INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

NETWORK ANALYSIS FOR IDENTIFICATION OF DIAGNOSTIC BIOMARKERS OF GLIOMA USING NGS DATA

Aqsa Naz[1], Naima Iqbal[1], Alisha Aftab[1], Falak Naz[1], Nafeesa Tahir[1], Saadia Momal Zafar[2]*, Rafia Anwer[3]*

[1]Faculty of Rehabilitation and Allied Health Sciences (FRAHS), Riphah International University, Islamabad, Pakistan.

[2]COMSATS University Islamabad (CUI), Pakistan.

[3]Faculty of Rehabilitation and Allied Health Sciences (FRAHS), Riphah International University, Faisalabad, Pakistan.

Corresponding author: Rafia Anwer, Faculty of Rehabilitation and Allied Health Sciences (FRAHS), Riphah International University Faisalabad, Pakistan Email: rafia.anwer@riphahfsd.edu.pk

Saadia Momal Zafar, COMSATS University Islamabad (CUI), Pakistan. Email: saadiamomal98@gmail.com

Acknowledgement: The authors acknowledge the CGGA database for providing open-access datasets used in this study.

Conflict of Interest: None

Grant Support & Financial Support: None

ABSTRACT

Background: Gliomas represent the most prevalent primary brain tumors, accounting for approximately 80% of cases worldwide, with an estimated 200,000 new diagnoses annually. Low-grade gliomas exhibit relatively slower progression and improved survival compared to high-grade variants; however, their clinical course remains unpredictable due to molecular heterogeneity. Current diagnostic approaches are limited by insufficient sensitivity and specificity, highlighting a critical need for reliable molecular biomarkers that enable early detection and accurate disease characterization.

Objective: To identify potential diagnostic biomarkers in low-grade glioma through integrative bioinformatics analysis of differentially expressed genes and associated molecular pathways.

Methods: A retrospective in silico study was conducted using RNA-seq datasets obtained from the Chinese Glioma Genome Atlas. Gene expression data from glioma and normal brain tissues were analyzed using R-based pipelines. Differential expression analysis was performed using thresholds of p-value <0.05 and log fold change $\geq \pm 1.5$. A total of 325 glioma samples and 20 normal samples were screened, from which 9 tumor and 9 normal samples were selected. Protein–protein interaction networks were constructed using STRING, and hub genes were identified through Cytoscape with CytoHubba. Functional enrichment analysis, including Gene Ontology and KEGG pathway analysis, was performed. Validation of key genes was conducted using the GEPIA platform.

Results: A total of 4,683 differentially expressed genes were identified, including 810 upregulated and 3,873 downregulated genes in glioma compared to normal tissue. Functional enrichment revealed significant involvement in axonogenesis, neuronal signaling, and membrane potential regulation. KEGG pathway analysis highlighted enrichment in PI3K–Akt, MAPK signaling, and focal adhesion pathways. Network analysis identified 15 hub genes, among which ATM, BCL2, BRCA1, CREBBP, EGFR, PIK3R1, EP300, IL1B, JUN, KRAS, NRAS, and PTEN were significantly dysregulated. Expression validation demonstrated consistent downregulation of these genes in tumor samples.

Conclusion: This study identified key differentially expressed genes and disrupted pathways associated with low-grade glioma, highlighting several hub genes with potential diagnostic relevance. These findings provide a foundation for the development of molecular biomarkers and may contribute to improved early detection and personalized management of glioma.

Keywords: Biomarkers; Differential Gene Expression; Glioma; Low-Grade Glioma; Molecular Pathways; Network Analysis; RNA Sequencing

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

INTRODUCTION

Brain tumors, also referred to as intracranial neoplasms, represent abnormal proliferations of cells within the brain and encompass a diverse spectrum of pathological entities classified broadly into benign and malignant forms based on their biological behavior (1). They may arise as primary tumors originating within the central nervous system or as secondary lesions metastasizing from extracranial sites. Although primary brain tumors account for less than 2% of adult malignancies, they contribute disproportionately to cancer-related morbidity and mortality due to their critical location and complex clinical course (2). Among these, gliomas constitute a heterogeneous group of tumors derived from glial cells, particularly astrocytes, which play essential roles in maintaining neuronal homeostasis, regulating cerebral blood flow, and supporting neural function. Genetic alterations involving key regulators such as IDH1/2, TP53, and ATRX disrupt normal cellular processes, driving uncontrolled proliferation and resistance to apoptosis, thereby contributing to glioma initiation and progression (3). Notably, gliomas account for approximately 30% of all primary brain tumors and nearly 80% of malignant variants, and they are associated with particularly poor clinical outcomes.

Clinically, gliomas present with a wide array of neurological, cognitive, and psychiatric manifestations depending on tumor location, size, and rate of progression, including seizures, headaches, cognitive decline, and behavioral disturbances (4,5). Despite advances in neurosurgical techniques, radiotherapy, and chemotherapy, the prognosis remains highly variable and is influenced by factors such as tumor grade, molecular profile, and patient characteristics (6). The World Health Organization (WHO) classification system integrates histological and molecular features to stratify gliomas into distinct subtypes, including astrocytomas and oligodendrogliomas, with grading reflecting tumor aggressiveness and expected clinical behavior. However, histopathological assessment alone is often insufficient due to inter-observer variability and overlapping morphological features, necessitating the incorporation of molecular markers for improved diagnostic accuracy. Furthermore, gliomas exhibit marked intra- and inter-tumoral heterogeneity, complicating disease characterization, treatment planning, and outcome prediction (7).

At the molecular level, gliomas are characterized by disruptions in key signaling pathways, including PI3K/AKT and MAPK, as well as alterations in genes such as IDH1/2, 1p/19q co-deletion, and MGMT methylation, which serve as important prognostic and predictive indicators. Advances in high-throughput technologies, particularly next-generation sequencing (NGS) and RNA sequencing (RNA-seq), have revolutionized the understanding of glioma biology by enabling comprehensive genomic and transcriptomic profiling. These approaches facilitate the identification of differentially expressed genes (DEGs), novel mutations, and molecular subtypes, thereby providing insights into tumor pathogenesis and potential therapeutic targets. Despite these developments, the translation of molecular findings into clinically reliable diagnostic tools remains limited, and many identified biomarkers lack sufficient sensitivity and specificity for routine clinical application (8).

Current diagnostic strategies rely heavily on neuroimaging modalities such as magnetic resonance imaging (MRI), followed by histological examination of biopsy or resected tissue. Although MRI remains the gold standard, it has notable limitations, including difficulty distinguishing tumor progression from treatment-related changes such as pseudo-progression, which occurs in approximately 20–30% of patients. Additionally, the invasive nature of tissue biopsy and the constraints imposed by the blood–brain barrier hinder the identification and validation of minimally invasive biomarkers. These challenges underscore a critical gap in glioma diagnostics, particularly in the early detection and accurate differentiation of tumor grades and subtypes.

In recent years, increasing attention has been directed toward the identification of molecular biomarkers that can enable early diagnosis, improve disease stratification, and guide personalized therapeutic strategies. Biomarkers derived from gene expression profiles, circulating tumor DNA, microRNAs, and other molecular entities offer promising avenues for non-invasive detection and monitoring of gliomas (9). However, most existing studies have predominantly focused on prognostic markers, with comparatively limited progress in identifying robust diagnostic biomarkers capable of distinguishing glioma from other intracranial pathologies and differentiating among its various stages. This limitation highlights a significant unmet clinical need for reliable, sensitive, and specific diagnostic indicators.

Given these considerations, the present study addresses the research question of whether differential gene expression profiling using RNA sequencing can identify novel and reliable diagnostic biomarkers for glioma. By systematically analyzing differentially expressed genes between glioma and normal brain tissues, followed by functional annotation, pathway analysis, and network-based identification of hub genes, this study aims to elucidate key molecular signatures associated with glioma pathogenesis. The objective is to establish a robust framework for the identification and validation of potential diagnostic biomarkers that may enhance early detection, improve diagnostic precision, and ultimately contribute to better clinical management of glioma patients.

METHODS

The present study was designed as a retrospective, in silico case–control investigation aimed at identifying potential diagnostic biomarkers in glioma through integrative bioinformatics analyses. Publicly available RNA sequencing (RNA-seq) datasets were retrieved from the Chinese Glioma Genome Atlas (CGGA), a well-established repository containing comprehensive genomic and clinical data of glioma patients. The study was conducted using advanced computational infrastructure and standardized analytical

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

pipelines to ensure reproducibility and robustness of findings. As the analysis was performed on de-identified, publicly accessible datasets, formal ethical approval and informed consent were considered exempt in accordance with international guidelines for secondary data analysis; however, the original studies contributing to the CGGA database had obtained appropriate institutional ethical approvals and participant consents.

The study population comprised human glioma samples and corresponding normal brain tissue samples extracted from the CGGA database. Inclusion criteria encompassed RNA-seq datasets containing gene expression profiles of glioma derived from human subjects, with clearly defined tumor and normal sample annotations. Only datasets with complete and high-quality gene expression information were considered eligible. Samples derived from cell lines, animal models, metastatic tumors, or those lacking sufficient clinical or molecular annotation were excluded to maintain dataset consistency and biological relevance. Following the application of these criteria, a subset of samples was selected for downstream analysis to ensure comparability between tumor and control groups.Data collection involved systematic extraction and preprocessing of RNA-seq read count data. Initially, datasets were screened and filtered to include only low-grade glioma (grade II) samples without prior therapeutic intervention, thereby minimizing treatment-related confounding effects (10,11). The dataset was curated using spreadsheet-based filtering methods, including functions such as COUNTIF, followed by further preprocessing using the Pandas library in Python to remove redundant or non-informative gene entries. A total of 18 samples, comprising nine glioma and nine normal brain tissue samples, were finalized for subsequent analysis based on data completeness and quality considerations.

Differential gene expression analysis was conducted using the EdgeR package within the R statistical environment, a widely validated tool for RNA-seq data analysis. The normalized read counts were modeled using the DGEList object, and statistical comparisons between glioma and normal samples were performed to identify differentially expressed genes (DEGs) (12). Genes meeting the threshold of adjusted p-value <0.05 were considered statistically significant. Further classification was based on log fold-change (logFC), where genes with logFC >1 were categorized as upregulated and those with logFC <1 as downregulated. Functional annotation of DEGs was carried out through Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis to elucidate their roles in biological processes, molecular functions, and cellular components, as well as their involvement in key signaling pathways associated with glioma pathogenesis.

To further explore the biological relevance of identified DEGs, protein–protein interaction networks were constructed using STRING database-derived interaction data and visualized in Cytoscape software. Network topology analysis was performed using the CytoHubba plugin to identify hub genes, defined as highly connected nodes with potential regulatory significance. A total of 15 hub genes were identified and considered critical in glioma development and progression (13). These candidate genes were subsequently validated using the Gene Expression Profiling Interactive Analysis (GEPIA) platform, which enabled cross-validation of gene expression patterns and assessment of their clinical relevance in independent datasets.Overall, the methodological framework integrated multiple analytical layers, including data preprocessing, differential expression analysis, functional enrichment, pathway mapping, network construction, and external validation, to provide a comprehensive understanding of the molecular landscape of glioma (14,15). This systematic approach was intended to enhance the reliability of biomarker identification and to support the discovery of novel diagnostic targets.

It is important to note certain methodological limitations and potential inconsistencies within this approach. The relatively small sample size (n=18) may limit the statistical power and generalizability of the findings. Additionally, the use of Excel-based filtering alongside programming tools may introduce variability and reduce reproducibility compared to fully automated pipelines. The classification criterion for downregulated genes (logFC <1) appears methodologically inconsistent, as standard practice typically defines downregulation as logFC <-1, which should be clarified. Furthermore, while in silico validation provides supportive evidence, the absence of experimental or clinical validation may restrict the translational applicability of the identified biomarkers. Addressing these aspects in future studies would strengthen the methodological rigor and clinical relevance of the findings.

RESULTS

The CGGA database was used to retrieve RNA-seq datasets relevant to low-grade glioma. A total of 325 glioma samples were initially identified from the mRNA-seq expression dataset generated from STAR read counts, of which 9 samples fulfilled the predefined eligibility criteria and were included in the final tumor group. For the control group, 20 non-glioma samples were screened, and 9 normal samples met the selection criteria and were retained for downstream analysis. Consequently, the final analytical dataset comprised 18 samples, including 9 glioma samples and 9 normal brain samples.Following data retrieval, normalization and quality assessment were performed before differential expression analysis. Distributional assessment by box plot showed that the median-centered expression values were comparable across the selected samples, indicating satisfactory normalization and cross-sample comparability. Density distribution analysis similarly demonstrated substantial overlap in the log-transformed expression profiles of tumor and normal samples, without evidence of marked batch effects or severe outliers. Minor variations in peak width and height were observed between samples, reflecting underlying biological variability in gene expression.

Differential expression analysis was performed based on adjusted p-value <0.05 and log2 fold-change thresholds. A total of 4,683 differentially expressed genes were identified between glioma and normal brain tissue. Among these, 3,873 genes were downregulated, whereas 810 genes were upregulated. A clear predominance of downregulated genes was observed, indicating widespread suppression of genes associated with normal neuronal activity, cellular regulation, and homeostatic signaling processes in glioma tissue.Among the identified differentially expressed genes, several genes demonstrated the highest degree of dysregulation and statistical significance. Notably, genes such as EGFR, PTEN, JUN, BCL2, BRCA1, ATM, PIK3R1, EP300, and CREBBP exhibited marked alterations in expression between glioma and normal samples, indicating their potential importance in glioma-associated molecular changes.The volcano plot demonstrated a clear separation of significantly dysregulated genes, with downregulated genes distributed on the negative log2 fold-change axis and upregulated genes on the positive axis, while non-significant genes clustered centrally. Heatmap analysis further confirmed distinct expression patterns between tumor and normal samples, with downregulated genes showing reduced expression across glioma samples and upregulated genes showing increased expression relative to controls.

Functional enrichment analysis of the differentially expressed genes revealed significant involvement in key biological processes, including axonogenesis, dendrite development, neuronal signaling, and regulation of membrane potential. These findings indicated that the dysregulated genes were primarily associated with neural development and synaptic function. Gene Ontology analysis demonstrated enrichment across biological processes, molecular functions, and cellular components, with higher gene ratios and lower adjusted p-values indicating strong functional associations.KEGG pathway analysis revealed that the differentially expressed genes were significantly enriched in multiple signaling pathways, including PI3K–Akt signaling, MAPK signaling, focal adhesion, axon guidance, and endocytosis pathways. These pathways are associated with cell proliferation, differentiation, survival, and cellular communication, reflecting the molecular alterations underlying glioma development.

Protein–protein interaction network analysis was performed to identify key regulatory genes within the dataset. A total of 15 hub genes were identified based on their degree of connectivity within the network. These included ATM, BCL2, BRCA1, CREBBP, EGFR, PIK3R1, EP300, IL1B, JUN, KRAS, NRAS, PTEN, H3C12, H3C13, and PIK3CA. These hub genes were centrally positioned within the interaction network and were associated with critical biological processes such as cell cycle regulation, apoptosis, DNA repair, signal transduction, and epigenetic modification.Further network analysis demonstrated that EP300 occupied a central position within the interaction network and was connected with multiple genes including EGFR, PTEN, KRAS, and BCL2. Additional highly interconnected genes such as ATM, BRCA1, CREBBP, and JUN also exhibited strong network associations, suggesting coordinated involvement in glioma-related molecular mechanisms.Validation analysis using GEPIA demonstrated consistent differential expression patterns between tumor and normal samples. The expression profiles confirmed the downregulation of key hub genes in glioma tissue, supporting the robustness and reproducibility of the identified molecular signatures.

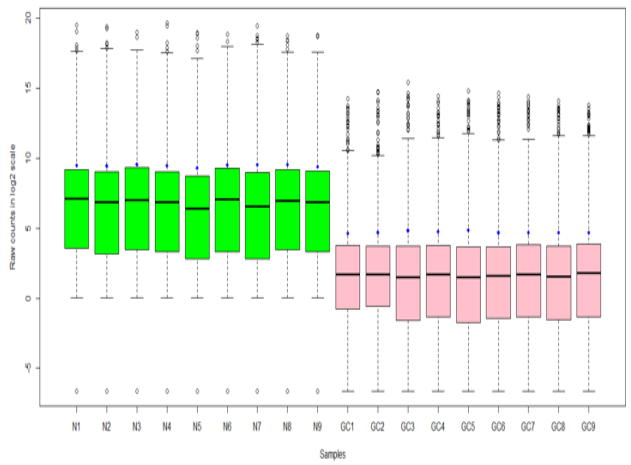

Figure 1: Box plot shows normalization of samples (Normal vs Tumor)

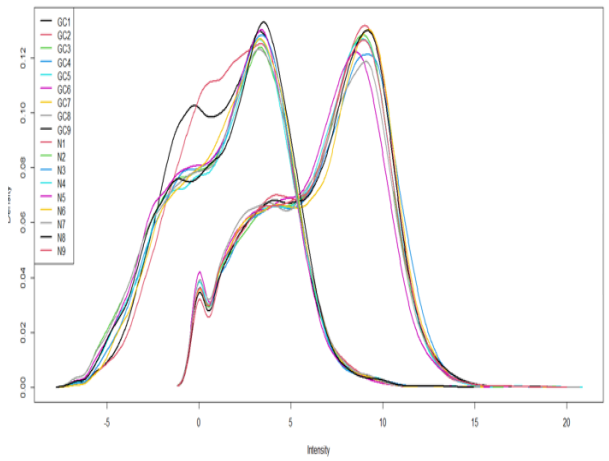

Figure 2: Density plot shows normalization of samples (Normal vs Tumor)

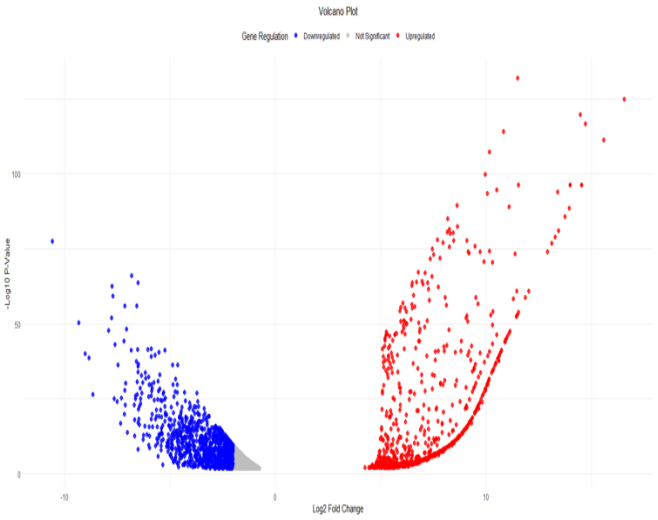

Figure 3: Volcano plot emphasized significantly upregulated and downregulated DEGs in LGG that are displayed according to the statistical significance P value vs amount of change (fold change) for the dataset. Red indicates upregulated genes, while blue

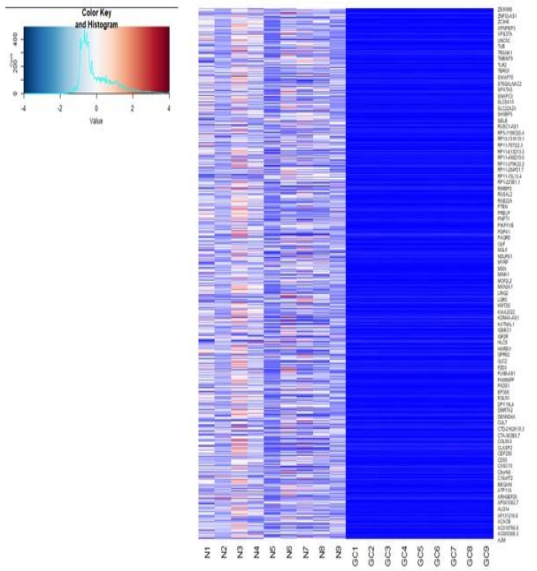

Figure 4: Heatmap of Downregulated genes, blue color shows expression of downregulated DEGs

Upregulated genes were more significantly expressed as compared to normal genes.

Out of total 4683 DEGs 810 DEGs were upregulated with comparison to the normal.

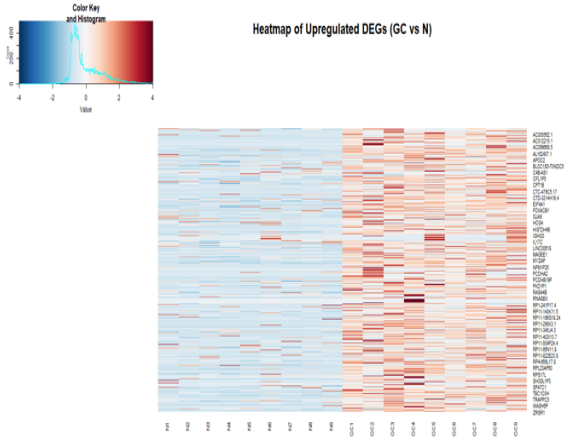

Figure 5: Heatmap of Upregulated genes, blue color shows expression of downregulated DEG

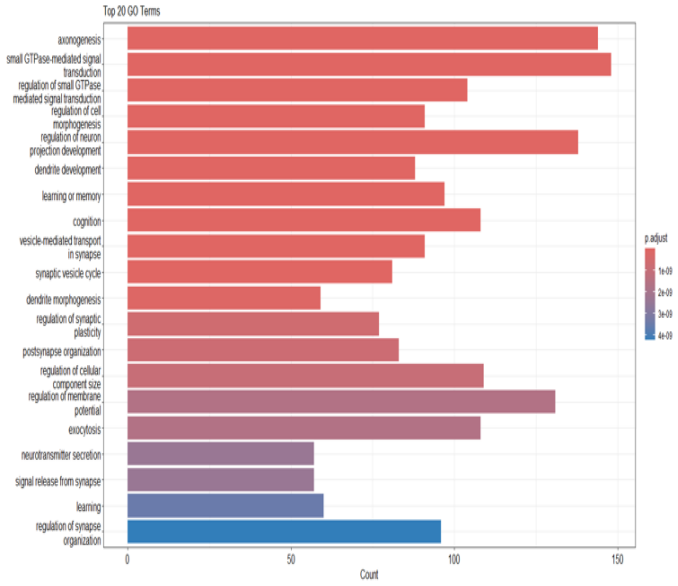

Figure 6: Box plot of Go term analysis DEGs by R

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

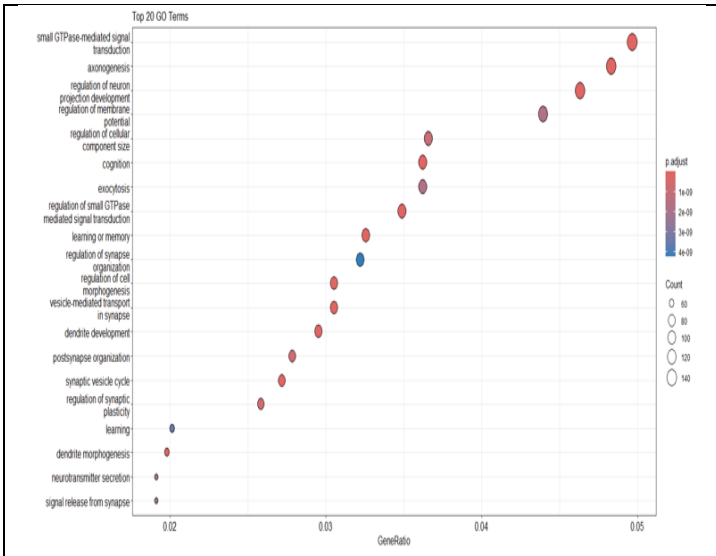

Figure 7: Dot plot of GO Term analysis of DEGs by R

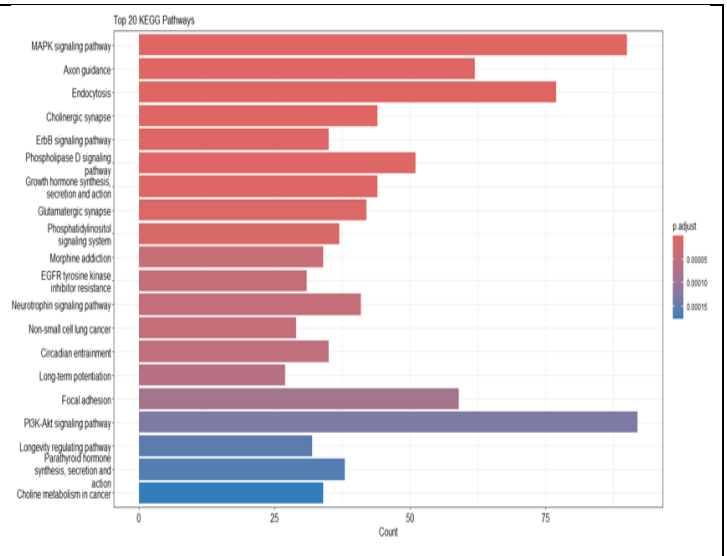

Figure 8: Box plot of KEGG Pathway analysis of DEGs by R

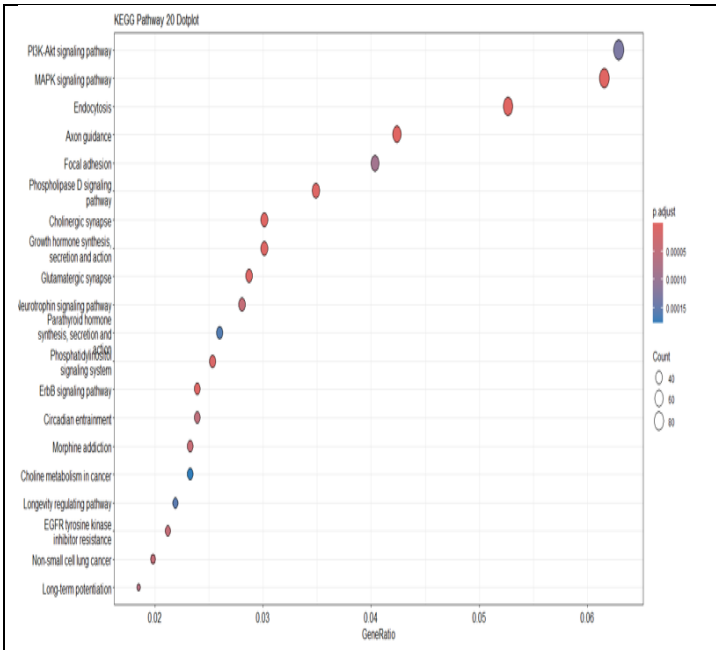

Figure 9: Dot plot of KEGG Pathway analysis of DEGs by R

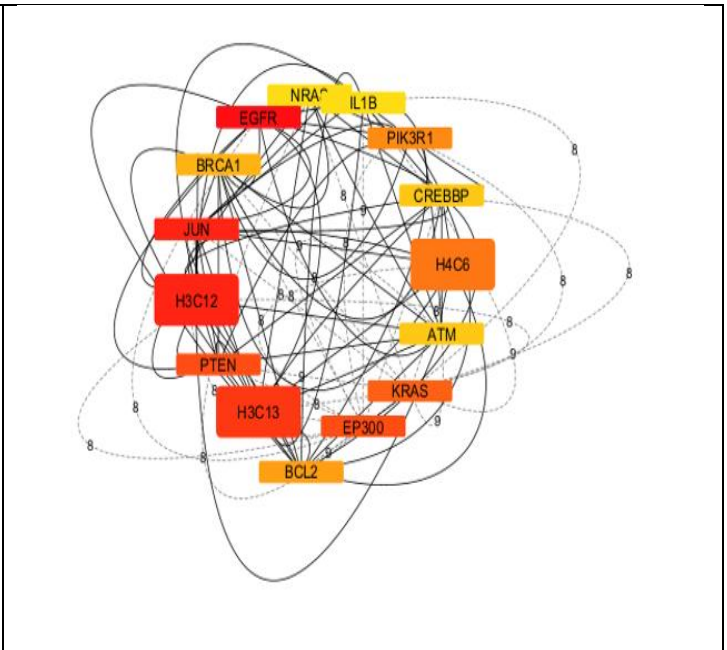

Figure 10: Identification of 15 hub genes using Cytohubba.

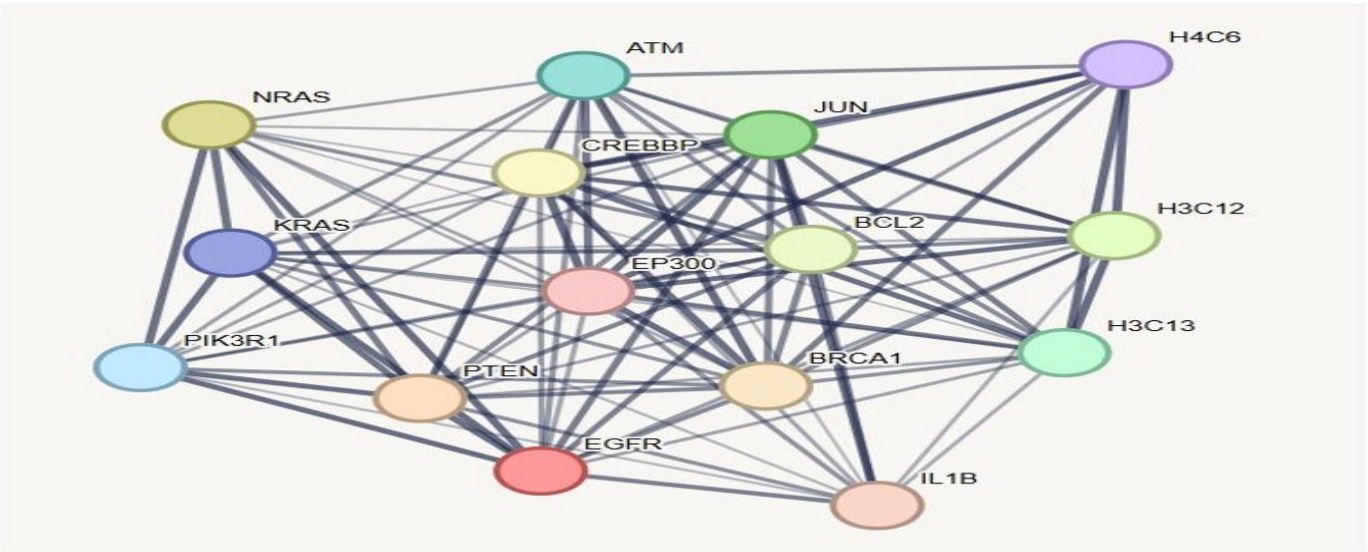

Figure 11: The protein-protein network analysis for identified DEGs

DISCUSSION

The present study provided a comprehensive transcriptomic analysis of low-grade glioma by identifying differentially expressed genes between normal and tumor tissues, thereby offering insights into the molecular alterations underlying glioma pathogenesis (16,17). A substantial number of differentially expressed genes were identified, indicating a pronounced shift in gene expression profiles in glioma, which is consistent with previous investigations reporting widespread transcriptomic dysregulation in brain tumors. The predominance of downregulated genes observed in this study suggested suppression of key regulatory and neuronal pathways, a finding that aligns with earlier reports highlighting loss of normal neuronal function and differentiation in glioma progression (18). Functional enrichment analyses further supported these observations, demonstrating that dysregulated genes were primarily involved in neuronal development, signal transduction, and membrane potential regulation, which have been consistently implicated in glioma biology in prior studies (19).

Pathway analysis revealed significant enrichment in signaling cascades such as PI3K–Akt and MAPK pathways, which are well-established drivers of tumor proliferation, survival, and resistance mechanisms in glioma (20). These findings corroborate earlier evidence indicating that aberrant activation of these pathways plays a central role in glioma development and progression. The protein–protein interaction network further highlighted the importance of interconnected molecular mechanisms, with EP300 identified as a central hub gene interacting with multiple oncogenic and tumor suppressor pathways. Similar network-based studies have emphasized the role of hub genes in orchestrating complex tumor biology, supporting the relevance of the current findings.

The identification of key hub genes including EGFR, PTEN, JUN, and BCL2 suggested their potential utility as diagnostic biomarkers. The observed downregulation of EGFR in low-grade glioma contrasted with its well-documented overexpression in high-grade gliomas, reinforcing its potential role in differentiating tumor grades (21). Likewise, alterations in PTEN and JUN have been associated with tumor progression and cellular signaling dysregulation, while BCL2 has been linked to apoptosis resistance and tumor survival, findings that are consistent with previously reported literature (22) Other identified genes such as BRCA1, ATM, PIK3R1, EP300, and CREBBP appeared to be more closely associated with tumor progression and genomic stability, suggesting a stronger prognostic or therapeutic relevance rather than purely diagnostic utility. This distinction highlights the complexity of biomarker identification in glioma, where individual genes may serve different clinical roles depending on their functional context.

The biological roles of inflammatory mediators such as IL1B and signaling regulators like KRAS and NRAS further underscored the multifactorial nature of glioma pathogenesis. Previous studies have demonstrated that inflammatory signaling and RAS pathway activation contribute to tumor microenvironment modulation and disease progression, which is consistent with the interaction patterns observed in the present analysis. Additionally, the involvement of histone-related genes such as H3C12 and H3C13 suggested a contribution of epigenetic dysregulation, aligning with emerging evidence that chromatin remodeling and histone modifications play a critical role in glioma heterogeneity and aggressiveness (23).

Despite these strengths, the study had several limitations that warrant careful consideration. The relatively small sample size may have limited the statistical power and generalizability of the findings. Furthermore, the reliance on a single dataset without extensive external

validation restricted the ability to confirm reproducibility across independent cohorts. The absence of quantitative diagnostic performance evaluation, such as ROC curve analysis derived from actual data, limited the ability to definitively establish the clinical diagnostic value of the identified biomarkers (24,25). Additionally, the study was based entirely on in silico analysis, and the lack of experimental validation using laboratory-based techniques reduced the translational applicability of the findings. Technical constraints, including limited computational resources and restricted data access, may have further influenced the analytical depth.

Notably, while several genes demonstrated potential diagnostic relevance, the findings also reflected an ongoing debate in the field regarding the distinction between diagnostic and prognostic biomarkers. Many of the identified genes have been previously reported as prognostic indicators or therapeutic targets, suggesting that their diagnostic specificity may be limited when considered individually. This underscores the importance of integrating multiple biomarkers and employing advanced analytical models to improve diagnostic accuracy. Future research should therefore focus on validating these findings in larger, multi-center cohorts, incorporating machine learning approaches to develop robust multigene diagnostic signatures, and performing functional and experimental validation to confirm biological significance. Additionally, integrating clinical parameters and longitudinal data may further enhance the applicability of these biomarkers in clinical practice.

The study contributed to the growing body of evidence supporting the role of transcriptomic profiling in glioma research and highlighted several candidate genes with potential clinical relevance. While the findings provided valuable insights into the molecular landscape of low-grade glioma, further validation and methodological refinement are essential before these biomarkers can be translated into routine clinical use.

CONCLUSION

The present study provided a comprehensive transcriptomic evaluation of low-grade glioma, successfully identifying key molecular alterations and dysregulated pathways that contribute to its pathogenesis. Through integrative bioinformatics analysis, several differentially expressed genes and functionally significant pathways were characterized, highlighting disruptions in neuronal regulation, signal transduction, and tumor-associated signaling networks. The identification of central hub genes, particularly those involved in cell proliferation, apoptosis, and epigenetic regulation, underscored their potential relevance in the molecular distinction of glioma from normal brain tissue. Notably, selected genes demonstrated promising utility as diagnostic biomarkers, with the potential to aid in early detection and improve disease stratification. The findings were further supported by validation analyses, reinforcing the consistency of observed expression patterns. Overall, this study contributes meaningful insights into the molecular landscape of low-grade glioma and establishes a foundation for future research aimed at developing reliable diagnostic tools and advancing personalized therapeutic strategies.

AUTHOR CONTRIBUTION

Author	Contribution
Aqsa Naz	Conceptualization, Methodology, Formal Analysis, Writing - Original Draft, Validation, Supervision
Naima Iqbal	Methodology, Investigation, Data Curation, Writing - Review & Editing
Alisha Aftab	Investigation, Data Curation, Formal Analysis, Software
Falak Naz	Software, Validation, Writing - Original Draft
Nafeesa Tahir	Formal Analysis, Writing - Review & Editing
Saadia Momal Zafar*	Supervision, Conceptualization, Review & Editing
Rafia Anwer*	Writing - Review & Editing, Assistance with Data Curation

REFERENCES

1. Di W, Fan W, Wu F, Shi Z, Wang Z, Yu M, et al. Clinical characterization and immunosuppressive regulation of CD161 (KLRB1) in glioma through 916 samples. Cancer Sci. 2022;113(2):756-69.

2. Xiulin J, Wang C, Guo J, Wang C, Pan C, Nie Z. Next-generation sequencing identifies HOXA6 as a novel oncogenic gene in low grade glioma. Aging (Albany NY). 2022;14(6):2819-54.

3. Kessler T, Schrimpf D, Doerner L, Hai L, Kaulen LD, Ito J, et al. Prognostic Markers of DNA Methylation and Next-Generation Sequencing in Progressive Glioblastoma from the EORTC-26101 Trial. Clin Cancer Res. 2023;29(19):3892-900.

4. White K, Connor K, Meylan M, Bougoüin A, Salvucci M, Bielle F, et al. Identification, validation and biological characterisation of novel glioblastoma tumour microenvironment subtypes: implications for precision immunotherapy. Ann Oncol. 2023;34(3):300-14.

5. Zhou Y, Xiao D, Jiang X, Nie C. EREG is the core onco-immunological biomarker of cuproptosis and mediates the cross-talk between VEGF and CD99 signaling in glioblastoma. J Transl Med. 2023;21(1):28.

6. Iser F, Hinz F, Hoffmann DC, Grassl N, Güngoör C, Meyer J, et al. Cerebrospinal Fluid cfDNA Sequencing for Classification of Central Nervous System Glioma. Clin Cancer Res. 2024;30(14):2974-85.

7. Leclair NK, Choudury A, Chen WC, Magill ST, McCortney K, Horbinski CM, et al. RNA splicing as a biomarker and phenotypic driver of meningioma DNA-methylation groups. Neuro Oncol. 2024;26(12):2222-36.

8. Li Z, Deng Z, Liu F, Li C, Yang K, Gong X, et al. Clinical sequencing reveals diagnostic, therapeutic, and prognostic biomarkers for adult-type diffuse gliomas. Heliyon. 2024;10(18):e37712.

9. Lu Y, Wang Z, Zhang D, Luo N, Yang H, Chen D, et al. Application of Circulating Tumor DNA in the Auxiliary Diagnosis and Prognosis Prediction of Glioma. Cell Mol Neurobiol. 2024;45(1):6.

10. Prince EW, Apps JR, Jeang J, Chee K, Medlin S, Jackson EM, et al. Unraveling the complexity of the senescence-associated secretory phenotype in adamantinomatous craniopharyngioma using multimodal machine learning analysis. Neuro Oncol. 2024;26(6):1109-23.

11. Zhou X, Ling Y, Cui J, Wang X, Long N, Teng W, et al. Mitochondrial RNA modification-based signature to predict prognosis of lower grade glioma: a multi-omics exploration and verification study. Sci Rep. 2024;14(1):12602.

12. Zou B, Li M, Zhang J, Gao Y, Huo X, Li J, et al. Application of a risk score model based on glycosylation-related genes in the prognosis and treatment of patients with low-grade glioma. Front Immunol. 2024;15:1467858.

13. Arora S, Nuechterlein N, Jensen M, Glatzer G, Sievers P, Varadharajan S, et al. Integrated transcriptomic landscape of medulloblastoma and ependymoma reveals novel tumor subtype-specific biology. bioRxiv. 2025.

14. Barcelos PM, Filgueiras IS, Nóbile AL, Usuda JN, Adri AS, de Alburquerque DG, et al. Gene regulatory networks analysis for the discovery of prognostic genes in gliomas. Sci Rep. 2025;15(1):14034.

15. Lan Z, Xiao Y, Liao Y, Li X, Zhang Y, Wang H, et al. NID2 Affects Prognosis of Glioma via Activating the Akt Signaling Pathway. Int J Mol Sci. 2025;26(8).

16. Latowska-Łysiak J, Zarębska Ż, Sajek MP, Grabowska A, Buratin A, Głodowicz P, et al. Transcriptome-wide analysis of circRNA and RBP profiles and their molecular relevance for GBM. Mol Oncol. 2025;19(8):2270-91.

17. Long S, Xu H, Li M, Wang L, Jiang J, Wei W, et al. Predicting podoplanin expression and prognostic significance in high-grade glioma based on TCGA TCIA radiomics. PLoS One. 2025;20(6):e0325964.

18. Rajakaruna P, Rios S, Elnahas H, Villanueva A, Uribe D, Leslie S, et al. Molecular Biomarkers of Glioma. Biomedicines. 2025;13(6).

19. Schmid S, Mirchia K, Tietze A, Liu I, Siewert C, Nückles J, et al. VGLL fusions define a new class of intraparenchymal central nervous system schwannoma. Neuro Oncol. 2025;27(4):1031-45.

20. van den Bent MJ, Franceschi E, Touat M, French PJ, Idbaih A, Lombardi G, et al. Updated EANO guideline on rational molecular testing of gliomas, glioneuronal, and neuronal tumors in adults for targeted therapy selection-Update 1. Neuro Oncol. 2025;27(2):331-7.

21. Wang W, Li J, He Q, Liu C, Wang S, Zheng Z, et al. Integrated Analysis to Reveal Heterogeneity of Tumor-Associated Neutrophils in Glioma. Cancer Med. 2025;14(5):e70745.

22. Yan Y, Chu Z, Zhong Q, Wang G. Single-cell sequencing combined with machine learning to identify glioma biomarkers and therapeutic targets. Front Oncol. 2025;15:1629102.

23. Zhang J, Liu G, Wang D, Bu C, Lv G, Zhang Z, et al. Dynamic Tumor in Situ Fluid Circulating Tumor DNA Postsurgery Effectively Predicts Recurrence and Clinical Benefits for Glioblastomas. Neurosurgery. 2025;97(3):671-80.

24. Zhao MY, Shen ZL, Dai H, Xu WY, Wang LN, Gu Y, et al. Single-cell sequencing elucidates the mechanism of NUSAP1 in glioma and its diagnostic and prognostic significance. Front Immunol. 2025;16:1512867.

25. Zhao YH, Cai YX, Pan ZY, Tang F, Ma C, Wang ZF, et al. Novel CHI3L1-Associated Angiogenic Phenotypes Define Glioma Microenvironments: Insights From Multi-Omics Integration. Cancer Sci. 2025;116(5):1433-48.