

Identification of Novel Cancer Stem Cell Markers in Glioblastoma by Comparing Tumor Cells with Stem-cell-like Cell Lines

G. Divya¹; D. Macrin^{2*}

¹Research Scholar, Department of Bioinformatics, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India.

^{2*}Project Guide, Department of Bioinformatics, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India.

^{2*}danniem.sse@saveetha.com

Abstract

Aim. The aim of this study is to identify differential gene expression for glioblastoma tumor cells, normoxic and hypoxic glioblastoma stem-like cell lines. Finding the upregulated and downregulated gene and pathway interactions. Analysis to find the differential expression genes and pathway interactions. **Materials and methods.** The gene expression profiling data from the microarray dataset GSE45117 from the Gene Expression Omnibus (GEO) database, as well as differentially expressed genes (DEGs) between the 2 categories, are used in this analysis. 4 Samples of Glioblastoma tumors were considered as group 1 and 4 samples of normoxic and Hypoxic glioblastoma stem-like cell lines were considered as group 2 in the GEO2R web tool that has been used to screen them. **Results.** The gene-gene interactions among the DEGs and the GGI network with 37 nodes and 13 edges. The stem-cell-like cell lines showed lower expression of endothelin-related genes such as EDN3 and EDNRA along with dysregulation of enzymes such as PDK1, PGK1 which points to dysregulation of cellular respiratory pathways. This effect in consensus with under expression of cell attachment genes such as COL2A1, COL5A2, COL15A1 denotes a strong shift toward metastasis. **Conclusion.** Thus, a computational pipeline for identifying the significant genes and pathways involved in the glioblastoma tumors and glioblastoma stem-like cell lines. This study provides a path towards discovering potential leads for the treatment of glioblastoma and aids in comprehending the underlying novel molecular mechanisms.

Key-words: Glioblastoma Cancer, Cancer Stem Cells, Cultured Cell Lines, Gene Expression, Gene Enrichment, Novel Molecular Markers, Molecular Biology, Genetic Analysis, Gene Expression.

1. Introduction

Glioblastoma cancer is the most common disease caused in the human brain (Ohgaki and Kleihues 2013). Cancer refers to neoplastic cells that appear to be immune and have a large clonogenic potential are called cancer stem cells. Glioblastoma stem-like cells can be successfully propagated in the media after being isolated from freshly resected human GBM (Bar et al. 2010). In this study, we'll aim for genes that influence cells and whether there's a way to treat them so that they don't progress as rapidly. We'll then analyze the pathway interactions in the cause of genes (Yi et al. 2016). In today's world, there are still no curative therapeutic options for glioblastoma, and the survival rate of patients who are diagnosed is very low. Identification of proteins and pathways involved in chemotherapeutic resistance detection using genomic and proteomic analysis (Shergalis et al. 2018). To determine differences in expression levels of glioblastoma cancer cells and normoxic stem-like cell lines. To identify the genetic markers for tumor differentiation. A study on biological processes which have been significantly altered of glioblastoma.

The metabolic linkage between the pentose phosphate pathway and glycolysis has also been found in glioblastoma stem-like cells (Kathagen et al. 2013). Cancer cells use glycolysis energy for rapid cell division. The research shows the FLK-1 provides a major role in the formation of VM in GBM. This study has shed light on modulating tumor aggressiveness and has also provided a basis for future research. Treatment (Francescone et al. 2012). The clinical role of SOX2 and new findings that may provide novel clinical applications for SOX2 as a prognostic marker, an indicator of metastasis, biomarker, or potential therapeutic target in some cancer types (Weina and Utikal 2014). Glioblastoma genes activated by Zeb1 are predicted to be tumor migration and invasion mediators, including the guanine nucleotide exchange factor Prex1, whose signaling pathway indicates shorter glioblastoma patient survival. (Rosmaninho et al. 2018).

Previously our team has a rich experience in working on various research projects across multiple disciplines (Sathish and Karthick 2020; Varghese, Ramesh, and Veeraiyan 2019; S.R. Samuel, Acharya, and Rao 2020; Venu, Raju, and Subramani 2019; M.S. Samuel et al. 2019; Venu, Subramani, and Raju 2019; Mehta et al. 2019; Sharma et al. 2019; Malli Sureshbabu et al. 2019; Krishnaswamy et al. 2020; Muthukrishnan et al. 2020; Gheena and Ezhilarasan 2019; Vignesh et al. 2019; Ke et al. 2019; Vijayakumar Jain et al. 2019; Jose, Ajitha, and Subbaiyan 2020). Now the growing trend in this area motivated us to pursue this project.

The Lacunae in this current study is that microarray data is available but no analysis is performed. Microarray gene expression data for glioblastoma tumor cells and stem-like cells are available for gene interactions. To identify differential gene expression for glioblastoma tumor cells and stem-like cells to find out upregulated downregulated gene interactions among them. Understand Functional Enrichment among genes of glioblastoma tumor and glioblastoma stem-like cells. They are obtained from microarray-based on the glioblastoma stem-like cells line which is used to find the differentially expressed genes. The samples are collected based on the tumor cells and tumor stem-like cells line (Normoxic and Hypoxic in 48 hrs) to identify differentially expressed genes. The methods used in bioinformatics to find out the identifying gene, and regulatory pathways associated networks in GBM disease. It provides the idea for explaining the molecular pathways underlying GBM and identifying diagnostic and therapeutic targets. In this study, we first assembled a list of genes linked to GBM from the GEO dataset (ID GSE45117). Then we use the Gene- Gene interactions (STRING), Funrich, cytohubba, and Cluego tools to perform bioinformatics analysis on these genes. This study aims to find out whether genes are expressed differently in glioblastoma tumor cells, normoxic and hypoxic glioblastoma stem-like cell lines. Identifying the interactions involving upregulated and downregulated genes and pathways. Identifying differential expression and pathway interactions.

2. Materials and Methods

2.1. Dataset

Table 1- The sample groups in GSE45117 were procured from the Gene Omnibus Expression database

S. No	No. of samples	Accession	Title	Source name	Cell type
1	4	GSM109744	Tumor 10	Glioblastoma Tumor 10	Glioblastoma Tumor
		GSM109744	Tumor 11	Glioblastoma Tumor 11	Glioblastoma Tumor
		GSM109744	Tumor 12	Glioblastoma Tumor 12	Glioblastoma Tumor
		GSM109744	Tumor 13	Glioblastoma Tumor 13	Glioblastoma Tumor
2	4	GSM109745	Normoxic Glioblastoma 10 Stem-like cell line	Normoxic Glioblastoma Stem-like cell line derived from tumor 10	Normoxic Glioblastoma Stem-like cell line derived from tumor 10
3	4	GSM109745	Normoxic Glioblastoma 11 Stem-like cell line,	Normoxic Glioblastoma Stem-like cell line derived from tumor 11,	Normoxic Glioblastoma Stem-like cell line derived from tumor 11

GEO – Gene expression omnibus is the freely distributed microarray and high throughput functional genomic data. The array Data and gene expression microarray array analysis dataset of GSE45117 were taken from the GEO database (Table. 1). GSE45117 contains 2 samples of (Glioblastoma tumor 4, normoxic, Normoxic cell lines exposed to hypoxia for 48 hrs 2, Hypoxic, hypoxic cell lines exposed to normoxic for 48 hrs). The GEOdatabase is classified based on the four groups of samples (g1 and g2).

2.2. Identification of Significant Genes

Significant genes are classified based on $0.05 < p$ value which is greater than 0.05 are taken as significant genes.

2.3. Classification of Upregulated and Downregulated Genes

Significant genes are regulated based on up-regulated and down-regulated genes. Upregulate as $>+3$ and down-regulated as < -3 .

2.4. Gene- Gene Interaction

For Gene-Gene interaction common genes are taken from the upregulated and downregulated. These common genes copy to the STRING database.

2.5. Gene Enrichment Analysis

STRING database the common genes are paste in the multiple proteins and homo sapiens as selected for function. The string interaction shows between the two co-expression genes (Mering and v. Mering 2003).

2.6. Refined Gene Enrichment Analysis

Gene enrichment analysis is used by Funrich. In Funrich all common genes are pasted in the data. Funrich analysis can be depicted graphically in the form of a bar chart. It has 9 types of enrichment analysis: cellular component, molecular function, biological process, biological pathway,

protein domain, site of expression, transcription factor, clinical phenotype, and COSMIC (Fonseka et al. 2020).

2.7. Overall Gene Enrichment

Cytoscape is complex visualizing molecular interaction networks and biological pathways. The string interaction is downloaded in tsv file format. The file is uploaded into Cytoscape and applies the function of CluePedia (ClueGO). CluePedia Cytoscape is used for finding out the pathways and to calculate the linear and nonlinear data of pathways. In CluePedia we have Ontologies/pathways, Clinvar, Corum-Corum, Chromosomal location, Biological pathways, Cellular component, Immune system process, Molecular function, Protein domains, KEGG, Reactome, Wikipathways (Shannon 2003).

2.8. Hub-Genes Identification (Cytohubba)

Cytohubba is the biological network that analyzes the network to find the hub genes in the network. Cytohubba is classified into topological and centralities. topological is classified into 10 types and centralities as 6 types. The file is uploaded in Cytohubba and the top 10 genes are selected. MCC is selected for network gene analysis (Chin et al. 2014).

2.9. Cluepedia /ClueGo

Cluepedia calculates the statistical value for linear and nonlinear between variables. Correlation for markers and to data. Pearson correlation, spearman's rank, Distance correlation, and maximal information coefficient (MIC). String node file is uploaded in the cluepedia for analysis(Shannon 2003; Chin et al. 2014; Bindea et al. 2009).

3. Results

3.1. Identification of DEGs from the Dataset

The gene expression profiles from the GSE45117 dataset from the GEO database have been used in our research. We obtained the differentially expressed genes (DEGs) from the dataset by comparing tumor samples with stem-like cell line samples using the GEO2R online platform.

P-values and $|\log_2FC|$ values are determined. The Rstudio web server shiny volt has been used to build a volcano plot (Fig. 1 and Fig. 2).

Fig. 1- Venn Diagram Representing the Common DEGs between Group 1 and Group 2, Out of Total Genes. An Adjusted p-Value ($P_{adj} < 0.05$) was Used and a Total of 32287 Genes were Found to be Common between the Groups. (g1: Tumor Cells, g2: Stem-like Cell Lines)

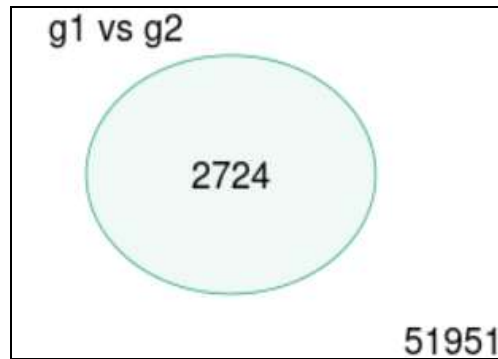
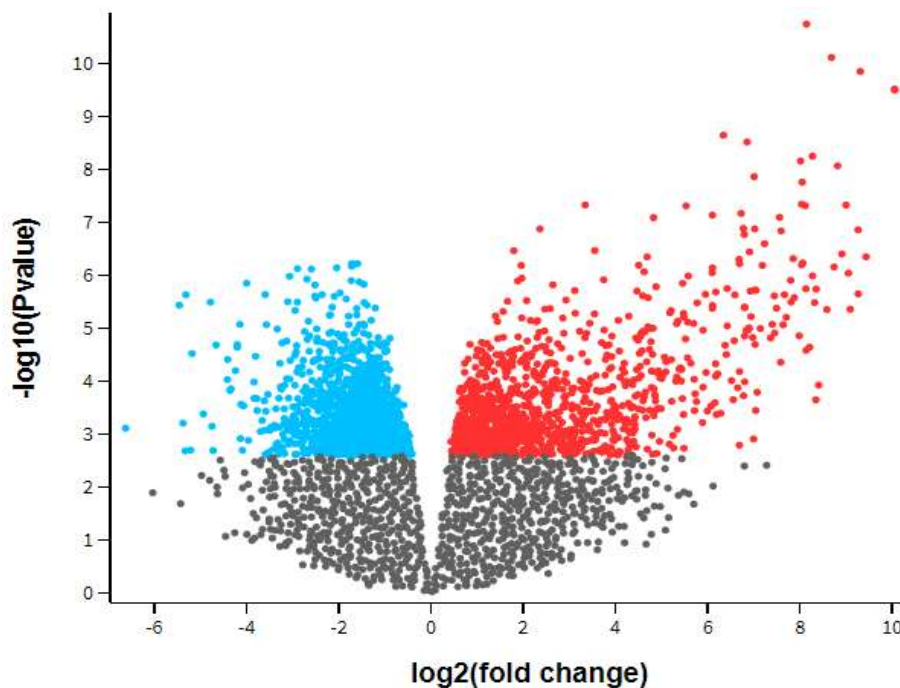


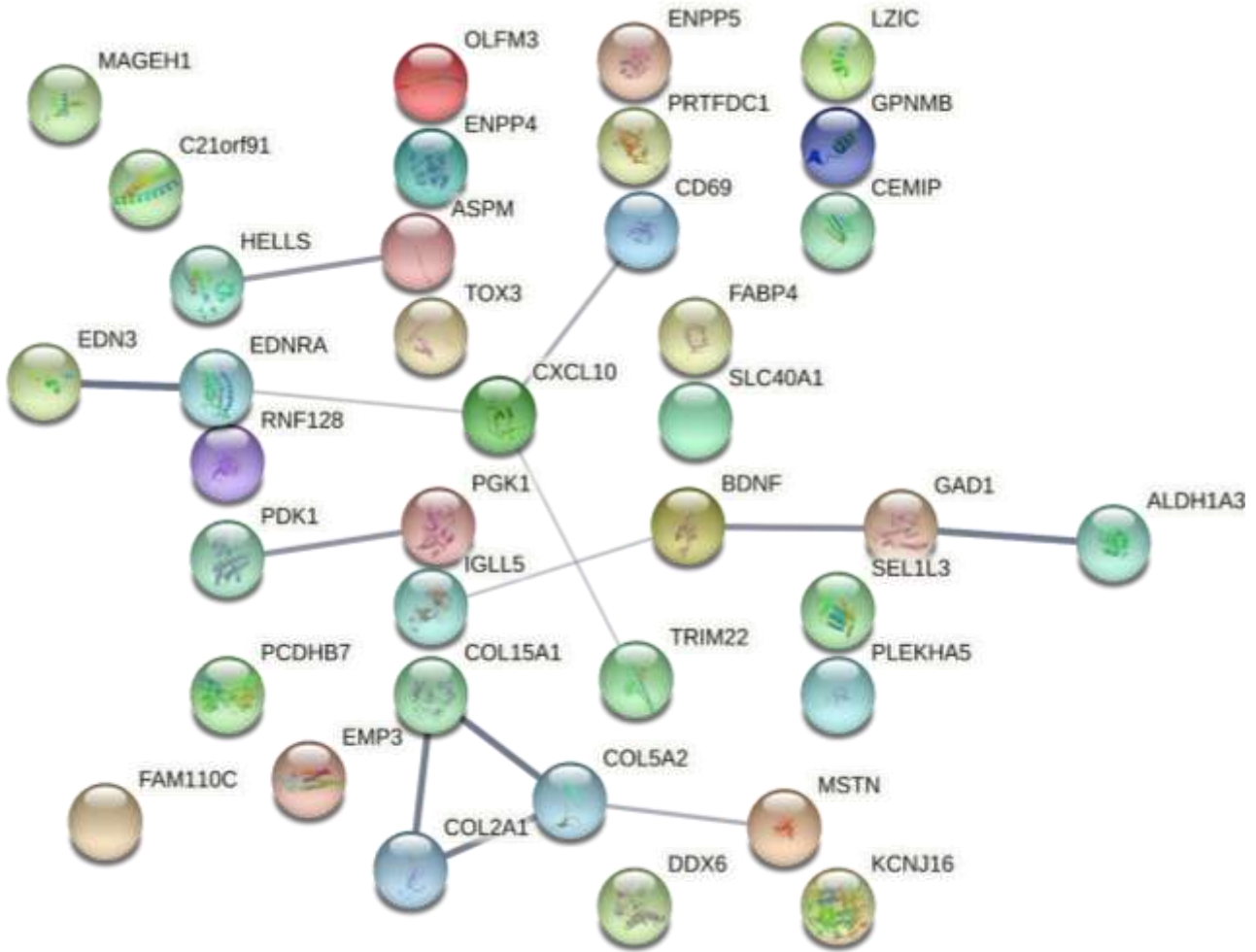
Fig. 2- Volcano Plot Showing Differentially Expressed Genes between Glioblastoma Tumors and Culture Stem-Cell-like Cells. **Red:** Overexpressed in Cultured Cell Line and **Blue:** Overexpressed in TUMOR CELLS



3.2. Gene-Gene Interaction

We simulated gene interactions using the STRING tool and plotted them using Cytoscape to evaluate the gene-gene interactions among the DEGs and the GGI network with 37 nodes and 13 edges (Fig.3). In this Fig. 3, we have found the co-expression genes were identified.

Fig. 3- STRING Showing Possible Interactions of Differentially Expressed Genes between Glioblastoma Tumor and Culture Stem-Cell-like Cells



3.3. Refine Gene Enrichment Analysis

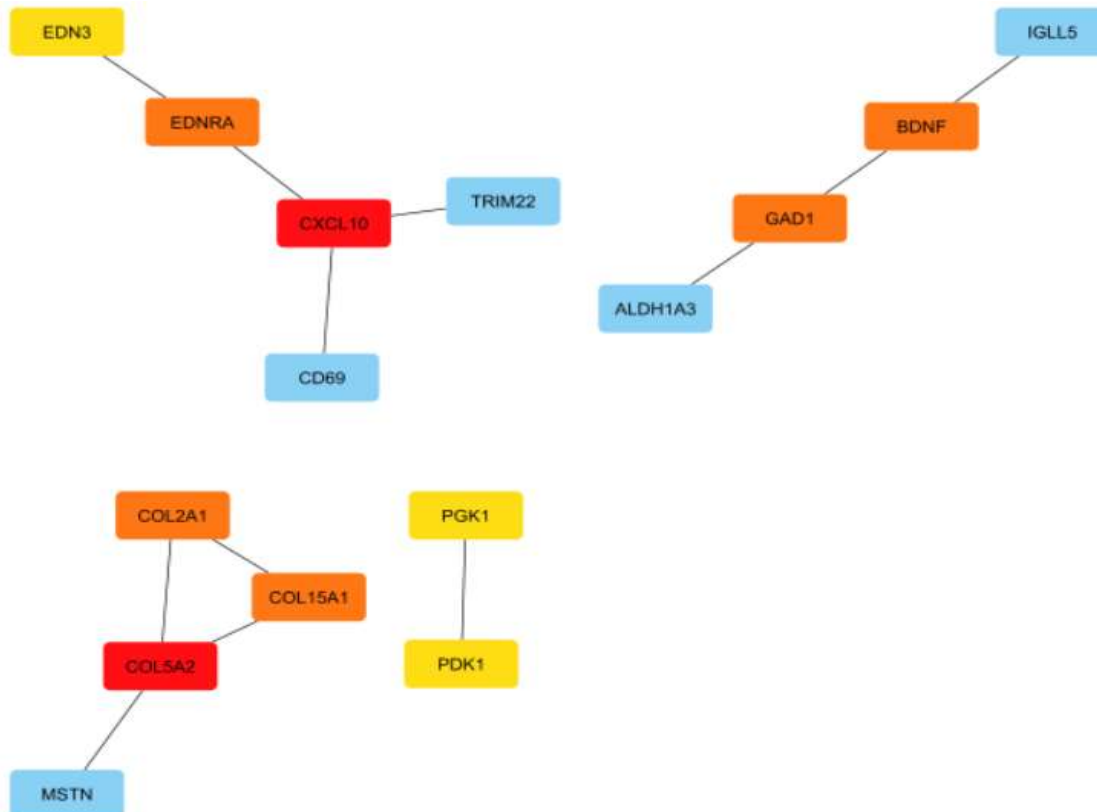
FUNRICH is the bioinformatics tool that is used to find out the genome, transcriptome, proteome, and metabolome of various systems (Shannon 2003). In the FunRich analysis common genes are identified 11 genes located in the extracellular region, 3 genes involved in the catalytic, 3 genes involved in the extracellular matrix structural constituent, 8 genes involved in the cell communication, 8 genes involved in the signal transduction, 6 genes involved in the integrin family cell surface interactions, 6 genes involved in the Beta 1 integrin cell surface interactions, 6 genes involved in the Endothelins, 17 genes involved in the signal peptide, 33 genes involved in the HUVEC , 12 genes involved in the SP1, 7 genes are involved in the Central nervous system, 36 genes involved in the large intestine. This data is represented in Table 2.

Table 2- Funrich Analysis is Done by Using Significant Genes Identified from GEO2R Analysis

Analysis	Analysis detail	No.of genes in the dataset
Cellular component	Extracellular	11
Molecular function	Catalytic Activity, Extracellular matrix structural constituent	3
Biological process	Cell communication, Signal transduction	8
Biological pathway	Integrin family cell surface interactions, Beta1 integrin cell surface interactions, Endothelins	6
Protein domain	signal peptide	17
Site of expression	HUVEC	33
Transcription factor	SP1	12
Clinical phenotype	Central Nervous System	7
COSMIC	large intestine	36

3.4. Hub Genes Classification

Fig. 4- HUB Genes Identification Using (a) Cytohubba Analysis was Performed to Identify the Gene Network from STRING Interactions. The Analysis Produced EDN3, EDNRA, CXCL10, COL5A2, PDK1, PGK1, COL15A1, COL2A1, and BDNF Genes as Highly Interacting Gene Clusters

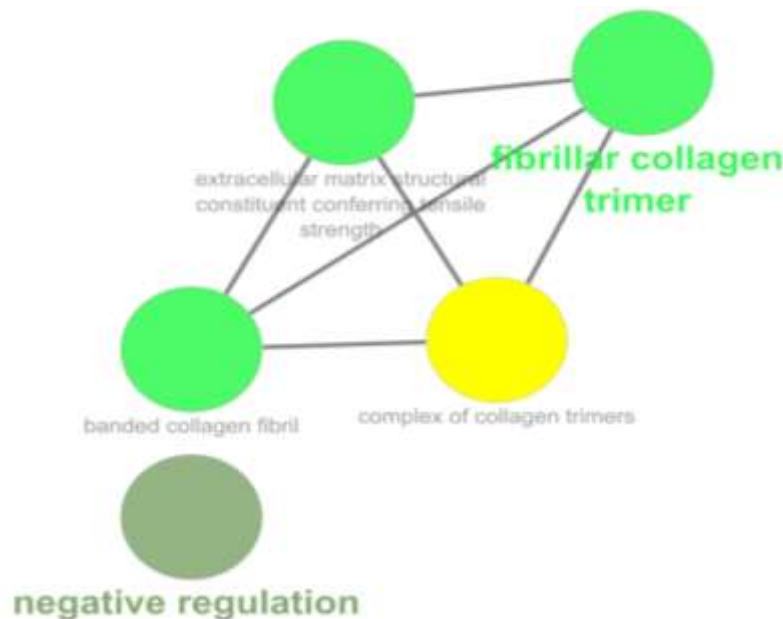


DEGs are represented as nodes, and DEG interactions are represented as edges. The densely connected regions within the gene network were identified using the Cytoscape CYTOHUBBA plugin. As a result, we can extract the DEGs gene network's top ten significant clusters (Fig. 4).

3.5. Cluepedia /ClueGo

Cluepedia calculates the statistical value for linear and nonlinear between variables. Correlation for markers and to data. Pearson correlation, spearman's rank, Distance correlation, and maximal information coefficient (MIC). The string node file is uploaded in the cluepedia for analysis (Fig. 5).

Fig. 5- Pathway Interaction between the Genes by Using the HUB Gene as Input in Cluepedia



4. Discussion

In this study, we observed the significant genes, upregulated and downregulated genes from the GEO database. The total number of significant genes present in the data is 33973 by the cutoff value ($P > 0.05$), a total number of upregulated genes are present 19 ($\log Fc > 3$) and downregulated genes are 25 ($\log Fc < -3$). The string interactions observed among the upregulated and downregulated genes are combined. 7 interactions were observed in a string (EDN3-EDNRA, HELLS-ASPM, PDK1-PGK, COL2A1-COL5A2, COL5A2-EMP3, BDNF-GAD1, GAD1- ALDH1A3). Funrich

analyses are done where 35.5% of genes are present in extracellular components. Cytohubba analysis was performed to identify the gene network from STRING interactions. The analysis produced EDN3, EDNRA, CXCL10, COL5A2, PDK1, PGK1, COL15A1, COL2A1, and BDNF genes as highly interacting gene clusters. Pathway interaction between the genes by using the HUB gene as input in Cluepedia.

The gene-gene interactions among the DEGs and the GGI network with 37 nodes and 13 edges. The strong interaction between the pathways EDN3 and EDNRA gene as the common pathway Peptide ligand-binding receptors, COL2A1, COL15A1, COL5A2 gene as the common pathway Collagen chain trimerization. In the FunRich analysis common genes are identified 11 genes located in the extracellular region, 3 genes involved in the catalytic, 3 genes involved in the extracellular matrix structural constituent, 8 genes involved in the cell communication, 8 genes involved in the signal transduction, 6 genes involved in the integrin family cell surface interactions, 6 genes involved in the Beta 1 integrin cell surface interactions, 6 genes involved in the Endothelins, 17 genes involved in the signal peptide, 33 genes involved in the HUVEC , 12 genes involved in the SP1, 7 genes are involved in the Central nervous system, 36 genes involved in the large intestine. Funrich analysis are done where 35.5% of genes are present in extracellular components. Cytohubba analysis was performed to identify the gene network from STRING interactions. The analysis produced EDN3, EDNRA, CXCL10, COL5A2, PDK1, PGK1, COL15A1, COL2A1, and BDNF genes as highly interacting gene clusters. The common pathways which are related to the disease are EDN3, EDNRA, COL2A1, COL15A1, and COL5A2.

The upregulated gene is EDN3- The interaction of genetic markers with anatomic clinical and histopathological types in Hirschsprung's disease (Benucci et al. 2001). EDNRA- Exercise-induced decreases in arterial stiffness in older people are regulated by mutations in endothelin-related genes. (Iemitsu et al. 2006). CXCL10 -Both human and viral interleukin regulate production from cytomegalovirus-stimulated microglia. (Cheeran et al. 2003). COL5A2 -Sequence analysis of COL5A2 gene in patients with spontaneous cervical artery diseases(Grond-Ginsbach et al. 2002). The downregulated genes are GAD1-Glutamate decarboxylase 1 as a candidate gene for autism: a population-based correlation study, (Buttenschøn et al. 2009) COL2A1 - Clinical outcome of congenital toxoplasmosis is based on genetic and epigenetic conditions at COL2A1 and ABCA4. (Jamieson et al. 2008). COL15A1-The human angiogenesis inhibitors restin and endostatin have new endogenous proteolytic types which have been identified and characterized. (John et al. 2005). PGK1-The promoter region of the human X-linked 3-phosphoglycerate kinase gene has also been

sequenced. (Singer-Sam et al. 1984). PDK1-The human pyruvate dehydrogenase kinase gene family is complex. (Gudi et al. 1995). Pathway interaction between the genes by using the HUB gene as input in Cluepedia. The stem-cell-like cell lines showed lower expression of endothelin-related genes such as EDN3 and EDNRA along with dysregulation of enzymes such as PDK1, PGK1 which points to dysregulation of cellular respiratory pathways. This effect in consensus with under expression of cell attachment genes such as COL2A1, COL5A2, COL15A1 denotes a strong shift toward metastasis. The outcome of this study suggests that the cancer-like cell population causes dysregulation of the respiratory pathway and increased metastasis thereby adversely affects therapeutic strategies.

Characterization of gene expression, genomic structure, and chromosomal localization of HELLS (Geiman, Durum, and Muegge 1998). Proliferation-associated SNF2-like gene (PASG): an SNF2 family member altered in leukemia(Lee et al. 2000). Mutations in ALDH1A3 represent a frequent cause of microphthalmia/anophthalmia in consanguineous families (Abouzeid et al. 2014). Protein-truncating mutations in ASPM cause variable reduction in brain size (Bond et al. 2003). Genomic organization of the human COL3A1 and COL5A2 genes: COL5A2 has evolved differently than the other minor fibrillar collagen genes (Välkkilä et al. 2001). The effect of depression, BDNF gene val66met polymorphism, and gender on serum BDNF levels (Ozan et al. 2010). GABA production by glutamic acid decarboxylase is regulated by a dynamic catalytic loop (Fenalti et al. 2007).

Our institution is passionate about high quality evidence based research and has excelled in various fields ((Vijayashree Priyadharsini 2019; Ezhilarasan, Apoorva, and Ashok Vardhan 2019; Ramesh et al. 2018; Mathew et al. 2020; Sridharan et al. 2019; Pc, Marimuthu, and Devadoss 2018; Ramadurai et al. 2019). We hope this study adds to this rich legacy.

The major limitation of our study is that these genes and pathways need to be confirmed by wet lab techniques such as western blot and RT-PCR before they can be clinically applied. This study provides a pathway towards discovering potential leads for the treatments of glioblastoma in comprehending the underlying molecular mechanisms.

5. Conclusion

Glioblastoma cancer is the most common type of brain cancer. Cancer can be defined as neoplastic cells that appear immune and have a high clonogenic potential. Are known as cancer stem

cells. After being isolated from recently resected human GBM, glioblastoma stem-like cells can be successfully propagated in the media. The significant genes are identified by upregulated and downregulated genes. Gene interactions are identified in the string database. Enrichment of genes is identified in Funrich. Hub genes are identified in cytohubba and pathway interactions are identified in cluepedia. The computational pipeline for identifying the significant genes and pathways involved in the glioblastoma tumors and glioblastoma stem-like cell lines. This study provides a path towards discovering potential leads for the treatment of glioblastoma and aids in comprehending the underlying novel molecular mechanisms.

Declarations

Conflict of Interest

The authors of this paper declare no conflict of interest.

Author Contribution

Author DG was involved in data collection, data analysis, manuscript writing. Author MD was involved in conceptualization, guidance, and critical review of a manuscript.

Acknowledgments

The authors would like to express their gratitude towards Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences (Formerly known as Saveetha University) for providing the necessary infrastructure to carry out this work successfully.

Funding

We thank the following organizations for providing financial support that enabled us to complete the study.

1. Finura Bioteks
2. Saveetha University

3. Saveetha Institute of Medical and Technical Sciences
4. Saveetha School of engineering

References

- Abouzeid, Hana, Tatiana Favez, Angélique Schmid, Céline Agosti, Mohammed Youssef, Iman Marzouk, Nihal El Shakankiry, Nader Bayoumi, Francis L. Munier, and Daniel F. Schorderet. 2014. "Mutations in ALDH1A3 Represent a Frequent Cause of Microphthalmia/anophthalmia in Consanguineous Families." *Human Mutation* 35 (8): 949–53.
- Bar, Eli E., Alex Lin, Vasiliki Mahairaki, William Matsui, and Charles G. Eberhart. 2010. "Hypoxia Increases the Expression of Stem-Cell Markers and Promotes Clonogenicity in Glioblastoma Neurospheres." *The American Journal of Pathology*. <https://doi.org/10.2353/ajpath.2010.091021>.
- Benucci, M., A. Mannoni, F. Li Gobbi, and U. Serni. 2001. "FRI0212 Correlation between Histopathological Findings, Sero-Immunological Profile and Markers of Disease Activity in Sicca Syndrome." *Speaker Abstracts 2001*. <https://doi.org/10.1136/annrheumdis-2001.305>.
- Bindea, Gabriela, Bernhard Mlecnik, Hubert Hackl, Pornpimol Charoentong, Marie Tosolini, Amos Kirilovsky, Wolf-Herman Fridman, Franck Pagès, Zlatko Trajanoski, and Jérôme Galon. 2009. "ClueGO: A Cytoscape Plug-in to Decipher Functionally Grouped Gene Ontology and Pathway Annotation Networks." *Bioinformatics* 25 (8): 1091–93.
- Bond, Jacquelyn, Sheila Scott, Daniel J. Hampshire, Kelly Springell, Peter Corry, Marc J. Abramowicz, Ganesh H. Mochida, et al. 2003. "Protein-Truncating Mutations in ASPM Cause Variable Reduction in Brain Size." *American Journal of Human Genetics* 73 (5): 1170–77.
- Buttenschøn, Henriette Nørmølle, Marlene Briciet Lauritsen, Agata El Daoud, Mads Hollegaard, Meta Jorgensen, Kristine Tvedegaard, David Hougaard, Anders Børghlum, Poul Thorsen, and Ole Mors. 2009. "A Population-Based Association Study of Glutamate Decarboxylase 1 as a Candidate Gene for Autism." *Journal of Neural Transmission*. <https://doi.org/10.1007/s00702-008-0142-4>.
- Cheeran, Maxim C-J, Maxim C. -J. Cheeran, Shuxian Hu, Wen S. Sheng, Phillip K. Peterson, and James R. Lokensgard. 2003. "CXCL10 Production from Cytomegalovirus-Stimulated Microglia Is Regulated by Both Human and Viral Interleukin-10." *Journal of Virology*.
<https://doi.org/10.1128/jvi.77.8.4502-4515.2003>.
- Chin, Chia-Hao, Shu-Hwa Chen, Hsin-Hung Wu, Chin-Wen Ho, Ming-Tat Ko, and Chung-Yen Lin. 2014. "cytoHubba: Identifying Hub Objects and Sub-Networks from Complex Interactome." *BMC Systems Biology*. <https://doi.org/10.1186/1752-0509-8-s4-s11>.
- Ezhilarasan, Devaraj, Velluru S. Apoorva, and Nandhigam Ashok Vardhan. 2019. "Syzygium Cumini Extract Induced Reactive Oxygen Species-Mediated Apoptosis in Human Oral Squamous Carcinoma Cells." *Journal of Oral Pathology & Medicine: Official Publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology* 48 (2): 115–21.
- Fenalti, Gustavo, Ruby H.P. Law, Ashley M. Buckle, Christopher Langendorf, Kellie Tuck, Carlos J. Rosado, Noel G. Faux, et al. 2007. "GABA Production by Glutamic Acid Decarboxylase Is Regulated by a Dynamic Catalytic Loop." *Nature Structural & Molecular Biology*.
<https://doi.org/10.1038/nsmb1228>.

- Fonseka, Pamali, Mohashin Pathan, Sai V. Chitti, Taeyoung Kang, and Suresh Mathivanan. 2020. "FunRich Enables Enrichment Analysis of OMICs Datasets." *Journal of Molecular Biology*. <https://doi.org/10.1016/j.jmb.2020.166747>.
- Francescone, Ralph, Steve Scully, Brooke Bentley, Wei Yan, Sherry L. Taylor, Dennis Oh, Luis Moral, and Rong Shao. 2012. "Glioblastoma-Derived Tumor Cells Induce Vasculogenic Mimicry through Flk-1 Protein Activation." *The Journal of Biological Chemistry* 287 (29): 24821–31.
- Geiman, T.M., S.K. Durum, and K. Muegge. 1998. "Characterization of Gene Expression, Genomic Structure, and Chromosomal Localization of Hells (Lsh)." *Genomics* 54 (3): 477–83.
- Gheena, S., and D. Ezhilarasan. 2019. "Syringic Acid Triggers Reactive Oxygen Species-Mediated Cytotoxicity in HepG2 Cells." *Human & Experimental Toxicology* 38 (6): 694–702.
- Grond-Ginsbach, C., F. Wigger, M. Morcher, F. von Pein, A. Grau, I. Hausser, and T. Brandt. 2002. "Sequence Analysis of the COL5A2 Gene in Patients with Spontaneous Cervical Artery Dissections." *Neurology* 58 (7): 1103–5.
- Gudi, R., M. M. Bowker-Kinley, N. Y. Kedishvili, Y. Zhao, and K. M. Popov. 1995. "Diversity of the Pyruvate Dehydrogenase Kinase Gene Family in Humans." *The Journal of Biological Chemistry* 270 (48): 28989–94.
- Iemitsu, Motoyuki, Seiji Maeda, Takeshi Otsuki, Jun Sugawara, Takumi Tanabe, Subrina Jesmin, Shinya Kuno, Ryuichi Ajisaka, Takashi Miyauchi, and Mitsuo Matsuda. 2006. "Polymorphism in Endothelin-Related Genes Limits Exercise-Induced Decreases in Arterial Stiffness in Older Subjects." *Hypertension*. <https://doi.org/10.1161/01.hyp.0000217520.44176.73>.
- Jamieson, Sarra E., Lee-Anne de Roubaix, Mario Cortina-Borja, Hooi Kuan Tan, Ernest J. Mui, Heather J. Cordell, Michael J. Kirisits, et al. 2008. "Genetic and Epigenetic Factors at COL2A1 and ABCA4 Influence Clinical Outcome in Congenital Toxoplasmosis." *PloS One* 3 (6): e2285.
- John, Harald, Kerstin Radtke, Ludger Ständker, and Wolf-Georg Forssmann. 2005. "Identification and Characterization of Novel Endogenous Proteolytic Forms of the Human Angiogenesis Inhibitors Restin and Endostatin." *Biochimica et Biophysica Acta* 1747 (2): 161–70.
- Jose, Jerry, Ajitha, and Haripriya Subbaiyan. 2020. "Different Treatment Modalities Followed by Dental Practitioners for Ellis Class 2 Fracture – A Questionnaire-Based Survey." *The Open Dentistry Journal* 14 (1): 59–65.
- Kathagen, Annegret, Alexander Schulte, Gerd Balcke, Heidi S. Phillips, Tobias Martens, Jakob Matschke, Hauke S. Günther, et al. 2013. "Hypoxia and Oxygenation Induce a Metabolic Switch between Pentose Phosphate Pathway and Glycolysis in Glioma Stem-like Cells." *Acta Neuropathologica*. <https://doi.org/10.1007/s00401-013-1173-y>.
- Ke, Yang, Mohammed Saleh Al Aboody, Wael Alturaiki, Suliman A. Alsagaby, Faiz Abdulaziz Alfaiz, Vishnu Priya Veeraraghavan, and Suresh Mickymaray. 2019. "Photosynthesized Gold Nanoparticles from *Catharanthus Roseus* Induces Caspase-Mediated Apoptosis in Cervical Cancer Cells (HeLa)." *Artificial Cells, Nanomedicine, and Biotechnology* 47 (1): 1938–46.
- Krishnaswamy, Haribabu, Sivaprakash Muthukrishnan, Sathish Thanikodi, Godwin Arockiaraj Antony, and Vijayan Venkatraman. 2020. "Investigation of Air Conditioning Temperature Variation by Modifying the Structure of Passenger Car Using Computational Fluid Dynamics." *Thermal Science* 24 (1 Part B): 495–98.

Lee, D.W., K. Zhang, Z. Q. Ning, E.H. Raabe, S. Tintner, R. Wieland, B.J. Wilkins, J.M. Kim, R.I. Blough, and R. J. Arceci. 2000. "Proliferation-Associated SNF2-like Gene (PASG): A SNF2 Family Member Altered in Leukemia." *Cancer Research* 60 (13): 3612–22.

Malli Sureshbabu, Nivedhitha, Kathiravan Selvarasu, Jayanth Kumar V, Mahalakshmi Nandakumar, and Deepak Selvam. 2019. "Concentrated Growth Factors as an Ingenious Biomaterial in Regeneration of Bony Defects after Periapical Surgery: A Report of Two Cases." *Case Reports in Dentistry* 2019 (January): 7046203.

Mathew, M.G., S.R. Samuel, A.J. Soni, and K.B. Roopa. 2020. "Evaluation of Adhesion of Streptococcus Mutans, Plaque Accumulation on Zirconia and Stainless Steel Crowns, and Surrounding Gingival Inflammation in Primary" *Clinical Oral Investigations*. <https://link.springer.com/article/10.1007/s00784-020-03204-9>.

Mehta, Meenu, Deeksha, Devesh Tewari, Gaurav Gupta, Rajendra Awasthi, Harjeet Singh, Parijat Pandey, et al. 2019. "Oligonucleotide Therapy: An Emerging Focus Area for Drug Delivery in Chronic Inflammatory Respiratory Diseases." *Chemico-Biological Interactions* 308 (August): 206–15.

Mering, C.V., and C.V. Mering. 2003. "STRING: A Database of Predicted Functional Associations between Proteins." *Nucleic Acids Research*. <https://doi.org/10.1093/nar/gkg034>.

Muthukrishnan, Sivaprakash, Haribabu Krishnaswamy, Sathish Thanikodi, Dinesh Sundaresan, and Vijayan Venkatraman. 2020. "Support Vector Machine for Modelling and Simulation of Heat Exchangers." *Thermal Science* 24 (1 Part B): 499–503.

Ohgaki, Hiroko, and Paul Kleihues. 2013. "The Definition of Primary and Secondary Glioblastoma." *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research* 19 (4): 764–72.

Ozan, Erol, Hamza Okur, Çağdaş Eker, Ozlem D. Eker, Ali Saffet Gönül, and Nurten Akarsu. 2010. "The Effect of Depression, BDNF Gene val66met Polymorphism and Gender on Serum BDNF Levels." *Brain Research Bulletin* 81 (1): 61–65.

Pc, J., T. Marimuthu, and P. Devadoss. 2018. "Prevalence and Measurement of Anterior Loop of the Mandibular Canal Using CBCT: A Cross Sectional Study." *Clinical Implant Dentistry and Related Research*. <https://europepmc.org/article/med/29624863>.

Ramadurai, Neeraja, Deepa Gurunathan, A. Victor Samuel, Emg Subramanian, and Steven J. L. Rodrigues. 2019. "Effectiveness of 2% Articaine as an Anesthetic Agent in Children: Randomized Controlled Trial." *Clinical Oral Investigations* 23 (9): 3543–50.

Ramesh, Asha, Sheeja Varghese, Nadathur D. Jayakumar, and Sankari Malaiappan. 2018. "Comparative Estimation of Sulfiredoxin Levels between Chronic Periodontitis and Healthy Patients - A Case-Control Study." *Journal of Periodontology* 89 (10): 1241–48.

Rosmaninho, Pedro, Susanne Mükusch, Valerio Piscopo, Vera Teixeira, Alexandre Asf Raposo, Rolf Warta, Romina Bennewitz, et al. 2018. "Zeb1 Potentiates Genome-Wide Gene Transcription with Lef1 to Promote Glioblastoma Cell Invasion." *The EMBO Journal* 37 (15). <https://doi.org/10.15252/emj.201797115>.

Samuel, Melvin S., Jayanta Bhattacharya, Sankalp Raj, Needhidasan Santhanam, Hemant Singh, and N. D. Pradeep Singh. 2019. "Efficient Removal of Chromium(VI) from Aqueous Solution Using Chitosan Grafted Graphene Oxide (CS-GO) Nanocomposite." *International Journal of Biological Macromolecules* 121 (January): 285–92.

- Samuel, Srinivasan Raj, Shashidhar Acharya, and Jeevika Chandrasekar Rao. 2020. "School Interventions-Based Prevention of Early-Childhood Caries among 3-5-Year-Old Children from Very Low Socioeconomic Status: Two-Year Randomized Trial." *Journal of Public Health Dentistry* 80 (1): 51–60.
- Sathish, T., and S. Karthick. 2020. "Wear Behaviour Analysis on Aluminium Alloy 7050 with Reinforced SiC through Taguchi Approach." *Journal of Japan Research Institute for Advanced Copper-Base Materials and Technologies* 9 (3): 3481–87.
- Shannon, P. 2003. "Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks." *Genome Research*. <https://doi.org/10.1101/gr.1239303>.
- Sharma, Parvarish, Meenu Mehta, Daljeet Singh Dhanjal, Simran Kaur, Gaurav Gupta, Harjeet Singh, Lakshmi Thangavelu, et al. 2019. "Emerging Trends in the Novel Drug Delivery Approaches for the Treatment of Lung Cancer." *Chemico-Biological Interactions* 309 (August): 108720.
- Shergalis, Andrea, Armand Bankhead 3rd, Urarika Luesakul, Nongnuj Muangsin, and Nouri Neamati. 2018. "Current Challenges and Opportunities in Treating Glioblastoma." *Pharmacological Reviews* 70 (3): 412–45.
- Singer-Sam, J., D.H. Keith, K. Tani, R.L. Simmer, L. Shively, S. Lindsay, A. Yoshida, and A.D. Riggs. 1984. "Sequence of the Promoter Region of the Gene for Human X-Linked 3-Phosphoglycerate Kinase." *Gene* 32 (3): 409–17.
- Sridharan, Gokul, Pratibha Ramani, Sangeeta Patankar, and Rajagopalan Vijayaraghavan. 2019. "Evaluation of Salivary Metabolomics in Oral Leukoplakia and Oral Squamous Cell Carcinoma." *Journal of Oral Pathology & Medicine: Official Publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology* 48 (4): 299–306.
- Välkkilä, Merja, Miia Melkonieni, Laura Kvist, Helena Kuivaniemi, Gerard Tromp, and Leena Ala-Kokko. 2001. "Genomic Organization of the Human COL3A1 and COL5A2 Genes: COL5A2 Has Evolved Differently than the Other Minor Fibrillar Collagen Genes." *Matrix Biology*. [https://doi.org/10.1016/s0945-053x\(01\)00145-7](https://doi.org/10.1016/s0945-053x(01)00145-7).
- Varghese, Sheeja Saji, Asha Ramesh, and Deepak Nallaswamy Veeraiyan. 2019. "Blended Module-Based Teaching in Biostatistics and Research Methodology: A Retrospective Study with Postgraduate Dental Students." *Journal of Dental Education* 83 (4): 445–50.
- Venu, Harish, V. Dhana Raju, and Lingesan Subramani. 2019. "Combined Effect of Influence of Nano Additives, Combustion Chamber Geometry and Injection Timing in a DI Diesel Engine Fuelled with Ternary (diesel-Biodiesel-Ethanol) Blends." *Energy* 174 (May): 386–406.
- Venu, Harish, Lingesan Subramani, and V. Dhana Raju. 2019. "Emission Reduction in a DI Diesel Engine Using Exhaust Gas Recirculation (EGR) of Palm Biodiesel Blended with TiO₂ Nano Additives." *Renewable Energy* 140 (September): 245–63.
- Vignesh, R., Ditto Sharmin, C. Vishnu Rekha, Sankar Annamalai, and Parisa Norouzi Baghkomeh. 2019. "Management of Complicated Crown-Root Fracture by Extra-Oral Fragment Reattachment and Intentional Reimplantation with 2 Years Review." *Contemporary Clinical Dentistry* 10 (2): 397–401.
- Vijayakumar Jain, S., M. R. Muthusekhar, M. F. Baig, P. Senthilnathan, S. Loganathan, P. U. Abdul Wahab, M. Madhulakshmi, and Yogaen Vohra. 2019. "Evaluation of Three-Dimensional Changes in Pharyngeal Airway Following Isolated Lefort One Osteotomy for the Correction of Vertical Maxillary Excess: A Prospective Study." *Journal of Maxillofacial and Oral Surgery* 18 (1): 139–46.

Vijayashree Priyadharsini, Jayaseelan. 2019. “In Silico Validation of the Non-Antibiotic Drugs Acetaminophen and Ibuprofen as Antibacterial Agents against Red Complex Pathogens.” *Journal of Periodontology* 90 (12): 1441–48.

Weina, Kasia, and Jochen Utikal. 2014. “SOX2 and Cancer: Current Research and Its Implications in the Clinic.” *Clinical and Translational Medicine* 3 (July): 19.

Yi, Yang, I-Yun Hsieh, Xiaojia Huang, Jie Li, and Wei Zhao. 2016. “Glioblastoma Stem-Like Cells: Characteristics, Microenvironment, and Therapy.” *Frontiers in Pharmacology* 7 (December): 477.