Bioinformatics - Driven Analysis of Vortioxetine's Impact on Glioblastoma: Identifying Key Differentially Expressed Genes for Treatment Strategies

ABSTRACT

Background

Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor in adults, characterized by rapid growth, invasive behavior, and poor survival outcomes. Current therapies, including surgery, radiation, and chemotherapy, provide limited benefit due to treatment resistance and the blood-brain barrier. Drug repurposing offers a promising strategy to overcome these challenges. This study investigates the effects of Vortioxetine, an antidepressant capable of crossing the blood-brain barrier, on gene expression in glioblastoma LN229 cells to identify potential biomarkers and therapeutic vulnerabilities.

Method

RNA sequencing data from the GEO dataset GSE214968 was analyzed using GEO2R to identify differentially expressed genes (DEGs) between untreated control (0-hour) and Vortioxetine-treated (24-hour) groups. A total of 9485 DEGs were identified and visualized through a volcano plot and Venn diagram. The top 50 DEGs (25 upregulated and 25 downregulated) were further analyzed using SRplot for Gene Ontology (GO) and KEGG pathway enrichment.

Results

RNA5S family genes showed significant downregulation, suggesting impaired ribosome biogenesis and protein synthesis in glioblastoma cells, potentially indicating therapeutic vulnerability. In contrast, genes such as NRARP, DLL3, COL6A3, and FREM2 were linked to pathways that support tumor progression, invasion, and resistance mechanisms. Pathways of interest included Ribosome biogenesis in eukaryotes, Ribosome, NOTCH signaling, and ECM-receptor interaction.

Conclusion

This study highlights the potential of Vortioxetine to disrupt ribosome biogenesis, presenting a novel therapeutic strategy for glioblastoma. Differentially expressed genes and enriched pathways identified in this analysis could serve as biomarkers or targets for future research to improve treatment outcomes in GBM.

Keywords

Glioblastoma Multiforme, Differentially Expressed Genes, Bioinformatics, Gene Ontology, KEGG Pathways, Ribosome Biogenesis, NOTCH Signaling, ECM-Receptor Interaction

INTRODUCTION

Glioblastoma Multiforme (GBM) is an aggressive brain tumor which originates from astrocytic glial cells. It grows rapidly, is highly invasive, and is the most common primary malignant central nervous system tumor in adults, accounting for 48.6 % of all such tumors. GBM is more prevalent amongst men and presents with symptoms such as headaches, seizures, and neurological decline. Treatment typically involves excessive surgery, radiation therapy, and chemotherapy; however outcomes remain poor, with a median survival rate of approximately 15 months (1).

The problem or challenge being addressed in this research is that GBM remains an incurable tumor. Achieving clear surgical margins is challenging due to the risks of damaging healthy brain tissues responsible for critical functions. GBM often exhibits resistance to radiation therapy, and excessive doses can lead to severe side effects, such as radiation necrosis and cognitive decline. Chemotherapy is also limited in effectiveness due to the Blood-Brain Barrier, which restricts many drugs from reaching the tumor. These challenges underscore the need for innovative treatment strategies, such as drug repurposing, to improve GBM outcomes (2).

This research investigates the potential application of Vortioxetine as a repurposed drug for the treatment of GBM. The repurposing of existing medication such as Vortioxetine, an antidepressant, is of primary interest. The question aims to understand the gene expression dynamics in response to Vortioxetine treatment and identify highly or lowly expressed genes that may provide insights into potential biomarkers for personalized targeted therapies for the patients (3).

Glioblastoma is a highly aggressive and deadly brain tumor originating from astrocytes, a type of glial cell that supports nerve cells. Classified as a grade IV glioma by the World Health Organization, GBM is the most malignant form of brain cancer and accounts for about half of all malignant brain tumors. The annual incidence rate is approximately 3.2 per 100,000 people worldwide and only 5.5% of patients survive beyond 5 years post-diagnosis. Its high morbidity and mortality rates make GBM a critical area of research (2).

What is known so far is that GBM is highly invasive, fast growing, and composed of heterogeneous cell populations, making it resistant to many treatment modalities. The Blood-Brain Barrier further complicates treatment by limiting drug delivery. Current treatment multidisciplinary team approaches, such as surgical resection, followed by radiotherapy and chemotherapy, have shown limited success. Researchers are exploring novel treatment

approaches for GBM, including immunotherapy, oncolytic viral therapy, and drug repurposing. Repurposed drugs, such as vortioxetine, are of particular interest due to established safety profiles, cost-effectiveness, and potential to contribute to the development of personalized gene targeted therapies (2).

So far, biomarker research in Glioblastoma has focused on identifying genetic and molecular targets to guide treatment and predict patient outcomes. Biomarkers such as EGFR amplification, MGMT promoter methylation, and IDH1 mutations have been extensively studied. These markers are crucial for understanding glioblastoma's heterogeneity and tailoring treatments to individual patients. However, the discovery of additional biomarkers remains essential for advancing personalized therapy. Bioinformatics tools, such as GEO2R, have proven instrumental in identifying novel biomarkers, as they allow researchers to analyze large data sets and correlate genetic expression with survival outcomes (4).

Glioblastoma's heterogeneity presents significant challenges for bioinformatics research. The tumor comprises a mix of cell types, each with unique genetic profiles, making it difficult to draw consistent conclusions about treatment responses. Additionally, the blood-brain barrier limits the ability of drug responsive genetic data. Bioinformatics studies also face challenges in data standardization and reproducibility, as data sets often come from diverse experimental conditions. Despite these hurdles, bioinformatics tools are invaluable for undercovering hidden patterns in gene expression and identifying potential therapeutic targets, which are otherwise hard to detect through traditional methods (1).

The primary goal of this research was to utilize bioinformatics tools to analyze the impact of vortioxetine on glioblastoma LN229 cells. The objective was to identify critical differentially expressed genes (DEGs) and pathways, with the ultimate aim of discovering novel therapeutic strategies and potential biomarkers for targeted therapies in glioblastoma patients (2).
The hypothesis of this research was that the treatment with antidepressant drug vortioxetine would result in significant difference in gene expression compared to untreated control samples. A specific prediction was made that some genes in the treated group would be upregulated (shoving higher gene expression) and some would be downregulated (lower expression).

This research is based on data available utilizing NCBI's GEO2R bioinformatic tools that supports data about vortioxetine leading to significant changes in the expression of specific genes in glioblastoma LN229 cells. These differentially expressed genes may play a critical role in tumor progression or suppression and serve as potential biomarkers for personalized therapies (3). NCBI GEO 2R is a Bioinformatic tool that is provided by the National Center for Biotechnology Information (NCBI) within the Gene Expression Omnibus (GEO) database. It was used to perform differential gene expression analysis by comparing two experimental conditions (control vs.treatment). It allowed us to determine top differentially expressed genes and visualise them with graphs such as Volcano Plot, Venn Diagram and others. Log2 fold change and p-values were selected for data manipulation to create a narrow-downed list, from 9485 differentially expressed genes, to the most important 50 up and down regulated genes for further analysis (6). In addition, SR plot, KEGG and GO bioinformatics was used to further analyze the results obtained from GEO2R. SR Plot is the bioinformatic tool that is used for functional analysis and is able to generate visual and graphical outputs of most important

selected genes based on their symbols (7). KEGG is a comprehensive database resource that helps to identify enriched biological pathways in gene lists for mapping and functional annotation (8)**.** GO is used to describe the biological processes, molecular functions, and cellular components in which gene products are involved. KEGG and GO work in synergy (9).

This research is important because Glioblastoma is highly resistant to conventional therapies, in part due to its genetic heterogeneity and the rapid evolution of treatment resistance mechanisms (5). This research is important because it utilizes bioinformatics to identify differentially expressed genes (DEG's) that could serve as biomarkers for a glioblastoma prognosis and treatment response. By focusing on vorteoxetine's impact on gene expression in glioblastoma cells, the study may uncover novel therapeutic targets that bypass existing resistance pathways. This knowledge has the potential to contribute to the design of personalized and more effective therapeutic approaches for glioblastoma, improving patient outcomes and survival rates.

METHODS

Data Collection Using Bioinformatics Tools

This research was conducted using the Gene Expression Omnibus (GEO) bioinformatics tool provided by the National Center for Biotechnology Information (NCBI). NCBI GEO2R is the powerful bioinformatic data tool that is a user-friendly data analysis platform which enables researchers to perform different gene expression analysis of datasets stored in GEO (6). The dataset **GSE214968,** titled *Effect of Vortioxetine treatment on gene expression of LN-229 glioblastoma cells over time*, was used to identify differentially expressed genes (DEGs) in glioblastoma following treatment with Voltioxetine over a 24-hour period. The GSE214968 dataset includes RNA sequencing data from LN229 glioblastoma cells (a commonly used cell line in cancer research) collected at baseline and across six time points after drug treatment (3).

To prepare the data for this research, two groups with samples were defined:
Control group - baseline samples collected at 0 hours with no treatment and Treatment group - samples collected 24 hours after treatment with Vortioxetine. The no-code GEO2R bioinformatics tool was then used to process these groups and generate initial results, including the identification of differentially expressed genes (DEGs). The programming language R was incorporated for data analysis and visualisation R script .

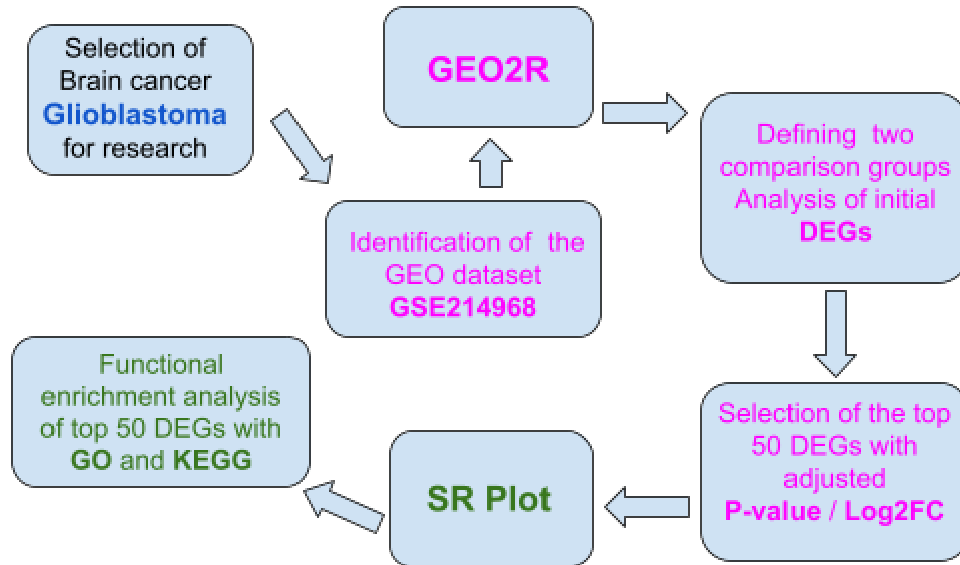

Figure 1. Research Methodology: The steps and bioinformatics tools used in this study.

Data Analysis of GEO2R Data

The data was analyzed and visualization graphs and outputs were generated. Two visual results, the Volcano Plot and Venn Diagram, were chosen. The Volcano Plot was analyzed to identify significantly differentially expressed genes, while the Venn Diagram was used to examine the number of gene expressions in the groups. Volcano plot analysis focused on identifying the highly expressed (Red) genes and low expression (Blue) genes. Venn Diagram analysis focused on visually highlighting a subset number of DEGs identified between the) 0-hour control and the 24-hour vortioxetine-treated glioblastoma samples.

Identification of the Top Differentially Expressed Genes

To identify the most significant differentially expressed genes (DEGs), further statistical analysis was performed. To support this selection process, a Venn diagram was utilized to visualize the distinctions in the data. The total list of 18326 genes generated with the GEO2R database was uploaded into Google Sheets. From this dataset a total of 9485 DEGS were identified based on the applied thresholds. A cut-off adjusted p-value (p.adj) of less than 0.05 and a log2 fold change (log2FC) threshold of +/-3 were used to filter the results. These criteria ensured the selection of the most statistically significant genes. Using these parameters, the study focused on a narrowed list of 50 DEGs, which included: 25 Upregulated Genes (log2FC > +3) and 25 Downregulated Genes (log2FC < -3)

Further Data Analysis Using SRPlot, KEGG, and GO Bioinformatics Tools

SRPlot, KEGG, and GO bioinformatics tool databases were utilized to analyze the functions of these top 50 differentially expressed genes (DEGs). These tools allowed for functional enrichment analysis and provided insights into the biological processes, cellular components, molecular functions and biological functions of the selected DEGs, with the ultimate goal of

uncovering their potential roles in glioblastoma. The gene symbols and corresponding log2 fold changes (log2 FC) were input into SR Plot online server, which generated a variety of graphical representations. These included results from Gene Ontology (GO) and KEGG pathways analysis, which summarized the functions of DEGs and mapped them to specific biological pathways relevant to glioblastoma. Three Ontologies, Pathway Enrichment Bar, and C Net Plot graphs were selected from Go and KEGG to demonstrate biological significance and the interconnected networks relevant to top 50 DEGs in glioblastoma cells. 14 genes that showed high enrichment and important functions in glioblastoma were chosen for further study. Ribosomal genes were identified as significantly downregulated in the CNET plot for Ribosome Biogenesis in Eukaryotes and Ribosome pathway.

RESULTS

Identification of Differentially Expressed Genes

The first step in identifying differentially expressed genes (DEGs) Involved the use of the GEO2R bioinformatics tool, provided by the National Center of Biotechnology Information (NCBI). This tool facilitated the comparison of two defined groups within the dataset: a control group (0-hour baseline samples) and a treatment group (24-hours post-treatment with Vortioxetine). GEO2R generated a comprehensive list of all genes and DEGs based on log2FC threshold set at 0 and adjusted p-value (p.adj) less than 0.05. From the initial analysis using GEO2R of 18326 genes, a total amount of 9485 were identified as differentially expressed between the control and treatment groups, reflecting the impact of Vortioxetine treatment on gene expression in glioblastoma LN229 cells. These DEGs were visualised using two key figures: a volcano plot and a Venn diagram. The volcano plot (Figure 2a) provides a visual representation of the DEGs identified. Red dots represent genes that are highly expressed genes (significantly upregulated genes - top right region of the plot - positive fold change, significant P- value). Blue dots represent lowly expressed genes (significantly downregulated genes - top left region of the plot - negative fold change significant, P- value). Black dots represent genes with no difference in expression (non-significant genes -close to 0 threshold (X and Y) of fold change and p-value). The Venn Diagram illustrates a total 18326 number of genes that were identified in glioblastoma LN229 cells after 24 hours of treatment with Vortioxetine, highlighting 9485 genes as differentially expressed genes (DEGs).

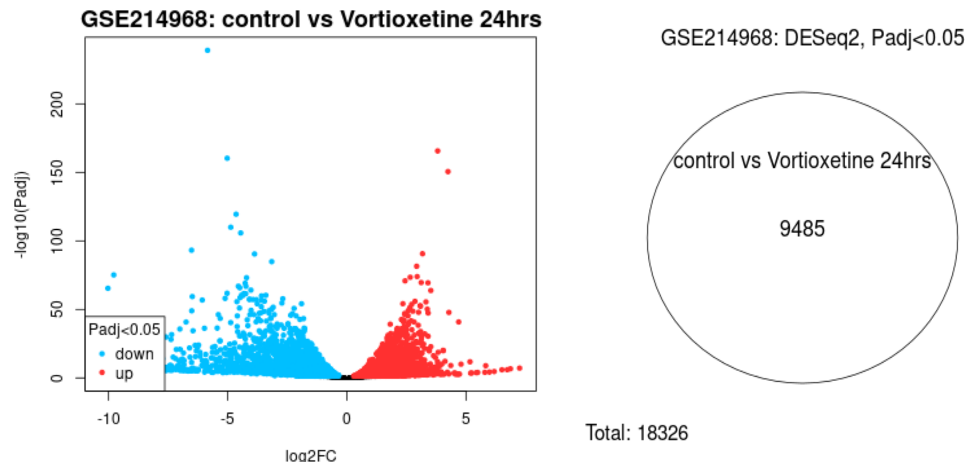

Figure 2. Differentially Expressed Genes in GEO2R: (a) Volcano Plot, (b) Venn Diagram visualize gene expression changes in glioblastoma LN229 cells after 24 hours treatment with Vortioxetine, using the control group (0-hour, untreated) as the baseline. (a) Genes with significant upregulation (higher expression) are highlighted in red, and those with significant downregulation (lower expression)are in blue. Black dots represent genes with minimal expression change. (b) illustrates a total 18326 genes number that were identified in glioblastoma LN229 cells after 24 hours of treatment with Vortioxetine, highlighting 9485 genes as differentially expressed genes (DEGs).

Identification of 50 Statistically Significant Differentially Expressed Genes (DEGs)

To refine the initial list of differentially expressed genes (DEGs), statistical thresholds were applied to identify the most significant genes from the GEO2R generated list of 9485 DEGs for future analysis. The selection criteria included: Log2 fold change (lig2FC) threshold:+/-3, Adjusted p-value (p.adj) at < 0.05. Using these criteria, a total of 50 statistically significant DRGs were identified, consisting of 25 upregulated genes (log2FC >+3) and 25 downregulated genes (logeFC <-3). This subset was selected based purely on statistical metrics and is summarized in a table, which included the gene symbol, name, log2FC and adjusted p-values.
full 50 data for further analysis log2fold +-

Potential Functions and Enrichment of the Identified Genes

To explore the biological roles and pathways associations of the 50 statistically significant differentially expressed genes (DEGs), functional enrichment analysis was performed using SRPlot, including GEne Ontology (GO) and KEGG pathway analysis. These analyses provided insights into the molecular, cellular, and biological mechanisms impacted by the identified DEGs, with particular emphasis on their relevance to glioblastoma biology. Gene Ontology (GO) enrichment analysis bar graph categorized the DEGs into three ontologies (Figure 3a). Higher enrichment score indicates stronger statistical significance and expressed in -log10(p-value). Biological Process (BP) highlighted processes such as somitogenesis, Notch signaling pathway, chemokine-mediated signaling pathway with enrichment score ranging from about 3.5 highest to

2 being the lowest. Cellular Components (CC) emphasized structural terms like Ribosome with significantly high enrichment score at about 12.

Finally, Molecular Function presented the most highly enriched score. Structural constituent of ribosome was about 13 in p-value, indicating that genes in this group are significantly present. The CCNet plot (Figure 3b) was analyzed to narrow down on some DEGs and the pathways they follow. The collagen containing extracellular matrix stood out as a significant pathway and connection with COL6A3 and FREM2 genes was noted.These genes are significantly upregulated and are known to to be a critical factors in tumor invasion and aggressiveness.

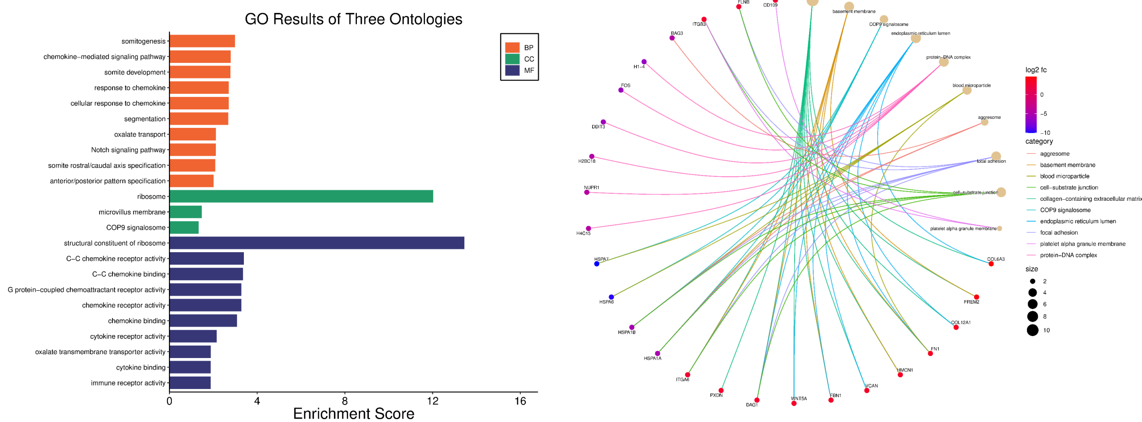

Figure 3. GO Pathway enrichment and DEGs in SR Plot: (a) Enrichment Three Ontologies Bar graph, (b) Cellular Component (CC) CNet plot provide insight into biological, cellular, and molecular roles and determine how likely it is that the observed association with specific DEGs occurred by chance. COL6A and FREM2 are significantly upregulated and show strong connection to the collagen-containing extracellular matrix pathway.

KEGG pathway analysis highlighted four key biological pathways enriched with DEGs. The Pathway Enrichment Bar graph (Figure 4a) shows Ribosome biogenesis in eukaryotes with the highest enrichment score 12.5, calculated -log10 p-value, closely followed by Ribosome pathway. In contrast, Notch signaling pathway and ECM-receptor interaction exhibit a significant drop to about 2-2.5 enrichment score level. Visualisation of CNet plot in KEGG pathway (Figure 4b) enables studying of these specific pathways and their association with individual statistically significant DEGs and highlighting their relevance to glioblastoma biology. Highly expressed COL6A3 and FREM2 in ECM-receptor interaction together with lowly expressed NRARP and DLL3 genes in Notch signaling pathway were identified from the GO analysis. New 10 DEGs in RNA5s family genes were depicted and identified as significantly downregulated in Ribosome and Ribosome biogenesis in eukaryotes pathways.

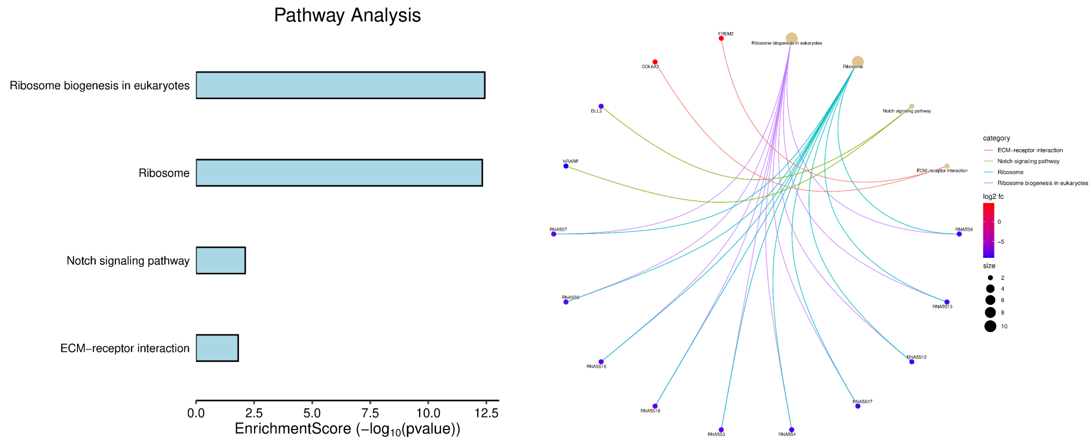

Figure 4: KEGG pathway enrichment and DEGs in SR Plot: (a) Pathway Enrichment Bar Graph, (b) CNet Plot provide comprehensive analysis of the biological pathways associated with the differentially expressed genes (DEGs). Among four significantly enriched pathways, the Ribosime and Ribosome biogenesis in eukaryotes pathways display the highest enrichment score. Additionally, 10 RNA5S family genes are prominently associated with these pathways.

The table below provides a comprehensive summary of the key differentially expressed genes (DEGs) identified in this study, their associated pathways, biological functions, and their relevance to glioblastoma. This table integrates findings of GO and KEGG enrichment analyses, highlighting both tumor-promoting and tumor-disrupting genes. By detailing the biological roles of these DEGs within their respective pathways, this table emphasizes their potential biomarkers or therapeutic targets in glioblastoma research.

Table 1. DEGs and Pathways identified in these Studies

Gene Code	Gene Name	Pathway	Functions	Glioblastoma Connection
RNA 5S (...7, 5, 15, 16, 3, 4, 17, 12, 13, 6)	Ribosomal RNA 5S	Ribosome biogenesis in eukaryotes	Essential for ribosome assembly and function; supports protein synthesis.	Low expression disrupts ribosome function, affecting protein synthesis (4).
RNA 5S (...7, 5, 15, 16, 3, 4, 17, 12, 13, 6)	Ribosomal RNA 5S	Ribosome	Integral to ribosome structure and efficient protein translation.	Reduced activity may impair protein translation in tumor cells (7).
NRARP	Notch Regulated Ankyrin Repeat Protein	NOTCH signaling pathway	Regulates NOTCH signaling; involved in cell differentiation.	Altered signaling influences tumor progression and differentiation (10).
DLL3	Delta like Protein 3	NOTCH signaling pathway	Ligand for NOTCH signaling; governs cell fate decisions.	Abnormal expression impacts glioblastoma cell proliferation (10).
COL6A3	Collagen VI alpha-3	ECM-receptor interaction	Provides structural support in ECM; promotes cell adhesion.	Overexpression facilitates tumor invasion and metastasis (12).
FREM2	FRAS-related extracellular matrix protein 2	ECM-receptor interaction	Mediates cell-ECM signaling; critical for tissue integrity.	Upregulation enhances glioblastoma cell migration and adhesion (12).

DISCUSSION

Summary of Findings

This study aimed to investigate the impact of vortioxetine on gene expression in glioblastoma LN229 cells using bioinformatics tools to identify differentially expressed genes (DEGs) and their associated pathways. Using GEO2R, a total of 1836 DEGs were identified, including 25 upregulated and 25 downregulated genes. KEGG pathway analysis highlighted four key pathways: Ribosome biogenesis in eukaryotes, Ribosome, Notch signaling pathway, and ECM-receptor interaction (8,12). Downregulation of the RNA5S family genes suggested disruption of ribosome biogenesis, potentially impairing protein synthesis in glioblastoma cells. Conversely, COL6A3 and FREM2 were highly upregulated, linking them to extracellular matrix remodeling and tumor progression (5,6). Go enrichment analysis further categorized these DEGs into biological processes, cellular components, and molecular functions, reinforcing their critical roles in glioblastoma biology.

Interpretation of Results

The findings underscore the dual nature of the DEGs in glioblastoma. The RNA5S family genes were strongly associated with the ribosome biogenesis pathways, and their significant downregulation suggests that Vortioxetine may disrupt ribosomal function and protein synthesis, presenting a potential therapeutic vulnerability (4, 7). On the other hand, the upregulation of COL6A3 and FREM2 in the ECM-receptor interaction pathway supports their role in extracellular matrix remodeling, a key process in glioblastoma cell adhesion, migration, and invasion (5, 6, 15). Furthermore, the identification of NRARP and DLL3 within the Notch signaling pathway highlights the importance of this pathway in tumor progression and glioblastoma stem-like cell maintenance (9, 13). Collectively, these findings align with the hypothesis that Vortioxetine affects key molecular pathways in glioblastoma, influencing both tumor-suppressive and tumor-promoting mechanisms.

Comparison with Previous Studies

The results are consistent with previous research emphasizing the importance of ribosome biogenesis in cancer. Upregulation of ribosomal activity has been shown to be critical for tumor growth, while its disruption can impair protein synthesis and reduce tumor viability (4, 7). Similarly, the involvement of COL6A3 and FREM2 in ECM remodeling aligns with studies demonstrating the role of the extracellular matrix in glioblastoma Invasion and metastasis (5, 6,15). The findings also corroborate earlier research on the role of the NOTCH signaling pathway in glioblastoma, particularly its regulation of stem-like cells (ability to self-renew, ability to differentiate into multiple cell types, and rapid growth and division) behavior and therapy resistance (9, 13). Unique to this study, however, is the impact of Vortioxetine on these pathways, offering new insights into its potential for repurposing as a glioblastoma therapy.

Implications

These findings have significant implications for glioblastoma research and treatment. The down regulation of RNA5S genes suggests that targeting ribosome biogenesis may be a viable therapeutic strategy, potentially limiting the tumor's ability to sustain high protein synthesis demands. Meanwhile, the up-regulated genes COL6A3 and FREM2 present potential targets for therapies aimed at reducing ECM-driven tumor invasiveness (5,6). Importantly, the ability of Vortioxetine to cross the blood-brain barrier (BBB), as highlighted in advancements in neurotechnology and drug delivery (17), makes it an attractive candidate for glioblastoma treatment. By modulating glioblastoma-related pathways and bypassing the BBB, Vortioxetine could offer a dual advantage in addressing the challenges of tumor resistance and drug delivery.

Limitations

A limitation of this study lies in its reliance on publicly available bioinformatics datasets derived from microarray experiments conducted by other researchers. While these datasets offer valuable insights, the findings may not fully capture the complexity of glioblastoma in clinical settings due to the absence of direct experimentation on human or animal samples (10,11). Additionally,

the use of a single glioblastoma cell LN229 limits the generalizability of the results to other glioblastoma subtypes or patient populations. The identified DEGs and pathways will require further validation in laboratory or clinical environments to confirm their roles and therapeutic potential (13).

Further Directions

Future research should focus on experimentally confirming the identified DEGs and pathways in laboratory or clinical environments. For instance, the downregulated RNA5S family genes could be investigated for their roles in ribosome biogenesis and its disruption in glioblastoma cells. Similarly, the upregulated genes COLA3 and FREM2 could be explored for their contributions to ECM remodeling and tumor invasion, with a view to developing anti-invasive therapies (5,6,16). Given Vortioxetine's ability to cross the BBB, further studies should assess its therapeutic efficacy in glioblastoma and its potential synergistic effects when combined with other treatments (17). Expanding research to include diverse glioblastoma cell lines and patient-derived samples would provide a more comprehensive understanding of these pathways' roles in glioblastoma biology (9, 11). Future research could expand on these findings to develop targeted therapies and biomarkers for improved glioblastoma management and patient survival (15, 16).

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