Research in Pharmaceutical Sciences, December 2025; 20(6): 889-902
Received: 08-03-2024
Peer Reviewed: 07-05-2024
Revised: 26-07-2024
Accepted: 16-09-2024
Published: 15-11-2025

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Exploring the diagnostic and prognostic potential of LCFAs' metabolic and biosynthetic pathways in glioblastoma multiforme using a bioinformatics approach

Ameneh Heydarian[1], Behnaz Yazdani[2], and Hajar Sirous[3,*]

[1]Department of Food Science and Technology, Tehran Medical Sciences, Islamic Azad University Pharmaceutical Sciences Branch, Tehran, Iran.
[2]Bioscience Department, Faculty of Science and Technology (FCT), Universitat de Vic - Universitat Central de Catalunya (Uvic-UCC), Vic, Spain.
[3]Bioinformatics Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

Abstract

Background and purpose: T-cell immunoglobulin and mucin-domain containing protein-3 (TIM-3)/ galectin-9 (Gal-9)/ autocrine loop in myeloid leukemia stem cells provokes inflammation through the NF-κB signaling pathway, which is influential in the expression of inflammatory factors. Interleukin1β (IL-1β) is a vital inflammatory cytokine that plays an important role in the proliferation and therapy resistance of acute myeloid leukemia (AML) cells. This study aimed to assess the effect of Gal-9 on IL-1β in the human leukemic U937 cell line.

Experimental approach: The U937 cells were cultured in different concentrations of Gal-9. Cell counting kit-8 was used to assess the effect of Gal-9 on human leukemic U937 cell proliferation. Also, its impact on the expression of *TIM-3, Gal-9, IL-1β, IL-1βR, IL-1βRAP,* and *NLRP3* genes and IL-1β protein was studied by RT-PCR and ELISA, respectively. Moreover, the effect of Gal-9 on the NF-κB signaling pathway was evaluated by western blotting.

Findings/Results: U937 cells were expanded in the presence of Gal-9 in a concentration-dependent manner. Following treatment of U937 cells with Gal-9, the gene expression of *Gal-9*, *IL-1B*, *IL-1BR*, and *IL-1BRAP* were significantly upregulated compared to the control group. The IL-1β concentration increased following Gal-9 treatment in a concentration-dependent manner, while following time its level significantly decreased. Furthermore, Gal-9 slightly increased NF-κB phosphorylation.

Conclusion and implications: Gal-9 increased IL-1β level as a critical inflammatory cytokine in the proliferation and resistance of AML cells to therapy. According to this finding, targeting and blocking the TIM-3/Gal-9 autocrine loop can suppress IL-1β production and facilitate AML treatment.

Keywords: AML; GAL-9; Inflammation; IL-1β, NF-κB.

INTRODUCTION

According to the classifications of the World Health Organization (WHO), glioblastoma multiforme (GBM) is a malignant form of brain tumor that is detected in the central nervous system and includes astrocytomas, oligodendrogliomas, and ependymomas (1). Most GBM cases are primary gliomas, originating from normal glial cells through a series of oncogenic events (2). GBM is one of the most common and aggressive forms of brain tumors in adults. Despite the advances achieved in treatment approaches of GBM in recent decades, the prognosis for patients with GBM is still very poor, with an overall survival of less than 12 months (1,3).

Access this article online	
	Website: http://rps.mui.ac.ir
	DOI: 10.4103/RPS.RPS_52_24

*Corresponding author: H. Sirous
Tel: +98-3137927065, Fax: +98-3136680011
Email: h_Sirous@pharm.mui.ac.ir

Treatment typically involves surgical resection of the tumor, radiotherapy, and chemotherapy (4). Lately, there has been a notable effort to gain a deeper understanding of the early molecular changes and altered biological pathways of these tumors to develop more precise and effective targeted therapeutic strategies and improve the sensitivity of tumor cells to chemotherapy and radiotherapy trials, such as investigating the changes in the cellular signal transduction pathways, cell cycle regulation, and metabolic adaptations (5,6).

Fatty acids (FAs) constitute about 50% to 60% of the total brain's dry mass and are important for the generation of the myelin sheath, providing the components of the lipid bilayer, and the cellular metabolism (7). While glucose is the primary substrate for the production of ATP molecules, the catabolism of FAs is also important for the generation of ATP molecules in the brain (8). High-fat diets have also been shown to elevate the number of glioma stem cells and promote the growth and self-renewal potentials of the tumor cells *in vivo* (9). While previous studies believed that GBM is dependent on aerobic glycolysis for energy maintenance, the new studies have suggested that when glucose levels are limited, GBM cells can alter their metabolism to enhance FA oxidation and adapt themselves to use FAs for their survival and energy demands (10).

FAs are categorized into small-chain FAs (SCFAs), medium-chain FAs (MCFAs), and long-chain FAs (LCFAs), concerning the length of the carbon chain in their structures (11). Researchers have shown that the levels of SCFAs decrease following glioma growth, such as propionate, butyrate, and acetate (12). MCFAs such as caprylic acid are utilized for energy production through β-oxidation pathways and are oxidized by medium-chain acyl-CoA dehydrogenase enzyme, whose expression was shown to be higher in GBM compared to normal brain samples (13,14).

Another study examining the expression of enzymes responsible for the synthesis and elongation of FAs in 28 GBM patient samples found that the synthesis of saturated FAs (SFAs) and polyunsaturated FAs (PUFAs) had different expression levels. Also, high expression of enzymes responsible for SFA synthesis in GBM tumors is associated with a lower survival rate, indicating the importance of this pathway in GBM (15). Despite various investigations into the importance of the metabolism of SCFAs and MCFAs in GBM cancer, the expression profiles of the genes involved in the metabolism of LCFAs in patients with GBM are poorly investigated.

Therefore, in this study, we used an integrative bioinformatic approach to analyze the expression patterns of genes involved in the metabolism of LCFAs using the RNAseq data of GBM cancer and normal brain tissue samples, and identify the best markers with the highest diagnostic and prognostic biomarker potential in patients with GBM cancer.

MATERIALS AND METHODS

Data collection and differential gene expression analysis

The RNA-seq count information of 169 cancer tissue samples from individuals diagnosed with GBM and 5 normal brain tissue samples from healthy individuals were obtained from the Cancer Genome Atlas Program (TCGA) and were analyzed, adhering to the criteria set in the Declaration of Helsinki for TCGA data access and usage guidelines (16). The RNAseq count data of the tissue samples underwent preprocessing and normalization and were transformed into logarithmic form (Log2 ratio) before identifying differentially expressed genes (DEGs) using the Limma, Voom, TCGAbiolinks, and edgeR packages in the R programming software (17-19). The DEGs with adjusted *P*-value smaller than 0.05 were considered statistically significant.

Gene set enrichment analysis

Gene set enrichment analysis (GSEA) is a powerful analytical technique commonly employed in bioinformatics to analyze and better interpret gene expression data. The GSEA technique operates by first ranking genes based on their differential expression between different groups of samples and evaluating enrichment to ascertain if a gene set or pathway is significantly associated with the phenotypic difference. Two gene sets associated with the metabolism of LCFAs were selected from the MSigDB database (https://www.gsea-msigdb.org/), and the normalized expression data of GBM samples were used as the expression matrix. According to the clinical data of the TCGA GBM patients, "cancer" and "normal" phenotypes were defined for the samples.

For this analysis, the GSEA software (version 4.0.3) by the BOARD Institute was used (20,21). The t-test method was selected as the ranking approach for the gene list while keeping the other parameters at the software's default settings. GSEA analysis calculates the ranking metric score, enrichment score, and false discovery rate (FDR) by assessing the correlation of each gene's expression level within the gene set across all samples. These scores indicate the enrichment level of individual genes within the specified gene set.

Gene ontology and protein-protein interaction analyses

Gene ontology (GO) analysis helps with understanding the biological functions, cellular components, and molecular processes of interest collection of genes. Functional enrichment or GO analysis was performed using the DAVID database (version 6.8, https://david-d.ncifcrf.gov/), an online platform that provides free access to tools for identifying enriched biological pathways and predicting gene function and localization. To better visualize the protein-protein interactions (PPI) between these gene sets and the DEGs in GBM cancer, the STRING database was utilized to calculate interaction scores and build a PPI network with a confidence score of 0.7. The STRING database is a comprehensive database of known and predicted PPIs. It includes interactions from a variety of sources, including experimental data, computational predictions, and text mining. The Cytoscape software (version 3.2.0) was used for better visualization of the PPI network, and the CytoNCA tool (version 2.1.6) was used for calculating the topological parameters of the network and identification of highly interacting protein nodes in the network (22-24).

Receiver operating characteristic test

The receiver operating characteristic (ROC) test is a graphical representation and one of the common methods for assessing the diagnostic potential of genes based on their expression levels in cancer and normal tissue samples. ROC analysis is employed to measure the ability of a biomarker to discriminate between two groups, such as healthy individuals and those with a disease. It quantifies the biomarker's ability to correctly identify positive cases (sensitivity) while avoiding misclassification of negative cases (specificity). For this test, the normalized expression data of GBM and normal brain tissue samples were used to perform the ROC test using GraphPad Prism software (version 10). The ROC test was performed for genes from the selected gene sets, and their expression levels were highly different between cancer and normal samples with adjusted P-values smaller than 0.01.

Survival analysis

The Kaplan-Meier method is a widely used non-parametric technique for survival analysis, particularly when working with time-to-event data. The survival analysis was done using the "survival" package in R programming software to assess the prognostic potential of differentially expressed genes from gene sets that presented an adjusted *P*-value smaller than 0.001. The samples were categorized into "high" and "low" groups using a threshold value determined by the median gene expression level among the samples. Genes with a two-sided *P*-value less than 0.01 were deemed statistically significant.

RESULTS

GSEA analysis results

The GSEA technique measures the degree of correlation between the expression level of gene sets and their enrichment in samples with cancer and normal phenotypes. The result of this analysis is reported by the normalized enrichment score (NES), which demonstrates the correlation between the phenotypes and gene sets' expression levels and ranks the best correlating genes based on a ranking metric known as the "Rank Metric Score". As shown in the Fig. 1A-C, The GSEA analysis results demonstrated that the LCFA biosynthetic gene set with 26 members is upregulated in normal phenotype with NES value of -1.08 and FDR q-value of 0.23 and 8 genes out of 26 genes were highly enriched, including proteolipid protein 1, acyl-CoA synthetase bubblegum family member 1 (ACSBG1), cytochrome P450 family 2 subfamily C member 8 (CYP2C8), acyl-CoA thioesterase 7 (ACOT7), arachidonate lipoxygenase 3 (ALOXE3), arachidonate 12-lipoxygenase (ALOX12B), cytochrome P450 family 2 subfamily E member 1 (CYP2E1), and cytochrome P450 family 1 subfamily A member 1 (CYP1A1) genes. The LCFA metabolic process gene set with 98 members was also predicted by GSEA analysis to be upregulated in the normal phenotype with an NES value of -1.33 and an FDR q-value of 0.08. Also, 31 genes out of 98 were classified as highly enriched, with the highest enrichment scores belonging to fatty acid amide hydrolase (FAAH), ALOX12B, CYP2E1, solute carrier family 27-member 2 (SLC27A2), cytochrome P450 family 2 subfamily A member 6 (CYP2A6), androgen-dependent TFPI regulating protein (ADTRP), and CYP1A1 genes. The rest of the enriched genes are shown in Fig. 1B and C.

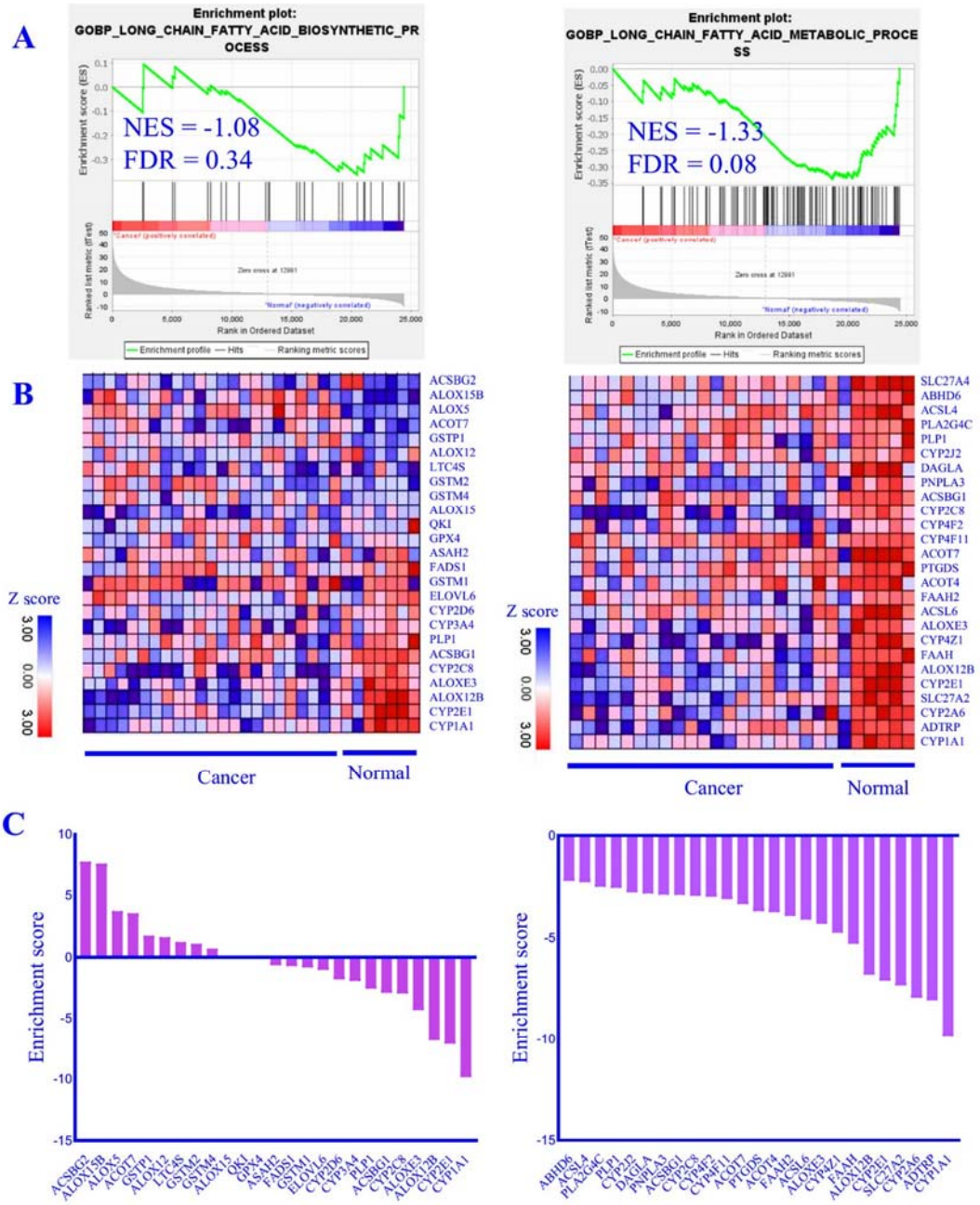

Fig. 1. Gene set enrichment analysis of LCFA metabolic and biosynthetic pathways in GBM. (A) enrichment plot depicting the enrichment score for the LCFA biosynthetic gene set in GBM tumor samples compared to normal brain tissue; (B) heatmap illustrating the expression levels of genes within the LCFA metabolic gene set in GBM and normal brain samples. (C) metric ranking scores for genes within the LCFA metabolic and biosynthetic gene sets, highlighting the relative contribution of individual genes to the overall enrichment scores. LCFA, Long-chain fatty acid; GBM, glioblastoma multiforme.

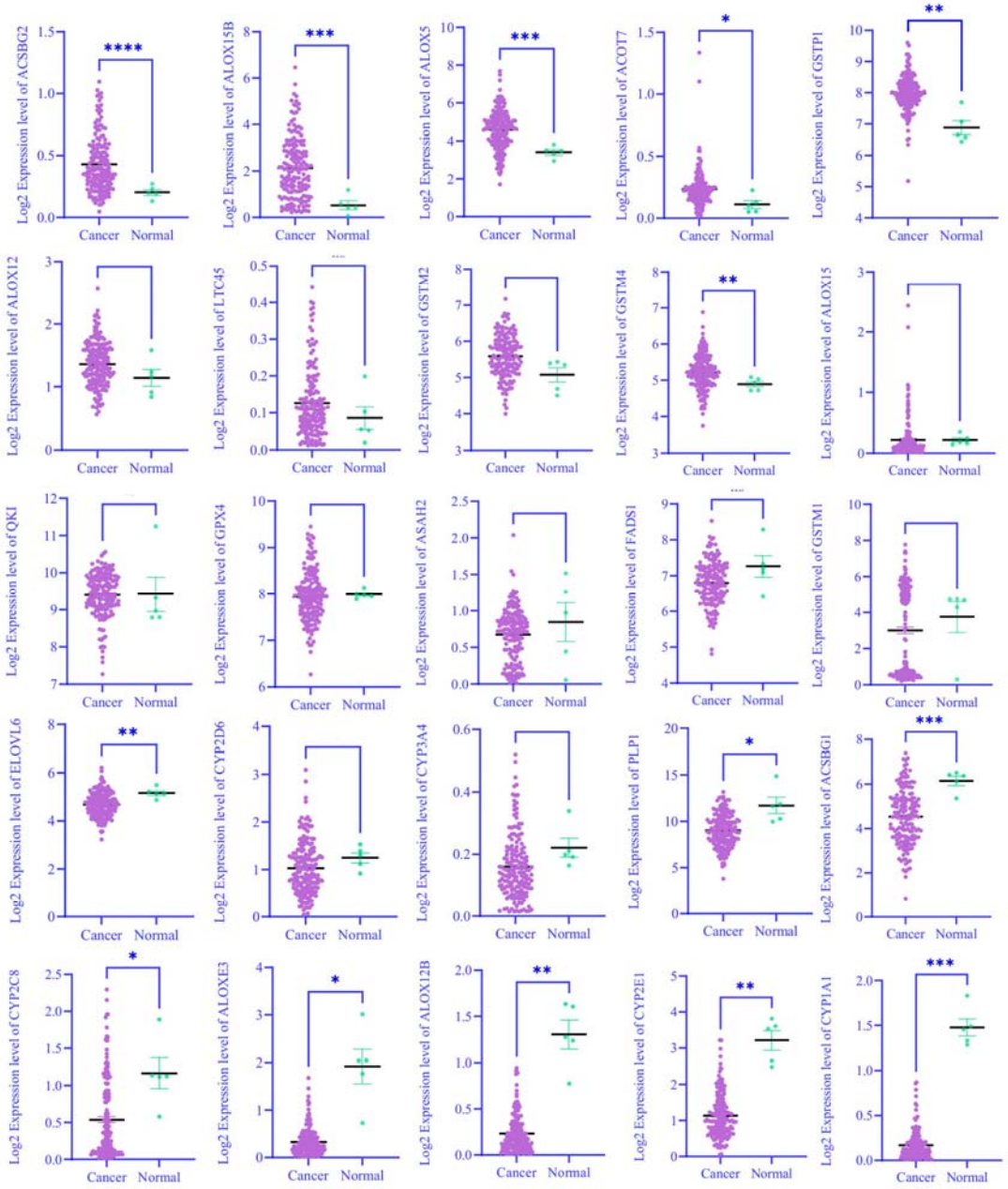

Fig 2. Differential gene expression analysis of the LCFAs biosynthetic pathway. The Log2 expression levels of genes belonging to the LCFAs biosynthetic process gene sets are compared between glioblastoma multiforme cancer and normal brain tissue expression data. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 indicate significant differences between the designated groups. LCFA, Long-chain fatty acid.

Differential expression analysis

In GSEA analysis, the top enriched genes with the best metric ranking scores from the LCFA biosynthetic and metabolic process gene sets were selected, and their expression levels were analyzed and compared between the GBM cancer tissues and normal brain tissue samples. As shown in the Figs. 2 and 3, the expression levels of Acyl-CoA synthetase Bubblegum family member 2 (ACSBG2; Log2FC = 1.03, adjusted P-value = 0.011), arachidonate 15-lipoxygenase type B (ALOX15B; Log2FC = 3.08, adjusted P-value = 0.0004), arachidonate 5-lipoxygenase (ALOX5; Log2FC = 1.25,

adjusted P-value = 0.011), ACSBG1 (Log2FC = -1.70, adjusted P-value = 0.004), CYP1A1 (Log2FC = -4.35, adjusted P-value = 8.66E-11), cytochrome P450 family 4 subfamily F member 11 (CYP4F11; Log2FC = -1.30, adjusted P-value = 0.019), ACOT7 (Log2FC = -2.41, adjusted P-value = 7.00E-12), prostaglandin D2 synthase (PTGDS; Log2FC = -3.56, adjusted P-value = 1.43E-6), CYP2A6 (Log2FC = -2.70, adjusted *P*-value = $6.56E^{-5}$), and ADTRP (Log_2FC = -3.35, adjusted *P*-value = $2.39E^{-6}$) genes were significantly different between GBM and normal patients.

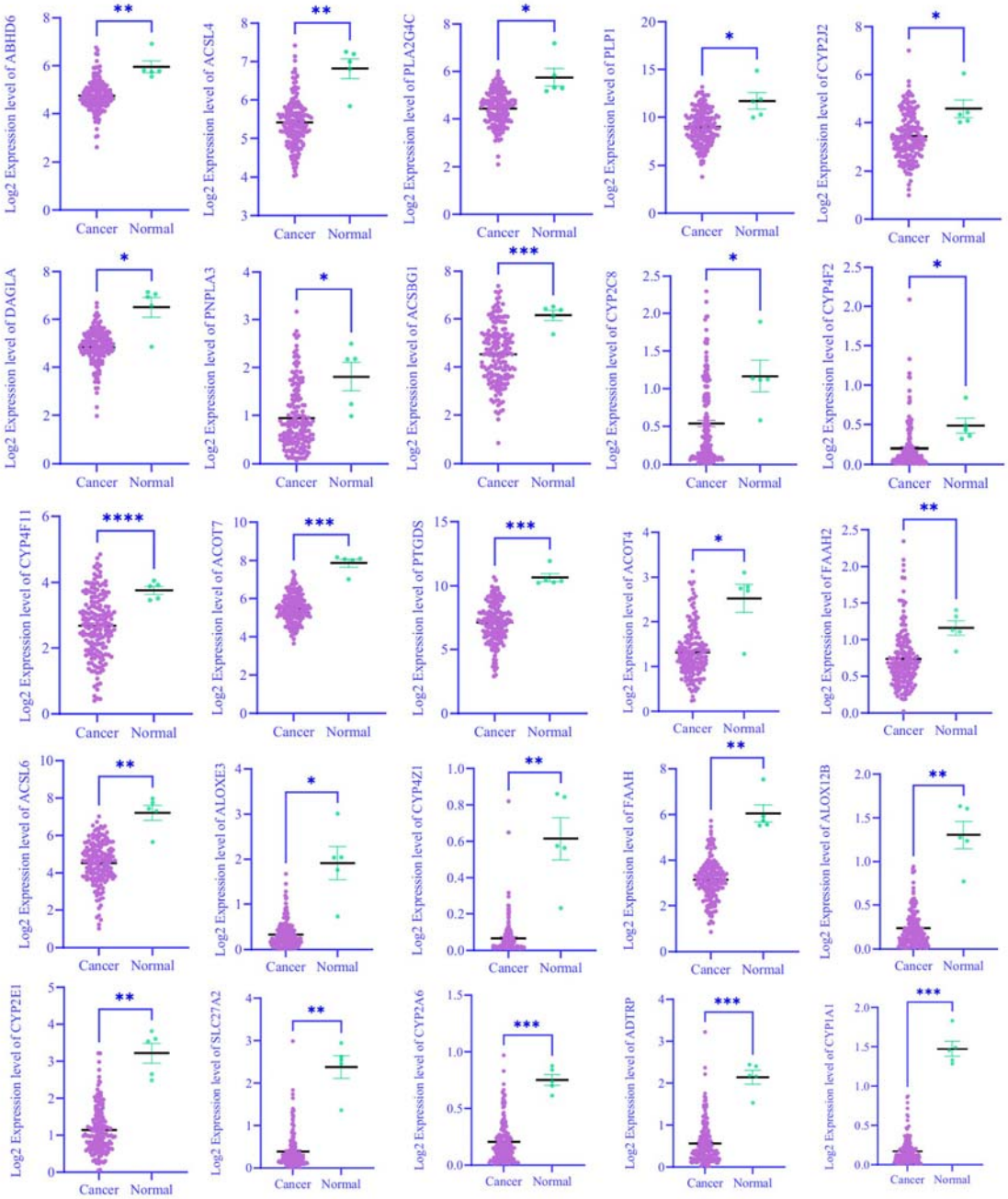

Fig. 3. Differential gene expression analysis of LCFAs metabolic process. The Log2 expression levels of genes belonging to the LCFA metabolic process gene set in glioblastoma multiforme cancer and normal brain tissue samples are displayed. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 indicate significant differences between the designated groups. LCFA, Long-chain fatty acid.

GO and PPI network analysis

GO and functional enrichment analysis are useful methods for better understanding the biological roles and molecular functions of interest genes. As shown in Fig. 4A, the biological pathways of the majority of the genes from the selected gene sets for GSEA analysis are predicted to be associated with cellular process (GO: 0009987), single-organism cellular process (GO: 0044763), and cellular metabolic process (GO: 0008152), while the molecular functions of most of these genes were predicted with catalytic activity (GO: 0003824) and ion binding (GO: 0043167) (Fig. 4B). The cellular components of most of the genes were also predicted to be in the cell part (GO: 0044464), intracellular part (GO: 0044424), and cytoplasm (GO: 0005737) (Fig. 4C). The PPI analysis between the genes involved in the metabolism of LCFAs and top DEGs in GBM cancer patients was performed to better understand the PPIs between these genes (Fig. 4D). According to the analysis of topological parameters of the constructed PPI network (Table 1), CYP2E1, prostaglandin-endoperoxide synthase 2 (PTGS2), Acyl-CoA synthetase long-chain family member 4 (ACSL4), CYP2C8, cytochrome P450 family 3 subfamily A member 4 (CYP3A4), and ACSL1 genes had the highest interaction degree and betweenness scores compared to other genes and are colored in red in the PPI network indicating their high interaction score.

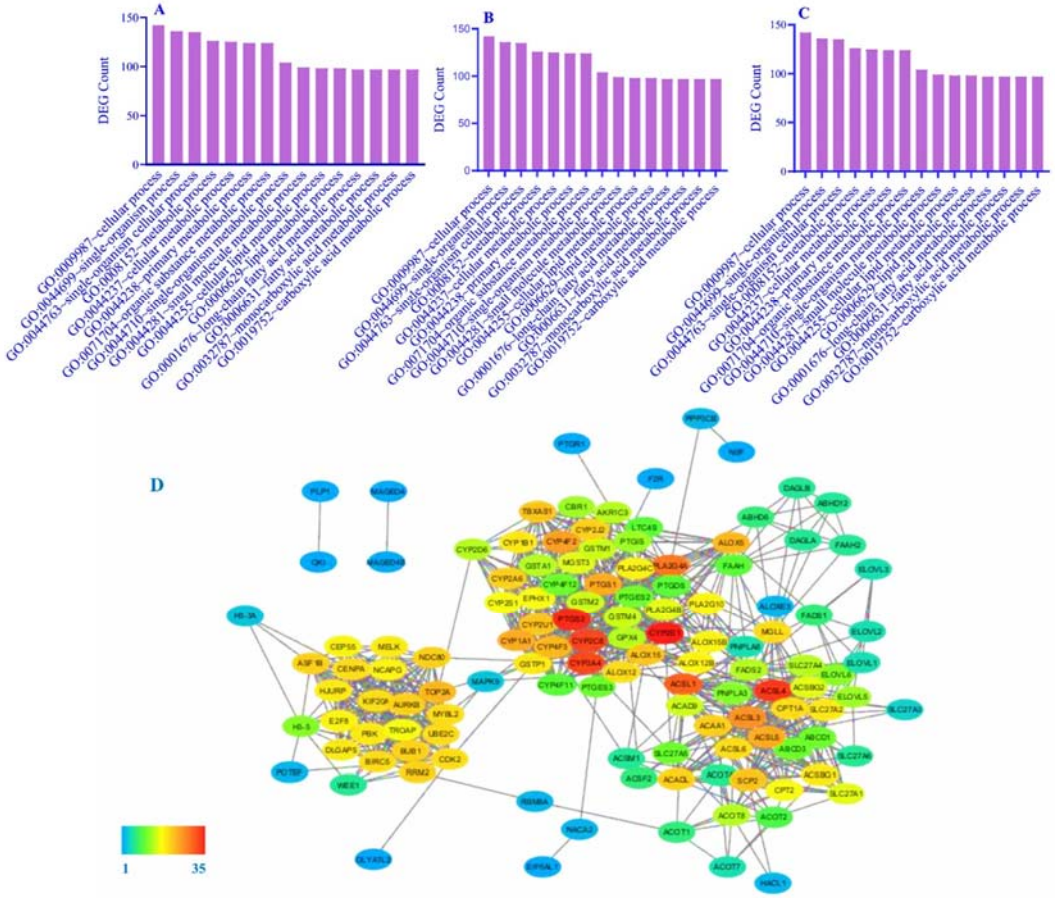

Fig. 4. GO and protein-protein interactions network analyses. (A-C) Demonstrating biological processes, molecular functions, and cellular components predicted from GO analysis results of the genes from LCFAs biosynthetic and metabolic pathways; (D) illustrates the protein-protein interaction results between the highly differentially expressed genes in glioblastoma multiforme cancer and the genes from LCFAs metabolic and biosynthetic pathway, which their color changes from blue to red according to their interaction degree scores calculated by CytoNCA application. GO, Gene Ontology; LCFA, long-chain fatty acid.

Table 1. Protein-protein interaction network analysis results obtained from by CytoNCA application in the Cytoscape software.

Gene ID	Degree	Betweenness	Closeness
CYP2E1	35	554	0.16
PTGS2	34	3831	0.17
ACSL4	33	1944	0.17
CYP2C8	32	398	0.17
CYP3A4	32	1159	0.17
ACSL1	30	857	0.16
PLA2G4A	28	1005	0.16
ACSL3	26	196	0.16
CYP4F2	25	95	0.16
PTGS1	24	331	0.16
CYP1A1	24	532	0.16
ACSL5	24	96	0.15
CYP4F3	23	69	0.16
ALOX5	23	359	0.16
TOP2A	23	508	0.15
CYP2U1	22	141	0.16
ALOX15	22	239	0.16
TBXAS1	22	124	0.16
SCP2	22	278	0.15

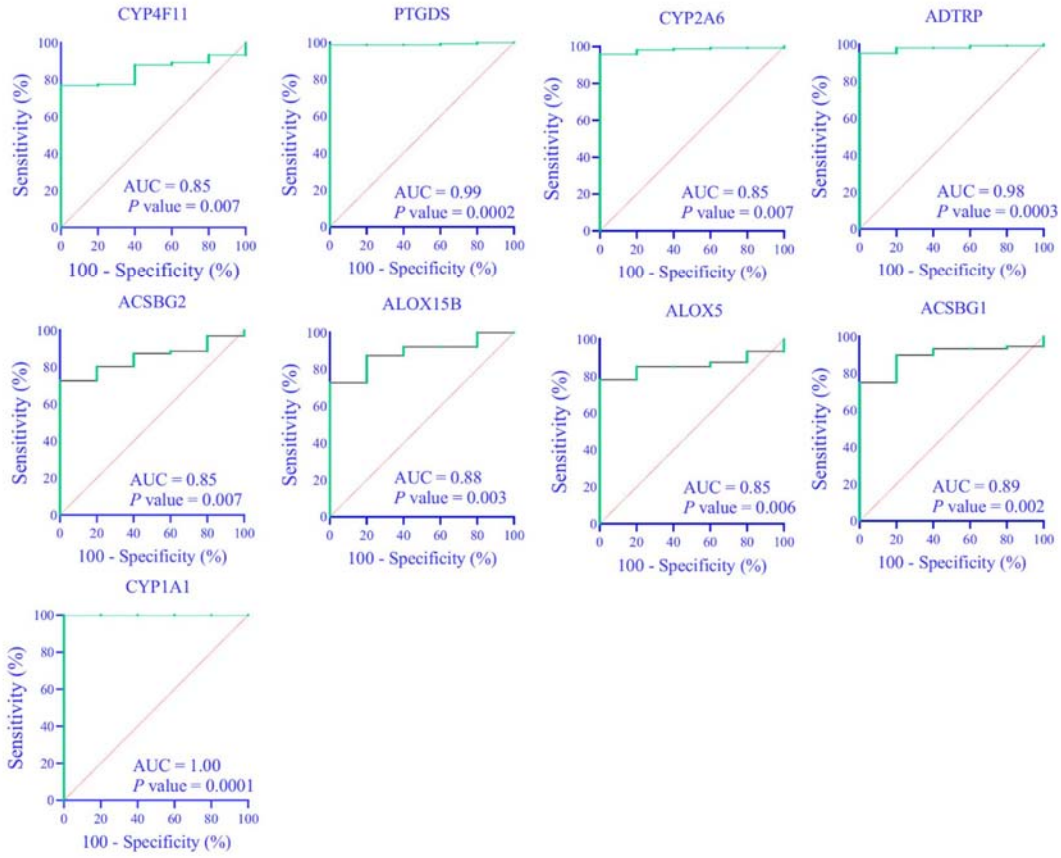

Fig. 5. ROC test. The diagnostic potential of the highly differentially expressed genes from LCFAs' metabolic and biosynthetic pathways in glioblastoma multiforme cancer and normal brain samples was analyzed using their normalized expression data, and AUC values were generated by taking an ROC test with GraphPad Prism software. The *p*-values smaller than 0.01 were considered statistically significant. ROC, Receiver operating characteristic; LCFA, long-chain fatty acid; AUC, area under the curve.

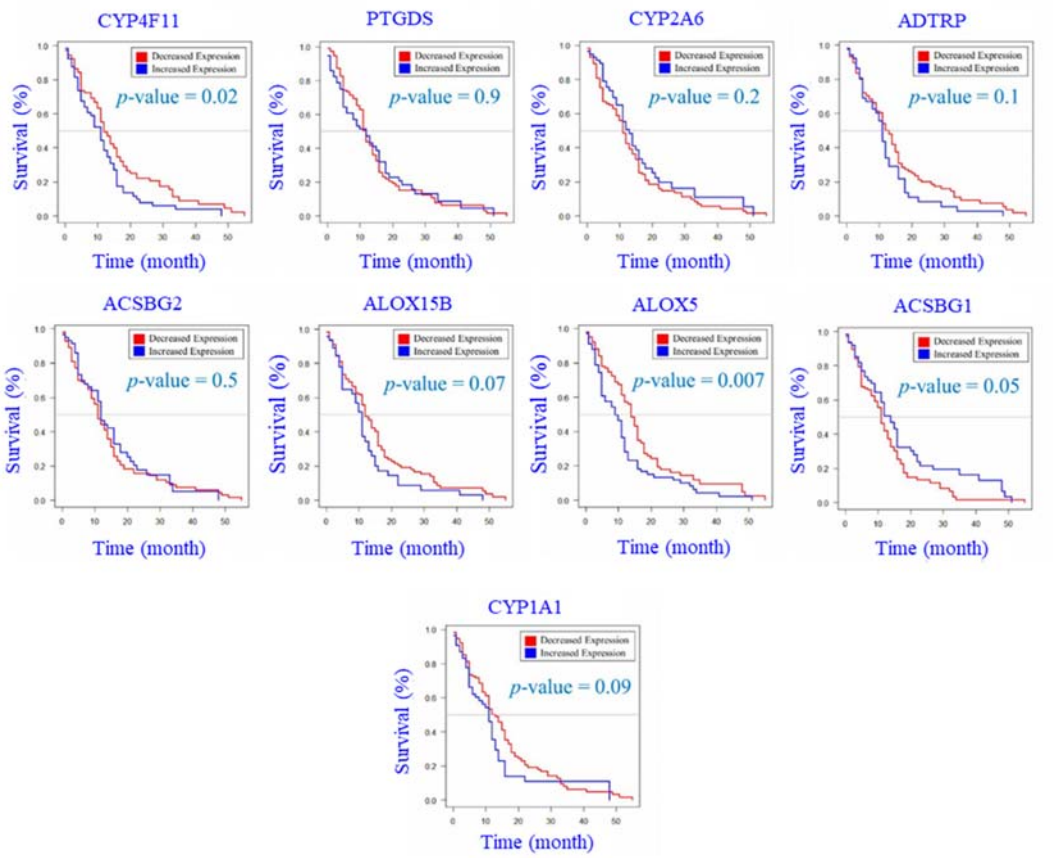

Fig. 6. Survival analysis. The prognostic potential of the highly differentially expressed genes from LCFAs' metabolic and biosynthetic pathways in glioblastoma multiforme cancer and normal brain samples was analyzed using the Kaplan-Meier method, which demonstrates the prognostic potential of each gene in the survival of patients with GBM cancer. *P*-values smaller than 0.05 were considered statistically significant.

The CYP1A1 gene as a potential diagnostic biomarker for the detection of GBM cancer

In cancer detection, the ROC test can be applied to assess the performance of specific genes as potential diagnostic biomarkers. By analyzing the area under the curve (AUC) values in the ROC graph, the accuracy and potential of a gene in distinguishing between cancerous and non-cancerous samples can be assessed. Genes with high AUC values close to 1 are considered good candidates to be investigated as diagnostic biomarkers. This information is crucial in identifying genes that have high diagnostic efficacy and can potentially be used for early cancer detection, prognosis, or treatment response prediction. As shown in Fig. 5, the results of the ROC test of top-selected differentially expressed genes from the metabolism of LCFAs pathways revealed statistically significant diagnostic potential, but CYP1A1, PTGDS, and ADTRP genes demonstrated significant diagnostic potential and the highest AUC values compared to the rest of the genes, and can be good candidates for the diagnosis of GBM cancer.

The ALOX5 gene is a good prognostic biomarker in GBM cancer

The Kaplan-Meier method is frequently applied to analyze the relationship between gene expression patterns and patient survival. Patients can be divided into "high-expressing" and "low-expressing" groups based on the expression levels of specific genes or gene signatures to estimate the probability of their survival for each group over time. This method is used to identify potential prognostic biomarkers that may help with the estimation of patients' survival according to their gene expression profile and better treatment

decisions for the patients who are predicted to be at higher risk. The prognostic potential of notably differentially expressed genes from pathways associated with the metabolism of LCFAs was analyzed in GBM cancer patients. As shown in Fig. 6, the expression level of the ALOX5 gene significantly correlated with patients' overall survival, and GBM patients who presented higher levels of the ALOX5 gene survived less compared to patients who had lower ALOX5 expression levels. The rest of the genes did not demonstrate a statistically significant correlation between their expression levels and the overall survival period in GBM patients.

DISCUSSION

An aggressive form of brain cancer that is known for poor prognosis and limited treatment options is GBM cancer (3). Altered lipid metabolism is one of the important hallmarks of resistant tumors. GBM cells can adapt their metabolic strategy to increase the oxidation of fatty acids and utilize them for optimum survival and energy requirements (10). *In vivo* experiments on high-fat diets have also been shown to promote the self-renewal and growth potential of GBM stem cells (9). Studying how fatty acids with varying carbon-chain lengths are metabolized can provide valuable insights into selecting more effective molecular targets for targeted therapies, treatment approaches, and dietary recommendations tailored to the metabolic characteristics of tumor cells.

While previous research has explored the role of SCFAs and MCFAs in GBM cancer, there is still a notable gap in understanding the influence and expression patterns of LCFAs metabolism in GBM patients and their survival. Therefore, in this study, we first analyzed the enrichment patterns of genes involved in the metabolism of LCFAs and compared their expression levels in normalized expression data of GBM cancer and normal brain samples. GSEA analysis suggested that the majority of the genes were downregulated in the cancer phenotype. Differential expression analysis demonstrated that ACSBG2, ALOX5, and ALOX15B genes were upregulated in GBM samples, while ACSBG1, CYP1A1, CYP4F11, ACOT7, PTGDS, CYP2A6, and ADTRP genes had significantly lower expression levels in the GBM patients compared to healthy individuals. Rui-han Pan also showed that the expression levels of the ALOX5 gene were higher in low-grade glioma samples, and its expression level can estimate the overall survival in GBM patients (25). Our survival analysis also confirmed the prognostic potential of the ALOX5 gene in GBM cancer patients compared to the rest of the DEGs involved in the metabolism of LCFAs, which demonstrated weak prognostic capability compared to the ALOX5 gene. The ALOX5 enzyme catalyzes the conversion of arachidonic acid, a PUFA, into 5-hydroperoxy eicosatetraenoic acid, which is a key step in the production of leukotrienes (26). One of the common lipoxygenase enzymes in human macrophage cells is ALOX15B enzyme, which has been shown to have a synergistic impact in the progression of lymphoma tumor cells (27,28), but its expression level in GBM cancer was not investigated previously, and our analysis suggests an upregulated level of the ALOX15B gene in GBM tissue samples.

ACSBG1 and ACSBG2 are two important enzymes that are responsible for the activation of LCFAs by conjugation with an acyl-CoA molecule, and without an activation step, FAs cannot enter many catabolic and metabolic reactions. In our analysis, the expression levels of ACSBG1 and ACSBG2 were lower and higher in GBM samples, respectively, compared to normal brain samples (29), which in a previous study we also saw a similar expression pattern of these two genes in colon adenocarcinoma patients as well (30). CYPs are a family of enzymes that are involved in the metabolism of a wide variety of endogenous and exogenous compounds. Differential expression analysis of LCFAs' metabolic and biosynthetic pathways demonstrated that three isoforms of CYP genes were notably downregulated in GBM samples, including CYP1A1, CYP4F11, and CYP2A6. The CYP1A1 gene also demonstrated great diagnostic biomarker potential compared to the rest of the genes. CYP enzymes can metabolize varying exogenous compounds, and each isoform acts specifically according to the

chemical and structural properties of the compound (31).

Despite multiple studies that have been performed on the function or expression levels of CYP enzymes in different cancers, their differential expression pattern in GBM cancer remains poorly understood. CYP1A1 is crucial for detoxifying environmental carcinogens and activating dietary compounds with cancer-preventative properties, and polymorphisms detected in this gene were associated with the risk of lung cancer as well (32,33).

LCFAs can also regulate the expression and activity of CYP1A1. CYP1A1 is one of the most well-studied CYPs, and its expression levels can be altered in various cancer cells following treatment with LCFAs. Higher expression level of the CYP4F11 isoform was also found by other studies to correlate with better survival in patients with hepatocellular carcinoma (34). Its expression level was also shown by Alnabulsi *et al*. to notably correlate with the survival of patients with colorectal cancer (35). Various compounds that are inhaled by tobacco smoke, such as nitrosamines, are metabolized by the CYP2A6 gene. Multiple studies have found different genetic variations in the CYP2A6 gene that correlated with the risk of lung cancer and esophageal cancer. CYP2A6 has also been suggested to be important in the progression of colorectal cancer as well (36,37).

ACOT7 has an important role in the regulation of the cell cycle by modulating p53/p21 signaling. While its expression level in GBM is poorly investigated, other studies have shown that its expression level was associated with the development and progression of breast cancer, lung cancer, hepatocellular carcinoma, and gastric cancer (38-40). The role of the PTGDS gene is important in the synthesis pathway of prostaglandin D2, and its expression level is correlated with the migration of proliferation of glioblastoma, the progression of cervical squamous cell carcinoma, and poor survival in patients with endometrial cancer (41-43). In this study, the diagnostic potential of PTGDS and ADTRP genes was high in the classification of GBM samples. The ADTRP gene regulates histone genes, apoptosis, and cell cycle progression, and has been suggested as an important gene in coronary artery disease (44). However, the differential expression level of ACSL4 in cancers, including GBM, remains poorly investigated and is still not fully understood. LCFAs' treatment of GBM cells can affect their lipid metabolism. A study has also noticed that treatment of GBM cells with LCFAs, such as oleic acid, was able to induce proliferation, affect lipid composition, and decrease the expression of fatty acid synthetase enzyme, which is involved in *de novo* lipogenesis (45). Notably, treatment of HepG2 cells with oleic acid has been shown to significantly alter CYP1A1 expression, leading to changes in the regulation of lipid peroxidation processes and potentially affecting overall cellular oxidative balance and lipid metabolism (46).

The majority of the genes in the selected gene sets for GSEA analysis are associated with biological pathways related to cellular processes and cellular metabolic processes. Additionally, most of these genes are predicted to have molecular functions related to catalytic activity and ion binding. Furthermore, the cellular components of these genes are predicted to be in the cell part, intracellular part, and cytoplasm. The PPI network analysis also revealed that CYP2E1, CYP3A4, CYP2C8, PTGS2, ACSL4, and ACSL1 genes had the highest interaction degrees with top DEGs in GBM cancer, and their interaction analysis can help with a better understanding of their biological functions. The genes CYP2E1, CYP3A4, and CYP2C8 contribute to the metabolism of long-chain fatty acids (LCFAs) and related lipids. For example, CYP2E1 is known to catalyze ω- and (ω-1)-hydroxylation of fatty acids, and CYP3A4 and CYP2C8 have been implicated more broadly in fatty acid/PUFA metabolism and oxylipin formation. CYP2C8 additionally metabolizes endogenous substrates such as arachidonic acid to epoxide derivatives (47,48). The PTGS2 gene encodes an enzyme that converts arachidonic acid to prostaglandin H2, a precursor to various prostaglandins and thromboxanes. Research has demonstrated that PTGS2 was capable of facilitating the proliferation of glioma tumor cells and improving their radio-tolerance (49).

ACSL1 and ACSL4 are involved in the activation of LCFAs to acyl-CoA esters, facilitating their uptake and metabolism. These metabolic reactions are important for maintaining cellular homeostasis and energy production in GBM cancer cells. ACSL1 has been specifically

associated with a shorter survival time in GBM patients, and inhibitors targeting ACSL1 have demonstrated the ability to reduce GBM tumor growth in both *in vivo* and *in vitro* models (50). Other studies have also reported that ACSL4 expression is downregulated in human glioma tissues and cells. Interestingly, ACSL4 has been found to protect glioma cells and exert anti-proliferative effects by activating the ferroptosis pathway (51).

In this study, we analyzed the enrichment and differential expression patterns of genes associated with the metabolism of LCFAs in GBM cancer samples using bioinformatic tools (52), and the genes with the best diagnostic or prognostic potential were detected, which demand further investigations to test these biomarker candidates in further experimental and clinical trials on GBM patients. These investigations are important to understand the true clinical relevance and potential of these biomarker candidates in the context of GBM patients, paving the way for the design of more effective diagnostic, prognostic, and therapeutic strategies in GBM cancer. Moving forward, it is imperative to further validate these potential biomarkers through rigorous experimental and clinical trials involving individuals affected by GBM.

CONCLUSION

In our investigation, we uncovered a notable upregulation of genes ACSBG2, ALOX5, and ALOX15B in GBM samples, suggesting their potential involvement in the disease process. Conversely, ACSBG1, CYP1A1, CYP4F11, ACOT7, PTGDS, CYP2A6, and ADTRP genes exhibited lower expression levels in GBM patients compared to their healthy counterparts. Notably, the genes CYP1A1 and ALOX5 emerged as outstanding biomarker candidates with notable potential for diagnosing and predicting the prognosis of GBM. Our findings highlight the significance of genes associated with LCFAs metabolism, revealing distinct expression patterns that may hold pivotal implications for the diagnosis and prognosis of GBM.

Acknowledgments

The authors would like to thank the Bioinformatics Research Center in Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

H. Sirous performed the study design. B. Yazdani analyzed the data. A. Heydarian, B. Yazdani, and H. Sirous interpreted the data, performed bioinformatics analysis, and wrote the manuscript. All authors have read and approved the finalized article. Each author has fulfilled the authorship criteria and affirmed that this article represents honest and original work.

Availability of data and materials

The dataset used for obtaining the transcription expression data of glioblastoma multiforme and normal brain tissue samples in the current study is available in TCGA (cancergenome.nih.gov/).

Ethics approval and consent to participate

The authors are responsible for thoroughly investigating and resolving any questions regarding the accuracy or integrity of all aspects of the work. This study was conducted in accordance with the Declaration of Helsinki.

REFERENCES

1. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, *et al*. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. 2007;114:97-109. DOI: 10.1007/s00401-007-0243-4.
2. Urbańska K, Sokołowska J, Szmidt M, Sysa P. Glioblastoma multiforme-an overview. Contemp Oncol (Pozn). 2014;18(5):307-312. DOI: 10.5114/wo.2014.40559.
3. Adamson C, Kanu OO, Mehta AI, Di C, Lin N, Mattox AK, *et al*. Glioblastoma multiforme: a review of where we have been and where we are going. Expert Opin Investig Drugs. 2009;18(8):1061-1083. DOI: 10.1517/13543780903052764.
4. Hanif F, Muzaffar K, Perveen K, Malhi SM, Simjee SU. Glioblastoma multiforme: a review of its epidemiology and pathogenesis through clinical presentation and treatment. Asian Pac J Cancer Prev. 2017;18(1):3-9. DOI: 10.22034/APJCP.2017.18.1.3.
5. Grossman SA, Batara JF. Current management of glioblastoma multiforme. Semin Oncol. 2004;31(5): 635-644. DOI: 10.1053/j.seminoncol.2004.07.005.
6. Batash R, Asna N, Schaffer P, Francis N, Schaffer M. Glioblastoma multiforme, diagnosis and treatment; recent

literature review. Curr Med Chem. 2017;24(27): 3002-3009.
DOI: 10.2174/0929867324666170516123206.
7. Nguyen LN, Ma D, Shui G, Wong P, Cazenave-Gassiot A, Zhang X, *et al*. Mfsd2a is a transporter for the essential omega-3 fatty acid docosahexaenoic acid. Nature. 2014;509(7501):503-506.
DOI: 10.1038/nature13241.
8. Dimas P, Montani L, Pereira JA, Moreno D, Trötzmüller M, Gerber J, *et al*. CNS myelination and remyelination depend on fatty acid synthesis by oligodendrocytes. Elife. 2019;8:e44702,1-29.
DOI: 10.7554/eLife.44702.
9. Hale JS, Otvos B, Sinyuk M, Alvarado AG, Hitomi M, Stoltz K, *et al*. Cancer stem cell-specific scavenger receptor CD36 drives glioblastoma progression. Stem Cells. 2014;32(7):1746-1758.
DOI: 10.1002/stem.1716.
10. Sperry J, Condro MC, Guo L, Braas D, Vanderveer-Harris N, Kim KK, *et al*. Glioblastoma utilizes fatty acids and ketone bodies for growth allowing progression during ketogenic diet therapy. Iscience. 2020;23(9):101453.
DOI: 10.1016/j.isci.2020.101453.
11. Rustan AC, Drevon CA. Fatty acids: structures and properties. eLS. 2005;1-7.
DOI: 10.1038/npg.els.0003894.
12. Dono A, Patrizz A, McCormack RM, Putluri N, Ganesh BP, Kaur B, *et al*. Glioma-induced alterations in fecal short-chain fatty acids and neurotransmitters. CNS Oncol. 2020;9(2):CNS57,1-14.
DOI: 10.2217/cns-2020-0007.
13. Puca F, Yu F, Bartolacci C, Pettazzoni P, Carugo A, Huang-Hobbs E, *et al*. Medium-chain acyl-CoA dehydrogenase protects mitochondria from lipid peroxidation in glioblastoma. Cancer Discov. 2021;11(11):2904-2923.
DOI: 10.1158/2159-8290.CD-20-1437.
14. Altinoz MA, Ozpinar A, Seyfried TN. Caprylic (octanoic) acid as a potential fatty acid chemotherapeutic for glioblastoma. Prostaglandins Leukot Essent Fatty Acids. 2020;159:102142.
DOI: 10.1016/j.plefa.2020.102142.
15. Korbecki J, Simińska D, Jeżewski D, Kojder K, Tomasiak P, Tarnowski M, *et al*. Glioblastoma multiforme tumors in women have a lower expression of fatty acid elongases *ELOVL2, ELOVL5, ELOVL6*, and *ELOVL7* than in men. Brain Sci. 2022;12(10):1356,1-22.
DOI: 10.3390/brainsci12101356.
16. Tomczak K, Czerwińska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn). 2015;2015(1):68-77.
DOI: 10.5114/wo.2014.47136.
17. Colaprico A, Silva TC, Olsen C, Garofano L, Cava C, Garolini D, *et al*. TCGAbiolinks: an R/bioconductor package for integrative analysis of TCGA data. Nucleic Acids Res. 2016;44(8):e71-e,1-11.
DOI: 10.1093/nar/gkv1507.
18. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2010;26(1):139-140.
DOI: 10.1093/bioinformatics/btp616.
19. Liu S, Wang Z, Zhu R, Wang F, Cheng Y, Liu Y. Three differential expression analysis methods for RNA sequencing: limma, EdgeR, DESeq2. J Vis Exp. 2021(175):e62528.
DOI: 10.3791/62528.
20. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, *et al*. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA. 2005;102(43):15545-15550.
DOI: 10.1073/pnas.0506580102.
21. Liberzon A, Subramanian A, Pinchback R, Thorvaldsdóttir H, Tamayo P, Mesirov JP. Molecular signatures database (MSigDB) 3.0. Bioinformatics. 2011;27(12):1739-1740.
DOI: 10.1093/bioinformatics/btr260.
22. Kohl M, Wiese S, Warscheid B. Cytoscape: software for visualization and analysis of biological networks. Methods Mol Biol. 2011;696:291-303.
DOI: 10.1007/978-1-60761-987-1_18.
23. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, *et al*. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498-2504.
DOI: 10.1101/gr.1239303.
24. Tang Y, Li M, Wang J, Pan Y, Wu FX. CytoNCA: a cytoscape plugin for centrality analysis and evaluation of protein interaction networks. Biosystems. 2015;127:67-72.
DOI: 10.1016/j.biosystems.2014.11.005.
25. Pan RH, Zhang X, Chen ZP, Liu YJ. Arachidonate lipoxygenase 5 is a novel prognostic biomarker and correlates with high tumor immune infiltration in low-grade glioma. Front Genet. 2023;14:1027690, 1-17.
DOI: 10.3389/fgene.2023.1027690.
26. Mehrabian M, Schulthess F, Nebohacova M, Castellani L, Zhou Z, Hartiala J, *et al*. Identification of ALOX5 as a gene regulating adiposity and pancreatic function. Diabetologia. 2008;51(6):978-988.
DOI: 10.1007/s00125-008-1002-3.
27. Liu P, Liu Y, Chen C, Liu T, Niu T. Alox15b gene contributes to lymphoma tumorigenesis via PI3K/AKT/mTOR pathway activation and has a synergistic effect with Alox5 gene. Am J Hematol. 2018;132:4112.
DOI: 10.1182/blood-2018-99-120130.
28. Wuest SJ, Horn T, Marti-Jaun J, Kühn H, Hersberger M. Association of polymorphisms in the ALOX15B gene with coronary artery disease. Clin Biochem. 2014;47(6):349-355.
DOI: 10.1016/j.clinbiochem.2013.12.013.
29. Mashek DG, Li LO, Coleman RA. Long-chain acyl-CoA synthetases and fatty acid channeling. Future Lipidol. 2007;2(4):465-476.
DOI: 10.2217/17460875.2.4.465.
30. Parsazad E, Esrafili F, Yazdani B, Ghafarzadeh S, Razmavar N, Sirous H. Integrative bioinformatics analysis of ACS enzymes as candidate prognostic and

diagnostic biomarkers in colon adenocarcinoma. Res Pharm Sci. 2023;18(4):413-429.
DOI: 10.4103/1735-5362.378088.
31. Honkakoski P, Negishi M. Regulation of cytochrome P450 (CYP) genes by nuclear receptors. Biochem J. 2000;347(2):321-337.
DOI: 10.1042/0264-6021:3470321.
32. Androutsopoulos VP, Tsatsakis AM, Spandidos DA. Cytochrome P450 CYP1A1: wider roles in cancer progression and prevention. BMC Cancer. 2009;9:187,1-17.
DOI: 10.1186/1471-2407-9-187.
33. Houlston R. CYP1A1 polymorphisms and lung cancer risk: a meta-analysis. Pharmacogenetics. 2000;10(2):105-114.
DOI: 10.1097/00008571-200003000-00002.
34. Eun HS, Cho SY, Lee BS, Seong IO, Kim KH. Profiling cytochrome P450 family 4 gene expression in human hepatocellular carcinoma. Mol Med Rep. 2018;18(6):4865-4876.
DOI: 10.3892/mmr.2018.9526.
35. Alnabulsi A, Swan R, Cash B, Alnabulsi A, Murray GI. The differential expression of omega-3 and omega-6 fatty acid metabolising enzymes in colorectal cancer and its prognostic significance. Br J Cancer. 2017;116(12):1612-1620.
DOI: 10.1038/bjc.2017.135.
36. Matsuda Y, Saoo K, Yamakawa K, Yokohira M, Suzuki S, Kuno T, *et al*. Overexpression of CYP2A6 in human colorectal tumors. Cancer Sci. 2007;98(10):1582-1585.
DOI: 10.1111/j.1349-7006.2007.00572.x.
37. Tan W, Chen GF, Xing DY, Song CY, Kadlubar FF, Lin DX. Frequency of CYP2A6 gene deletion and its relation to risk of lung and esophageal cancer in the Chinese population. Int J Cancer. 2001;95(2):96-101.
DOI: 10.1002/1097-0215(20010320)95:2<96::aid-ijc1017>3.0.co;2-2.
38. Feng H, Liu X. Interaction between ACOT7 and LncRNA NMRAL2P via methylation regulates gastric cancer progression. Yonsei Med J. 2020;61(6):471-481.
DOI: 10.3349/ymj.2020.61.6.471.
39. Sun X, Zhang Q, Wang M, Yao L, Li X, Cao H, *et al*. Acyl-CoA thioesterase 7 is oncogenic in breast cancer by promoting oxidative phosphorylation via PGC1α. Genes Dis. 2023;11(5):101149.
DOI: 10.1016/j.gendis.2023.101149.
40. Zheng C, Zhang G, Xie K, Diao Y, Luo C, Wang Y, *et al*. Pan-cancer analysis and experimental validation identify ACOT7 as a novel oncogene and potential therapeutic target in lung adenocarcinoma. Cancers (Basel). 2022;14(18):4522,1-19.
DOI: 10.3390/cancers14184522
41. Zou R, Zheng M, Tan M, Xu H, Luan N, Zhu L. Decreased PTGDS expression predicting poor survival of endometrial cancer by integrating weighted gene co-expression network analysis and immunohistochemical validation. Cancer Manag Res. 2020;12:5057-5075.
DOI: 10.2147/CMAR.S255753.
42. Jiang P, Cao Y, Gao F, Sun W, Liu J, Ma Z, *et al*. SNX10 and PTGDS are associated with the progression and prognosis of cervical squamous cell carcinoma. BMC Cancer. 2021;21(1):694,1-14.
DOI: 10.1186/s12885-021-08212-w.
43. Wu W, Li H, Wang Z, Dai Z, Liang X, Luo P, *et al*. The tertiary lymphoid structure-related signature identified PTGDS in regulating PD-L1 and promoting the proliferation and migration of glioblastoma. Heliyon. 2024;10(1):e23915,1-18.
DOI: 10.1016/j.heliyon.2023.e23915.
44. Luo C, Wang F, Qin S, Chen Q, Wang QK. Coronary artery disease susceptibility gene ADTRP regulates cell cycle progression, proliferation, and apoptosis by global gene expression regulation. Physiol Genomics. 2016;48(8):554-564.
DOI: 10.1152/physiolgenomics.00028.2016.
45. Taïb B, Aboussalah AM, Moniruzzaman M, Chen S, Haughey NJ, Kim SF, *et al*. Lipid accumulation and oxidation in glioblastoma multiforme. Sci Rep. 2019;9(1):19593.
DOI: 10.1038/s41598-019-55985-z.
46. Huang B, Bao J, Cao YR, Gao HF, Jin Y. Cytochrome P450 1A1 (CYP1A1) catalyzes lipid peroxidation of oleic acid-induced HepG2 cells. Biochemistry (Mosc). 2018;83(5):595-602.
DOI: 10.1134/S0006297918050127.
47. Eccles JA, Baldwin WS. Detoxification cytochrome P450s (CYPs) in families 1-3 produce functional oxylipins from polyunsaturated fatty acids. *Cells.* 2022;12(1):82,1-30.
DOI: 10.3390/cells12010082.
48. Lucas D, Goulitquer S, Marienhagen J, Fer M, Dreano Y, Schwaneberg U, *et al*. Stereoselective epoxidation of the last double bond of polyunsaturated fatty acids by human cytochromes P450. J Lipid Res. 2010;51(5):1125-1133.
DOI: 10.1194/jlr.M003061.
49. Tan C, Liu L, Liu X, Qi L, Wang W, Zhao G, *et al.* Activation of PTGS2/NF-κB signaling pathway enhances radiation resistance of glioma. Cancer Med. 2019;8(3):1175-1185.
DOI: 10.1002/cam4.1971.
50. Xu C, Zhao J, Song J, Xiao M, Cui X, Xin L, *et al*. lncRNA PRADX is a mesenchymal glioblastoma biomarker for cellular metabolism targeted therapy. Front Oncol. 2022;12:888922,1-14.
DOI: 10.3389/fonc.2022.888922.
51. Cheng J, Fan YQ, Liu BH, Zhou H, Wang JM, Chen QX. ACSL4 suppresses glioma cells proliferation *via* activating ferroptosis. Oncol Rep. 2020;43(1): 147-158.
DOI: 10.3892/or.2019.7419.
52. Rezvani Sichani A, Rezvani Sichani Z, Yazdani B, Azizmohammad Looha M, Sirous H. A bioinformatics approach of specificity protein transcription factors in head and neck squamous cell carcinoma. Res Pharm Sci. 2024;19(3):287-302.
DOI: 10.4103/RPS.RPS_171_23.