Evaluation of Secretome Biomarkers in Glioblastoma Cancer Stem Cells: A Bioinformatics Analysis

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Abstract

Glioblastoma (GBM) is a malignant brain tumor that frequently occurs alongside other central nervous systems (CNS) conditions. Glutamate release and aberrant cellular behavior are shared features of both CNS diseases and GBM cells. Neither their origin nor the ways in which CNS disorders affect the development or behavior of GBM are well understood. Using data from the Gene Expression Omnibus (GEO) and the Cancer Genome Atlas (TCGA) datasets–where both healthy and cancerous samples were analyzed–we used a quantitative analytical framework to identify differentially expressed genes (DEGs) and cell signaling pathways that might be related to GBM. Then, we performed gene ontology studies and hub protein identifications to estimate the roles of these DEGs after finding disease-gene connection networks and signaling pathways. Using the GEPIA Proportional Hazard Model and the Kaplan-Meier estimator, we widened our analysis to identify the important genes that may play a role in both progression and the survival of patients with GBM. Totally, 890 DEGs, including 475 and 415 up- and down-regulated were identified, respectively. Our results revealed *SQLE*, *DHCR7*, *delta-1 phospholipase C* (*PLCD1*), and *MINPP1* genes are high expression, and the *Enolase 2* (*ENO2*) and *hexokinase-1* (*HK1*) genes are low expressions. Hence, our findings suggest novel mechanisms that affect the occurrence of GBM development, growth, and/or establishment and may also serve as secretory biomarkers for GBM prognosis and possible targets for therapy.

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Abstract

Glioblastoma (GBM) is a malignant brain tumor that frequently occurs alongside other central nervous systems (CNS) conditions. Glutamate release and aberrant cellular behavior are shared features of both CNS diseases and GBM cells. Neither their origin nor the ways in which CNS disorders affect the development or behavior of GBM are well understood. Using data from the Gene Expression Omnibus (GEO) and the Cancer Genome Atlas (TCGA) datasets–where both healthy and cancerous samples were analyzed–we used a quantitative analytical framework to identify differentially expressed genes (DEGs) and cell signaling pathways that might be related to GBM. Then, we performed gene ontology studies and hub protein identifications to estimate the roles of these DEGs after finding disease-gene connection networks and signaling pathways. Using the GEPIA Proportional Hazard Model and the Kaplan-Meier estimator, we widened our analysis to identify the important genes that may play a role in both progression and the survival of patients with GBM. Totally, 890 DEGs, including 475 and 415 up- and down-regulated were identified, respectively. Our results revealed *SQLE* , *DHCR7* ,*delta-1 phospholipase C* (*PLCD1*), and *MINPP1* genes are high expression, and the *Enolase 2* (*ENO2*) and*hexokinase-1* (*HK1*) genes are low expressions. Hence, our findings suggest novel mechanisms that affect the occurrence of GBM development, growth, and/or establishment and may also serve as secretory biomarkers for GBM prognosis and possible targets for therapy.

Keywords : Glioblastoma, Cancer Stem Cells, Biomarker, Gene Expression Profiles, Bioinformatics Analysis

Abbreviations

GBM, Glioblastoma; CSCs, cancer stem cells; ECM, extracellular matrix; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BPs, biological processes; MFs, molecular functions; CCs, cellular components; MAPK, Mitogen-activated protein kinase; ATP, adenosine triphosphate; ITM2A, integral membrane protein 2A; FLRT3, fibronectin leucine-rich transmembrane protein 3; NOG, noggin; TAMs, tumor-associated macrophages; ARS2, arsenite-resistant protein-2; MAGL, monoacylglycerol lipase; PGE2, prostaglandin E2; EMT, epithelial mesenchymal transition; GSCs, glioma stem cells; MTT, molecular target therapy; PGBs, potential glioma biomarkers; *HDAC3, histone deacetylase 3* ; *ALDH1A3, Aldehyde dehydrogenase 1A3* ; USP9X, ubiquitin-9-specific protease; *PLCD1, delta-1 phospholipase C;ENO2, Enolase 2; HK1* , *hexokinase-1* ; DHCR7, 7-dehydrocholesterol reductase; ESCC, esophageal squamous cell carcinoma; 2,3-BPG, 2,3-bisphosphoglycerate; HCC, hepatocellular carcinoma; HBV, hepatitis B virus;

1. Introduction

Glioblastoma (GBM) is the most common and, unfortunately, most malignant brain tumor, which is more common in men [1]. There is no clear boundary between the tumor and the brain tissue, and the tumor cells penetrate the normal brain tissue over long distances, so complementary therapies are often used to control the growth and spread of this cell [2]. These complementary therapies, as mentioned, include radiation therapy and chemotherapy [2]. Meanwhile, cancer stem cells (CSCs) play a significant role in resistance to treatment and can even cause the recurrence of high-intensity disease and secondary tumors [3, 4]. Therefore, finding signal pathways and proteins, especially secretory biomarkers, can be a new key to treating the GBM microenvironment [4]. Although CSCs make up about one percent of the total tumor cell population, they could lead to the recurrence of the disease and/or the formation of secondary tumors [5-7].

Over the past decade, bioinformatics knowledge has been instrumental in finding biomarkers from the genome to the proteome in various cancers [8-10]. In this study, using continuous and integrated bioinformatics analyses, we investigated the gene expression profile of GBM CSCs and isolated specific pathways and proteins that participated in the secretion, extracellular matrix (ECM), and microenvironment of GBM.

2. Methods and Materials

2.1. GBM stem cell gene expression profile datasets

In this study, the GSE146698 database from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo) was selected. This dataset contains six samples that included two groups of three members in the form of GBM tissue-derived stem cells and a control cell. The platform used in this dataset was GPL17077 Agilent-039494 SurePrint G3 Human GE v2 8x60K Microarray 039381.

2.2. Preparation of gene expression profile data for additional analysis

The GSE146698 dataset was isolated using GEO2R analysis, then genes with $P<0.05$, logFC <-1, and logFC> 1 were isolated, and then genes with high- and low-expression were classified. Finally, it was prepared for other analyses (Figure-1).

2.3. Determined signaling pathways and the gene ontology (GO)

From the GSE146698 dataset, genes subject to differential expression were analyzed separately with the Enrichr database (https://amp.pharm.mssm.edu/Enrichr), and with the help of Kyoto Encyclopedia of Genes and Genomes (KEGG), the related signal pathways and its genes were selected. In addition, by using the Enrichr and PANTHER databases (http://www.pantherdb.org/geneListAnalysis.do), biological processes (BPs), molecular functions (MFs), and cellular components (CCs) were evaluated. In this study, genes with high expression were more important due to selecting appropriate biomarkers, especially in CCs. The next step evaluated ten genes with the highest and lowest expressions separately. Also, $P<0.05$ was considered to study the signal pathway and GOs.

2.4. Investigation of the relationship between proteins

Selected genes from the signal and GO pathways were uploaded to the network analyst and section STRINGS database (https://string-db.org), and a network of linkages between proteins was obtained. To better display the data, we used Cytoscape software (version 3.7.1).

2.5. Evaluation of gene candidates in clinical data

After selecting the most important communication proteins in this section, we entered them in the GEPIA database and evaluated them with clinical data on gene expression and survival (Figure-2).

2.6. Ethical Consideration

This study was approved by the Research Ethics Committees of the Neuroscience Institute (approval code: IR.TUMS.NI.REC.1401.023).

3. Results

3.1. Apoptosis, microenvironments pathways, ECM receptor, and Mitogen-activated protein kinase (MAPK) pathway

Analysis of the GSE146698 gene expression profile showed that 1250 high-expression and 1030 low-expression genes could play a role in the critical pathways of GBM progression. Apoptosis, insulin receptor, P53 signaling, microenvironments signaling, ferroptosis, RAP1, MAPK, HIF1, TNF, focal adhesion, and prostaglandin pathways showed as a significant molecular mechanism (Figure-3 and Table-1).

3.2. GO Analysis

In the BPs of cell cycle pathways, processing of cellular processes, positive self-regulation in microenvironments pathways, regulation of biosynthetic processes and intracellular transitions, MFs of phosphates, freezled junctions, integrin junctions, endothelial vascular growth factor, and cytokine activity was present. The same study was performed for low-expression genes that regulate phosphorus metabolism, regulate cell motility, respond to cellular stress, and organic molecules. Organization of cellular organs and modification of macromolecule structures were involved in BPs. NADH dehydrogenase and adenosine triphosphate (ATP) activities were involved in MFs (Figure-4).

3.3. Protein network analysis

We examined the genes with high- and low-expression at this stage that were involved in critical microenvironment pathways. Accordingly, 129 nodes and 236 edges are formed in the protein network with increased expression, and 67 nodes and 175 edges in the protein network with low expression (Figure-5).

3.4. Evaluation of candidate genes in clinical data related to GBM CSCs

In this section, genes and protein products related to the activity of GBM CSCs were evaluated. Accordingly, *integral membrane protein 2A* (*ITM2A*), *fibronectin leucine-rich transmembrane protein 3* (*FLRT3*), noggin (*NOG*), and *SEMA3D* genes increased expression. Interestingly, all of these genes throughout approximately the same period (about 20 months), showed a significant reduction in survival that reaches below

4. Discussion

GBM has been a significant concern for physicians and neurosurgeons. Despite heavy and high-dose radiation therapy and chemotherapy, which eventually remove the tumor tissue with a complicated surgery, recurrence occurs [8]. One of the leading causes of the recurrence is cancer cells related to GBM tumor tissue, which usually remain in the patient's body during the surgical procedure. These cells can become resistant to chemotherapy and radiation therapy [9]. Due to the two main characteristics of stem cells, namely differentiation and self-regeneration, a small population of GBM CSCs could be able to form large tumors, and the origin of all these tumor cells in GBM CSCs [10]. Also, therapies previously performed on the patient do not have the initial effectiveness, and the combinations of chemotherapy drugs or the type of radiation therapy should be changed [10, 11]. Also, a person cannot get GBM themselves and risk other cancers [12-14]; hence, it is essential to find biomarkers associated with GBM CSCs. Interaction between GBM CSCs and tumor-associated macrophages (TAMs) promotes the development of GBM; however, the exact molecular mechanisms between these two cell types are unclear [13-16]. Here, Yin *et al* . [17] showed that arsenite-resistant protein-2 (ARS2), a zinc finger protein essential for early mammalian growth, plays a vital role in maintaining GBM CSCs and M2-like TAM polarity. Also, ARS2 directly activates MGLL–a novel transcription target gene that encodes monoacylglycerol lipase (MAGL)– to regulate the self-regeneration and tumorigenesis of GBM CSCs through the production of prostaglandin E2 (PGE2) as well as CSCs β-catenin activation and TAM polarity, which stimulates the pseudo M2 [17]. Also, Yin *et al* . [17] identified M2-like signature genes by which the MAGL-specific inhibitor, JZL184, significantly increased survival in the mouse xenograft transplant model by blocking PGE2 production. Overall, the results of Yin *et al* . [17] study revealed that blocking the association between GBM CSCs and TAMs by targeting ARS2 / MAGL signaling offers a potential new treatment option for GBM patients [14]. Multivariate analyses of global expression profiles are valid indicators of the prognosis of various diseases, including brain cancer. To identify candidates for prognostic markers of GBM, Takashima *et al* . [18] performed multivariate analyses on the status

of epithelial (EPI)-mesenchymal (MES) transition (EMT), glioma (GLI) stem cells (GSCs), molecular target therapy (MTT), and potential glioma biomarkers (PGBs) using patients' clinical information and expression data. Random proportional hazard regression analysis and Cox test showed significant variable values for *DSG3* , *CLDN1* ,*CDH11* , *FN1* , *histone deacetylase 3*(*HDAC3*)/*PTEN* , *L1CAM* , *OLIG2* , *TIMP4* ,*IGFBP2* , and *GFAP* [18]. The analyses also included prognostic prediction formulas that could distinguish between the survival curves of GBM patients [18]. In addition to the genes listed, *HDAC1* , *FLT1* , *EGFR* , *MGMT* , *PGF* ,*STAT3* , *SIRT1* , and *GADD45A* form complex genetic communication networks [18]. Also, survival curve analysis indicated that MES^{high} , $MES^{high}GLI^{low}$, $GSC^{high}GLI^{low}$, $MES^{high}MTT^{low}$, and PGB^{high}showed poor prognosis, while MES^{middle} ,GSC^{low} , and PGB^{low}were related to good prognosis [18]. These results suggest that EMT and GSC evaluation make it possible to predict the prognosis of GBM, which contributes to the development of new therapies and new marker candidates for the prognosis of GBM [15, 18]. The mesenchymal subtype of GBM CSCs represents a subset of cancer cells that are made due to their highly aggressive nature and resistance to conventional therapies [19]. *Aldehyde dehydrogenase 1A3* (*ALDH1A3*) has recently been suggested as a critical determinant for maintaining the characteristics of mesenchymal GBM CSCs [19]. However, the general mechanisms of *ALDH1A3* ectopic expression remain unknown. Chen *et al* . [19] identified the ubiquitin-9-specific protease (USP9X) as a deubiquitinase with *ALDH1A3* in mesenchymal GBM CSCs.*USP9X* interacted with *ALDH1A3* and polyubicoetilate, and stabilized. In addition, they showed that FACS-classified USP9Xhi cells were enriched for GBM CSCs with high *ALDH1A3* activity and tumorigenic solid capacity [19]. Decreased USP9X significantly reduced *ALDH1A3* , leading to loss of autoimmune capacity and tumorigenesis of GBM CSCs, which could be highly associated with*ALDH1A3* expression [19]. In addition, it suggested that the*USP9X* *WP1130* inhibitor degraded *ALDH1A3* and showed significant therapeutic efficacy in orthopedic xenograft transplant models derived from GBM CSCs [19]. Evidence indicated that*USP9X* was strongly correlated with *ALDH1A3* expression in human GBM prototypes and had a high prognostic significance for patients with the mesenchymal subtype [19]. Hence, these findings suggest*USP9X* as a key deubiquitinase for ALDH1A3 protein stabilization and a potential target for GBM CSCs-based therapy [19]. Despite all these studies, the study of microenvironment pathways that are the energy source of GBM CSCs activity has been underestimated. Our study focused on bioinformatics, selecting important biomarkers related to GBM CSCs secretory. We found that the *SQLE* , 7-dehydrocholesterol reductase (DHCR7), *delta-1 phospholipase C* (*PLCD1*), and*MINPP1* genes are high expression, and the *Enolase 2*(*ENO2*) and *hexokinase-1* (*HK1*) genes are low expressions. The DHCR7 could reduce the double bonding of C7-C8-cholesta-5,7-diane-3-beta-ol (7-dehydrocholesterol/7-DHC) and cholesta-5,7,24-trine-3-beta-ol–two intermediates in the pathway of cholesterol production– via the cholesterol biosynthetic pathway [20, 21]. This gene has not been identified directly in GBM CSCs but has been studied in some cancers. Vitamin D deficiency has been reported to be associated with cancer risk [21]. *DHCR7* and *CYP2R1*are vital components of vitamin D metabolizing enzymes [21]. Therefore, researchers are investigating the association of vitamin D with the polymorphisms of the *DHCR7* and *CYP2R1* genes and cancer susceptibility. Nevertheless, the results are contradictory. Wen*et al* . conducted an integrated study on the association of the*DHCR7* and *CYP2R1* SNPs with cancer susceptibility [21]. They calculated the association of each SNP with cancer risk by using odds ratios. Twelve designed case-control studies covering 23,780 cases, and 27,307 controls were finally identified in the meta-analysis of five SNPs (DHCR7 rs12785878, rs1790349 SNP, CYP2R1 rs1074116579, SYP2R1 rs1074116579, and rs1074126574) [21]. Also, they indicated that the DHCR7 rs12785878 SNP was significantly associated with cancer risk in the entire target population [21]. DHCR7 rs1790349 SNP was analyzed to increase the risk of cancer in Caucasians [21]. In addition, the CYP2R1 rs12794714-A allele was associated with a lower risk of colon cancer, and SNPs rs12785878, rs1790349, and rs12794714 might be potential biomarkers for cancer susceptibility [21].

The second messenger molecules, diacylglycerol and inositol 1,4,5-triphosphate, are activated by phosphatidylcholine-specific phospholipase C enzymes for the growth of trophoblasts and the placenta. The use of laser spectroscopy has increased dramatically in the last few decades, and applications are now being found in experimental and theoretical development in areas such as biomolecular research and medical practice. To evaluate the feasibility of a random instrument to mark the effect of *PLCD1* gene therapy on breast cancer, the properties of random lasers in two groups of human breast xenograft transplant tissues were in-

vestigated [22, 23]. Both coherent and incoherent random laser regimes were observed in the AdHu5-EGFP and AdHu5-PLCD1 groups, respectively [23]. Intrinsic disruption of breast tumor tissues produces more light scattering randomly distributed in the tumor specimen, leading to random coherent laser propagation [23]. Hematoxylin and eosin staining confirmed that the spatial arrangement of breast tumor cells is more disordered than their organized structure in the tumor tissues receiving PLCD1 treatment [23]. The *DLC1* , a tumor suppressor gene regulated in many types of cancer by genetic and non-genetic mechanisms, encodes a protein whose RhoGAP activities and scaffolding contribute to tumor suppressor functions [24]. Besides binding to Caveolin-1, the role of the DLC1 START domain (STAR-associated fat transfer; DLC1-START) is unknown [24]. Other START domains have a critical function that involves binding lipids, but the hypothetical lipid ligand for DLC1-START is unknown [24]. Sanchez-Solana *et al* . [24] identified PS as a lipid ligand for DLC1-START and showed that DLC1-START binds to the PLCD1 protein in addition to Caveolin-1.PS binding contributes to DLC1 interactions with Caveolin-1 and PLCD1 [24]. The importance of these activities for tumorigenesis was identified in seven cancer-associated DLC1-START mutations, each of that reduced tumor suppressive function [24]. He *et al* . [25] found that *PLCD1* expression in esophageal squamous cell carcinoma (ESCC) cells was significantly reduced compared to normal esophageal epithelial cells. In addition, positive regulation of PLCD1 reduced the capacity of TE-1 and EC18 cells to proliferate, invade, and migrate [25]. It was found that*PLCD1* activity was negatively correlated with the expression of -catenin, C-myc, cyclin D1, MMP9, and MMP7 [25]. Finally, PLCD1 activity was confirmed to inhibit ESCC proliferation in vivo [25].

MINPP1 acts as a 5-phosphoinositide and 5-phosphoinositide 6-phosphatase and regulates the cellular levels of inositol pentose phosphate and inositol hexachord phosphate [26]. It also acts as a 2,3-bisphosphoglycerate 3-phosphatase, mediated by the dephosphorylation of 2,3-bisphosphoglycerate (2,3-BPG) to produce phospho-D-glycerate without the formation of 3-phosphoglycerate [26]. Chen *et al* . [27] found that a gene, *MINPP1* , via the glycolytic bypass microenvironments pathway, had an essential biological function in hepatocellular carcinoma (HCC). Also, *MINPP1* is regulated in HCC and could prevent tumor cells from proliferating and migrating [27]. Meanwhile, miRNA-30b-5p is a stimulus for tumor cell proliferation via a glycolytic bypass in HCC [27]. More importantly, miRNA-30b-5p could significantly reduce *MINPP1* expression [27]. Microenvironment tests showed that the miRNA-30b-5p/MINPP1 axis could accelerate the conversion of glucose to lactate and 2,3-BPG. In HCC cells, miRNA-30b-5p and MINPP1 cannot regulate glycolytic bypass to promote tumorigenesis [27]. However, when the hepatitis B virus (HBV) entered these cells, miRNA-30b-5p and *MINPP1* significantly amplified, increased tumor cell migration, and promoted glycolytic bypass. Indeed, it showed that HBV infection increases miRNA-30b-5p expression through the interaction of HBV P protein with FOXO3 [27]. Bioinformatics analysis of a large cohort dataset showed that high *MINPP1* expression was associated with optimal survival of hepatitis C virus-positive patients with HCC, leading to slower disease progression [27].

The HK1 catalyzes the phosphorylation of hexoses such as D-glucose, D-glucosamine, D-fructose, D-mannose, and 2-deoxy-D-glucose to hexose 6-phosphate (e.g., D-glucose 6-phosphate and D-glucosamine 6-phosphate) [28]. Also, it plays a role in innate immunity and inflammation by acting as a pattern-recognition receptor for bacterial peptidoglycans [28]. When released into the cytosol, the bacterial peptidoglycan N-acetyl-D-glucosamine component inhibits HK1 activity, causing it to detach from the outer mitochondrial membrane and thereby activating NLRP3 inflammation [29, 30]. Zheng *et al* . [31] showed that *HK1* expression was expressed by HK1-cDNA and inhibited by si-HK1 in DLD1 and HCT8. Ectopic expression of *HK1* increased cell migration and invasion in colorectal cancer cells and increased lung metastasis in rat models [31]. In addition, HK1-induced migration and invasion of colorectal cancer cells activate Snail2 expression, stimulating epithelial-mesenchymal transmission and cancer progression [31]. Tumors often show abnormal energy metabolism that relies heavily on glycolysis to produce ATP, known as the Warburg effect or aerobic glycolysis [32]. Therefore, clarifying these energy changes in malignant tumors is essential for more effective cancer treatments. Tseng *et al* . [32] investigated the effects of the first and rate-limiting quenching of the glycolytic HK isoenzymes HK1 and HK2 on tumor progression [32]. There was an inverse correlation between*HK1* and *HK2* expressions in human cancer cells [32]. Indeed, only *HK1* caused an epithelial-

mesenchymal phenotypic change in cervical cancer cells, which accelerated tumor growth and metastasis in both in vitro and in vivo assays—remarkably, quenching HK1 impaired aerobic respiration and increased glycolysis while did not affect ATP production [32]. These microenvironment changes were associated with higher HK2 and lactate dehydrogenase-1 expression but lower citrate synthase levels [32]. In particular, the failure of HK1 caused aberrant energy metabolism, which was almost related to the overexpression of *HK2* [32]. In addition, HK1-quenched cells showed strong glucose-dependent growth and inhibition of 2-deoxyglucose (2-DG)-induced cell proliferation [32]. These results indicate that silencing HK1, but not HK2, alters energy metabolism and induces an EMT phenotype, which increases tumor malignancy but increases the sensitivity of cancer cells to the inhibition of 2-DG [32]. In addition, it also suggests that glycolytic inhibitors should only be used to treat cancers with high glycolytic activity [33]. Li*et al* . found that *HK1* expression was increased in ovarian cancer tissues and associated with clinical features patients [33]. Survival curve analysis showed that patients with ovarian cancer had poor survival in the *HK1* overexpression group [33]. In addition, univariate and multivariate analyses indicated that HK1 might be an independent biomarker for the poor prognosis of patients with ovarian cancer [33]. Indeed, HK1 reduces glucose uptake, lactic acid production, ATP production, invasion, and migration via MAPK/ERK signaling [33].

ENO2 has neurotrophic and neuroprotective properties on various neurons in the central nervous system [34]. It binds to cultured neocortical neurons in a calcium-dependent manner, increasing cell survival [34]. ENO2 levels dramatically increase in cardiovascular attacks, brain trauma, brain tumors, and Creutzfeldt-Jakob disease [34]. Studies have shown that ENO2 is regulated in various tumor tissues, including cervical cancer, esophageal cancer, renal cancer, leukemia, melanoma, pancreatic cancer, and sarcoma [34, 35]. In addition, ENO2 overexpression was negatively correlated with the overall survival of lung cancer patients. Subsequently, the predictive potential of ENO2 was much more pronounced in patients with lung adenocarcinoma [35]. ENO2 is a key glycolytic enzyme in the microenvironment's process of glycolysis. Zheng *et al* . observed a significant overexpression of*ENO2* in pancreatic tissues, and its expression was associated with metastasis as well as poor prognosis in patients with pancreatic cancer [36]. K394 was identified as a considerable acetylation site in ENO2 that regulates enzymatic activity, cellular metabolism, and progression [36]. Also, via destroying ENO2, tumor growth and liver metastasis could inhibit [36]. In contrast to mutant K394 mimetic acetylation, wild-type *ENO2* expression could reduce tumor malignancy [36]. Most HDAC3 and factors associated with P300 and CBP were ENO2 deacetylase and acetyltransferase, respectively [36]. They also showed that HDAC3-mediated deacetylation leads to the activation of ENO2 and increased glycolysis [36]. Significantly, insulin-like growth factor-1 decreases K394 acetylation, stimulates ENO2 activity in a dose- and time-dependent manner, and facilitates the PI3K/AKT/mTOR pathway of HDAC3 phosphorylation in S424—which reduced K394 steel and activated ENO2 [36]. In addition, Zheng *et al* . [36] revealed a new mechanism by which acetylation negatively regulates ENO2 activity in pancreatic cancer metastasis by modulating glycolysis. Hence, insulin-like growth factor-1-induced ENO2 deacetylation blockade can be a promising strategy to prevent the development of pancreatic cancer.

In the current study, via the GEPIA database (dependent on the TCGA clinical database), we found that *ITM2A* , *FLRT3* ,*NOG* , and *SEMA3D* expressions significantly increased. All genes are relatively directly related to the mortality rate of patients with GBM and reach less than 10% in about 20 months, which calls for their high importance for laboratory and clinical evaluations. These genes are still unclear in GBM CSCs and need further clarification.

6. Disclosure

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Conflict of Interest:

The authors have no conflict of interest.

Ethics Statement:

- This study was approved by the Research Ethics Committees of the Neuroscience Institute, Brain and Spinal Cord Injury Research Center (approval code: IR.TUMS.NI.REC.1401.023).

- Informed Consent: N/A

- Registry and the Registration No. of the study/trial: N/A

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Author Contribution:

Conceptualisation: M.H., S.M.G., E.J. Data curation: H.A.T., A.G., M.H., S.F. Formal Analysis: E.J., A.B. Funding Acquisition: Not applicable. Investigation: E.J., A.B., S.M.G. Methodology: All. Project Administration: J.H., M.R., A.C. Supervision: M.H. Validation: All. Writing original draft: E.J., H.A.T., A.G., M.H., S.F., M.T. Writing review & editing: All.

Data availability statement

The analysis code and datasets used here are available from the corresponding author on request.

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Figures legends

Figure 1 : Schematic pathway of how to perform bioinformatics analysis

Figure 2 : General information on the expression profile of the GSE146698 dataset gene associated with GBM CSCs. Accordingly, the Vulcano diagram (**A**) shows the gene expression differentiation between the two groups. Also, the PCA diagram (**B**) indicates that the density and placement of the samples of each group next to each other are good similarities between the data, which are a good option for analysis. **C** : The box diagram of the samples of each group.**D** : Degree of differentiation of gene expression in each group.Figure 3: Evaluation of signal pathways marked for low- (**A**) and high-experesion (**B**) genes.

Figure 4 : The communication network between biological processes in GBM CSCs with high- and low-expressions was plotted using the shiny GO database. Accordingly, in this network, the size of the circles and the color intensity between them show more significance.**A** : upregulated genes, **B** : downregulated genes.**C** and **E** : upregulated biological processes and molecular functions. **D** and **F** : downregulated biological processes and molecular functions.

Figure 3 : Evaluation of signal pathways marked for low- (A) and high-experesion (B) genes

Figure 5 : Protein network between high- (**A**) and low-expression (**B**) genes. Phosphatidylinositol pathways, cholesterol biosynthesis, carbon cycle metabolism in cancer, and glycolysis have been identified.

Figure 6 : Expression and survival curves in candidate genes with high and low expression are identified, related to *SEMA3D*(**A**), *NOG* (**B**), *FLRT3* (**C**), and*ITM2A* (**D**) genes.

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