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RESEARCH ARTICLE

DIFFERENTIAL EFFECTS OF SERTRALINE AND PENFLURIDOL ON EMT AND ECM REMODELING IN GLIOBLASTOMA CELL LINES

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ABSTRACT

Purpose: Glioblastoma multiforme (GBM) is an aggressive brain tumor with poor prognosis due to rapid recurrence, chemoresistance, and limited efficacy of standard therapies. Epithelial-to-mesenchymal transition (EMT) and matrix metalloproteinase (MMP)-mediated extracellular matrix (ECM) remodeling are critical processes in GBM progression and metastasis. The aim of this study is to examine the potential effects of sertraline and penfluridol on the EMT process and gelatinase activity in human glioblastoma cell lines.
Material and Methods: U87 and U251 human glioblastoma cells were treated with sertraline and penfluridol at previously identified IC50 doses. Protein levels of EMT markers, E-cadherin, vimentin, Snail, Slug, Twist1, phospho-Akt (p-Akt), and tissue inhibitor of metalloproteinases-2 (TIMP-2), were evaluated using Western blotting. Additionally, the impact of sertraline and penfluridol on the release and activity of MMP-2 and MMP-9 were assessed through gelatin zymography.
Results: Both sertraline and penfluridol significantly reduced vimentin expression in U251 cells, indicating inhibition of the mesenchymal phenotype. Conversely, these drugs increased vimentin levels in U87 cells, highlighting cell line-specific differences. Sertraline and penfluridol also increased TIMP-2 levels in U251 cells but not in U87 cells. Neither drug altered MMP-2 or MMP-9 activity in either cell line, suggesting that their effects on ECM remodeling may be mediated through TIMP-2 upregulation rather than direct modulation of gelatinase activity.
Conclusion: These findings suggest that sertraline and penfluridol potentially inhibit EMT and reduce ECM degradation in U251 cells but exert contrasting effects in U87 cells. This highlights the heterogeneity of GBM tumors and the importance of personalized therapeutic approaches.

Keywords: Sertraline, Penfluridol, Glioblastoma, Epithelial-mesenchymal transition, Matrix metalloproteinases

INTRODUCTION

Glioblastoma multiforme (GBM), also known as astrocytoma grade IV, is the most common and aggressive primary brain neoplasm in adults. GBM arises from diffuse astrocytomas or anaplastic astrocytomas and is typically located in deep white matter, the basal ganglia, or the thalamus. Despite multimodal treatment involving surgical resection, radiotherapy, and chemotherapy with temozolomide (TMZ), the prognosis for GBM remains poor, with an average survival of less than 15 months. The limited efficacy of standard GBM treatments is attributed to

several factors, including rapid tumor recurrence, the development of chemoresistance, and the inability of most chemotherapeutic agents to cross the blood-brain barrier (BBB) (1). These limitations underscore the urgent need for novel therapeutic strategies in the treatment of GBM.

Repositioning FDA-approved antipsychotic and antidepressant agents, commonly used in the management of psychiatric disorders, shows potential for the treatment of GBM, due to their long clinical history, proven reliability, and ability to cross the blood-brain barrier (BBB). Sertraline, a selective serotonin reuptake inhibitor (SSRI) antidepressant is widely used to treat depression, anxiety, and obsessive-compulsive disorder. Sertraline, in particular, has minimal side effect profile and is commonly utilized as a reliable and effective antidepressant/anxiolytic agent for patients with metastatic cancer who concurrently exhibit depression (2-4). Penfluridol, a long acting antipsychotic medication used in the treatment of schizophrenia (5, 6). Both sertraline and penfluridol have emerged as promising candidates for cancer therapy due to their ability to exhibit anti-tumorigenic activities by inhibiting the cell cycle, suppressing tumor growth, and promoting apoptosis in various cancer types, including glioblastoma (7), colon (8), liver (9), breast (10, 11), and pancreatic cancers (12, 13). Sertraline has been shown to inhibit the translationally controlled tumor protein (TCTP), an intracellular chaperone that contributes to GBM drug resistance by impairing p53 function (14). Penfluridol, on the other hand, has been shown to induce apoptosis in glioblastoma cells through the inhibition of Akt-mediated GLI1 expression, which is crucial for GBM cell survival and proliferation (7). Despite the promising anti-cancer properties of sertraline and penfluridol observed in various cancer types, their specific effects on EMT and ECM remodeling in GBM remain largely unexplored.

Epithelial-mesenchymal transition (EMT) is a critical process in cancer metastasis, facilitates tumor cell migration and invasion. During EMT, epithelial markers such as E-cadherin are downregulated, while mesenchymal markers such as vimentin are upregulated, driven by transcription factors (Snail, Slug, Twist1, ZEB1/2) (15). EMT is often accompanied by extracellular matrix (ECM) remodeling mediated by matrix metalloproteinases (MMPs) (16). Dysregulation of MMPs, particularly MMP-2 and MMP-9, and their endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs) correlate with tumor aggressiveness, promoting angiogenesis, metastasis, and therapy resistance (17).

GBM is characterized by its highly invasive nature, which is closely linked to EMT-like processes. While GBM cells do not undergo a classic EMT due to their non-epithelial origin, they do exhibit EMT-like changes that contribute to their aggressive behavior (18). The ability of sertraline and penfluridol to penetrate the BBB positions them uniquely to potentially modulate these processes directly within the brain microenvironment. If these drugs can inhibit EMT-like changes and MMP activity in GBM, they could enhance the efficacy of existing treatments by reducing tumor invasiveness and therapy resistance. This study aims to investigate potential inhibitory effects of sertraline and penfluridol on EMT process and MMP activity in glioblastoma cells lines (U251 and U87). In this context, we evaluated key EMT markers (E-cadherin, vimentin, Snail, Slug, Twist1) and ECM modulators (MMP-2, MMP-9, TIMP-2) to elucidate their roles in glioblastoma progression. By focusing on these molecular targets, we aim to provide a mechanistic understanding of how sertraline and penfluridol might modulate GBM cell behavior, potentially opening new avenues for therapeutic intervention.

MATERIALS AND METHODS

Cell Culture and Drug Preparation

The human glioblastoma cell lines, U87 and U251, (kindly provided by Dr. Erdoğan Pekcan Erkan) was cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, US) supplemented with 10% fetal bovine serum (FBS) (Gibco, US), 2mM L-glutamine (Gibco, US), 1mM sodium pyruvate (Gibco, US), and100U/mL penicillin - 100µg/mL streptomycin (Gibco, US). The cells were maintained at 37°C in a humidified incubator with a 5% CO_2 atmosphere.

Sertraline (Cat: S6319, Sigma, US) and penfluridol (Cat: P3371, Sigma, US), supplied in lyophilized form, were each dissolved in DMSO to create a primary stock solution at 38 mM and stored at -20°C. Intermediate stock solutions of 2 mM sertraline or penfluridol were prepared by diluting the primary stock solutions with DMSO. For all experiments, 0.1 % DMSO corresponding to the applied maximum doses served as the solvent control (DMSO control).

Cell Viability

The WST-1 cell viability assay was carried out to confirm previously determined the IC_{50} doses of sertraline and penfluridol in U251 and U87 cell lines (19). In the prior study, the effects of these drugs on cell viability were assessed by treating the cells with concentrations ranging from 1 μM to 40 μM and incubating for 24, 48, or 72 hours to establish the IC50 values. In this study, we specifically seeded 2.5 x 10^4 cells into a 96-well plate and allowed them to adhere for 24 hours. Cells were treated with sertraline (12μM for U251, 10μM for U87), penfluridol (5μM for U251, 6.5μM for U87) and solvent control containing 0.1 % DMSO for 72h. Following treatment, WST-1 reagent (Roche Diagnostics, US) was added, and absorbance was obtained at 450 nm (background correction: 620 nm). The percentage of cell viability was calculated relative to the solvent control group.

Western Blotting

After treatment with sertraline, penfluridol and solvent control (0.1 % DMSO) for 72h, the expression levels of target proteins (E-cadherin, vimentin, Slug, Snail, Twist1, pAkt, and TIMP-2) were evaluated using Western blotting. At the end of the treatment period, the cells were lysed in RIPA buffer (Cell Signaling, US) containing protease/phosphatase inhibitors (2 μg/ml aprotinin, 5 μg/ml leupeptin, 1 μg/ml pepstatin A, 1 mM PMSF, 10 mM NaF and 1mM sodium orthovanadate). The total protein concentration was determined using a Bicinchoninic Acid (BCA) total protein assay kit (Pierce, US). Next, 30 μg of total protein was separated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a polyvinylidene fluoride (PVDF) membrane. After blocking the membranes in 1X TBS-T containing 5% non-fat dry milk for 1 hour at room temperature, they were incubated overnight at +4°C with primary antibodies against E-cadherin (1:1000) (Cat: 3195, Cell Signaling, US), vimentin (1:3000) (Cat: 5741, Cell Signaling, US), Snail (1:1000) (Cat: 3895, Cell Signaling, US), Slug (1:1000) (Cat: 9585, Cell Signaling, US), Twist1 (1:1000) (Cat: 46702, Cell Signaling, US), phosho-Akt (p-Akt - Ser473) (1:1000) (Cat: 4060, Cell Signaling, US), TIMP-2 (1:1000) (Cat: 5738, Cell Signaling, US), and α-actinin (1:5000) (Cat: 3134, Cell Signaling, US). Following washings with 1X TBS-T, the membranes were incubated for 1 hour at room temperature with HRP-conjugated secondary antibodies. Images were captured using an enhanced chemiluminescence (ECL) solution (Merck, US) and a UVP gel documentation system (UVP Ltd, UK). The densitometric analyses were performed using the UVP Bioimaging system with LabWorks 4.6 Image Acquisition software (UVP Ltd, UK), and each target protein was normalized to the corresponding reference protein, α-actinin.

Gelatin Zymography

The activity levels of MMP-2 and MMP-9, released from cells treated with sertraline and penfluridol, were evaluated using gelatin zymography. Following 48 hours of treatment with the drugs, the cells were transferred to serum-free media and incubated for an additional 24 hours. The media was then collected and concentrated using concentrator tubes (Millipore, US) at 4°C. The total protein concentration in the concentrated media was determined with BCA total protein analysis method (Pierce, US). Subsequently, 15 μg of the concentrated media was applied to polyacrylamide gels containing 1 mg/ml gelatin, and electrophoresis was performed at a constant 110V at +4°C. After electrophoresis, the gels were washed twice with Triton X-100 and incubated in an activation buffer (50 mM Tris-base, 50 mM NaCl, 1 mM $CaCl_2$, and 0.05% Brij 35 (pH 7.6)) at 37°C for 48 hours. Following Coomassie Blue staining and several washings with distilled water, the gels were visualized using a white light UVP gel documentation system (UVP Ltd., UK), and the densities of the lytic bands was determined using the UVP Bioimaging system with LabWorks 4.6 Image Acquisition software (UVP Ltd, UK). The gelatinolytic activity was calculated based on the formula "band density (area (mm^2) x optical density (O.D/mm^2))/ μg protein"

Statistical Analysis

Data were analyzed using Graph Pad Prism 10.2.3 software (Graph Pad Inc, US) and represented as means ± standard deviations (SD). Experiments were conducted as at least three independent biological replicates. First, the normality of the data distribution was confirmed with the Shapiro–Wilk test. Next, statistical significance was assessed with one-way ANOVA with Tukey test for the multiple group comparisons. For continuous data that do not follow a normal distribution, the Kruskal-Wallis test was utilized for comparisons among three or more groups, with Dunn's test employed for post hoc analyses.

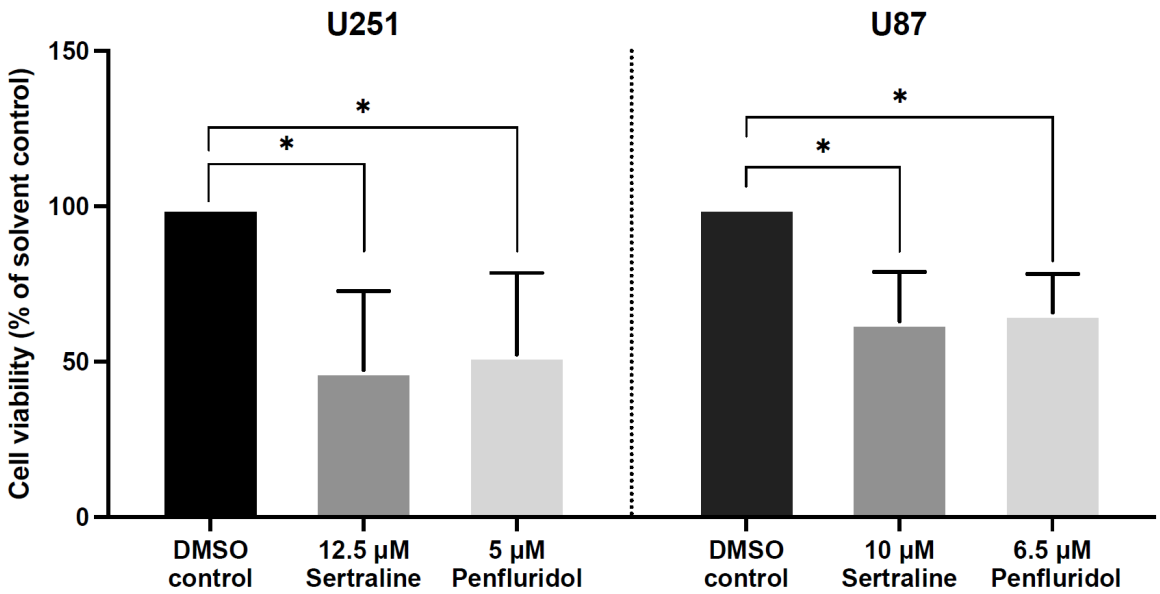

Figure 1. Cell viability of U251 and U87 glioblastoma cells treated with sertraline and penfluridol. U251 and U87 cells were treated with sertraline (12.5 µM for U251, 10 µM for U87) or penfluridol (5 µM for U251, 6.5 µM for U87) for 72 hours. Cell viability was assessed using WST-1 assay. Data are presented as percentage of viable cells compared to DMSO control (%0.1 DMSO). Bars represent mean ± SD (n=3).

Statistical significance was defined as follows: *p < 0.05; **p < 0.01.

RESULTS

Effects of Sertraline and Penfluridol on EMT Markers in U87 and U251 Cells.

In our previous study (19), we established the 72-hour IC_{50} values for sertraline (12.5 µM for U251, 10 µM for U87) and penfluridol (5 µM for U251, 6.5 µM for U87) in glioblastoma cell lines. To confirm these values, we conducted WST-1 cell viability assays. Our results demonstrated that sertraline at 12.5 µM reduced U251 cell viability to 47.3%, while penfluridol at 5 µM decreased it to 52.4% (p< 0.05, n= 3, Figure 1). In U87 cells, 10 µM sertraline and 6.5 µM penfluridol reduced viability to 63.3% and 65.97%, respectively (p< 0.05, n= 3, Figure 1). These concentrations were subsequently used in further experiments.

Next, we investigated the effects of sertraline and penfluridol on EMT markers in human glioblastoma cell lines. Western blot analysis revealed that both drugs significantly decreased vimentin levels in U251 cells (p < 0.01, n = 3, Figure 2a, b), suggesting a suppression of mesenchymal phenotype. However, pAkt (Ser473) levels were slightly reduced, but this decrease was not statistically significant (p > 0.05, n = 3, Figure 2a, b). Additionally, Slug, Snail and Twist1 levels remained unchanged (p > 0.05, n = 3, Figure 2a, b). Due to E-cadherin being undetectable or barely detectable in U251, we could not able to analyze the effects of agents on this marker in U251 cells (Figure 2a).

Conversely, a significant increase in vimentin levels was observed in U87 cells treated with either sertraline or penfluridol (p < 0.05, n = 3, Figure 2c, d). E-cadherin expression showed a slight but non-significant decreased, while Snail, Slug, Twist1, and pAkt (Ser473) levels were unaffected in response to both drugs in U87 cell lines (p > 0.05, n = 3, Figure 2c, d).

These results indicate that sertraline and penfluridol exert opposing effects on vimentin expression in U251 and U87 cells, highlighting cell line-specific responses. Further studies are required to elucidate the molecular mechanisms driving these differential responses and their potential relevance to glioblastoma therapy.

Effects of Sertraline and Penfluridol on Gelatinase Activity in U87 and U251 Cells.

In this study, we focused on the effects of sertraline and penfluridol on key regulators of ECM remodeling, including TIMP-2, MMP-2 and MMP-9 in human

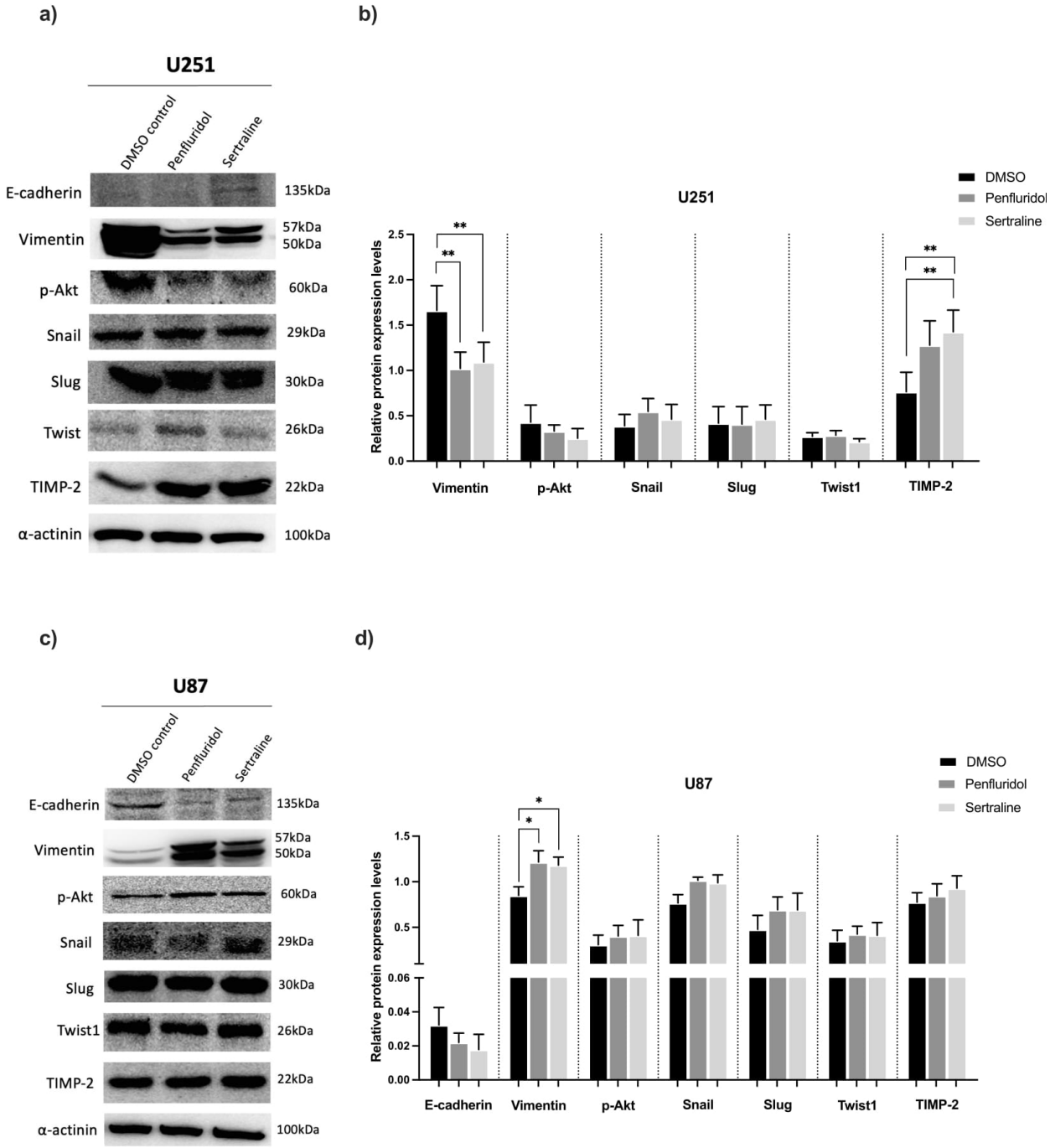

Figure 2. Effects of sertraline and penfluridol on EMT markers and TIMP-2 in U251 and U87 glioblastoma cells. (a) Representative Western blot images of EMT markers and TIMP-2 in U251 cells treated with sertraline (12.5 μM) or penfluridol (5 μM) for 72 hours. (b) Quantification of protein levels in U251 cells. Data are presented as mean ± SD (n=3). **p < 0.01 compared DMSO control (%0.1 DMSO). (c) Representative Western blot images of EMT markers and TIMP-2 in U87 cells treated with sertraline (10 μM) or penfluridol (6.5 μM) for 72 hours. (d) Quantification of protein levels in U87 cells. Data are presented as mean ± SD (n=3). *p < 0.05 compared to DMSO control (%0.1 DMSO).

glioblastoma cell lines. Western blot analysis revealed a significant increase in TIMP-2 protein levels in U251 cells treated with both sertraline ($p < 0.05$, $n = 4$, Figure 2a, b) and penfluridol ($p < 0.05$, $n = 4$, Figure 2a, b). In contrast, neither drug elicited statistically significant change in TIMP-2 levels in U87 cells ($p > 0.05$, $n = 4$, Figure 2c, d).

To further assess the impact on ECM degradation, we examined the activity levels of gelatinases, MMP-2 and MMP-9, using gelatin zymography. Our results

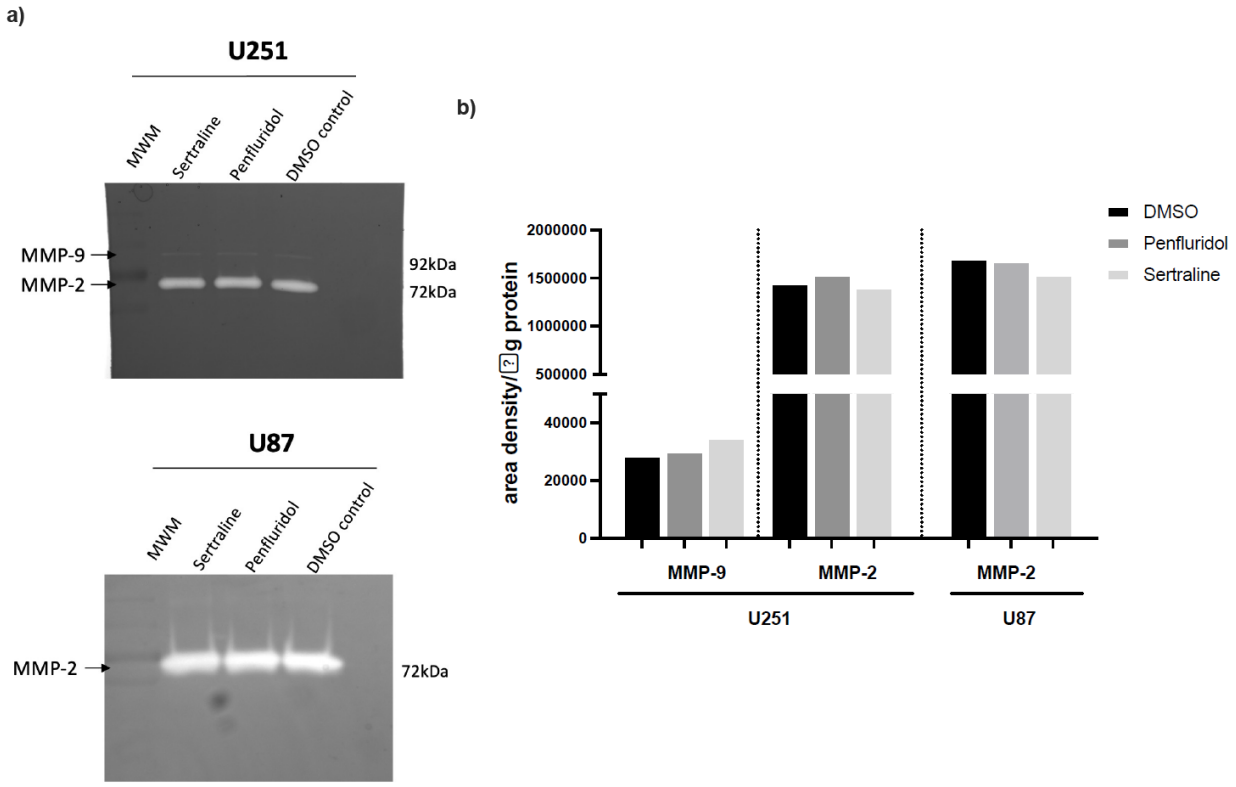

Figure 3. Effects of sertraline and penfluridol on MMP-2 and MMP-9 activity in U251 and U87 glioblastoma cells. Representative gelatin zymography images showing MMP-2 and MMP-9 activity in (a) U251 and (b) U87 cells treated with sertraline or penfluridol for 72 hours (n=1).

indicated that neither sertraline nor penfluridol altered the enzymatic activity of MMP-2 or MMP-9 in either cell line (n = 1, Figure 3). However, this experiment was replicated only once, limiting the statistical power of the findings.

These results suggest that sertraline and penfluridol may modulate ECM dynamics in glioblastoma cells via the upregulation of TIMP-2, particularly in U251 cells, rather than through direct suppression of gelatinase activity.

DISCUSSION

Epithelial-to-mesenchymal transition (EMT) is critical driver of cancer metastasis, enabling epithelial cells to lose polarity and adhesion, acquiring invasive and migratory capacities characteristic of mesenchymal cells. EMT often involves the upregulation of matrix metalloproteinases (MMPs), which degrade extracellular matrix (ECM) components, further enhancing invasiveness (20). This study explored the effects of sertraline and penfluridol on EMT markers and ECM remodeling in glioblastoma cell lines U251 and U87.

Our results demonstrate that both sertraline and penfluridol treatments led to a significant reduction in vimentin levels in U251 cells, suggesting suppression of mesenchymal characteristics. The absence or minimal detection of E-cadherin in U251 cells is characteristic of this mesenchymal-like glioblastoma subtype (21, 22), which presents a limitation of this study by restricting our ability to evaluate the EMT status in these cells. Therefore, we recommend employing more sensitive techniques, such as immunofluorescence (IF), to better evaluate E-cadherin expression in future studies.

Conversely, U87 cells exhibited a paradoxical response: a significant increase in vimentin, and a slight but not significant decrease in E-cadherin. This divergence is particularly noteworthy given our previous observations of enhanced invasiveness in U87 cells compared to U251 cells (19). Moreover, U87 cells are characterized as more proliferative and display a neuronal-like phenotype, while U251 cells have a slower proliferation rate and exhibit mesenchymal-like features (23).

To further explore these differences, we suggest conducting genomic and epigenomic profiling of the sertraline- or penfluridol-treated U87 and U251 cell lines, incorporating whole-exome sequencing, RNA-sequencing, and methylation analyses. This multi-omics approach will help identify key genetic and epigenetic differences between the cell lines and allow for correlations between these findings and the observed variations in their responsiveness to sertraline and penfluridol.

The differential responses observed between U251 and U87 cells underscore the intrinsic heterogeneity of glioblastoma tumors and their variable responsiveness to therapeutic interventions, emphasizing the necessity for personalized treatment strategies. These discrepancies suggest fundamental variations in EMT signaling cascades between these cell lines, warranting further mechanistic investigations. Additionally, the largely unchanged levels of EMT markers, Snail, Slug, and Twist1, indicate that these transcription factors may not be central to the observed effects. Future studies could explore alternative factors, such as Zeb1 and Zeb2, which could provide deeper insights into the mechanisms driving vimentin expression and EMT in glioblastoma. Investigating other EMT and mesenchymal-epithelial transition (MET) related factors, including ZO-1, fibronectin, and N-cadherin, could further enrich our understanding of these processes.

We also evaluated the role of sertraline and penfluridol on ECM remodeling in glioblastoma by assessing TIMP-2, MMP-2, and MMP-9. Neither sertraline nor penfluridol affected the activity of MMP-2 or MMP-9 in either cell line, suggesting that the anti-invasive effects of drugs may not involve direct modulation of gelatinase activity. It is important to note that this result is based on a single biological replicate; thus, further studies are necessary to validate these findings and to explore the potential involvement of other MMPs in ECM remodeling. Notably, both drugs significantly upregulated TIMP-2 levels in U251 cells, suggesting a potential role for this endogenous MMP inhibitor in mediating ECM dynamics. In contrast, TIMP-2 levels remained unchanged in U87 cells, highlighting the cell line-specific responses to sertraline and penfluridol.

To our knowledge, this is the first study to demonstrate the modulatory effects of sertraline and penfluridol on TIMP-2 expression in glioblastoma cell lines. These findings underscore the complexity of glioblastoma ECM regulation and suggest that the drugs' effects on tumor invasiveness may depend on distinct molecular contexts within different cell types.

Previous studies corroborate our findings by highlighting the anti-invasive and anti-metastatic potential of penfluridol in various cancer types, including breast (10, 24), lung (25), and glioblastoma (7) cancer. Hung et al. demonstrated that penfluridol suppresses uPA/uPAR/TGF-β signaling, resulted in reduced MMP-12 activity and EMT inhibition in lung adenocarcinoma cells (26). Additionally, penfluridol decreased MMP-9 expression in vivo and in vitro and inhibited invasion and migration in A549 lung cancer cells (25). In patient specific glioma sphere-forming cells, penfluridol downregulates stemness and invasion markers, including Integrin α6, Zeb-1, N-cadherin (27).

Limited studies have reported the potential of sertraline as an anti-invasive agent in cancer, but its effects in the context of glioblastoma have not been well-explored. Sertraline has been shown to lateral cell motility in breast cancer cells (28), and to target EMT markers in prostate cancer stem cells by downregulating transcription factors such as TCF8 and LEF1 (29). In HT-29 colon cancer cells, sertraline was reported to induce the expression of EMT-related markers, including β-catenin, and, E-cadherin (30). These findings from other cancer types appear to align with our observation of reduced mesenchymal marker expression in the U251 glioblastoma cell line following sertraline treatment.

However, it is important to note that the regulation of EMT in glioblastoma is known to be highly complex and context-dependent, potentially differing from the mechanisms observed in other cancer models. While our study is the first to reveal the effect of sertraline on the invasive process in glioblastoma, further investigation is required to elucidate the precise molecular mechanisms by which sertraline may differentially modulate EMT regulation in distinct glioblastoma subtypes.

CONCLUSION

This study demonstrates the potential of sertraline and penfluridol to modulate EMT and ECM dynamics in glioblastoma cells, with significant inhibitory effects observed in U251 cells. However, the contrasting effects in U87 cells highlight the critical need for personalized therapeutic strategies tailored to the molecular characteristics of individual tumors. It is essential to conduct thorough molecular

characterization of GBM subtypes to better understand these differential responses, as they may arise from distinct molecular profiles, variations in EMT signaling pathways, or intrinsic differences in drug uptake and metabolism.

Future research should prioritize the development of combination therapies that target complementary pathways to enhance treatment efficacy and overcome resistance in glioblastoma. Specifically, integrating sertraline and penfluridol with existing therapies could provide significant benefits. By incorporating molecular profiling into these therapeutic strategies, we can better understand the unique characteristics of individual tumors, ultimately advancing the clinical applicability of these drugs and improving clinical outcomes for patients with glioblastoma.

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Author contributions: DK: Conception, Design, Biological Material Responsibility, Data Collecton and Processing, Analysis - Interpretation, Literature Review, Writing. MS: Design, Biological Material Responsibility, Data Collecton and Processing, Analysis – Interpretatio, Literature Review. ŞİS: Biological Material Responsibility, Data Collecton and Processing, Analysis – Interpretation, Literature Review. GO: Supervision, Fundings, Biological Material Responsibility, Analysis – Interpretation, Literature Review, Critical Review. All authors reviewed and approved the final version.

Conflict of interest: The authors declare no conflict of interest.

Ethical approval: Ethical approval was obtained from the Health Sciences Research Ethics Committee of Izmir University of Economics, Izmir, Turkey (Date: 17/09/2024, No: B.30.2.İEÜSB.0.05.05-20-320).

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