

L-Sarcosine Reduced the Mobility of Human Glioblastoma Cells, with Potential Involvement of Vimentin

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Background: Glioblastoma multiforme (GBM) is a highly aggressive brain tumor with poor prognosis, highlighting the need for novel therapeutic approaches. Vimentin plays a critical role in cancer cell motility, with elevated expression observed in various cancers. This study aimed to evaluate the effects of L-sarcosine (L-S) on the migration and adhesion of U-87 cells, with a particular focus on vimentin.

Methods: *VIM* expression and its prognostic significance were assessed in GBM tissue samples and cells. Molecular docking was performed to elucidate the binding interactions between L-S and vimentin, with emphasis on phosphorylation sites. Experimentally, the effects of L-S on viability, proliferation, apoptosis, migration, adhesion, and gene expression were evaluated in U-87 cells.

Results and Conclusion: Upregulation of *VIM* was detected in both GBM tissues and U-87 cells. Molecular docking demonstrated that L-S interacts with vimentin at key residues involved in filament stabilization, including Ser39. In vitro assays showed that L-S significantly inhibited U-87 cell migration ($p < 0.01$), enhanced cell adhesion to the ECM ($p < 0.01$), and modulated *VIM* expression ($p < 0.05$). Collectively, these findings underscore the potential of L-S as an effective anti-migratory agent and highlight innovative therapeutic strategies targeting intermediate filaments.

Keywords: L-sarcosine, glioblastoma, vimentin, migration, adhesion

Introduction

Glioblastoma multiforme (GBM) remains one of the most aggressive and lethal human cancers, characterized by poor prognosis and treatment resistance.¹ According to the latest statistics, the median overall survival for GBM patients is approximately 10.4 to 19 months, depending on various factors such as age, treatment protocols, and molecular characteristics of the tumor.² The five-year survival rate also remains low, estimated at 5–10%.^{3,4} The current standard of care for GBM primarily involves a multimodal approach combining maximal safe surgical resection, radiotherapy, and chemotherapy. Despite advances in these therapies, the prognosis for GBM patients remains poor due to several intrinsic and extrinsic factors. The tumor's critical location often limits the feasibility of complete surgical removal, as GBM exhibits aggressive infiltration into surrounding brain tissue. Additionally, the blood–brain barrier (BBB) poses a significant obstacle by restricting the penetration of many therapeutic agents. Tumor heterogeneity and the immunosuppressive microenvironment further contribute to resistance against conventional treatments.^{5–7} To overcome these challenges, novel targeted strategies such as molecular inhibitors, immunotherapies, and nanoparticle-based drug delivery systems have been developed.^{8,9} Nonetheless, issues including poor stability, potential toxicity, and limited therapeutic efficacy persist in nanomedicine, underscoring the ongoing need for innovative therapeutic agents to improve GBM patient outcomes.¹⁰

Intermediate filaments play crucial roles in maintaining cellular structure and supporting tissue integrity. Vimentin, a type III intermediate filament, is generally expressed in cells of mesenchymal origin, but its expression in epithelial cells and association

with metastatic behavior has also been confirmed.^{11–13} This protein plays important roles in maintenance of cellular integrity and resistance to stress, and vimentin knockout mice show impaired wound healing ability.¹⁴ Vimentin has been shown to regulate cell migration via interaction with paxillin, integrin $\alpha 6 \beta 4$ and myosin II.¹⁵ Phosphorylation of specific amino acid residues in vimentin critically regulates filament dynamics and cellular functions, particularly in cell motility and cancer progression. For example, phosphorylation at Ser39 by Akt1 and PAK enhances cancer cell motility and promotes filament assembly. Additionally, phosphorylation at Ser56 and Ser83 drives mitotic filament disassembly, while phosphorylation at Ser7 and Ser34 by PKC facilitates integrin trafficking. These post-translational modifications underscore the essential roles of these residues in modulating vimentin function and its involvement in tumor aggressiveness and metastatic processes.^{16–22} In an insightful study, siRNA-mediated knockdown of vimentin (*VIM*) was shown to impair GBM cell migration. Furthermore, treatment of GBM-derived cells with glycogen synthase kinase-3 (GSK-3) inhibitors caused rapid downregulation of *VIM*, suggesting that anti-migratory compounds targeting GSK-3 influence vimentin cytoskeletal dynamics, thereby contributing to their anti-invasive effects.²³ Another study reported that vimentin forms complexes with molecules such as Nogo receptor (NgR), modulating TGF β 1-mediated migration and invasion in GBM cells; knockdown of *VIM* in this context also suppressed cell migration and invasion.²⁴ In addition, the use of anti-vimentin nanobodies has been found to reduce GBM cell invasion, concomitant with increased expression of the tight junction protein ZO-1, indicating that targeting vimentin can enhance cell-cell adhesion and limit invasiveness.²⁵

L-sarcolysine (L-S), also known as Melphalan, is an alkylating agent primarily used to treat hematological malignancies and has demonstrated synergistic effects with other anticancer drugs.^{26,27} Although the therapeutic efficacy of L-S spans several cancers,^{28–31} its effects on GBM cell viability and metastasis have not yet been reported. L-S exerts its effect by forming DNA adducts and activating apoptotic pathways.³² In melanoma cells, L-S has been shown to reduce cell motility, particularly under hypoxic condition, by inhibiting the expression of carbonic anhydrase XII and the phosphorylation of FAK, both of which are essential for cell migration and invasion.³³ Despite its potent anticancer activity and demonstrated benefits in progression-free and overall survival, high doses of L-S are associated with increased toxicity, including renal dysfunction.^{34,35}

To our knowledge, the effect of L-S on the motility of GBM cells has not been previously investigated. Therefore, the potential of L-S as an anti-migration agent against GBM was explored, with a particular focus on its interaction with vimentin in U-87 cells. *VIM* expression was evaluated in GBM tissue samples and cells, and molecular docking was performed to characterize the binding interactions between L-S and vimentin. Experimental validation was carried out by assessing the effects of L-S on U-87 cell viability, apoptosis, migration, adhesion, and gene expression, providing a comprehensive evaluation of its anti-metastatic potential.

Methods

Analysis of *VIM* Expression and Its Prognostic Significance in GBM

VIM expression in GBM was evaluated in comparison to normal tissue using GEPIA2 (<http://gepia2.cancer-pku.cn/>), where boxplot analysis was employed to compare log2-transformed TPM (transcripts per million) expression levels between 163 tumor samples and 207 normal samples. Additionally, the prognostic significance of *VIM* expression was assessed; Kaplan-Meier survival curve was generated to analyze the impact of *VIM* expression levels on patient survival over time.

VIM expression was further analyzed in GBM and U-87 cells using microarray data from GEO database (<http://www.ncbi.nlm.nih.gov/geo>). For differential expression analysis, GSE4290 dataset was selected, which includes 77 GBM samples and 23 non-tumor control samples. Although the number of non-tumor controls was smaller compared to GBM samples, this dataset provides a relevant comparison. Additionally, GSE4536 dataset was examined, comprising gene expression profiles of U-87 cells and neural stem cells. Data analysis was performed using GEO2R, with *p* values adjusted using the “ggplot2” package in R. A significance threshold of *p* < 0.05 and log2 fold change (log2FC) > 0.5 were applied.

Molecular Docking

To investigate the interactions between L-S and vimentin, molecular docking was performed using the 3D structure of vimentin retrieved from AlphaFold (UniProt ID: P08670; Average pLDDT=77.3). The 3D structures of L-S (CID:

460612) and Simvastatin (CID: 54454), which serves as a reference vimentin inhibitor, were retrieved in SDF format from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). Docking was carried out on Proteins Plus server (<https://proteins.plus/>), which optimizes protonation states and hydrogen atom coordinates while assessing binding affinities and orientations between the protein and ligand. Docking was performed using the JAMDA algorithm with a site radius of 6.5 Å and high-precision parameters. The docking score represents the predicted binding free energy in arbitrary units specific to the JAMDA scoring function, where more negative values indicate stronger predicted binding affinity. Intermolecular interactions in the best docking poses were visualized using PoseEdit in both 2D and 3D formats.^{36–38}

Cell Culture and Treatment

Human GBM cells (U-87 cell line) and normal fibroblasts (HFF-3 cell line) were sourced from Pasteur Institute (Tehran, Iran), and cultured using Dulbecco's modified Eagle's medium-high glucose (Capricorn) supplemented with 10% fetal bovine serum (neoFroxx). Cells were maintained at 37°C in a humidified incubator with 5% CO₂, and subcultured by 0.25% trypsin-1 mM EDTA (Biowest). For treatment, stock solutions of L-S (Sigma) were prepared using dimethyl sulfoxide (DMSO) as solvent, and final concentrations (25, 50, and 100 µM) were prepared freshly using complete medium.

Viability Assay

Cell viability was assessed using alamarBlue reagent. Briefly, U-87 and HFF-3 cells were cultured in 96-well plates at densities of 8000 and 10,000 cells per well, respectively. Then after, cells were treated with increasing concentrations of L-S for 24, 48 and 72 h. Cell viability was assessed at the end of each time point by adding alamarBlue solution (0.1 mg/mL, Sigma), followed by 3 h incubation at 37°C. Optical density (OD) was measured at 600 nm using microplate spectrophotometer (BioTek Epoch), and cell viability (%) was calculated.

Apoptosis Assay

To detect apoptosis in U-87 cells following L-S treatment, annexin V-FITC and propidium iodide (PI) staining was performed. Briefly, U-87 cells were treated with 100 µM L-S, while untreated and DMSO treated cells were considered as controls. After 48 h treatment, 100,000 cells per sample were collected, centrifuged at 1200 rpm for 4 min and washed twice with phosphate-buffered saline (PBS). The cell pellets were stained with annexin V-FITC and PI (Sigma) and flow cytometry analysis was conducted using BD FACSCalibur instrument, acquiring data from 10,000 cells per sample. FL1-H and FL2-H channels were used to detect annexin V-FITC and PI fluorescence, respectively. Gating was performed using FlowJo V10 software to distinguish live cells (annexin V⁻/PI⁻), necrotic cells (PI⁺ only), early apoptotic cells (annexin V⁺ only), and late apoptotic cells (annexin V⁺/PI⁺). Experiments were conducted in triplicate to ensure reproducibility.

Migration Assay

To determine the effects of L-S on the migration ability of GBM cells, scratch assay was performed. To achieve this, U-87 cells were seeded in 24-well plates and cultured for 24 h to form a monolayer. Then, a straight scratch was made using a micropipette tip, followed by PBS washing and treatment with 100 µM L-S in serum-free medium. To note, untreated and DMSO treated cells were considered as controls. Photomicrographs were captured at 0 and 24 h, and the gap area was quantified using ImageJ software to calculate the rate of cell migration.

Adhesion Assay

To investigate whether L-S could affect the adhesion ability of U-87 cells, adhesion assay was performed. Briefly, 96-well plates were coated with fibronectin (5 µg/mL, Solarbio) and incubated at 4°C overnight for polymerization. Plates were then blocked with 0.2% bovine serum albumin (Biosera) at 37°C for 1 h. L-S-treated U-87 cells were harvested and seeded onto the coated wells, while untreated and DMSO treated cells were considered as controls. Following 1 h incubation at 37°C, non-adherent cells were removed with PBS, and alamarBlue was used to calculate viability as previously described.³⁹

Gene Expression Analysis

The expression of candidate genes was analyzed by qPCR. In summary, total RNA was extracted from cells treated with L-S and their respective controls using isopropanol and chloroform according to the manufacturer's protocol (DENA ZIST Asia). cDNA synthesis was then performed with oligo-dT, random hexamers and M-MuLV reverse transcriptase following the manufacturer's instruction (Pars Toos). To verify the fidelity of synthesized cDNAs, PCR was conducted on all samples using primers for TATA box binding protein (TBP) as a housekeeping control, and the PCR products were analyzed by electrophoresis on 1% agarose gel. Quantification of *VIM* and *P53* expression was then performed using SYBR green-based qPCR kit (Pars Toos) on LightCycler 96 System (Roche). Primer sequences are shown in Table 1. The PCR cycling conditions were as follows: 95°C for 12 min, followed by 40 cycles of denaturation at 95°C for 15 sec, annealing at 55°C for 30 sec and extension at 72°C for 30 sec. Data analysis was performed using LinReg PCR software, and relative gene expression was calculated using the standard curve method.

Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's test in GraphPad Prism 10.4. Results are expressed as mean $\pm$ standard deviation (SD), and p value < 0.05 and < 0.01 were considered statistically significant.

Results

Analysis of *VIM* expression in GBM revealed significant upregulation compared to normal tissue. Boxplot analysis using GEPIA2 demonstrated a marked increase in log2-transformed TPM expression levels, with tumor samples exhibiting significantly higher *VIM* expression than normal samples ($p < 0.05$, Figure 1A). Additionally, survival analysis by Kaplan-Meier curve indicated that *VIM* expression levels affect patient survival over time (Figure 1B). These findings were corroborated by microarray data from GSE4290 dataset, where *VIM* expression was elevated in GBM samples relative to non-tumor controls ($\log_2FC > 1$, adjusted p value < 0.05 ; Figure 1C). Similarly, analysis of GSE4536 dataset revealed upregulation of *VIM* in U-87 cells compared to neural stem cell lines (Figure 1D). Collectively, these data confirm *VIM* overexpression in GBM and highlight its prognostic value.

Molecular docking analysis revealed that L-S interacts with vimentin at key residues involved in filament stabilization, forming hydrogen bonds with Ser37 and Ser39, the latter being a known phosphorylation site that modulates filament assembly (Figure 2A). The docking scores were calculated using the JAMDA scoring function, an empirical method specific to the ProteinsPlus platform, where more negative values indicate stronger predicted binding affinity in arbitrary units. Although not directly comparable to binding energies expressed in kcal/mol by other algorithms, the JAMDA score of -1.381 suggests favorable binding for L-S with vimentin in this context. For comparison, Simvastatin, a known vimentin inhibitor, docked to the same binding pocket displayed hydrogen bonding with Ser39 and a similar JAMDA score of -1.479 , indicating comparable binding strength under the same scoring metric (Figure 2B).

Viability assay was conducted on U-87 cells and normal cells to assess the effects of L-S over time. As presented in Figure 3A, results of alamarBlue assay showed that L-S at concentrations of 25 μ M and 50 μ M did not significantly impact U-87 cell viability over 24, 48 and 72 h. At concentration of 100 μ M, L-S reduced viability to 88.7%, 89.6% and 82.6% after 24, 48 and 72 h, respectively. IC_{50} values calculated via nonlinear regression were 246.2, 200 and 183.1 μ M at 24, 48 and 72 h, respectively. Additionally, cell counting revealed that 100 μ M L-S considerably decreased the number of U-87 cells after 48 h (Figure 3B). For HFF-3 cells, L-S concentrations of 25 μ M and 50 μ M showed no significant effect on viability after 48 h. However, upon treatment with 100 μ M L-S, viability of HFF-3 cells reduced to 87%

Table 1 Sequence of Primers and Product Length Used for qPCR

Gene Name	Forward (5' to 3')	Reverse (5' to 3')	Product Size (bp)
TBP	ACAACAGCCTGCCACC	GAATAGGCTGTGGGGTCAG	120
P53	GTTCCGAGAGCTGAATGAGG	TTATGGCGGGAGGTAGACTG	123
VIM	GCGTGACGTACGTCAGCAATATGA	GTTCCAGGGACTCATTGGTTCCTT	218

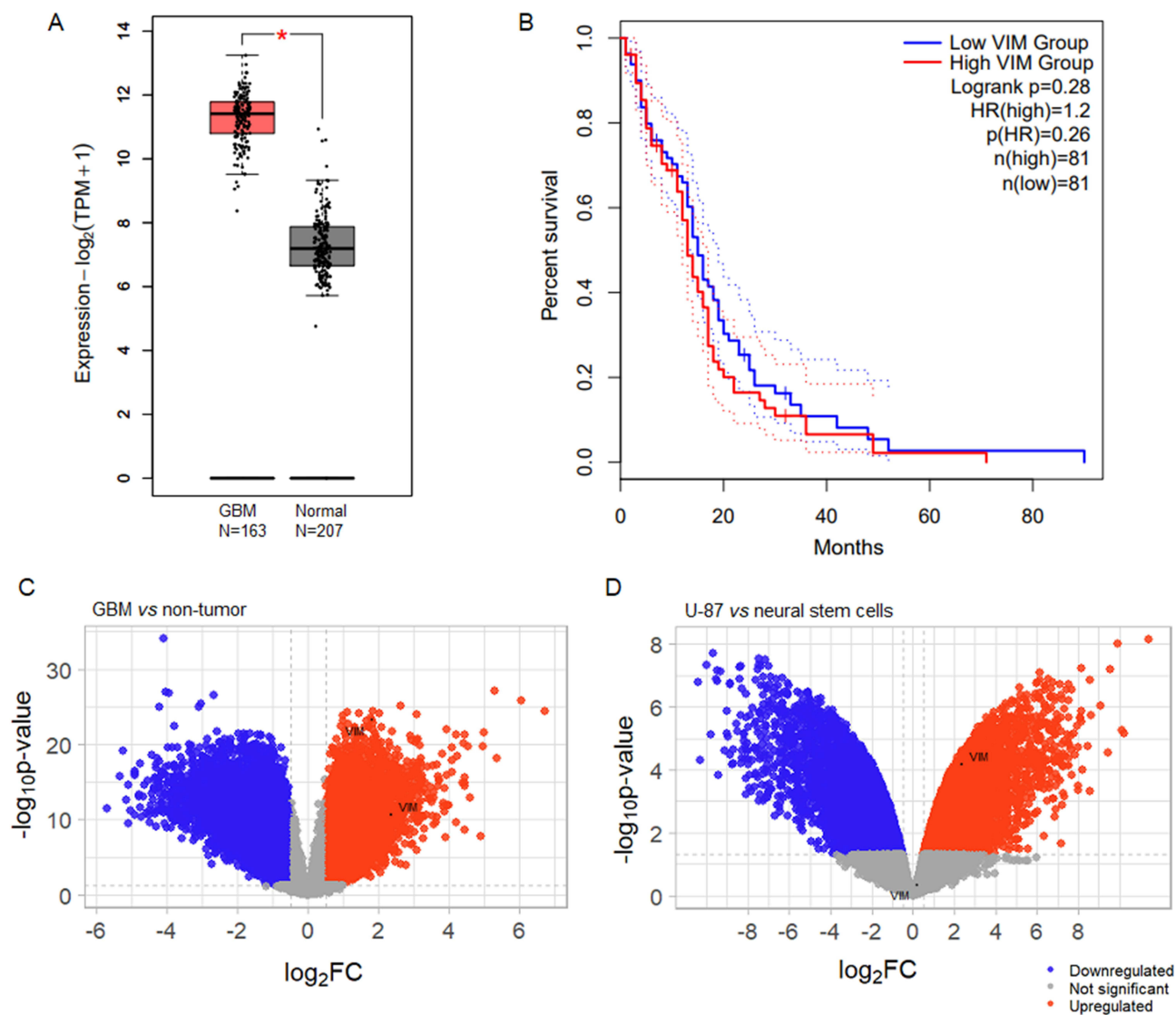


Figure 1 Validating the expression of *VIM* in GBM samples and U-87 cells. Differential expression of *VIM* in GBM and normal samples in GEPIA2 (A). Kaplan-Meier analysis of *VIM* expression (high, N = 81; low, N = 81) in GBM (B). Volcano plots resulting from the differential expression analysis between 77 GBM samples and 23 non-tumor samples - GSE4290 (C), and between U-87 cells and neural stem cells - GSE4536 (D). * $p < 0.05$, $\log_2\text{FC} > 0.5$.

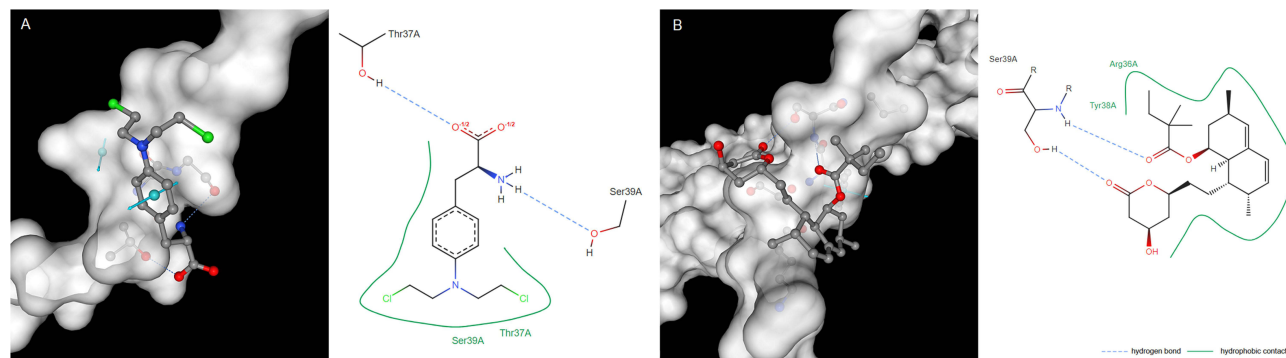


Figure 2 Molecular docking diagrams generated for predicting the interaction of L-S (A) and Simvastatin (B) with vimentin. The docking positions are illustrated in 3D and 2D.

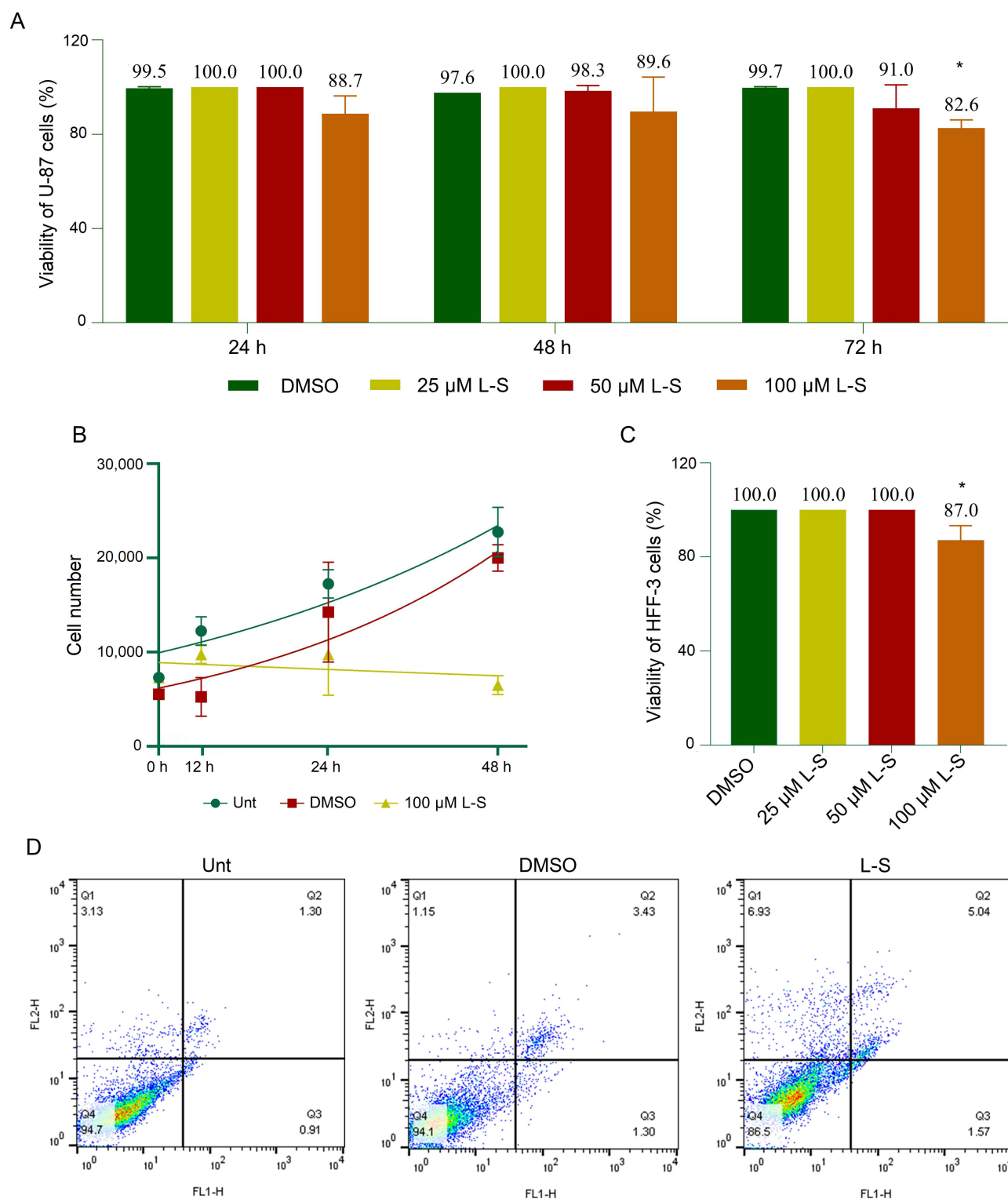


Figure 3 Results of viability assay for U-87 cells treated with L-S (25, 50, and 100 μM) over 24, 48, and 72 h, compared to untreated and DMSO controls (**A**). Dose-response curve illustrating number of U-87 cells at different L-S concentrations and time points (**B**). Results of viability assay for HFF-3 cells treated with L-S (25, 50, and 100 μM) over 48 h (**C**). Apoptosis detection after treatment with L-S (**D**). Flow cytometry was performed after 48 h treatment with 100 μM L-S, and cells were categorized as alive (Q4; negative for both annexin V-FITC and PI), necrotic (Q1; positive for PI only), and early or late apoptotic (Q2 and Q3; positive for annexin V-FITC). Data are presented as mean ± SD; **p* < 0.05 compared to control.

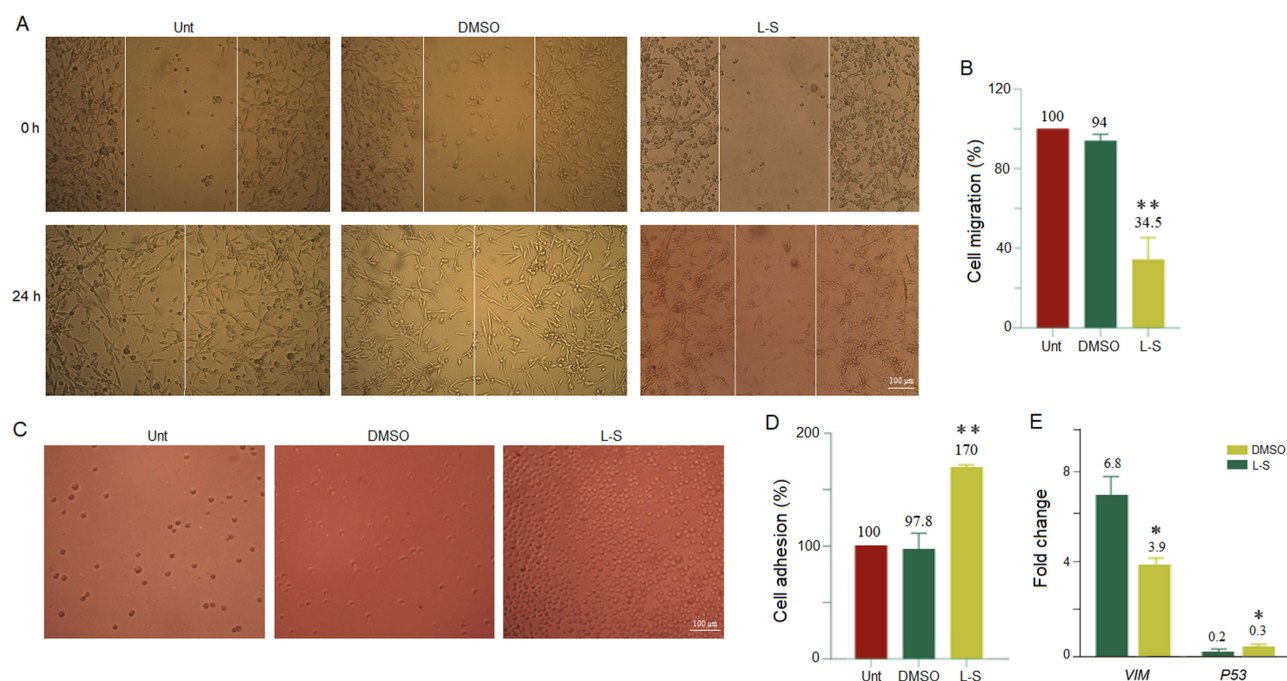


Figure 4 Assessment of L-S on the migration, adhesion and gene expression of U-87 cells upon treatment with L-S. Migration assay demonstrating cell migration at 0 h and 24 h (A), with corresponding quantification showing significant reduction in migration upon L-S treatment (B). Cell adhesion assay showing increased ECM adhesion in L-S-treated cells compared to controls (C), with quantitative analysis (D). qPCR analysis demonstrating fold changes in *VIM* and *P53* expression following L-S treatment compared to DMSO control (E). Data are presented as mean $\pm$ SD; * $p < 0.05$ and ** $p < 0.0001$, compared to control.

(Figure 3C). Detection of apoptosis in U-87 cells showed a slight increase in the late apoptotic cell population upon treatment with 100 μ M L-S (5.04%), compared to 3.43% and 1.3% in DMSO and untreated cells, respectively (Figure 3D). These results collectively indicate that 100 μ M L-S modestly reduces viability and induces limited apoptosis at 48 h. Based on these findings, a concentration of 100 μ M L-S, half of its IC_{50} in U-87 cells at 48 h, was selected for subsequent experiments.

Migration assay was conducted to assess the effect of L-S on U-87 cell motility. Phase-contrast images taken before and 24 h after treatment with 100 μ M L-S showed significant reduction in migration compared to controls (Figure 4A). Statistical analysis confirmed that L-S significantly ($p < 0.01$) reduced U-87 cell migration (Figure 4B). Specifically, untreated and DMSO treated cells exhibited migration rate of 100% and 94%, respectively, whereas L-S treatment reduced migration to 34.5% at 24 h. Adhesion assay was also performed to evaluate the effect of L-S on cell adhesion to the ECM (Figure 4C). Treatment with 100 μ M L-S for 24 h significantly ($p < 0.01$) increased U-87 cell adhesion compared to controls. Specifically, L-S treatment increased adhesion by 170%, while adhesion rate in untreated and DMSO control groups were 100% and 97.8%, respectively (Figure 4D). Gene expression analysis using qPCR revealed that 100 μ M L-S significantly ($p < 0.05$) reduced the expression of *VIM*, while also increasing *P53* expression (Figure 4E). These findings collectively suggest that L-S can inhibit GBM cell migration and enhance cell-ECM interactions, potentially limiting tumor progression.

Discussion

L-S is an L-phenylalanine nitrogen mustard DNA-alkylating agent employed in the treatment of a variety of hematological malignancies and solid tumors^{40–42}. Although the anticancer effects of L-S are well-established, its anti-metastatic potential remains poorly understood. Importantly, to date, the impact of L-S on GBM cell viability, migration, and adhesion has not been explored. Our study addresses this critical gap by demonstrating, for the first time, the significant effects of L-S on these key processes in U-87 cells, thereby providing novel insights into its potential therapeutic applications in GBM management.

Findings of the present study revealed upregulation of *VIM* in GBM tissue samples, as well as U-87 cells, and highlight its prognostic value. Additionally, molecular docking demonstrated that L-S interacts with vimentin at critical residues potentially involved in filament stabilization. Results of in vitro experiments demonstrated that 100 μ M L-S slightly reduced cell viability and induced apoptosis at 48 h; however, it significantly inhibited the migration of U-87 cells and enhanced cell adhesion to the ECM. Notably, viability of normal cells treated with 100 μ M L-S remained higher compared to GBM cells. These findings suggest that, while L-S modestly induces cytotoxicity in both GBM and normal cells, its effects on metastatic behavior were more pronounced. Additionally, L-S modulated gene expression by decreasing *VIM*, a marker of cell motility. Collectively, these results indicate that L-S predominantly targets metastasis-related processes, likely through downregulation of vimentin expression and activity, rather than inducing cell death or broadly impairing viability.

Vimentin is an intermediate filament protein involved in cell signaling, migration, and invasion across various cancer types.^{11–13,43} A few numbers of small-molecule inhibitors targeting vimentin have shown therapeutic potential in diverse diseases. For example, Withaferin A reduced vimentin levels, thereby affecting multiple cellular processes.⁴⁴ Additionally, Simvastatin induces cell death in vimentin-expressing cancer cells, and FiVe1 disrupts vimentin networks in mesenchymal cancers, leading to mitotic catastrophe.^{45,46} In the present study, L-S was found to downregulate *VIM* expression, which correlated with reduced cell motility and enhanced cell adhesion. The negative regulation of vimentin in response to L-S may involve several molecular pathways. One potential mechanism is the modulation of the PI3K/Akt pathway, which is known to regulate vimentin expression. The ability of L-S to induce oxidative stress could lead to decreased Akt activity, resulting in reduced vimentin levels. Additionally, downregulation of vimentin may be mediated by reactive oxygen species (ROS); since L-S increases ROS levels, it can significantly influence various cellular responses, including the modulation of cytoskeletal proteins like vimentin.^{47,48} Collectively, our observations provide valuable insights into the mechanisms by which L-S may exert its effects, highlighting the therapeutic potential of targeting cell migration in GBM. A limitation of the current study is the absence of direct experimental validation of L-S binding to vimentin, such as co-immunoprecipitation or biophysical assay, which are essential to confirm protein-ligand interactions. While molecular docking and qPCR analyses provide strong evidence, future studies incorporating these functional assays will be critical to validate and extend our findings. Furthermore, studies involving a broader range of GBM cell lines, normal neural cell types, as also GBM models, such as orthotopic xenografts, are warranted to refine L-S as a targeted therapy.

In conclusion, this study establishes L-S as a promising anti-metastatic agent for GBM treatment, with vimentin playing a central role in its therapeutic efficacy. L-S significantly inhibits GBM cell migration and enhances ECM adhesion by downregulating vimentin expression, a key driver of tumor invasiveness. These findings underscore the potential of targeting vimentin to curb GBM metastasis, while also emphasizing the need for further investigation into optimal dosing strategies and safety profiles to support the clinical development of L-S as a targeted therapy.

Data Sharing Statement

The data that support the findings of this study are available on request from the corresponding author.

Ethical Approval

This study used publicly available human data and was exempt from ethical review according to applicable institutional guidelines.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflict of interest.

References

1. Torp SH, Solheim O, Skjulsvik AJ. The WHO 2021 Classification of central nervous system tumours: a practical update on what neurosurgeons need to know-a minireview. *Acta Neurochir.* **2022**;164(9):2453–2464. doi:10.1007/s00701-022-05301-y
2. Mustafa YH, Keogh RJ, Barry S, O'Reilly S, Gleeson J, Bambury RM. Single institution analysis of glioblastoma multiforme (GBM) outcomes based on year of diagnosis: a 17-year review. *Neuro Oncol.* **2024**;26(Supplement_2):S1–S10.
3. Blakstad H, Brekke J, Rahman MA, et al. Survival in a consecutive series of 467 glioblastoma patients: association with prognostic factors and treatment at recurrence at two independent institutions. *PLoS One.* **2023**;18(2):e0281166. doi:10.1371/journal.pone.0281166
4. Fekete B, Werlenius K, Tisell M, et al. What predicts survival in glioblastoma? A population-based study of changes in clinical management and outcome. *Front Surg.* **2023**;10:1249366. doi:10.3389/fsurg.2023.1249366
5. Pineda E, Domenech M, Hernández A, Comas S, Balaña C. Recurrent glioblastoma: ongoing clinical challenges and future prospects. *Onco Targets Ther.* **2023**;16:71–86. doi:10.2147/OTT.S366371
6. Wu W, Klockow JL, Zhang M, et al. Glioblastoma multiforme (GBM): an overview of current therapies and mechanisms of resistance. *Pharmacol Res.* **2021**;171:105780. doi:10.1016/j.phrs.2021.105780
7. Kotecha R, Odia Y, Khosla AA, Ahluwalia MS. Key Clinical principles in the management of glioblastoma. *JCO Oncol Pract.* **2023**;19(4):180–189. doi:10.1200/OP.22.00476
8. Habeeb M, Vengateswaran HT, Kumbhar ST, You HW, Asane GS, Aher KB. Nano–bio interactions: understanding their dynamic connections. In: Kesharwani P, Jain NK, editors. *Theranostics Nanomat in Drug Delivery*. Academic Press; **2025**:11–25. doi:10.1016/B978-0-443-22044-9.00025-5
9. Vengateswaran HT, Habeeb M, Aher KB, Bhavar GB, Suseela P, Navabshah I. Radiant revolution with carbon dots transforming medicine. *Futur Trends Chem Mater Sci Nano Technol.* **2024**;3(5):59–70. doi:10.58532/V3BECSS5P1CH6
10. Habeeb M, Vengateswaran HT, You HW, Saddhona K, Aher KB, Bhavar GB. Nanomedicine facilitated cell signaling blockade: difficulties and strategies to overcome glioblastoma. *J Mater Chem B.* **2024**;12(7):1677–1705. doi:10.1039/d3tb02485g
11. Zhao J, Zhang L, Dong X, Liu L, Huo L, Chen H. High expression of vimentin is associated with progression and a poor outcome in glioblastoma. *Appl Immunohistochem Mol Morphol.* **2018**;26(5):337–344. doi:10.1097/PAI.0000000000000420
12. Hirano H, Maeda H, Takeuchi Y, et al. Lymphatic invasion of micropapillary cancer cells is associated with a poor prognosis of pathological stage IA lung adenocarcinomas. *Oncol Lett.* **2014**;8(3):1107–1111. doi:10.3892/ol.2014.2284
13. Liu LK, Jiang XY, Zhou XX, Wang DM, Song XL, Jiang HB. Upregulation of vimentin and aberrant expression of E-cadherin/beta-catenin complex in oral squamous cell carcinomas: correlation with the clinicopathological features and patient outcome. *Mod Pathol.* **2010**;23(2):213–224. doi:10.1038/modpathol.2009.160
14. Cheng F, Shen Y, Mohanasundaram P, et al. Vimentin coordinates fibroblast proliferation and keratinocyte differentiation in wound healing via TGF- β -Slug signaling. *Proc Natl Acad Sci U S A.* **2016**;113(30):E4320–E4327. doi:10.1073/pnas.1519197113
15. Menko AS, Bleaken BM, Libowitz AA, Zhang L, Stepp MA, Walker JL. A central role for vimentin in regulating repair function during healing of the lens epithelium. *Mol Biol Cell.* **2014**;25(6):776–790. doi:10.1091/mbc.E12-12-0900
16. Paulin D, Lilienbaum A, Kardjian S, Agbulut O, Li Z. Vimentin: regulation and pathogenesis. *Biochimie.* **2022**;197:96–112. doi:10.1016/j.biochi.2022.02.003
17. Zhu QS, Rosenblatt K, Huang KL, et al. Vimentin is a novel AKT1 target mediating motility and invasion. *Oncogene.* **2011**;30(4):457–470. doi:10.1038/onc.2010.421
18. Wang R, Li QF, Anfinogenova Y, Tang DD. Dissociation of Crk-associated substrate from the vimentin network is regulated by p21-activated kinase on ACh activation of airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol.* **2007**;292(1):L240–L248. doi:10.1152/ajplung.00199.2006
19. Ivaska J, Vuoriluoto K, Huovinen T, Izawa I, Inagaki M, Parker PJ. PKCepsilon-mediated phosphorylation of vimentin controls integrin recycling and motility. *EMBO J.* **2005**;24(22):3834–3845. doi:10.1038/sj.emboj.7600847
20. Yamaguchi T, Goto H, Yokoyama T, et al. Phosphorylation by Cdk1 induces Plk1-mediated vimentin phosphorylation during mitosis. *J Cell Biol.* **2005**;171(3):431–436. doi:10.1083/jcb.200504091
21. Eriksson JE, He T, Trejo-Skalli AV, et al. Specific in vivo phosphorylation sites determine the assembly dynamics of vimentin intermediate filaments. *J Cell Sci.* **2004**;117(Pt 6):919–932. doi:10.1242/jcs.00906
22. Tang DD, Bai Y, Gunst SJ. Silencing of p21-activated kinase attenuates vimentin phosphorylation on Ser-56 and reorientation of the vimentin network during stimulation of smooth muscle cells by 5-hydroxytryptamine. *Biochem J.* **2005**;388(Pt 3):773–783. doi:10.1042/BJ20050065
23. Nowicki MO, Hayes JL, Chiocca EA, Lawler SE. Proteomic analysis implicates vimentin in glioblastoma cell migration. *Cancers.* **2019**;11(4):466. doi:10.3390/cancers11040466
24. Kang YH, Han SR, Jeon H, et al. Nogo receptor-vimentin interaction: a novel mechanism for the invasive activity of glioblastoma multiforme. *Exp Mol Med.* **2019**;51(10):1–15. doi:10.1038/s12276-019-0332-1
25. Zottel A, Novak M, Šamec N, et al. Anti-vimentin nanobody decreases glioblastoma cell invasion in vitro and in vivo. *Cancers.* **2023**;15(3):573. doi:10.3390/cancers15030573
26. Costa BA, Mouhieddine TH, Ortiz RJ, Richter J. Revisiting the role of alkylating agents in multiple myeloma: up-to-date evidence and future perspectives. *Crit Rev Oncol Hematol.* **2023**;187:104040. doi:10.1016/j.critrevonc.2023.104040
27. Khodadadi F, Sadeghian MH, Delbari Z, Kazemi M, Koohpaykar H, Rassouli FB. Melphalan improves toxicity of arsenic trioxide in adult T-cell leukemia/lymphoma cells. *Biochem Biophys Rep.* **2025**;43:102109. doi:10.1016/j.bbrep.2025.102109
28. Pocza A, Rogalska A, Marczak A. Treatment of multiple myeloma and the role of melphalan in the era of modern therapies-current research and clinical approaches. *J Clin Med.* **2021**;10(9):1841. doi:10.3390/jcm10091841
29. Bayraktar UD, Bashir Q, Qazilbash M, Champlin RE, Ciurea SO. Fifty years of melphalan use in hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant.* **2013**;19(3):344–356. doi:10.1016/j.bbmt.2012.08.011
30. Piver MS. Treatment of ovarian cancer at the crossroads: 50 years after single-agent melphalan chemotherapy. *Oncology.* **2006**;20(10):1156, 1158.

31. Yamazaki F, Yamasaki K, Kiyotani C, et al. Thiotepa-melphalan myeloablative therapy for high-risk neuroblastoma. *Pediatr Blood Cancer*. 2021;68(6):e28896. doi:10.1002/pbc.28896
32. Poczta A, Krzeczynski P, Ionov M, et al. Newly synthesized melphalan analogs induce DNA damage and mitotic catastrophe in hematological malignant cancer cells. *Int J Mol Sci*. 2022;23(22):14258. doi:10.3390/ijms232214258
33. Giuntini G, Monaci S, Cau Y, Mori M, Naldini A, Carraro F. Inhibition of melanoma cell migration and invasion targeting the hypoxic tumor associated CAXII. *Cancers*. 2020;12(10):3018. doi:10.3390/cancers12103018
34. Shah G, Giral S, Dahi P. Optimizing high dose melphalan. *Blood Rev*. 2024;64:101162. doi:10.1016/j.blre.2023.101162
35. Attal M, Lauwers-Cances V, Hulin C, et al. Lenalidomide, bortezomib, and dexamethasone with transplantation for myeloma. *New Eng J Med*. 2017;376(14):1311–1320. doi:10.1056/NEJMoa1611750
36. Schöning-Stierand K, Diedrich K, Ehrh C, et al. ProteinsPlus: a comprehensive collection of web-based molecular modeling tools. *Nucleic Acids Res*. 2022;50(W1):W611–W615. doi:10.1093/nar/gkac305
37. Flachsenberg F, Meyder A, Sommer K, Penner P, Rarey M. A Consistent scheme for gradient-based optimization of protein-ligand poses. *J Chem Inf Model*. 2020;60(12):6502–6522. doi:10.1021/acs.jcim.0c01095
38. Diedrich K, Krause B, Berg O, Rarey M. PoseEdit: enhanced ligand binding mode communication by interactive 2D diagrams. *J Comput Aided Mol Des*. 2023;37(10):491–503. doi:10.1007/s10822-023-00522-4
39. Al-Nasiry S, Geusens N, Hanssens M, Luyten C, Pijnenborg R. The use of Alamar Blue assay for quantitative analysis of viability, migration and invasion of choriocarcinoma cells. *Hum Reprod*. 2007;22(5):1304–1309. doi:10.1093/humrep/dem011
40. Meng X, Ye L, Yang Z, Xiang R, Wang J. Adsorption behavior of melphalan anti-ovarian cancer drug onto boron nitride nanostructures. Studying MTT assay: in vitro cellular toxicity and viability. *Chem Pap*. 2021;75(4):1469–1474. doi:10.1007/s11696-020-01378-5
41. Kuczmarski M, Ding ZC, Zhou G. Immunostimulatory effects of melphalan and usefulness in adoptive cell therapy with antitumor CD4+ T cells. *Crit Rev Immunol*. 2016;36(2):179–191. doi:10.1615/CritRevImmunol.2016017507
42. Lin Z, Chu B, Qu Y, et al. Liposome-encapsulated melphalan exhibits potent antimyeloma activity and reduced toxicity. *ACS Omega*. 2022;8(1):1693–1701. doi:10.1021/acsomega.2c07555
43. Chen MH-S, Yip GW-C, Tse GM-K, et al. Expression of basal keratins and vimentin in breast cancers of young women correlates with adverse pathologic parameters. *Mod Pathol*. 2008;21:1183–1191. doi:10.1038/modpathol.2008.90
44. Pietraszkiewicz A, Hampton C, Caplash S, et al. Desmin deficiency is not sufficient to prevent corneal fibrosis. *Exp Eye Res*. 2019;180:155–163. doi:10.1016/j.exer.2018.12.019
45. Trogden KP, Battaglia RA, Kabiraj P, Madden VJ, Herrmann H, Snider NT. An image-based small-molecule screen identifies vimentin as a pharmacologically relevant target of simvastatin in cancer cells. *FASEB J*. 2018;32(5):2841–2854. doi:10.1096/fj.201700663R
46. Bollong MJ, Pietilä M, Pearson AD, et al. A vimentin binding small molecule leads to mitotic disruption in mesenchymal cancers. *Proc Natl Acad Sci U S A*. 2017;114(46):E9903–E9912. doi:10.1073/pnas.1716009114
47. Zub KA, Sousa MM, Sarno A, et al. Modulation of cell metabolic pathways and oxidative stress signaling contribute to acquired melphalan resistance in multiple myeloma cells. *PLoS One*. 2015;10(3):e0119857. doi:10.1371/journal.pone.0119857
48. Jiang H, Gao M, Shen Z, et al. Blocking PI3K/Akt signaling attenuates metastasis of nasopharyngeal carcinoma cells through induction of mesenchymal-epithelial reverting transition. *Oncol Rep*. 2014;32(2):559–566. doi:10.3892/or.2014.3220

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