Original Article

Molecular pathways in glioblastoma-derived stem cells to identify effective drug agents: A bioinformatics study

Tahereh Mirzaei[1], Seyed Amir Sheikholeslami[2], Ahmad Bereimipour[3,4], Arsalan Jalili[3], Alireza Zali[5], Sheida Sharbati[6], Vahid Kaveh[7], Sina Salari[8]

[1]Nargund College of Pharmacy, Rajiv Gandhi University of Health Sciences, Bengaluru, Karnataka, India, [2]Department of Hematology and Oncology, Imam Hossein Hospital, [5]Functional Neurosurgery Research Center, Shohada Tajrish Comprehensive Neurosurgical Center of Excellence, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [6]Department of Pharmaceutics, School of Pharmacy, [8]Department of Medical Oncology, Hematology, Taleghani Hospital, Shahid Beheshti University of Medical Sciences, [3]Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran, [4]Faculty of Sciences and Advanced Technologies in Biology, University of Science and Culture, [7]Department of Medical Oncology, Hematology, Iran University of Medical Sciences, Tehran, Iran

Abstract

Background and Aim: Glioblastoma multiform (GBM) is considered as one of the malignant brain tumors that affect a wide range of people every year. Cancer stem cells, as essential factors, are resistant to chemotherapy drugs and complicate treatments. Therefore, finding critical molecular pathways in GBM-derived stem cells, and selecting the appropriate drug agents can prove more effective treatment approaches for GBM. **Method:** In this study, using RNA-Seq data, we performed continuous bioinformatics analyses and examined the up-and down-regulated genes from GBM-derived stem cells samples. Afterward, we separated the signaling pathways using the KEGG database and measured the protein interactions with the STRING database. Then, using the Drug matrix database, we nominated drugs that could affect these genes. **Results:** The first 20 pathways on tumorigenesis and 41 up-regulated and 73 down-regulated genes were selected. These genes were most active in the pathways involved in cell division, metabolism, cytoskeleton, cell adhesion molecules, and extracellular space. We then examined the candidate genes and the approach of the drugs that target these genes. Chlorambucil, cyclosporine A, doxorubicin, and etoposide were selected as the drug agents. **Conclusion:** Using integrated bioinformatics analyses, it was found that prominent genes in the cell cycle and cytoskeletal pathways are more expressed in cancer stem cells and that Chlorambucil, cyclosporine A, doxorubicin, and etoposide can be effective compounds to attenuate these cells.

Keywords: Cancer stem cells, chemo resistance, drug compounds, glioblastoma, RNA- seq analysis

Address for correspondence: Dr. Sina Salari,
Department of Medical Oncology, Hematology, Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
E-mail: s.salari@sbmu.ac.ir
Dr. Vahid Kaveh,
Department of Medical Oncology, Hematology, Iran University of Medical Sciences, Tehran, Iran.
E-mail: kaveh.v@iums.ac.ir

Received: 17-07-2021 **Revised:** 12-10-2021
Accepted: 14-01-2022 **Published:** 30-06-2022

Access this article online

Quick Response Code:

Website: www.jfmpc.com

DOI: 10.4103/jfmpc.jfmpc_1436_21

Introduction

Glioblastoma multiform (GBM) is a malignant brain tumor that affects many people every year. Despite the new treatment options, its mortality is high. Moreover, due to the late diagnosis of asymptomatic patients in the early phase, cancer advances to an advanced stage, and as a result, treatment is difficult or even ineffective.[1,2] Another challenge is the blood-brain barrier (BBB) that makes it difficult for the medication to pass through. In

How to cite this article: Mirzaei T, Sheikholeslami SA, Bereimipour A, Jalili A, Zali A, Sharbati S, *et al*. Molecular pathways in glioblastoma-derived stem cells to identify effective drug agents: A bioinformatics study. J Family Med Prim Care 2022;11:2856-64.

addition, tumor cells have shown resistance to drug compounds during several chemotherapy sessions, and one of the main factors for this is the stem cells derived from cancerous tissues.[3]

Cancer stem cells constitute 1% of the total population of cancer cells. Despite chemotherapy, these cells can survive due to their small size and form new tumor cells, which due to their differentiation and automotive properties, are more resistant to drugs.[4,5] In one study, it was found that temozolomide was ineffective against GBM-derived stem cells.[2] Therefore, new strategies in the study of signaling pathways and molecules involved in cancer stem cells may help in identifying more suitable drug compounds to overcome cancer stem cells.

In the past few years, bioinformatics has proven to be very effective in identifying essential elements in cell physiology and their pathways that play a beneficial role in predicting molecular functions and the nature of genes and protein products.[6-8] Therefore, this study aimed to investigate the pathways of cancer stem cells derived from GBM patients. Finally, our attempt was to select the required genes in the development and progression of GBM and to determine the effective drug agents in this regard.

Methods and Materials

Selection of databases

In this study, we selected the RNA-Seq dataset (GSE92459) from the SRA database. This dataset contained 21 samples from stem cells derived from GBM patients and several cells as a control sample.

Classifying the data and performing bioinformatics analyses

In this step, we uploaded the GSE92459 to the Biojupies database and then separated the signaling pathways from the wiki pathway database and the gene ontology from the Enrichr database. In this section, all routes were classified according to the *P* value <0.05.

Investigation of protein association

In this section, we isolated the genes that were closely related to tumorigenesis and the progression of GBM, loaded them into the STRING database; and finally, isolated the protein network of the upregulated genes.

Choosing effective medications

Following bioinformatics analysis, we entered the critical genes, both in terms of ontology genes and the relationship between their proteins, in the Drug matrix database and then listed the drugs associated with cancer pathways along with their dosage.

Examining and plotting essential genes in the pathway of various cancers

In this section, essential genes associated with cancer were isolated and uploaded to the GEPIA database for testing with other common cancers and GBM. Also, a Kaplan Meyer box plot diagram was drawn to show the survival rate.

Results

Tumor-dependent neuronal migration, axon guidance, and cell cycle phase-dependent pathways were significantly expressed.

After bioinformatics analysis, mitotic prometaphase, resolution of sister chromatid cohesion, cell cycle, mitotic, gastric cancer network 1, cell cycle, M phase, and mitotic anaphase pathways showed up-regulation. The neuronal system, GABA synthesis, release, reuptake and degradation, axon guidance, neurotransmitter release cycle, and extracellular matrix organization showed down-regulation when GBM-derived stem cells were compared to normal cells [Table 1, Figure 1].

Investigation of gene ontology

We evaluated three general approaches to molecular functions, biological processes, and cellular components after examining the gene expression profile. Accordingly, in terms of molecular procedures and biological processes, transcriptional activator activity, RNA polymerase II transcription regulatory region sequence-specific binding (GO: 0001228), spindle (GO: 0005819), RNA polymerase II regulatory region sequence-specific DNA binding (GO: 0000977), mitotic sister chromatid segregation (GO: 0000070), antigen processing and presentation of exogenous peptide antigen via MHC class II (GO: 0019886), microtubule cytoskeleton (GO: 0015630), and chromosome, centromeric region (GO: 0000775) showed up-regulation, while nervous

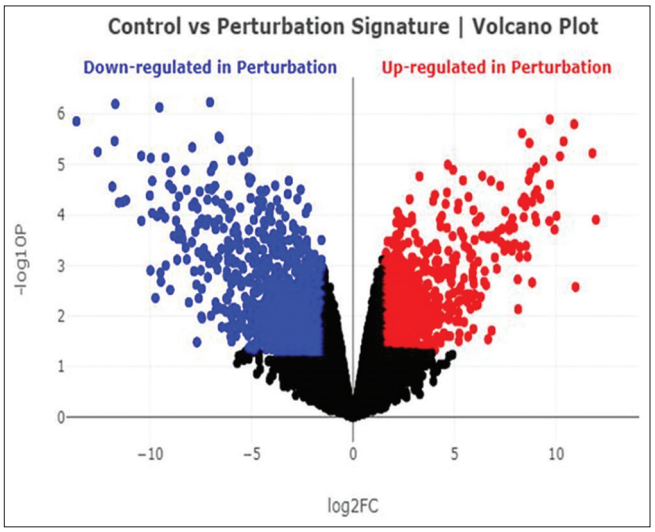

Figure 1: Volcano Plot. The figure contains an interactive scatter plot that displays the log2-fold changes and statistical significance of each gene calculated by performing a differential gene expression analysis. Every point in the plot represents a gene. Red points indicate significantly up-regulated genes; blue points indicate down-regulated genes

Table 1: Top 10 up/downregulated signaling pathways

Pathways	*P*	Genes
Upregulated pathways		
Mitotic Prometaphase	1.60E-10	ZWILCH, PLK1, CDCA8, SMC4, CENPA, NCAPH, SKA1, CDC20, CCNB2, CENPE, CCNB1, KIF18A, CENPF, BIRC5, KIF2C, CENPN, BUB1, MAD2L1
Resolution of Sister Chromatid Cohesion	3.07E-09	ZWILCH, PLK1, CDCA8, CENPA, SKA1, CDC20, CCNB2, CENPE, CCNB1, KIF18A, CENPF, BIRC5, KIF2C, CENPN, BUB1, MAD2L1
Cell Cycle, Mitotic	2.21E-08	TOP2A, ZWILCH, CDCA8, FOXM1, SMC4, CENPA, NCAPH, SKA1, AURKA, CDC20, CCNB2, CCNB1, PTTG1, NEK2, MYBL2, BUB1, NEK9, CDKN2C, BORA, UBE2C, PLK1, KIF23, CDC25C, CCNA2, CENPE, TPX2, CENPF, KIF18A, DBF4, BIRC5, CENPN, KIF2C, KIF20A, MAD2L1
Gastric Cancer Network 1	2.28E-08	TOP2A, TPX2, CENPF, UBE2C, MYBL2, ECT2, E2F7, AURKA, KIF15
Polo-like kinase mediated events	5.51E-08	CCNB2, CENPF, CCNB1, PLK1, MYBL2, FOXM1, CDC25C
Cell Cycle	1.12E-07	TOP2A, ZWILCH, CDCA8, FOXM1, SMC4, CENPA, NCAPH, SKA1, AURKA, CDC20, CCNB2, CCNB1, TERT, PTTG1, MYBL2, NEK2, NBN, BUB1, NEK9, CDKN2C, BORA, UBE2C, PLK1, KIF23, CDC25C, CCNA2, CENPE, TPX2, CENPF, KIF18A, DBF4, DKC1, BIRC5, CENPN, KIF2C, KIF20A, MAD2L1
RHO GTPases Activate Formins	1.63E-07	ZWILCH, PLK1, CDCA8, CENPA, SKA1, CDC20, CENPE, KIF18A, CENPF, DIAPH 3, BIRC5, KIF2C, CENPN, BUB1, MAD2L1
M Phase	3.05E-07	NEK9, ZWILCH, UBE2C, PLK1, CDCA8, KIF23, SMC4, CENPA, NCAPH, SKA1, CDC20, CENPE, CCNB2, CENPF, CCNB1, KIF18A, PTTG1, BIRC5, KIF2C, CENPN, KIF20A, BUB1, MAD2L1
MHC class II antigen presentation	1.76E-06	SEC23A, SEC24A, KIF23, KIF15, CENPE, KIF18A, RACGAP1, KIF4A, KIF2C, KIF20A, OSBPL1A, SEC24D, SEC31A
Activation of HOX genes during differentiation	2.16E-06	EGR2, HOXA3, HOXB4, HOXA2, HOXB3, HOXC4, HOXA1, PAX6, HOXB2, HOXD4, HOXD3, HOXA4
Downregulated pathways		
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	2.81E-07	CACNB2, GJA1, CACNB3, TCF7L1, CACNB4, ITGB5, CDH2, JUP, CACNA2D1, CACNA1C, SLC8A1, ITGA9
Transmission across Chemical Synapses	3.75E-07	GABRB3, GRIA1, BCHE, SNAP25, SLC32A1, KCNJ12, CACNA2D1, GABRA4, GAD1, CHRNA7, CACFD1, RASGRF2, GAD2, SLC6A1, SYN2, GRIP2, GRIN2D, CACNB2, CACNB3, CACNB4
Neuronal System	2.29E-06	GABRB3, GRIA1, BCHE, SNAP25, KCND1, KCNH5, SLC32A1, KCNJ12, CACNA2D1, GABRA4, GAD1, CHRNA7, CACFD1, RASGRF2, GAD2, KCNAB2, SLC6A1, SYN2, GRIP2, GRIN2D, CACNB2, CACNB3, CACNB4
Phase 1 - inactivation of fast Na+channels	3.37E-06	CACNB2, CACNB3, KCND1, CACNB4, CACNA2D1, KCNIP4, CACNA1C
Depolarization of the Presynaptic Terminal Triggers the Opening of Calcium Channels	1.04E-05	CACNB2, CACNB3, CACNB4, CACFD1, CACNA2D1
NCAM1 interactions	3.14E-05	CACNA1I, CACNB2, CACNB3, CACNB4, ST8SIA2, GFRA1, CACNA1C
Nicotine addiction	5.34E-05	GABRB3, GRIA1, SLC32A1, CHRNA7, GABRA4,
GABA synthesis, release, reuptake and degradation	8.33E-05	SNAP25, SLC32A1, GAD1, GAD2, SLC6A1
Mecp2 and Associated Rett Syndrome	0.000177379	GRIA1, RBFOX1, DLX5, GAD1, OPRK1, FGF3, CDON
Phase 2 - plateau phase	0.000407637	CACNB2, CACNB3, CACNB4, CACNA2D1, CACNA1C

system development (GO: 0007399), chemical synaptic transmission (GO: 0007268), calcium ion transport into the cytosol (GO: 0060402), anterograde trans-synaptic signaling (GO: 0098916), neuron projection morphogenesis (GO: 0048812), amyloid-beta binding (GO: 0001540), dendrite (GO: 0030425), and Wnt-activated receptor activity (GO: 0042813) demonstrated down-regulation. The presence of protein products of genes in different cell locations can also be seen in [Figure 2].

Correlation between protein networks

We examined 41 up-regulated genes that showed a more critical role and formed 54 nodes and 73 edges in the protein network. This network showed acceptable communication in cell adhesion molecules, TNF, and P53 signaling pathways [Figure 3].

Evaluation of drugs that can be effective in treatments of GBM

We isolated important genes that play a significant role in the main processes of tumorigenesis and GBM progression. Azathiprine (20 mg/kg), doxorubicin (3 mg/kg), cyclosporin A (350 mg/kg), chlorambucil (0.6 mg/kg), etoposide (100 mg/kg), leflunomide (30 mg/kg), thioguanine (12 mg/kg), cyclophosphamide (25 mg/kg), daunorubicin (3.25 mg/kg), and clobetasol propionate (17 mg/kg) were the top 10 drugs with high significance *P* value [Table 2].

Discussion

In this study, the main purpose was to find essential signaling pathways in GBM-derived stem cells to select effective

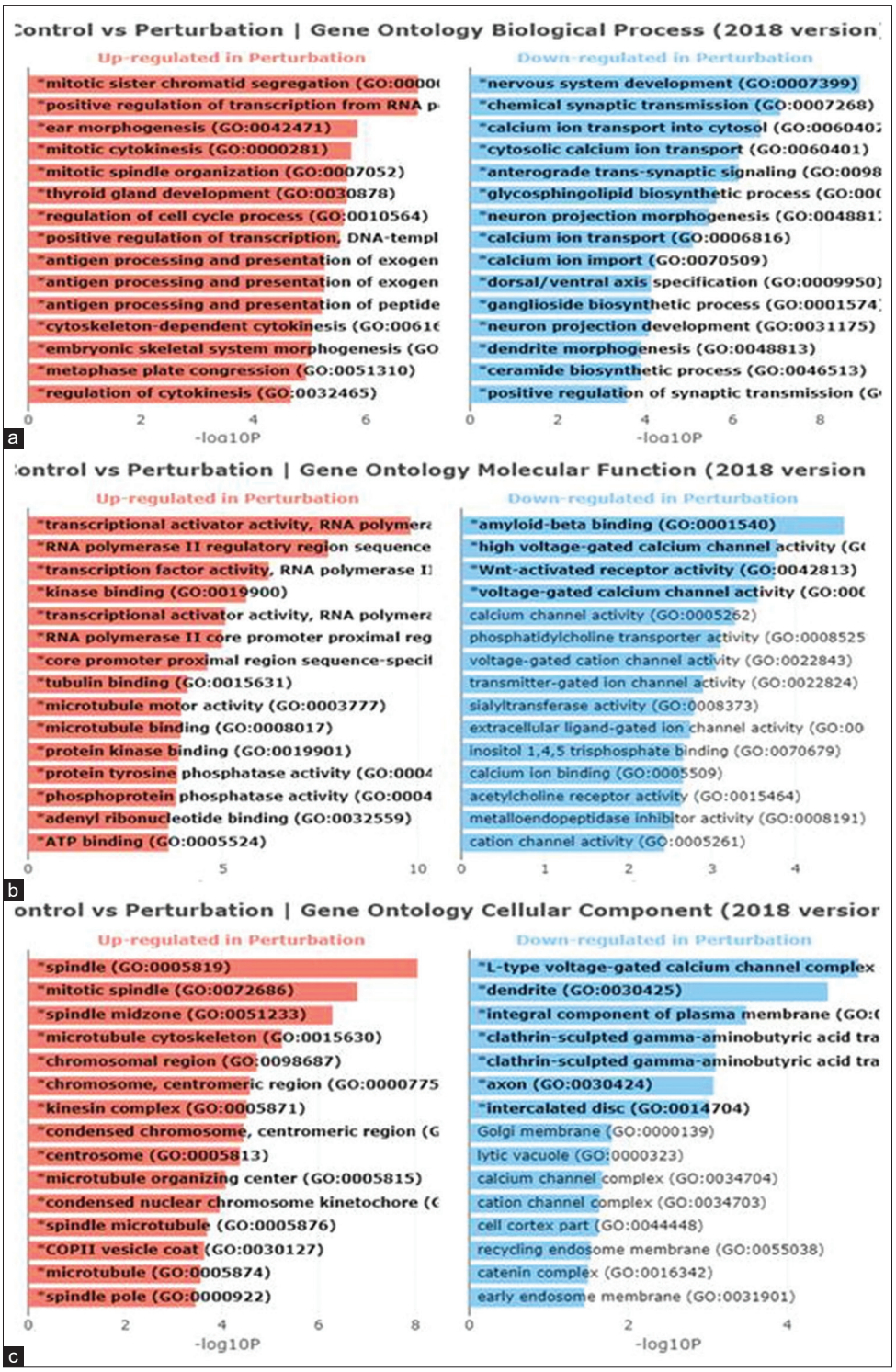

Figure 2: Gene ontology enrichment analysis results. The figure contains interactive bar charts displaying the results of the gene ontology enrichment analysis generated using Enrichr. The x-axis indicates the -log10 (*P*-value) for each term. Significant terms are highlighted in bold. Additional information about enrichment results is available by hovering over each bar. a: Biological processes; b: Molecular functions; c: Cellular component

drugs by finding important genes and molecules in these pathways.

For the past decade, researchers have been interested in studying cancer stem cells that are necessary for the treatment of a wide range of cancers. Studies on brain cancers have shown that cancer stem cells have a high potential for tumorigenesis under hypoxia.[9] Safari *et al.*[10] demonstrated that GBM-derived stem cells have a high expression of O6-methylguanine methyltransferase, which plays a vital role in chemotherapy resistance. In addition, Warrier *et al.*[11] showed that GBM-derived stem cells have a significant expression for ABC family genes highly resistant to chemotherapy.

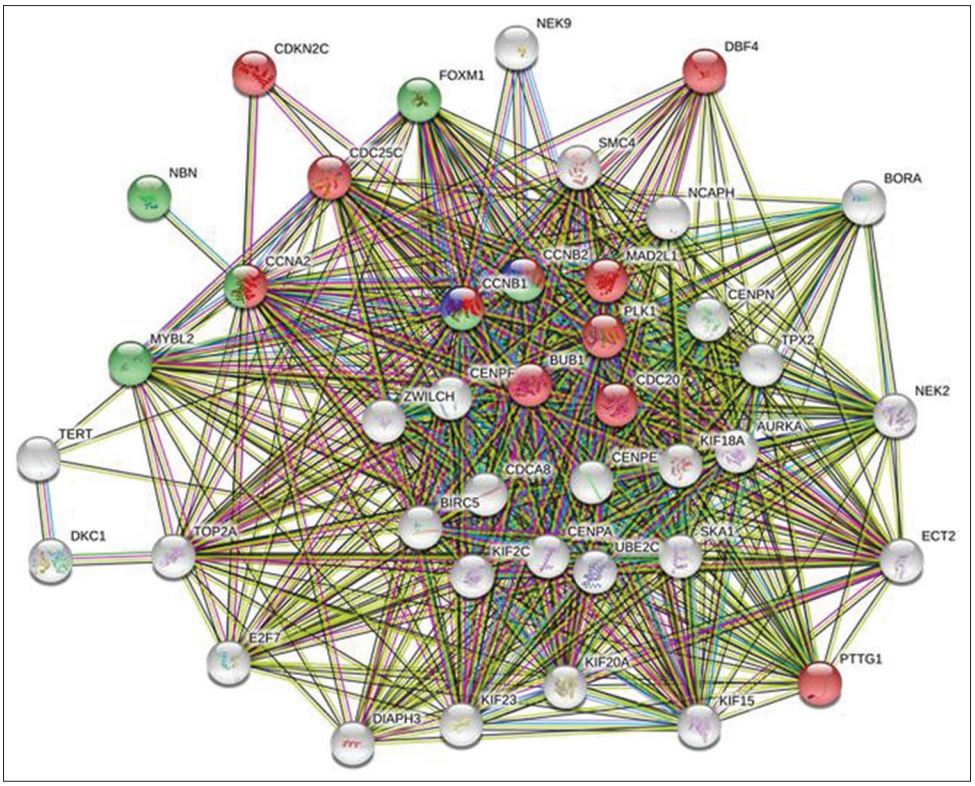

Figure 3: Correlations between protein products of up-regulated genes. Cell cycle (red), cellular senescence (green), and P53 (blue) signaling pathway

Table 2: Candidate drugs associated with up-regulated genes

Drugs and dosage	*P*	Genes
Azathioprine (20 mg/kg)	1.01E-06	CCNA2;CENPE; CDKN2C; CDCA8;MYBL2;FOXM1;KIF15
Doxorubicin (3 mg/kg)	2.17E-05	CCNA2;CDKN2C; DKC1;CDCA8;MYBL2;KIF15
Cyclosporin A (350 mg/kg)	2.35E-05	CCNA2;NEK9;CDKN2C; CDCA8;MYBL2;KIF15
Chlorambucil (0.6 mg/kg)	2.40E-05	CCNA2;CENPE; CDKN2C; CDCA8;MYBL2;KIF15
Etoposide (100 mg/kg)	2.85E-05	CCNA2;CENPE; CDKN2C; CDCA8;MYBL2;KIF15
Leflunomide (30 mg/kg)	3.37E-05	CCNA2;CENPE; CDCA8;MYBL2;FOXM1;KIF15
Thioguanine (12 mg/kg)	4.11E-05	CCNA2;CENPE; CDKN2C; CDCA8;MYBL2;KIF15
Cyclophosphamide (25 mg/kg)	4.34E-05	CCNA2;CDKN2C; DKC1;CDCA8;MYBL2;KIF15
Daunorubicin (3.25 mg/kg)	1.96E-04	CCNA2;CDKN2C; CDCA8;MYBL2;KIF15
Clobetasol propionate (17 mg/kg)	2.00E-04	CCNA2;CDKN2C; CDCA8;MYBL2;KIF15

Limited drugs have been used to address the GBM-derived stem cells that have not been very successful, including temozolomide, carboplatin, and 1,3-bis (2-chloroethyl)-1-nitroso-urea BCNU.[12]

In the current study, we isolated the most relevant and significant genes with high differential expression to find more accurate pathways of GBM-derived stem cells by bioinformatics analysis on RNA-Seq data.

CCNA2 is an essential gene in cell division that plays a significant role in the transition of G1/S to G2/M. A microarray data study showed that TAF7/CCNA2 could play a high pathogenic role in GBM and there is a close relationship between them.[13] Another survey found that miR-219 could control the CCNA2 gene and play a significant role in inhibiting cancer cells' growth [Figure 4a].[14]

CDKN2C is another candidate gene in this study, which was part of the INK4 family and is associated with CDK4/6, and acts by inhibiting CDK activity on cell division in the G1 phase. Various studies were performed on this gene, the high expression of which has been noticed and proven in the sample of GBM compared to normal brain tissue.[15] In the GBM xenograft model, CDKN2C expression was significantly higher than in the control group, playing a pathogenic role in GBM.[16] Previous studies on the GBM cell line using microarray analysis showed that 25 genes could be sensitive to chemotherapy drugs, including CDKN2C.[17,18] Evidence demonstrated that CDKN2C is not directly involved in the development of tumor cells but may be included in the event of cancer by affecting cyclin D.[19] Mutations in CDKN2C can also be involved in the development of cancer cells [Figure 4b].[20]

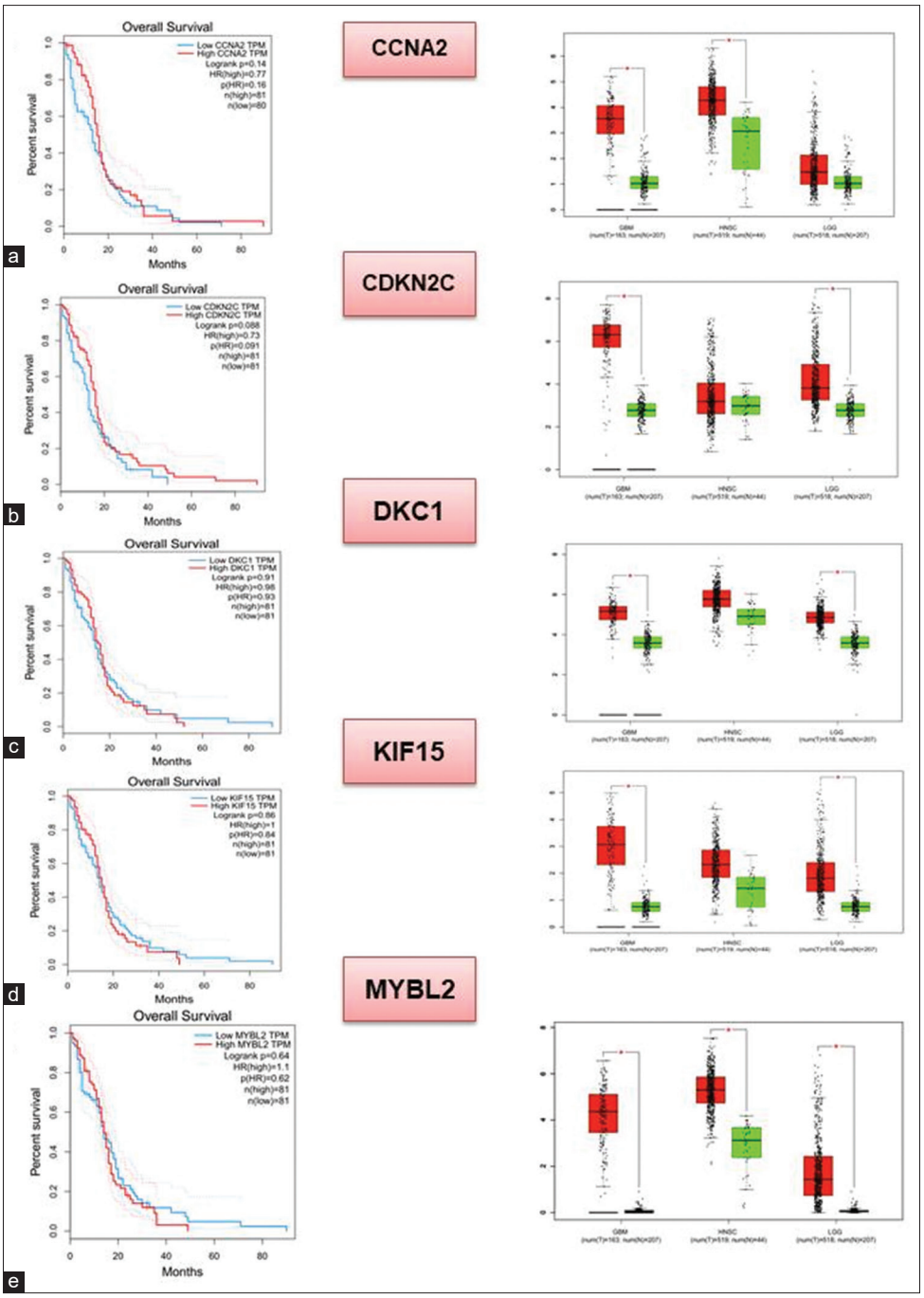

Figure 4: The expression of candidate genes in GBM has been compared by the GEPIA database (right) and the survival plot of these genes is also shown on the left. a: CCNA2; b: CDKN2C; c: DKC1; d: KIF15; e: MYBL2

DKC1 is one of the most important genes involved in telomerase, which plays a significant role in apoptosis and cellular aging. These genes have very high expression in GBM, the product of which can protect telomerase and cause cancer cell death. In a study using viral vectors on GBM, it was found that DKC1 -4.5 LogFC decreased expression and positively affected the apoptosis of cancer cells.[21] Miao *et al.*[22] showed increased abnormal expression, increased angiogenesis, division, and migration of cadherin N, HIF-1, and MMP2-mediated cancer cells. DKC1 expression has been reported in several other cancers. High expression of this gene is hazardous in lung cancer cells.[23] Also, by affecting the HIF-1 gene, it played a role in increasing the angiogenesis of colorectal cancer and its invasion to other tissues.[24] In addition, studies have been developed on the progression of prostate cancer [Figure 4c].[25]

The molecular dynamics of the cytoskeleton' also play an essential role in stimulating the activity of cancer cells to grow and proliferate, as well as in their invasion. KIF15 was one of the critical items in this study that was identified. Wang *et al.*[26]

showed that the product of this gene in GBM cells could increase cell division in the G1 phase, in which inhibition or extinction can also reduce tumorigenesis. PBK is a member of the MAPK family and is a mitogen activator. In GBM, PBK can increase cell proliferation by interacting with KIF15.[27] Also, Terribas *et al.*[28] indicated that KIF11/15/25 has a high expression in neural sheath tumors and plays a vital role in the survival of these cells. Moreover, KIF15 plays a crucial role in increasing cell division and tumorigenesis of cancer cells by acting on the MEK/ERK pathway [Figure 4d].[29]

Another significant gene identified in this study was MYBL2, which is effective as a nuclear transcription factor in cell cycle regulation. An in-silico study of GBM data showed that several genes, including MYBL2, play a significant role in cancer survival and division.[30] Zhang *et al.*[31] have found that by increasing MYBL2 expression the miR- 30e increases and the growth and invasion of glioblastoma cells are decreased. A clinical trial on GBM patients showed that MYBL2 is downstream of the AKT/FOXM1 pathway genes involved in cell division and apoptosis inhibition. When AKT inhibitors are active, and FOXM1 is silent, MYBL2 expression decreases and eventually results in cell cycle inhibition and apoptosis induction, indicating that the AKT, FOXM1, and MYBL2 are related to each other.[32] Another in-silico study by gene network analysis showed that FOXM1 and MYBL2 play a vital role in cancer cell growth and proliferation.[33] In gastric adenocarcinoma, it was found that high expression of MYBL2 causes cancer cells to differentiate and invade lymph nodes, the inhibition of which can be a binding antitumor effect [Figure 4e].[34]

The next step is to identify drug agents that are able to inhibit or reduce the expression of mentioned genes that have an influential role in attenuating cancer stem cells. Chlorambucil is used as a chemotherapy drug, which is mainly used for leukemia cancers. In a study of 297 patients, the effects of chlorambucil and almethosumab were evaluated, with 55% and 43% of cure rates for leukemia, respectively.[35] Hu *et al.*[36] studied chlorambucil with drug delivery and tissue engineering approach, which showed that when chlorambucil is combined with 1, 6-Hexanediamine hydrochloride (HDH) micelles, it has a high permeability into the cancerous tissue and physiological barriers and could have an acceptable therapeutic effect. Millard *et al.*[37] showed that chlorambucil could specifically affect the energy production pathways in mitochondria and increase the death of pancreatic and breast cancer cells by more than 80% by acting on mtDNA. Luo *et al.*[38] revealed that chlorambucil could increase the path of oxidative stress and be used as a viable treatment option for breast cancer.

Cyclosporine A is considered an immunosuppressive drug, and also plays a significant role in various cancers. A study of the C6 GBM cell line found that cyclophilin A can develop drug resistance in tumor cells. Cyclosporine A with sanglifehrin combined with cisplatin can reduce the expression of cyclophilin A and increase the apoptosis and the reactive oxygen species pathway.[39] A study on the T98G GBM cell line found that cyclosporine A with an effect on the morphine tolerance pathway could affect the NO/ERK pathway and ultimately play an inhibitory role in GBM cell division.[40] Sliwa *et al.*[41] demonstrated a new route of GBM with the use of cyclosporine A. Microglia in brain tissue could contribute to higher cell proliferation and tumorigenesis by activating the PI3K/AKT pathway in conditions where the tumor tends to invade the brain. Cyclosporine A with effect on this pathway and inhibiting microglia activity prevents the invasion and progression of the disease. Cyclosporine A induces apoptosis in gastric cancer by inhibiting the NF-KB pathway by Dastaxel.[42] In breast cancer, cyclosporine A reduces the drug resistance in this cancer by decreasing the expression of ABCG2.[43]

Doxorubicin is another chemotherapy drug that can induce apoptosis with high potency. Most studies of these drugs are used in tissue engineering and pass through the BBB in the form of micelles or nanoparticles of this drug, which can have an acceptable effect in reducing tumorigenesis.[44-47] It has also been used in other cancers, such as lung cancer.[48]

Etoposide is a very successful drug in chemotherapy. In a study of a mouse model, researchers showed that low doses of etoposide effectively induced apoptosis.[49] For this drug to have a better effect on brain tumors, the approach of drug delivery with lipid particles and nanoparticles has been a good option so far.[50-52]

Conclusion

In conclusion, it can be argued that the use of appropriate drug regimens for GBM can be more effective in destroying cancer stem cells, especially in the GBM, and that etoposide, doxorubicin, cyclosporine A, and chlorambucil can be used and have good synergistic effectiveness.

Acknowledgements

The authors would like to thank Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran for providing the possibility of doing the study and helpful assistance.

Notes on Contributors

T.M. A.J., A.B., S.A.S.E., conception and design, acquisition of data, or analysis and interpretation of data; S.S., V.K., A.Z., drafting the article or revising it critically for important intellectual content. All authors revised final approval of the version to be submitted for publication

Financial support and sponsorship

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

Conflicts of interest

There are no conflicts of interest.

References

1. Hosseini MM, Karimi A, Behroozaghdam M, Javidi MA, Ghiasvand S, Bereimipour A, *et al.* Cytotoxic and apoptogenic effects of cyanidin-3-glucoside on the glioblastoma cell line. World Neurosurg 2017;108:94-100.
2. Shergalis A, Bankhead A, Luesakul U, Muangsin N, Neamati N, Campus N, *et al.* Current challenges and opportunities in treating. Pharmacol Rev 2018;70:412-45.
3. Ghosh D, Nandi S, Bhattacharjee S. Combination therapy to checkmate Glioblastoma : Clinical challenges and advances. Clin Transl Med 2018;7:33.
4. Altaner C. Glioblastoma and stem cells. Neoplasma 2008;55:369-74.
5. Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, *et al.* Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. Mol Cancer 2006;5:67.
6. Wu D, Rice CM, Wang X. Cancer bioinformatics : A new approach to systems clinical medicine. BMC Bioinformatics 2012;13:71.
7. Nalini V, Segu R, Deepa PR, Khetan V, Vasudevan M, Krishnakumar S. Bioinformatics and biology insights molecular insights on post-chemotherapy retinoblastoma by microarray gene expression analysis. Bioinform Biol Insights 2013;7:289-306.
8. Beta M, Vasudevan M, Vetrivel U, Khetan V. Bioinformatics and biology insights identification and insilico analysis of retinoblastoma serum microRNA profile and gene targets towards prediction of novel serum biomarkers. Bioinform Biol Insights 2013;7:21-34.
9. Crowder SW, Balikov DA, Hwang Y-S, Sung H-J. Cancer stem cells under hypoxia as a chemoresistance factor in the breast and brain. Curr Pathobiol Rep 2014;2:33-40.
10. Safari M, Khoshnevisan A. Cancer stem cells and chemoresistance in glioblastoma multiform : A review article. Stem Cells 2015;10:271-85.
11. Warrier S, Pavanram P, Raina D, Arvind M. Study of chemoresistant CD133+cancer stem cells from human glioblastoma cell line U138MG using multiple assays. Cell Biol Int 2012;36:1137-43.
12. Nakai E, Park K, Yawata T, Chihara T, Kumazawa A, Nakabayashi H, *et al.* Enhanced MDR1 expression and chemoresistance of cancer stem cells derived cellular and molecular biology enhanced MDR1 expression and chemoresistance of cancer stem cells derived from glioblastoma. Cancer Invest 2009;27:901-8.
13. Yang L, Zeng W, Sun H, Huang F, Yang C, Cai X, *et al.* Bioinformatical analysis of gene expression omnibus database associates TAF7/CCNB1, TAF7/CCNA2, and GTF2E2/CDC20 pathways with glioblastoma development and prognosis. World Neurosurg 2020;138:e492-514.
14. Ma Q. MiR-219-5p suppresses cell proliferation and cell cycle progression in esophageal squamous cell carcinoma by targeting. Cell Mol Biol Lett 2019;24:4.
15. Scrideli CA, Carlotti CG Jr, Okamoto OK, Andrade VS, Cortez MAA, Motta FJN, *et al.* Gene expression profile analysis of primary glioblastomas and non-neoplastic brain tissue : Identification of potential target genes by oligonucleotide microarray and real-time quantitative PCR. J Neurooncol 2008;88:281-91.
16. Cen L, Carlson BL, Schroeder MA, Ostrem JL, Kitange GJ, Mladek AC, *et al.* p16-Cdk4-Rb axis controls sensitivity to a cyclin-dependent kinase inhibitor PD0332991 in glioblastoma xenograft cells. Neuro Oncol 2012;14:870-81.
17. Zhao Z, Liu Y, He H, Chen X, Chen J, Lu Y. Candidate genes influencing sensitivity and resistance of human glioblastoma to Semustine. Brain Res Bull 2011;86:189-94.
18. de Tayrac M, Etcheverry A, Aubry M, Saïkali S, Hamlat A, Quillien V, *et al.* Integrative genome-wide analysis reveals a robust genomic glioblastoma signature associated with copy number driving changes in gene expression. Genes Chromosomes Cancer 2009;48:55-68.
19. Iolascon A, Giordani L, Moretti A, Basso G, Borriello A, Ragione FD. Analysis of $CDKN_2A$, $CDKN_2B$, $CDKN_2C$, and cyclin ds gene status in hepatoblastoma. Hepatology 1998;27:989-95.
20. Gluick T, Yuan Z, Libutti SK, Marx SJ. Mutations in CDKN2C (p18) and CDKN2D (p19) may cause sporadic parathyroid adenoma. Endocr Relat Cancer 2013;20:L27-9.
21. Ng SSM, Gao Y, Chau DHW, Li GHY, Lai LH, Huang PT, *et al.* A novel glioblastoma cancer gene therapy using AAV-mediated long-term expression of human TERT C-terminal polypeptide. Cancer Gene Ther 2007;14:561-72.
22. Miao F, Chu K, Chen H, Zhang M, Shi P, Bai J, *et al.* Proliferation, migration and invasion Increased DKC1 expression in glioma and its significance in tumor cell proliferation, migration and invasion. Invest New Drugs 2019;37:1177-86.
23. Penzo M, Ludovini V, Treré D, Siggillino A, Bellezza G, Crinò L, *et al.* Dyskerin and TERC expression may condition survival in lung cancer patients. Oncotarget 2015;6:21755-60.
24. Hou P, Shi P, Jiang T, Yin H, Chu S, Shi M, *et al.* DKC1 enhances angiogenesis by promoting HIF-1 α transcription and facilitates metastasis in colorectal cancer. Br J Cancer 2020;122:668-79.
25. Sieron P, Hader C, Hatina J, Engers R, Wlazlinski A, Müller M, *et al.* DKC1 overexpression associated with prostate cancer progression. Br J Cancer 2009;101:1410-6.
26. Wang Q, Han BIN, Huang WU, Qi C, Liu F. Identification of KIF15 as a potential therapeutic target and prognostic factor for glioma. Oncol Rep 2020;43:1035-44.
27. Stangeland B. PBK/TOPK as a potential therapeutic target in glioblastoma and other malignancies. Mol Biol 2015;4:151.
28. Terribas E, Fernández M, Mazuelas H, Fernández-rodríguez J, Biayna J, Blanco I, *et al.* KIF11 and KIF15 mitotic kinesins are potential therapeutic vulnerabilities for malignant peripheral nerve sheath tumors. Neurooncol Adv 2020;2(Suppl 1):i62-74.
29. Wang J, Guo X, Xie C, Jiang J. KIF15 promotes pancreatic cancer proliferation via the MEK – ERK signalling pathway. Br J Cancer 2017;117:245-55.
30. Jiang L, Zhong M, Chen T, Zhu X, Yang H, Lv K. Gene regulation network analysis reveals core genes associated with survival in glioblastoma multiforme. J Cell Mol Med 2020;24:10075-87.
31. Zhang K, Fu G, Pan G, Li C, Shen L, Hu R, *et al.* Demethylzeylasteral inhibits glioma growth by regulating the miR-30e-5p/MYBL2 axis. Cell Death Dis 2018;9:1035.
32. Zhang X, Lv Q, Huang Y, Zhang L, Zhou H. Akt/FoxM1 signaling pathway-mediated upregulation of MYBL2 promotes progression of human glioma. J Exp Clin Cancer Res 2017;36:105.

33. Ahmed F. Integrated network analysis reveals FOXM1 and MYBL2 as key regulators of cell proliferation in non-small cell lung cancer. Front Oncol 2019;9:1011.
34. Jia Y, Gao Y, Li J, Chang Z, Yan J, Qin Y. Prognostic implications of MYBL2 in resected Chinese gastric adenocarcinoma patients. Onco Targets Ther 2019;12:1129-35.
35. Hillmen P, Skotnicki AB, Robak T, Jaksic B, Dmoszynska A, Wu J, *et al.* Alemtuzumab compared with chlorambucil as first-line therapy for chronic lymphocytic leukemia. J Clin Oncol 2007;25:5616-23.
36. Hu X, Liu R, Zhang D, Zhang J, Li Z, Luan Y. Rational design of an amphiphilic chlorambucil prodrug realizing self-assembled micelles for efficient anticancer therapy. ACS Biomater Sci Eng 2018;4:973-80.
37. Millard M, Gallagher JD, Olenyuk BZ, Neamati N. A selective mitochondrial-targeted chlorambucil with remarkable cytotoxicity in breast and pancreatic cancers. J Med Chem 2013;56:9170-9.
38. Luo C, Zhou Y, Zhou T, Xing L, Cui F, Sun M, *et al.* Reactive oxygen species-responsive nanoprodrug with quinone methides-mediated GSH depletion for improved chlorambucil breast cancers therapy. J Control Release 2018;274:56-68.
39. Han X, Yoon SH, Ding YAN, Choi TAEGYU, Choi WONJ, Kim YUNH, *et al.* Cyclosporin A and sanglifehrin A enhance chemotherapeutic effect of cisplatin in C6 glioma cells. Oncol Rep 2010;23:1053-62.
40. Rashki A, Mumtaz F, Jazayeri F, Shadboorestan A, Esmaeili J, Mehr SE, *et al.* Cyclosporin A attenuating morphine tolerance through inhibiting NO/ERK signaling pathway in human glioblastoma cell line: The involvement of calcineurin. EXCLI J 2018;17:1137-51.
41. Sliwa M, Markovic D, Gabrusiewicz K, Synowitz M, Glass R, Zawadzka M, *et al.* The invasion promoting effect of microglia on glioblastoma cells is inhibited by cyclosporin A. Brain 2007;130:476-89.
42. Nakahara C, Nakamura K, Yamanaka N, Baba E, Wada M, Matsunaga H, *et al.* Cyclosporin-A enhances docetaxel-induced apoptosis through inhibition of nuclear factor-kappaB activation in human gastric carcinoma cells. Clin Cancer Res 2003;9:5409-16.
43. Gupta A, Dai Y, Vethanayagam RR, Hebert ME, Thummel KE, Unadkat JD, *et al.* Cyclosporin A, tacrolimus and sirolimus are potent inhibitors of the human breast cancer resistance protein (ABCG2) and reverse resistance to mitoxantrone and topotecan. Cancer Chemother Pharmacol 2006;58:374-83.
44. Koukourakis MI, Koukouraki S, Fezoulidis I, Kelekis N, Kyrias G, Archimandritis S, *et al.* High intratumoural accumulation of stealth liposomal doxorubicin (Caelyx) in glioblastomas and in metastatic brain tumours. Br J Cancer 2000;83:1281-6.
45. Chang M, Yang C-S, Huang D-M. Aptamer-conjugated DNA icosahedral nanoparticles as a carrier of doxorubicin for cancer therapy. ACS Nano 2011;5:6156-63.
46. Steiniger SC, Kreuter J, Khalansky AS, Skidan IN, Bobruskin AI, Smirnova ZS, *et al.* Chemotherapy of glioblastoma in rats using doxorubicin-loaded nanoparticles. Int J Cancer 2004;109:759-67.
47. Wohlfart S, Khalansky AS, Gelperina S, Maksimenko O, Bernreuther C, Glatzel M, *et al.* Efficient chemotherapy of rat glioblastoma using doxorubicin-loaded PLGA nanoparticles with different stabilizers. PLoS One 2011;6:e19121.
48. Hong Y, Che S, Hui B, Yang Y, Wang X, Zhang X. Lung cancer therapy using doxorubicin and curcumin combination : Targeted prodrug based, pH sensitive nanomedicine. Biomed Pharmacother 2019;112:108614.
49. Cheema TA, Kanai R, Kim GW, Wakimoto H, Passer B, Rabkin SD, *et al.* Enhanced antitumor efficacy of low-dose etoposide with oncolytic herpes simplex virus in human glioblastoma stem cell xenografts. Clin Cancer Res 2011;17:7383-93.
50. Das CM, Aguilera D, Vasquez H, Prasad P, Zhang M, Wolff JE, *et al.* Valproic acid induces p21 and topoisomerase-II (α/β) expression and synergistically enhances etoposide cytotoxicity in human glioblastoma cell lines. J Neurooncol 2007;85:159-70.
51. Jiang H, Pei L, Liu N, Li J, Li Z, Zhang S. Etoposide-loaded nanostructured lipid carriers for gastric cancer therapy cancer therapy. Drug Deliv 2016;23:1379-82.
52. Kuo Y, Chen Y. Targeting delivery of etoposide to inhibit the growth of human glioblastoma multiforme using lactoferrin- and folic acid-grafted poly (lactide- co -glycolide) nanoparticles. Int J Pharm 2015;479:138-49.