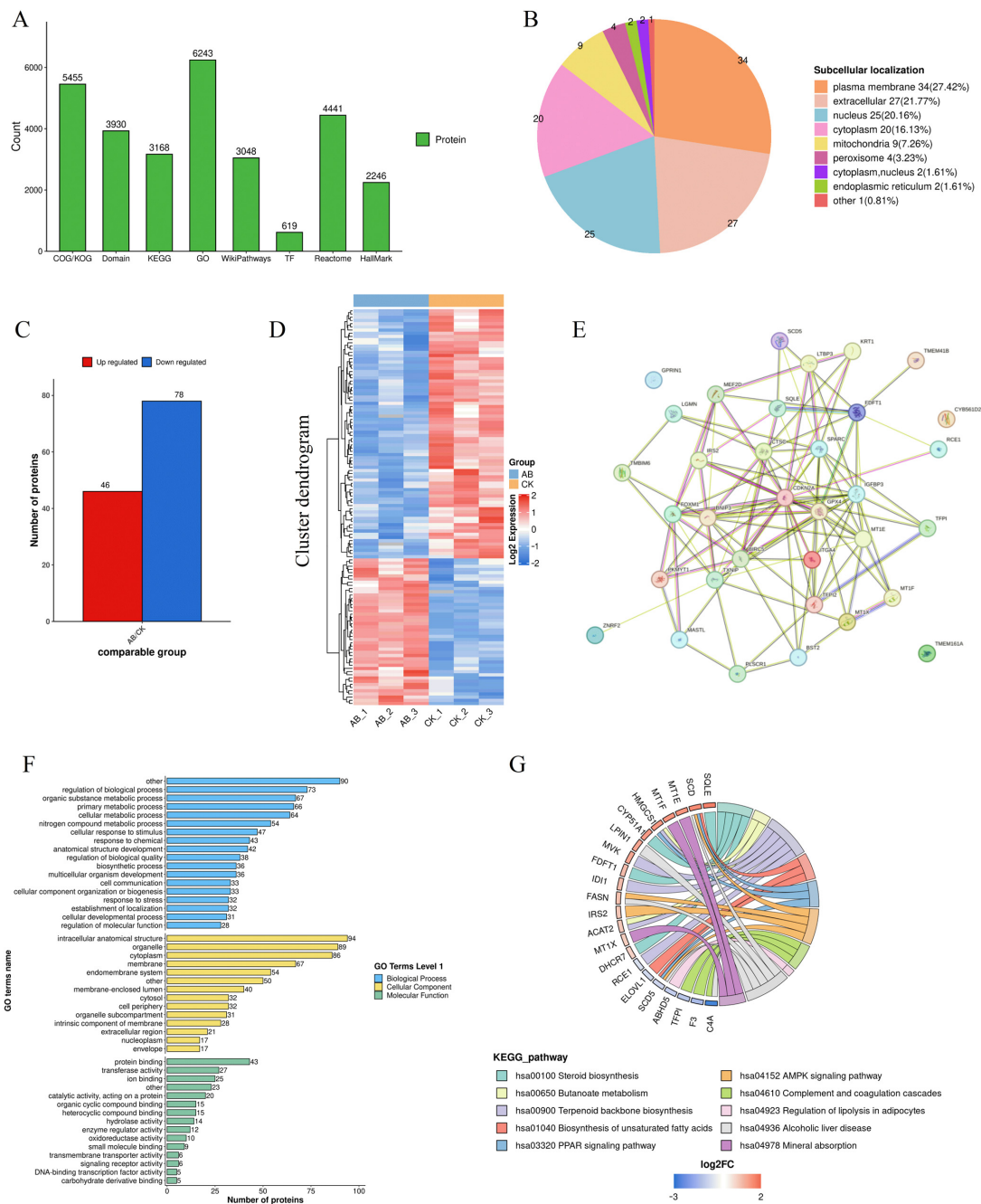


Figure 5. Proteomic profiling reveals Alisol B-induced differential protein expression and pathway enrichment. (A) Overview of protein functional annotations. Each bar represents the number of proteins annotated to specific functional categories. (B) Subcellular localization of DEPs. (C) Number of the up-regulated and down-regulated proteins. Red bar: up-regulated proteins; blue bar: down-regulated proteins. (D) Heatmap of differentially expressed proteins. AB: 20 μ M Alisol B-treated group; CK: control group. Rows represent individual proteins, and columns represent samples. Color scale from blue (low expression) to red (high expression) indicates relative protein abundance. (E) Protein-protein interaction network of cancer-related DEPs. Nodes represent proteins, and edges represent interactions. Node size reflects degree of connectivity. (F) Gene Ontology classification of DEPs. Bars represent the number of proteins enriched in biological processes, cellular components, and molecular functions. Different colors distinguish the three GO categories. (G) Chord diagram of enriched KEGG pathways. The right semicircle displays enriched KEGG pathways, with connecting lines representing associated differentially expressed proteins. Proteins are ordered clockwise according to descending $\log_2$ (fold-change) values. Each chord connects proteins to their associated pathways. Different colors represent distinct KEGG pathways.

From the 124 differentially expressed proteins (DEPs), we prioritized key tumor-associated targets, including upregulated (e.g., FDFT1, SQLE) and downregulated (e.g., forkhead box M1 (FOXM1), baculoviral IAP repeat containing 5 (BIRC5), stearoyl-CoA desaturase 5 (SCD5), integrin subunit alpha 4 (ITGA4)) molecules (Figure 5C,D). Among these, proteins such as FOXM1, BIRC5, and FDFT1 were highlighted for further investigation due to their established roles as master regulators of cell cycle, apoptosis resistance, and cholesterol biosynthesis in cancer, respectively. A protein–protein interaction network of these cancer-related DEPs revealed interconnected modules (Figure 5E), providing a topological framework for the multi-omics integration presented in Section 2.8.

Gene Ontology enrichment analysis indicated that DEPs were involved in processes such as regulation of biological processes, organic substance metabolism, and protein binding (Figure 5F). KEGG pathway analysis further showed significant enrichment in steroid biosynthesis, terpenoid backbone biosynthesis, and metabolic pathways (Figure 5G).

2.7. Acetyl-Proteomic Analysis Identifies Extensive Lysine Acetylation Reprogramming

Comprehensive lysine acetyl-proteomic profiling identified 1006 acetylated proteins in total. Functional annotation revealed their primary involvement in metabolic regulation and epigenetic processes (Figure 6A). Differential acetylation analysis identified 91 significantly altered lysine acetylation sites (35 upregulated, 56 downregulated) corresponding to 80 differentially acetylated proteins (DAPs; Figure 6B). A volcano plot visually highlights these key altered acetylation sites (Figure 6C). Subcellular localization analysis demonstrated that these DAPs were predominantly distributed in the cytoplasm (29%), nucleus (27%), and mitochondria (12%) (Figure 6D).

Key metabolic and regulatory proteins exhibited altered acetylation, including hypoacetylated FASN and FDFT1, and hyperacetylated nucleophosmin 1 (NPM1) and heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1) (Figure 6E). Gene Ontology (GO) enrichment analysis of DAPs implicated sterol biosynthesis, RNA processing, and organelle organization (Figure 6F–H). Reactome pathway analysis corroborated enrichment in cholesterol biosynthesis regulation and tumor protein p53 (TP53)-mediated cell cycle gene transcription (Figure 6I).

Notably, the number of significantly altered acetylation sites and proteins exceeded the number of DEPs, highlighting acetylation as a prevalent layer of regulation that may fine-tune protein function independently of abundance changes.

2.8. Integrated Multi-Omics Analysis Reveals Multi-Network Reprogramming

Integration of transcriptomic, proteomic, and acetyl-proteomic data provided a systems-level view of Alisol B's action (Figure 7A). Among 56 molecules altered in at least two omics layers, fatty acid synthase (FASN) was the only molecule dysregulated across all three platforms, underscoring its central role. Forty-one molecules overlapped between DEGs and DEPs (e.g., FOXM1, BIRC5), indicating coordinated transcriptional and translational responses. In contrast, only two proteins (FASN and FDFT1) were common to DEPs and DAPs, suggesting acetylation often operates independently of protein abundance changes.

Network analysis of the 56 overlapping molecules highlighted key regulatory patterns (Figure 7B). Triple-omics nodes like FASN and cyclin dependent kinase 1 (CDK1) showed complex fine-tuning. Correlation analysis revealed co-regulated clusters (e.g., lipid synthesis genes) and antagonistic relationships between metabolic and survival programs (Figure 7C). Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis identified terpenoid backbone biosynthesis, apoptosis, and central carbon metabolism as primary targets (Figure 7D).

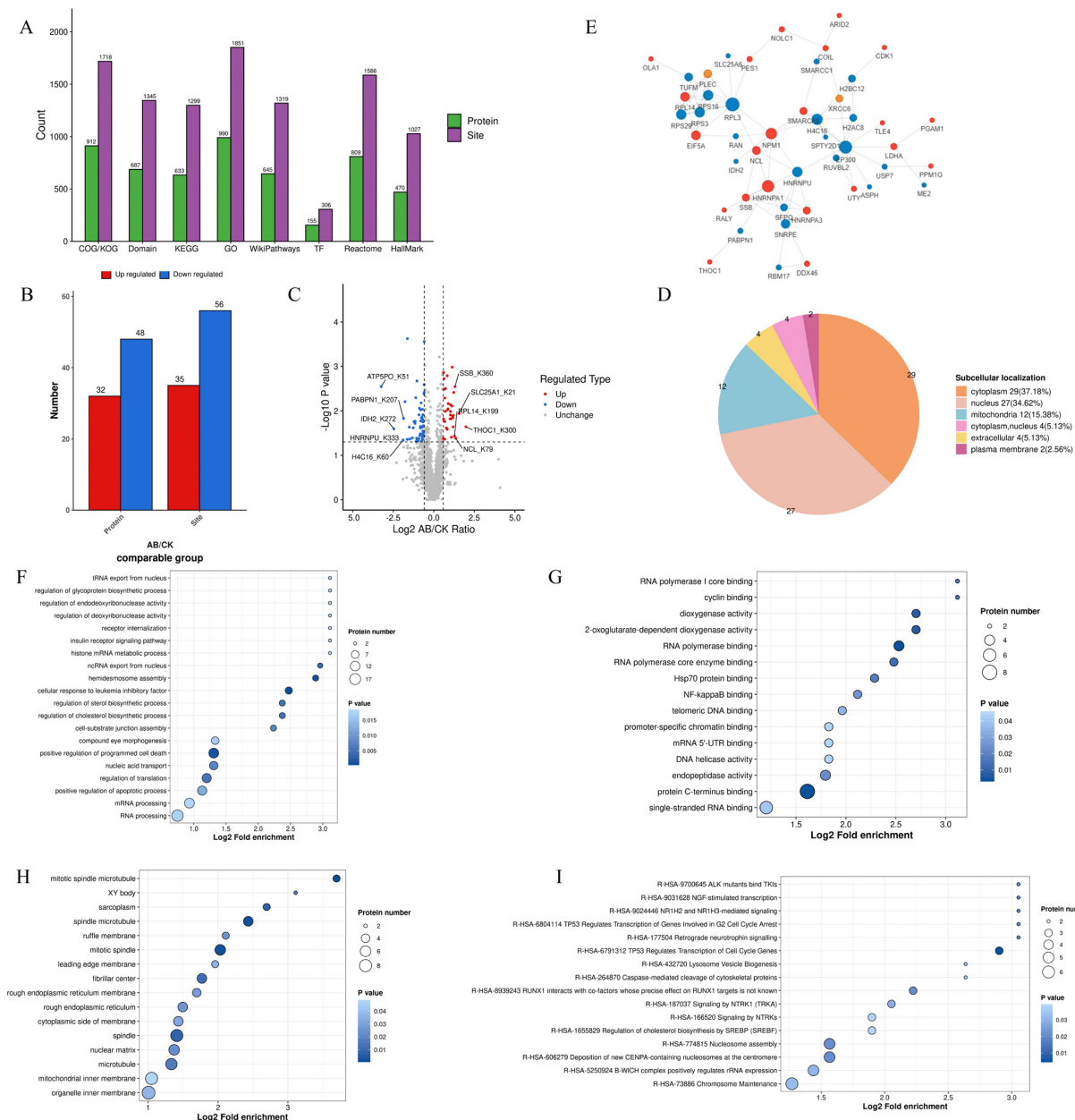


Figure 6. Acetyl-proteomic profiling identifies Alisol B-induced reprogramming of lysine acetylation. **(A)** Acetylated protein function annotation. Green bars represent the number of acetylated proteins, and purple bars represent the number of acetylation sites identified in each functional category. **(B)** Number of the up-regulated and down-regulated acetylation sites (red: up-regulated, blue: down-regulated, AB: 20 μ M Alisol B-treated group; CK: control group). **(C)** Volcano plot of differentially acetylated sites. The x-axis represents Log2(fold change), and the y-axis represents $-\text{Log}_{10}(p \text{ value})$. Red indicates up-regulated sites, blue indicates down-regulated sites, and gray denotes non-significant sites. The top five up-regulated and down-regulated sites are labeled. **(D)** Subcellular localization and distribution of DAPs. **(E)** PPI network of DAPs, with node size proportional to degree centrality and color indicating acetylation change (red: up, blue: down). **(F–H)** GO classification of DAPs (**(F)**: Biological Process, **(G)**: Molecular Function, **(H)**: Cellular Component). The y-axis shows GO terms, the x-axis shows Log2(fold enrichment), bubble color indicates enrichment significance ($p \text{ value}$), and bubble size corresponds to the number of mapped proteins. **(I)** Bubble chart of top enriched Reactome pathways. The y-axis shows pathway descriptions, the x-axis shows Log2(fold enrichment), bubble color indicates enrichment significance ($p \text{ value}$), and bubble size corresponds to the number of mapped proteins.

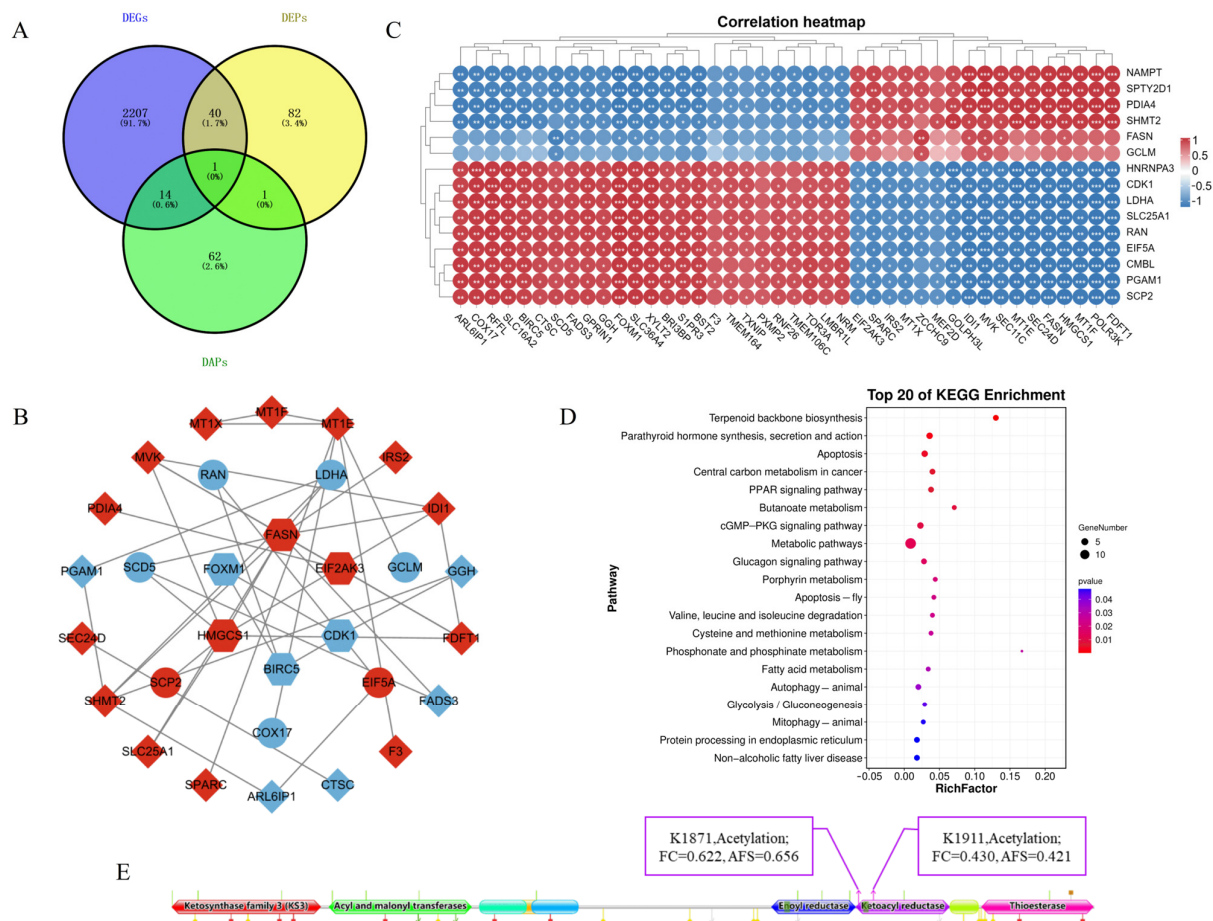


Figure 7. Integrated multi-omics analysis reveals molecular reprogramming. **(A)** Venn diagram of overlapping molecules across omics datasets. DEGs: Differentially Expressed Genes, DEPs: Differentially Expressed Proteins, DAPs: Differentially Acetylated Proteins. Numbers indicate unique and shared molecules. **(B)** PPI network of overlapping molecules. Node shapes indicate omics overlap: circle (single-omics), diamond (two-omics overlap), hexagon (triple-omics overlap). Node colors reflect regulation direction: red (up-regulated), blue (down-regulated). **(C)** Correlation heatmap among key DEPs and DAPs. Red: positive correlations; blue: negative correlations. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. **(D)** Bubble plot of enriched KEGG pathways. The x-axis represents the p value of enrichment. Bubble size corresponds to the number of overlapping molecules enriched in each pathway. **(E)** Domain architecture of FASN with identified acetylation sites. Green lines indicate known sites; magenta arrowheads highlight two hypoacetylated sites (K1871 and K1911) within the beta-ketoacyl reductase domain identified in this study. FC: Fold Change; AFS: Acetylation Functional Score.

Acetyl-proteomic profiling identified functionally relevant hypoacetylation sites on FASN (K1871 and K1911) within its beta-ketoacyl reductase domain (Figure 7E), which may impair its activity. The observed discordant regulation of the cholesterol pathway—transcriptional upregulation of early enzymes versus protein-level suppression of terminal enzymes—is consistent with a model of toxic intermediate accumulation. While these acetylation changes are consistent with targeted regulatory rewiring, we cannot completely exclude the contribution of stress-induced, non-specific acetylation alterations secondary to ER stress or metabolic crisis.

2.9. Orthogonal Validation of Key Multi-Omics Targets

Western blot analysis confirmed the dose-dependent suppression of key proteins predicted by multi-omics analysis in T98G cells (Figure 8A–I). This included downregulation of

the oncogenic axis components BIRC5, FOXM1, and ITGA4, the lipid metabolism regulator SCD5, and crucially, the terminal cholesterol biosynthesis enzymes 7-dehydrocholesterol reductase (DHCR7) and cytochrome P450 family 51 subfamily A member 1 (CYP51A1).

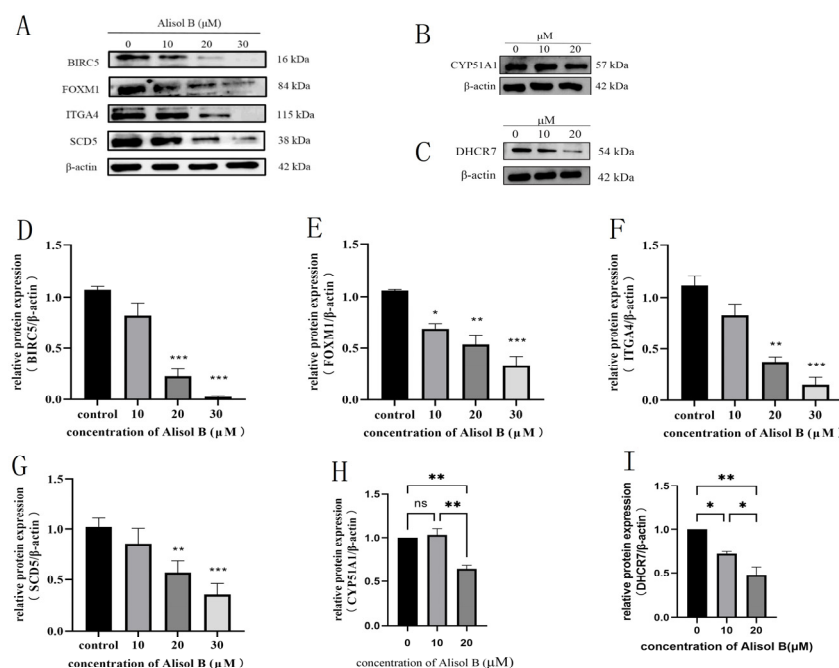


Figure 8. Protein-level validation of multi-omics targets ($n = 3$ independent biological replicates). (A–I) Representative Western blots of key proteins in T98G cells after Alisol B treatment. β -Actin served as a loading control. (D–I) Densitometric quantification from three independent experiments (mean $\pm$ SD). The black bar represents the untreated control group (0 μ M), and gray bars represent groups treated with Alisol B at the concentrations indicated on the x-axis. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant.

Consistent with the protein data, Alisol B treatment significantly downregulated BIRC5, FOXM1, and ITGA4 mRNA levels in tumor-bearing zebrafish (Figure S1). The downregulation of these transcripts in the xenograft model—where human tumor cells are the only source of these human-specific transcripts—indicates that this effect is largely tumor-cell autonomous.

Collectively, this orthogonal validation delineates a coherent network-targeted mechanism: Alisol B simultaneously disrupts an oncogenic survival axis, a key adhesion molecule, and lipid synthesis pathways, while specifically blocking the terminal steps of cholesterol biosynthesis.

3. Discussion

Glioblastoma remains a therapeutic impasse due to its intrinsic resistance and adaptive plasticity [36,37]. This study elucidates a sophisticated, multi-layered anti-tumor mechanism of the natural triterpenoid Alisol B against temozolomide (TMZ)-resistant GBM, moving beyond previous reports focusing on the inhibition of the MAPK signaling pathway [20] or the modulation of systemic lipid metabolism in non-oncological contexts [38]. By integrating transcriptomic, proteomic, and acetyl-proteomic datasets with orthogonal biochemical validation, we reveal that Alisol B does not merely inhibit a single pathway but orchestrates a systems-level catastrophe for the cancer cell, simultaneously targeting epigenetic regulation, core metabolism, and essential oncogenic signaling.

3.1. Epigenetic and Metabolic Synergistic Reprogramming: A Core Mechanism

Our integrated acetylome and proteome analysis positions epigenetic rewiring as a pivotal upstream event in Alisol B's action. The extensive reprogramming of lysine acetylation on histones and key metabolic enzymes (notably including FASN and the cholesterol synthesis enzyme FDFT1) directly connects to the dysregulation of core metabolic pathways, exemplifying the dynamic crosstalk between epigenetic and metabolic regulation—a key pathophysiological feature of gliomas highlighted in the Special Issue introduction [9]. A central and novel finding of our work is the induction of a discordant sterol metabolic state. Cancer cells, including GBM, are notoriously dependent on *de novo* cholesterol synthesis for membrane integrity, signaling, and proliferation [39–42]. Alisol B appears to hijack this dependency. While the cell perceives a sterol deficit—likely through sterol regulatory element-binding protein (SREBP) activation—and responds by transcriptionally upregulating the early mevalonate pathway (3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1), mevalonate kinase (MVK)), the acetylation modification of FDFT1 and the specific post-transcriptional silencing of the very last steps of cholesterol production (DHCR7, CYP51A1) by Alisol B create a unique metabolic bottleneck. This protein-level validation supports a model in which this discordant regulation leads to the accumulation of non-productive, and potentially toxic, sterol intermediates such as 7-dehydrocholesterol and lanosterol [43–45]. While our data confirm the terminal blockade and are consistent with this hypothesis, direct metabolite profiling in future studies will be required to definitively establish this causal link. Such intermediates can disrupt endoplasmic reticulum membrane fluidity and function, directly contributing to the observed severe ER stress [46,47], and may also exert cytotoxic effects through oxysterol formation or disruption of protein prenylation [48].

Furthermore, our acetyl-proteomic analysis identified a hypoacetylation of FASN at functionally relevant sites within its beta-ketoacyl reductase domain (K1871, K1911). Together with the validated downregulation of SCD5, this reveals a coordinated assault on lipid metabolism at both the post-translational and transcriptional levels.

3.2. Synergistic Disruption of the Oncogenic Network

This metabolic reprogramming likely acts synergistically with the direct suppression of the BIRC5-FOXM1-ITGA4 axis. Data from both *in vitro* and *in vivo* models consistently show that BIRC5 (Survivin) and FOXM1 are master regulators of cell cycle progression and chemo-resistance [49], while ITGA4, a key mediator of cell adhesion and migration, promotes tumor progression; its downregulation has been directly linked to inhibited cell migration and induced apoptosis [50]. Their co-downregulation, confirmed here from mRNA to protein, strikes at the heart of GBM's proliferative, therapy-evasive, and infiltrative nature.

The extensive rewiring of the lysine acetylome, particularly on metabolic enzymes and histones, extends our understanding of epigenetic regulation in glioma pathophysiology. While our study establishes a strong correlation between these modifications (e.g., FASN hypoacetylation) and downstream metabolic inhibition, the findings also introduce a novel epigenetic dimension to the metabolic dysregulation of IDH-wildtype GBM. This expands beyond the well-characterized DNA and histone methylation alterations in IDH-mutant tumors, suggesting that acetylation-mediated epigenetic plasticity is a critical and targetable vulnerability across glioma subtypes [9,24].

3.3. Implications for Therapeutic Strategy and Future Perspectives

Collectively, our multi-omics dissection provides a mechanistic exemplar of how a natural compound can co-opt and disrupt the core pathophysiological modules of IDH-

wildtype glioblastoma. The terminal blockade of cholesterol synthesis—a unique form of metabolic reprogramming—creates a lethal “metabolic trap” that exploits the tumor’s addiction to de novo lipogenesis while potentially bypassing compensatory feedback. When synergized with the direct dismantling of the cell-cycle engine (BIRC5-FOXM1-CDK1 axis) and pro-survival signaling, this coordinated multi-front attack illustrates a polypharmacology blueprint that directly addresses the heterogeneity and adaptive resistance that render single-target therapies ineffective in GBM [10].

Building on this blueprint, our findings transcend the mechanistic profiling of a single compound and offer a broader therapeutic paradigm. The coordinated multi-pathway efficacy of Alisol B validates the concept of simultaneously attacking the interconnected pathophysiological triad of gliomas: epigenetic plasticity, metabolic reprogramming, and dysregulated cell-cycle/survival signaling. Moreover, the extensive acetylome reprogramming positions epigenetic modulation as a potent upstream lever to preempt tumor adaptation.

Thus, this study provides a molecular pathophysiology framework that validates the simultaneous targeting of these core processes as a viable therapeutic strategy. Our work underscores that deconstructing natural products can yield precise insights for designing next-generation, polypharmacological treatment regimens [9]. These findings not only elucidate the polypharmacology of Alisol B but also provide a rational framework for designing novel combinatorial regimens or developing polypharmacological agents that co-opt the interconnected vulnerabilities of resistant glioblastoma.

3.4. Limitations and Future Perspectives

This study provides a comprehensive mechanistic map, but it also has limitations that chart the course for future research. First, direct mass spectrometry-based quantification of sterol intermediates (e.g., 7-dehydrocholesterol (7-DHC), lanosterol) is required to conclusively prove their accumulation. Second, functional rescue experiments, such as supplementing with cholesterol or specific sterol intermediates, would definitively link the metabolic blockade to cell death. Third, while the zebrafish model demonstrated efficacy, evaluating Alisol B in immunocompetent rodent GBM models, both as a monotherapy and in combination with TMZ or radiotherapy, is essential to assess its therapeutic potential and potential immune modulatory effects. Finally, a thorough investigation of its pharmacokinetics, blood–brain barrier penetration, and toxicity profile in non-tumor tissues is a prerequisite for translational development.

4. Materials and Methods

4.1. Ethical Approval

The zebrafish xenograft model was selected for this study due to its unique advantages for real-time, high-resolution *in vivo* imaging of tumor cell proliferation and dissemination, its optical transparency during early development, and its conserved vertebrate lipid metabolic pathways. This model allows for rapid, cost-effective assessment of Alisol B’s anti-tumor efficacy in a living vertebrate system, bridging the gap between *in vitro* findings and future validation in mammalian models. All animal experiments were conducted in compliance with the guidelines established by the Institutional Animal Care and Use Committee (IACUC) of Fujian Academy of Medical Sciences. The study protocol received formal approval from the Institutional Animal Ethics Committee of the same academy under the approval number DL2023-02.

4.2. Cell Culture

The human glioblastoma T98G cell line (Wuhan Punosai Life Technology Co., Ltd., Wuhan, China) was routinely cultured in high-glucose Dulbecco’s Modified Eagle Medium

(DMEM; PM150410, Procell Life Science & Technology Co., Ltd., Wuhan, China) supplemented with 10% (*v/v*) heat-inactivated fetal bovine serum (FSP500, Suzhou ExCell Biotechnology Co., Ltd., Suzhou, China) and 100 U/mL penicillin-streptomycin (HyClone, SV30010, Logan, UT, USA). Cells were maintained at 37 °C in a humidified incubator with a 5% CO₂ atmosphere (Galaxy 170 S, Eppendorf AG, Hamburg, Germany). The culture medium was refreshed every 48 h, and cells were passaged upon reaching 80–90% confluency.

4.3. CCK-8 Assay

Cell viability was assessed using the Cell Counting Kit-8 (CCK-8) assay. Briefly, T98G cells were collected, resuspended, and counted to prepare a single-cell suspension at a density of 1×10^4 cells/mL. A 100 µL aliquot of the suspension was seeded into each well of a 96-well plate and incubated for 24 h under standard conditions (37 °C, 5% CO₂) to allow adherence. After confirming cell attachment under a microscope, the medium in each well was replaced with fresh medium containing the specified concentrations of the drug (with drug-free medium used for the control group). Following another 24 h treatment period, 10 µL of CCK-8 reagent was added to each well, and the plate was further incubated for 1 h in the dark. The absorbance at 450 nm (with a reference wavelength of 650 nm) was then measured using a microplate reader. Cell viability for each treatment group was calculated as a percentage relative to the untreated control. Each experiment was performed with three independent biological replicates ($n = 3$).

4.4. Wound Healing Assay

To evaluate cell migratory capacity, a wound healing assay was performed. T98G cells were seeded into 6-well plates at an initial density of 5×10^5 cells/mL and cultured until they reached complete confluence. Two straight, perpendicular wounds were then introduced into the monolayer using a sterile pipette tip. After gently washing with phosphate buffered saline (PBS) to remove dislodged cells, fresh serum-free Minimum Essential Medium (MEM) medium containing the corresponding drug treatments was added to the wells, with control wells receiving medium only. The initial wound area (T_0) was immediately recorded under a microscope. After incubating the plates for 24 h, the same wound fields were imaged again to determine the remaining area at T_{24} . The cell migration rate was calculated using the following formula: Migration rate (%) = $1 - (\text{Scratch area at } T_{24} / \text{Scratch area at } T_0) \times 100\%$. The quantitative analysis of wound areas and subsequent statistical comparisons were conducted using ImageJ (version 1.53t) and GraphPad Prism software (version 9.5.0), respectively. Each condition was tested in three independent biological replicates.

4.5. Cell Transwell Invasion Assay

Cell invasion was assessed using Transwell chambers pre-coated with Matrigel. Briefly, after overnight thawing at 4 °C, Matrigel was diluted 1:8 in serum-free medium on ice and applied to the upper chamber (100 µL per well) to form a basement membrane gel through a 3 h incubation at 37 °C. Following hydration with serum-free medium, cells were harvested, resuspended in serum-free medium at a density of $2.5\text{--}3 \times 10^5$ cells/mL, and seeded into the upper chambers (200 µL per well). The lower chambers were filled with 700 µL of complete medium containing 10% serum and the indicated drug treatments. After 24 h of incubation, non-invasive cells on the upper surface of the membrane were removed with cotton swabs. Invasive cells on the lower membrane surface were fixed with 4% paraformaldehyde, stained with 0.1% crystal violet, and quantified by counting three random fields per chamber under a microscope. ImageJ software (version 1.53t) was used

to calculate the relative invasion rate for each group. Experiments were repeated three times independently (biological replicates, $n = 3$).

4.6. Cell Transwell Migration Assay

Cell migration was assessed using Transwell chambers. After 24 h serum starvation, cells were harvested and resuspended in serum-free medium at a density of $2\text{--}2.5 \times 10^5$ cells/mL. For each well, 700 μL of complete medium containing 10% serum and the respective drug treatment was placed in the lower compartment, while 200 μL of cell suspension was added to the upper chamber. Following 24 h incubation, non-migrated cells on the upper membrane surface were removed with cotton swabs. Migrated cells on the lower surface were fixed with 4% paraformaldehyde, stained with 0.1% crystal violet, and quantified by counting three random microscopic fields per membrane using ImageJ software (version 1.53t) to calculate the migration rate. Three independent biological replicates were performed.

4.7. Cell Apoptosis Assay

Cell apoptosis was evaluated via Annexin V-FITC and propidium iodide (PI) staining followed by flow cytometry. After 24 h treatment with drugs at specified concentrations in MEM medium, both adherent and floating cells were collected. Cells were detached using trypsin (without EDTA), neutralized with complete medium, washed with PBS, and counted. For staining, approximately $1\text{--}5 \times 10^5$ cells were resuspended in 100 μL of $1 \times$ Annexin V binding buffer. According to the standard protocol, 2.5 μL of Annexin V-FITC and 2.5 μL of PI were added sequentially, followed by a 15 min incubation in the dark at room temperature. The reaction was terminated by adding 400 μL of binding buffer. All samples were kept on ice and protected from light, and flow cytometry analysis was completed within 60 min.

4.8. Determination of LC_{50} of Alisol B in a Zebrafish Xenograft Glioma Model

Collected embryos were disinfected with 0.003% sodium hypochlorite (NaClO), and 24 hpf embryos were selected and placed in six-well plates (20 embryos/well). The experiment included a blank control group and six concentration gradients of Alisol B (15, 20, 25, 30, 35, and 40 μM), each with three replicates. Stock solutions were serially diluted to achieve the desired concentrations in a final volume of 4 mL per well. Culture plates were incubated in darkness for 48 h in a temperature-controlled incubator set at 28.5°C , after which embryo mortality was recorded. The LC_{50} was calculated using probit analysis. A dose–response relationship was established by nonlinear regression, and the LC_{50} value was determined using GraphPad Prism software (version 9.5.0).

Prior to each experimental session, the injection volume was calibrated using a standardized droplet measurement protocol. Tumor cell suspensions were dispensed into mineral oil-coated slides using the microinjection system, and droplet diameters were measured with a calibrated eyepiece reticle under a stereomicroscope. Volumes were calculated using the spherical volume formula ($V = 4/3\pi r^3$), with injection parameters adjusted to consistently deliver 1–2 nL per embryo. This calibration was repeated at the beginning of each session and every 2 h during prolonged experiments to account for potential needle variations. Mineral oil prevented droplet evaporation ($<5\%$ volume variation), and needles were replaced every 50 injections to maintain precision. Embryos receiving injections with $>10\%$ volume deviation were excluded from analysis.

4.9. Construction of Zebrafish T98G Glioblastoma Xenograft Model

To establish the xenograft model, healthy 24 hpf AB strain zebrafish embryos were selected and maintained in egg water containing 0.003% 1-phenyl 2-thiourea (PTU) at

28.5 °C to inhibit pigmentation. T98G glioblastoma cells were cultured, harvested using trypsin, and labeled with 5 µM Dil fluorescent dye. At 48 hpf, embryos were anesthetized with 0.02% tricaine and microinjected with 150–200 cells into either the yolk sac or the perivitelline cavity using a calibrated microinjection system (Femtojetv 4I/Eppendorf, 10 nL/injection). Injected embryos were immediately transferred to anesthetic-free egg water for recovery. For each experimental group, a minimum of 30 embryos were injected to account for potential technical losses during microinjection. Sample sizes were determined based on established protocols in zebrafish xenograft studies and preliminary experiments showing consistent and significant effects with similar group sizes. Only larvae that survived the injection procedure, exhibited normal development, and contained a confirmed fluorescent tumor graft at the 2 h post-injection checkpoint were included in the final analysis. The exact number of experimental units (n) included per group is reported in the corresponding figure legends. During subsequent imaging and quantitative analysis of tumor proliferation and migration, embryos were randomly assigned codes, and investigators were blinded to the treatment groups to minimize potential bias.

4.10. Evaluation of Alisol B Anti-Proliferative Effects in Zebrafish Xenograft Model

To evaluate the anti-proliferative effects of Alisol B, zebrafish larvae with yolk sac-transplanted tumor cells were divided into three groups ($n \geq 10$ /group) receiving either E3 medium (control), 1/20 LC₅₀ Alisol B, or 1/10 LC₅₀ Alisol B (4 mL/group). The larvae were maintained at 35 °C for 48 h with daily solution replacement, and tumor growth was monitored by quantifying the fluorescent area of transplanted cells using stereomicroscopy at 2 and 48 h post-transplantation.

4.11. Evaluation of Anti-Migratory Effects of Alisol B in Zebrafish Xenograft Model

To evaluate the effects of Alisol B on tumor cell migration, zebrafish larvae with yolk sac-transplanted tumor cells were divided into three treatment groups ($n \geq 10$ /group): control (4 mL E3 medium), 1/20 LC₅₀ Alisol B, and 1/10 LC₅₀ Alisol B. The larvae were maintained at 35 °C for 24 h with daily solution replacement, and tumor cell migration was assessed by quantifying the fluorescent area of tumor cells in the tail region using fluorescence stereomicroscopy at 2 and 24 h post-transplantation.

4.12. Proteomic and Lysine Acetylome Analysis

To comprehensively profile protein expression and post-translational modifications induced by Alisol B, integrated proteome and lysine acetylome profiling was conducted on T98G cells. Analyses were performed on three independent biological replicates per condition to ensure statistical robustness. Proteins were extracted using a urea-based lysis buffer supplemented with deacetylase inhibitors (3 µM trichostatin A (TSA), 50 mM nicotinamide (NAM)) for acetylome preservation. After tryptic digestion and desalting, peptides were divided for parallel processing: one portion for whole proteome analysis and the other subjected to immunoaffinity enrichment of acetylated peptides using anti-acetyllysine antibody beads (PTM Bio Co., Ltd., Hangzhou, China).

The LC-MS/MS detection and data acquisition were performed by Jingjie PTM BioLab Co., Ltd. (Hangzhou, China). Both whole proteome and enriched acetylated peptides were analyzed under identical conditions using a NanoElute ultra-high performance liquid chromatography (UHPLC) system coupled to a timsTOF Pro 2 mass spectrometer (Bruker Daltonics GmbH & Co. KG, Bremen, Germany) operated in data-independent acquisition—parallel accumulation serial fragmentation (dia-PASEF) mode to ensure direct quantitative comparability.

4.13. Transcriptomic Data Source

The transcriptomic data used in the integrated multi-omics analysis were derived from our previously published study, in which T98G cells were treated with 20 μ M Alisol B and subjected to RNA sequencing. This concentration was selected based on its sub-cytotoxic efficacy (approximately IC_{50}) observed in preliminary phenotypic assays, aiming to capture pathway modulation prior to extensive cell death. The detailed experimental procedures, bioinformatics analysis, and the full dataset of differentially expressed genes (DEGs) have been described in detail [20]. In the present study, this dataset was integrated with newly generated proteomic and acetyl-proteomic data for multi-omics analysis.

4.14. Bioinformatics Methods

The goal of bioinformatics analysis was to identify significantly altered molecules and infer their biological functions. The initial identification of proteins and acetylated peptides was conducted by searching MS/MS spectra against the appropriate databases. Positive identifications were filtered by applying a 1% false discovery rate (FDR) cutoff at both the peptide and protein levels, and only proteins supported by at least one unique peptide were retained for further analysis. For the acetylome dataset, lysine acetylation was specified as a variable modification during the database search. Acetylation sites with a localization probability score of 0.75 or higher were classified as confidently localized. Sites meeting the criteria of an absolute $\log_2$ fold change $> \log_2(1.5)$ and a statistical significance of $p < 0.05$ were defined as differentially acetylated sites (DAS), and their corresponding proteins were designated as differentially acetylated proteins (DAPs). Functional enrichment analyses, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses, were subsequently performed on the DAcP list to infer biological implications.

Protein functional annotation was performed using an integrated workflow for all identified proteins, with a specific focus on those carrying differentially acetylated sites. This analysis encompassed Gene Ontology (GO) classification (cellular components, molecular functions, biological processes) via evolutionary genealogy of genes: Non-supervised Orthologous Groups - mapper (eggNOG-mapper); protein domain prediction using Pfam Search Tool (PfamScan) against the Protein Families database (Pfam); pathway enrichment analysis through KEGG (Basic Local Alignment Search Tool for proteins (blastp), $e\text{-value} \leq 1 \times 10^{-4}$), Reactome Pathway Database (Reactome), and WikiPathways Database (WikiPathways); subcellular localization prediction with Wolf PSORT (Protein Subcellular Localization Prediction Tool); orthologous group classification via EggNOG (Clusters of Orthologous Groups of proteins (COG)/eukaryotic Orthologous Groups (KOG)); and specialized annotations including Hallmark gene sets and transcription factors (TRRUST for human). Functional enrichment analysis of differentially expressed proteins (DEPs) and proteins harboring DAS was conducted using Fisher's exact test, with all identified proteins serving as the background. Terms demonstrating a fold enrichment > 1.5 and a $p\text{-value} < 0.05$ were deemed significant. Enriched terms from GO, KEGG, and other databases were subjected to hierarchical clustering and visualized as a heatmap following a $-\log_{10}(p\text{-value})$ transformation.

Acetylation Site Functional Scoring. To assess the potential functional importance of identified differentially acetylated lysine sites, each site was assigned an Acetylation Functional Score (AFS) using the published Lysine Acetylation Functional Evaluating Model (LAFEM) [51]. This machine learning model predicts the functional relevance of an acetylation site on a scale from 0 to 1 based on eight molecular features, including solvent-accessible surface area, sequence homology, and disorder probability. A higher AFS indicates a greater predicted likelihood that acetylation at that site regulates protein

function. The protein domain topology schematic in this study was automatically generated using the ProToDeviseR software (<https://matrinet.shinyapps.io/ProToDeviseR/>, accessed on 7 January 2026, version 4.4.0). ProToDeviseR is an R package for the automated generation of protein topology diagrams [52]. Domain annotations integrated into the schematic were obtained from the Pfam database [53].

4.15. Statistical Analysis

Data were statistically analyzed with GraphPad Prism (version 9.5). For comparisons among multiple groups, one-way analysis of variance (ANOVA) was employed, followed by Tukey's test for post hoc analysis. Continuous data are expressed as mean $\pm$ standard deviation (SD). Differences were considered statistically significant at $p < 0.05$ (two-tailed).

5. Conclusions

In conclusion, this integrated multi-omics study delineates a novel, multi-mechanistic action of the natural triterpenoid Alisol B against therapy-resistant, IDH-wildtype glioblastoma. We demonstrate that Alisol B orchestrates multi-pathway disruption for the cancer cell by: (i) inducing extensive epigenetic reprogramming via lysine acetylation, (ii) triggering a unique dysfunctional metabolic state through terminal blockade of cholesterol synthesis, and (iii) dismantling the core oncogenic BIRC5-FOXM1-ITGA4 axis. These findings elucidate the polypharmacology of Alisol B. More importantly, they provide a rational framework for future therapeutic development, paving the way for novel combinatorial regimens or polypharmacological agents designed to co-opt the interconnected vulnerabilities of resistant glioblastoma.

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References

1. Foltyn-Dumitru, M.; Banan, R.; Schell, M.; Mahmutoglu, M.A.; Kessler, T.; Wick, W.; Brugnara, G.; Bendszus, M.; Sahm, F.; Vollmuth, P. Histopathological and Molecular Characteristics of IDH-Wildtype Glioblastoma without Contrast Enhancement: Implications for Clinical Outcomes. *Neuro Oncol.* **2025**, *27*, 1878–1887. [[CrossRef](#)]
2. Dakal, T.C.; Kakde, G.S.; Maurya, P.K. Genomic, Epigenomic and Transcriptomic Landscape of Glioblastoma. *Metab. Brain Dis.* **2024**, *39*, 1591–1611. [[CrossRef](#)] [[PubMed](#)]

3. Stepka, J.; Dotka, M.; Kosiński, M.; Suchecki, P.; Hobot, M.; Piotrowski, I. The Role of Systemic Therapies in the Treatment of Grades 1-4 Gliomas. *Cureus* **2024**, *16*, e70532. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Costa, A.L.; Doncevic, D.; Wu, Y.; Yang, L.; Man, K.H.; Spreng, A.-S.; Winter, H.; Fletcher, M.N.C.; Radlwimmer, B.; Herrmann, C. A New IDH-Independent Hypermethylation Phenotype Is Associated with Astrocyte-like Cell State in Glioblastoma. *Genome Biol.* **2025**, *26*, 192. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Siddaway, R.; Milos, S.; Vadivel, A.K.A.; Dobson, T.H.W.; Swaminathan, J.; Ryall, S.; Pajovic, S.; Patel, P.G.; Nazarian, J.; Becher, O.; et al. Splicing Is an Alternate Oncogenic Pathway Activation Mechanism in Glioma. *Nat. Commun.* **2022**, *13*, 588. [\[CrossRef\]](#)
6. Cortes Ballen, A.I.; Amosu, M.; Ravinder, S.; Chan, J.; Derin, E.; Slika, H.; Tyler, B. Metabolic Reprogramming in Glioblastoma Multiforme: A Review of Pathways and Therapeutic Targets. *Cells* **2024**, *13*, 1574. [\[CrossRef\]](#)
7. Chava, S.; Wajapeyee, N. NSD Proteins in Anti-Tumor Immunity and Their Therapeutic Targeting by Protein Degraders. *Cell Mol. Life Sci.* **2025**, *82*, 268. [\[CrossRef\]](#)
8. Anguraj Vadivel, A.K.; Pajovic, S.; Siddaway, R.; Zhu, S.; Sbergio, S.-G.; Matic, O.; Phillips, L.; Bu, Y.J.; Nitz, M.; Hawkins, C. The Proteomic Landscape of Diffuse Midline Glioma Highlights the Therapeutic Potential of Non-Histone Protein Methyltransferases. *Neuro Oncol.* **2025**, *27*, 1829–1846. [\[CrossRef\]](#)
9. Natarajan, S.K.; Pun, M.; Haggerty-Skeans, J.; Venneti, S. Integration and Intersection of Cancer Metabolism with Epigenetic Pathways in Gliomas. *Annu. Rev. Pathol.* **2026**, *21*, 447–477. [\[CrossRef\]](#)
10. Arieu-Bonnet, J.; Berges, R.; Montero, M.-P.; Mouysset, B.; Piris, P.; Muller, K.; Pinna, G.; Failes, T.W.; Arndt, G.M.; Morando, P.; et al. Combination Drug Screen Targeting Glioblastoma Core Vulnerabilities Reveals Pharmacological Synergisms. *EBioMedicine* **2023**, *95*, 104752. [\[CrossRef\]](#)
11. Ma, J.; Zhang, Y.; Du, J.; Chen, J.; Sun, J.; Ma, X.; Zeng, J.; Efferth, T. High-Fat Diet-Associated Digestive Cancers: Mechanisms, Natural Product-Based Therapies, and Drug Development. *Phytomedicine* **2025**, *150*, 157574. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Bayoudh, A.; Tarhouni, N.; Ben Mansour, R.; Mekrazi, S.; Sadraoui, R.; Kriaa, K.; Ahmed, Z.; Soussi, A.; Kallel, I.; Hadrich, B. Integrated Network Pharmacology and Molecular Dynamics Reveal Multi-Target Anticancer Mechanisms of *Myrtus communis* Essential Oils. *Pharmaceuticals* **2025**, *18*, 1542. [\[CrossRef\]](#)
13. Qi, Y.; Yan, J.; Huang, X.; Jiang, X.; Li, R.; Wan, J.; Li, Y.; Miao, Z.; Song, Z.; Liu, Y.; et al. Targeting Tumor-Associated Macrophage Polarization with Traditional Chinese Medicine Active Ingredients: Dual Reversal of Chemoresistance and Immunosuppression in Tumor Microenvironment. *Pharmacol. Res.* **2025**, *216*, 107788. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Xu, T.; Zhang, W.; Zhang, Y.; Song, F.; Huang, P. Ophiopogonin D' Inhibited Tumour Growth and Metastasis of Anaplastic Thyroid Cancer by Modulating JUN/RGS4 Signalling. *J. Cell Mol. Med.* **2024**, *28*, e70014. [\[CrossRef\]](#)
15. Zhao, Z.-X.; Zou, Q.-Y.; Ma, Y.-H.; Morris-Natschke, S.L.; Li, X.-Y.; Shi, L.-C.; Ma, G.-X.; Xu, X.-D.; Yang, M.-H.; Zhao, Z.-J.; et al. Recent Progress on Triterpenoid Derivatives and Their Anticancer Potential. *Phytochemistry* **2025**, *229*, 114257. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Ma, J.-J.; Zhang, L.-F.; Xu, D.-P.; Wang, Z.; Meng, Y.-Q. Design, Synthesis and Antitumor Activity of Pentacyclic Triterpenoid Ursolic Acid Derivatives and Oleanolic Acid Derivatives Based on Multi-Target. *J. Asian Nat. Prod. Res.* **2025**, *27*, 1429–1446. [\[CrossRef\]](#)
17. Lin, W.; Sun, F.; Zhang, Y.; Xu, X.; Lu, X.; Li, L.; Xu, R. Comparative Transcriptome and Metabolite Profiling of Four Tissues from *Alisma orientale* (Sam.) Juzep Reveals Its Inflorescence Developmental and Medicinal Characteristics. *Sci. Rep.* **2019**, *9*, 12310. [\[CrossRef\]](#)
18. Zhang, A.; Sheng, Y.; Zou, M. Antiproliferative Activity of Alisol B in MDA-MB-231 Cells Is Mediated by Apoptosis, Dysregulation of Mitochondrial Functions, Cell Cycle Arrest and Generation of Reactive Oxygen Species. *Biomed. Pharmacother.* **2017**, *87*, 110–117. [\[CrossRef\]](#)
19. Jang, E.; Lee, J.-H. Promising Anticancer Activities of *Alismatis rhizome* and Its Triterpenes via P38 and PI3K/Akt/mTOR Signaling Pathways. *Nutrients* **2021**, *13*, 2455. [\[CrossRef\]](#)
20. Wen, X.; Xu, X.; Zhang, Y.; Lu, X.; Li, L.; Jiang, R.; Lin, W. Study on the Mechanism of Inhibitory Effect of Alismol B on Glioma Cell Proliferation Based on Transcriptome and In Vitro Experiments. *J. Chin. Med. Mater.* **2023**, *46*, 2030–2038. [\[CrossRef\]](#)
21. François, C.M.; Pihl, T.; Dunoyer de Segonzac, M.; Héroult, C.; Hudry, B. Metabolic Regulation of Proteome Stability via N-Terminal Acetylation Controls Male Germline Stem Cell Differentiation and Reproduction. *Nat. Commun.* **2023**, *14*, 6737. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Han, R.; Wu, Y.; Yang, Y.; Wang, Q.; Ding, T.; Zhang, X.; Yang, J. Dynamic Proteomic and Acetylomic Profiling of Mechanically Induced Cancer Stemness in Breast Cancer Cells. *Proteomics* **2025**, *25*, e202400409. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Wang, W.W.; Singha Roy, S.J.; Parker, C.G. Targeted Protein Acetylation Through Chemically Induced Proximity. *Acc. Chem. Res.* **2025**, *58*, 2695–2707. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Shvedunova, M.; Akhtar, A. Modulation of Cellular Processes by Histone and Non-Histone Protein Acetylation. *Nat. Rev. Mol. Cell Biol.* **2022**, *23*, 329–349. [\[CrossRef\]](#)

25. Facchini, G.; Ignarro, R.S.; Rodrigues-Silva, E.; Vieira, A.S.; Lopes-Cendes, I.; Castilho, R.F.; Rogerio, F. Toxic Effects of Phytol and Retinol on Human Glioblastoma Cells Are Associated with Modulation of Cholesterol and Fatty Acid Biosynthetic Pathways. *J. Neurooncol.* **2018**, *136*, 435–443. [\[CrossRef\]](#)
26. Choo, M.; Mai, V.-H.; Kim, H.S.; Kim, D.-H.; Ku, J.-L.; Lee, S.K.; Park, C.-K.; An, Y.J.; Park, S. Involvement of Cell Shape and Lipid Metabolism in Glioblastoma Resistance to Temozolomide. *Acta Pharmacol. Sin.* **2023**, *44*, 670–679. [\[CrossRef\]](#)
27. Ye, M.; Hu, C.; Chen, T.; Yu, P.; Chen, J.; Lu, F.; Xu, L.; Zhong, Y.; Yan, L.; Kan, J.; et al. FABP5 Suppresses Colorectal Cancer Progression via mTOR-Mediated Autophagy by Decreasing FASN Expression. *Int. J. Biol. Sci.* **2023**, *19*, 3115–3127. [\[CrossRef\]](#)
28. Mo, H.; Shao, J.; Li, Z.; Zeng, P.; Yin, X.; Huang, Y.; Wang, P.; Liao, J. FDFT1 Maintains Glioblastoma Stem Cells through Activation of the Akt Pathway. *Stem Cell Res. Ther.* **2024**, *15*, 492. [\[CrossRef\]](#)
29. Marzook, N.B.; Song, O.-R.; Baumgärtel, L.; Bernitz, N.; Mkandawire, T.T.; Watson, L.C.; Nunes, V.; Warchal, S.; MacRae, J.I.; Howell, M.; et al. The Essential Host Genome for Cryptosporidium Survival Exposes Metabolic Dependencies That Can Be Leveraged for Treatment. *Cell* **2025**, *188*, 5947–5961.e15. [\[CrossRef\]](#)
30. De Martino, M.; Daviaud, C.; Minns, H.E.; Lazarian, A.; Wacker, A.; Costa, A.P.; Attarwala, N.; Chen, Q.; Choi, S.-W.; Rabadán, R.; et al. Radiation Therapy Promotes Unsaturated Fatty Acids to Maintain Survival of Glioblastoma. *Cancer Lett.* **2023**, *570*, 216329. [\[CrossRef\]](#)
31. van Bree, N.; Oppelt, A.-S.; Lindström, S.; Zhou, L.; Boutin, L.; Coyle, B.; Swartling, F.J.; Johnsen, J.I.; Bräutigam, L.; Wilhelm, M. Development of an Orthotopic Medulloblastoma Zebrafish Model for Rapid Drug Testing. *Neuro Oncol.* **2025**, *27*, 779–794. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Guo, X.; Guo, Z.; Sun, D.; Zou, L.; Ou, J.; Yu, L.; Lu, Z.; Cao, H.; Liu, J. Single extract of Forsythia Suspense versus the prepared drug in pieces: Comparison of their anti-inflammatory, antitumor and antibacterial effects in zebrafish. *Nan Fang Yi Ke Da Xue Xue Bao* **2024**, *44*, 594–604. [\[CrossRef\]](#)
33. Murali Shankar, N.; Ortiz-Montero, P.; Kurzyukova, A.; Rackwitz, W.; Künzel, S.R.; Wels, W.S.; Tonn, T.; Knopf, F.; Eitler, J. Preclinical Assessment of CAR-NK Cell-Mediated Killing Efficacy and Pharmacokinetics in a Rapid Zebrafish Xenograft Model of Metastatic Breast Cancer. *Front. Immunol.* **2023**, *14*, 1254821. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Sturtzel, C.; Grissenberger, S.; Bozatzi, P.; Scheuringer, E.; Wenninger-Weinzierl, A.; Zajec, Z.; Dernovšek, J.; Pascoal, S.; Gehl, V.; Kutsch, A.; et al. Refined High-Content Imaging-Based Phenotypic Drug Screening in Zebrafish Xenografts. *NPJ Precis. Oncol.* **2023**, *7*, 44. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Weiss, A.; D'Amata, C.; Pearson, B.J.; Hayes, M.N. A Syngeneic Spontaneous Zebrafish Model of Tp53-Deficient, EGFRvIII, and PI3KCAH1047R-Driven Glioblastoma Reveals Inhibitory Roles for Inflammation during Tumor Initiation and Relapse In Vivo. *Elife* **2024**, *13*, RP93077. [\[CrossRef\]](#)
36. Ullrich, V.; Ertmer, S.; Baginska, A.; Dorsch, M.; Gull, H.H.; Cima, I.; Berger, P.; Dobersalske, C.; Langer, S.; Meyer, L.; et al. KDM5B Predicts Temozolomide-Resistant Subclones in Glioblastoma. *iScience* **2024**, *27*, 108596. [\[CrossRef\]](#)
37. Wang, J.; Li, K.; Wang, Y.; Zhang, J.; Peng, X.; Ji, N. Niche-Specific Epigenetic Interventions in the Spatially Heterogeneous Glioblastoma Microenvironmental Landscape: Strategies for Radiotherapy Enhancement. *Front. Mol. Biosci.* **2025**, *12*, 1704311. [\[CrossRef\]](#)
38. Gao, G.; Zhao, J.; Ding, J.; Liu, S.; Shen, Y.; Liu, C.; Ma, H.; Fu, Y.; Xu, J.; Sun, Y.; et al. Alisol B Regulates AMPK/mTOR/SREBPs via Directly Targeting VDAC1 to Alleviate Hyperlipidemia. *Phytomedicine* **2024**, *128*, 155313. [\[CrossRef\]](#)
39. Guo, X.; Zhou, S.; Yang, Z.; Li, Z.-A.; Hu, W.; Dai, L.; Liang, W.; Wang, X. Cholesterol Metabolism and Its Implication in Glioblastoma Therapy. *J. Cancer* **2022**, *13*, 1745–1757. [\[CrossRef\]](#)
40. Chung, J.; Saad, J.; Kafri, A.; Rossignol, J.; Verbrugge, M.; Bakke, J. Metabolism of Glioblastoma: A Review of Metabolic Adaptations and Metabolic Therapeutic Interventions. *Front. Oncol.* **2025**, *15*, 1712576. [\[CrossRef\]](#)
41. Zhao, L.; Qiu, Z.; Yang, Z.; Xu, L.; Pearce, T.M.; Wu, Q.; Yang, K.; Li, F.; Saulnier, O.; Fei, F.; et al. Lymphatic Endothelial-like Cells Promote Glioblastoma Stem Cell Growth through Cytokine-Driven Cholesterol Metabolism. *Nat. Cancer* **2024**, *5*, 147–166. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Gu, D.; Zhou, F.; You, H.; Gao, J.; Kang, T.; Dixit, D.; Wu, Q.; Yang, K.; Ci, S.; Shan, D.; et al. Sterol Regulatory Element-Binding Protein 2 Maintains Glioblastoma Stem Cells by Keeping the Balance Between Cholesterol Biosynthesis and Uptake. *Neuro Oncol.* **2023**, *25*, 1578–1591. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Li, Y.; Ran, Q.; Duan, Q.; Jin, J.; Wang, Y.; Yu, L.; Wang, C.; Zhu, Z.; Chen, X.; Weng, L.; et al. 7-Dehydrocholesterol Dictates Ferroptosis Sensitivity. *Nature* **2024**, *626*, 411–418. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Dong, Y.; Zhang, J.; Wang, Y.; Zhang, Y.; Rappaport, D.; Yang, Z.; Han, M.; Liu, Y.; Fu, Z.; Zhao, X.; et al. Intracavitary Spraying of Nanoregulator-Encased Hydrogel Modulates Cholesterol Metabolism of Glioma-Supportive Macrophage for Postoperative Glioblastoma Immunotherapy. *Adv. Mater.* **2024**, *36*, e2311109. [\[CrossRef\]](#)
45. Skubic, C.; Trček, H.; Nassib, P.; Kreft, T.; Walakira, A.; Pohar, K.; Petek, S.; Režen, T.; Ihan, A.; Rozman, D. Knockouts of CYP51A1, DHCR24, or SC5D from Cholesterol Synthesis Reveal Pathways Modulated by Sterol Intermediates. *iScience* **2024**, *27*, 110651. [\[CrossRef\]](#)

46. Lee, B.-S.; Park, Y.-I.; Liu, H.; Kim, S.G.; Kim, H.J.; Choi, J.-H.; Rho, S.H.; Padilla, J.; Roh, J.; Woo, H.G.; et al. The Role of 7-Dehydrocholesterol in Inducing ER Stress and Apoptosis of Head and Neck Squamous Cell Carcinoma. *Cancer Lett.* **2025**, *628*, 217842. [[CrossRef](#)]
47. Sørensen, D.M.; Holen, H.W.; Pedersen, J.T.; Martens, H.J.; Silvestro, D.; Stanchev, L.D.; Costa, S.R.; Günther Pomorski, T.; López-Marqués, R.L.; Palmgren, M. The P5A ATPase Spflp Is Stimulated by Phosphatidylinositol 4-Phosphate and Influences Cellular Sterol Homeostasis. *Mol. Biol. Cell* **2019**, *30*, 1069–1084. [[CrossRef](#)]
48. Zhou, X.; Wang, G.; Tian, C.; Du, L.; Prochownik, E.V.; Li, Y. Inhibition of DUSP18 Impairs Cholesterol Biosynthesis and Promotes Anti-Tumor Immunity in Colorectal Cancer. *Nat. Commun.* **2024**, *15*, 5851. [[CrossRef](#)]
49. Sun, Y.; Wang, Z.; Na, L.; Dong, D.; Wang, W.; Zhao, C. FZD5 Contributes to TNBC Proliferation, DNA Damage Repair and Stemness. *Cell Death Dis.* **2020**, *11*, 1060. [[CrossRef](#)]
50. Zhang, J.; Wang, G.; Liu, J.; Tang, F.; Wang, S.; Li, Y. ITGA4 as a Potential Prognostic and Immunotherapeutic Biomarker in Human Cancer and Its Clinical Significance in Gastric Cancer: An Integrated Analysis and Validation. *Front. Oncol.* **2025**, *15*, 1513622. [[CrossRef](#)]
51. Liang, J.-Z.; Li, D.-H.; Xiao, Y.-C.; Shi, F.-J.; Zhong, T.; Liao, Q.-Y.; Wang, Y.; He, Q.-Y. LAFEM: A Scoring Model to Evaluate Functional Landscape of Lysine Acetylome. *Mol. Cell Proteom.* **2024**, *23*, 100700. [[CrossRef](#)]
52. Petrov, P.; Izzì, V. ProToDeviseR: An Automated Protein Topology Scheme Generator. *BMC Bioinform.* **2025**, *26*, 71. [[CrossRef](#)]
53. Mistry, J.; Chuguransky, S.; Williams, L.; Qureshi, M.; Salazar, G.A.; Sonnhammer, E.L.L.; Tosatto, S.C.E.; Paladin, L.; Raj, S.; Richardson, L.J.; et al. Pfam: The Protein Families Database in 2021. *Nucleic Acids Res.* **2021**, *49*, D412–D419. [[CrossRef](#)]

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