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Research paper

Immunogenomic Profiling Reveals a Suppressive Tumor Microenvironment and Immune Evasion Signatures In Glioblastoma

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Abstract

Glioblastoma (GBM) is an aggressive brain tumor known for its poor prognosis and resistance to immunotherapy, largely due to a profoundly immunosuppressive tumor microenvironment. Understanding the immunogenomic landscape of GBM is critical for identifying biomarkers and therapeutic targets. Transcriptomic data from 45 GBM and 8 normal neural stem cell (NSC) samples were analyzed to iden-tify differentially expressed genes (DEGs). Functional enrichment analysis (GO and KEGG), immune cell infiltration scoring (xCell), and immune checkpoint gene profiling were performed to characterize the tumor immune microenvironment. A total of 15,405 DEGs were iden-tified, including 2,044 upregulated and 13,361 downregulated genes. Notably, AGBL5, FDPS, SLC25A3, NONO, ZDHHC16, and NUTF2 were among the most significantly upregulated genes in GBM. GO and KEGG analyses revealed enrichment of cell cycle and ECM remodeling pathways among upregulated genes, and suppression of immune receptor and cytokine signaling pathways among down-regulated genes. Immune profiling showed increased infiltration of immunosuppressive M2 macrophages and reduced NK and dendritic cell presence in GBM. Furthermore, immune checkpoint genes CD274 (PD-L1), CTLA4, and LAG3 were significantly upregulated, indicating a tumor environment favoring immune evasion. These findings reveal that GBM promotes tumor progression and immune evasion through coordinated gene expression changes and cellular reprogramming of the tumor microenvironment. The identified genes and immune signa-tures represent potential candidates for further investigation as biomarkers and therapeutic targets for overcoming GBM-associated immu-nosuppression.

Keywords: *Glioblastoma; Immunogenomics; Immune Evasion; Immune Suppression; Tumor-Infiltrating Immune Cells; Tumor Microenvironment.*

1. Introduction

Glioblastoma (GBM) is the most common malignant primary brain tumour, accounting for nearly 57% of all gliomas and 48% of primary malignant tumours of the central nervous system [1]. Despite advances in multimodal management, including maximal safe surgical resection, radiotherapy, systemic therapies such as chemotherapy and targeted agents, and comprehensive supportive care, the prognosis remains dismal, with long-term survival still uncommon [2]. Progressive neurologic decline and deterioration in quality of life further compound the disease burden, profoundly affecting patients, clinicians, and families. Advancements in precision oncology and immunotherapy are opening new opportunities to develop more effective therapeutic strategies against this highly aggressive malignancy [3]. Glioblastomas typically present at a median age of 64 years, though they can occur across the lifespan, including in children. Their incidence is slightly higher in men than in women (1.6:1) and is more common in Caucasians compared with other ethnic groups [4], [5]. GBMs are broadly classified as either primary (de novo), arising without a known precursor lesion, or secondary, evolving from a pre-existing lower-grade glioma. Most GBMs are primary, and these cases usually occur in older patients and are associated with a poorer prognosis than secondary GBMs [4]. Immunotherapy has shown significant promise in managing several solid tumours, such as melanoma, renal cell carcinoma, and non-small cell lung cancer, leading to interest in its application for GBM treatment [5]. The ability of the immune system to attack cancer cells upon identification is known as immunosurveillance, which leads to emerging tumour cells, forming the foundation of modern cancer immunotherapeutic strategies. The immunosurveillance concept was first proposed by William Coley in the 1890s and later expanded by Ehrlich, Thomas, and Burnet in the mid-20th century. This theory evolved with the recognition that tumours can escape detection through immunoediting, resulting in the evasion of the immune system by cancer cells [9]. Cancer immunotherapy seeks to counteract these escape mechanisms and restore effective antitumor immunity, a goal advanced significantly by the development of immune checkpoint inhibitors [4]. Immunotherapies have leveraged immune checkpoints, which are physiological regulators that maintain self-tolerance, such as PD-1/PD-L1 and CTLA-4, to suppress the host's antitumor immunity. Immune checkpoint inhibitors (ICIs),

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monoclonal antibodies that block these suppressive signals, prevent tumour-driven immune downregulation and have transformed treatment for several solid cancers. Anti-PD1 therapies have improved survival in hepatocellular carcinoma, non-small cell lung cancer, renal cell carcinoma, melanoma, and urothelial cancer, while anti-CTLA4 therapy has shown clear benefits in metastatic melanoma and is being explored in other malignancies [5]. Despite the success of ICIs in several malignancies, they are not universally effective across all solid tumours, and many patients experience limited benefit alongside significant toxicity. In a 2019 retrospective cross-sectional study evaluating six anti-CTLA4 and anti-PD1 agents, it was reported that only 43.6% of cancer patients in 2018 were eligible for immunotherapy, and the estimated overall response rate was just 12.46%, with substantial variability across tumor types. Patient-specific factors and tumor biology further influence these outcomes [6]. Phase III trials conducted between 2015 and 2019 demonstrated response rates ranging from 19% to 60%, with higher rates typically seen in carefully selected populations or in studies combining ICIs with other treatments such as chemotherapy. These variations underscore the many barriers that still limit the effectiveness of immunotherapy in solid tumors [4]. One of the major impediments to the successful management of GBM is GBM's ability to circumvent the host's immune response through a range of cellular, molecular, and epigenetic mechanisms that impair immune surveillance and induce immunotherapy-refractory responses. Key to this immunosuppression is the reprogramming of the TME to promote immunosuppressive cells, specifically tumor-associated macrophages (TAMs), regulatory T cells (Tregs), and myeloid-derived suppressor cells (MDSCs), that together dampen the activation of and cytotoxic activity of T cells [10]. Notably, the accumulation of M2-polarized macrophages is strongly associated with glioblastoma progression, as these cells promote tumor growth, suppress antitumor immune responses, and correlate with poor clinical outcomes [11], [12]. At the molecular level, GBM tumors utilize immune checkpoint pathways like PD-1/PD-L1 and CTLA-4 to further suppress the action of cytotoxic T lymphocytes, leading to a condition of T cell exhaustion and functional dysfunction [13], [14]. Recent work has also emphasized the contribution of glioma-intrinsic pathways in promoting immune evasion. GBM stem-like cells, for instance, are subjected to epigenetic immunoediting, where persistent transcriptional programs are established that preferentially direct the recruitment of suppressive myeloid cells while suppressing immunogenicity [15]. In addition to remodeling the local tumor environment's immune landscape, such mechanisms also make the tumor refractory to immune checkpoint inhibitors [12]. Concurrent with this, glioblastoma-derived extracellular vesicles that are PD-L1-positive suppress the activation of the T cell systemically, showing that GBM immune suppression is not contained to the confines of the tumor [12]. Although several biomarkers of immune evasion and suppression, CD274 (PD-L1), CTLA4, LAG3, CD163, and THEMIS2, have been implicated in GBM [10], [11], an urgent need still exists for profiling methods where transcriptomic information is integrated with an analysis of infiltrating immune cells to determine resilient immunomodulatory signatures. Such methods are capable of revealing the major mechanisms of immune escape and pinpointing key targets for precision immunotherapy. This paper aims to examine immunogenomic characterization of GBM versus normal stem cell (NSC) tissue through differential gene expression, gene ontology (GO), KEGG pathway enrichment, immune cell infiltration scoring, and checkpoint molecule analysis. Our goal is to identify specific immunosuppressive biomarkers and outline the molecular mechanisms that could result in GBM immune evasion, to guide the biomarker-based therapeutic approaches.

2. Methods

2.1. Data acquisition and preprocessing

Transcriptomic data for GBM and both control sample and normal stem cell (NSC) samples were downloaded from the NCBI Gene Expression Omnibus databases [16] (Ascension No. GSE119834). The raw gene expression counts were normalized using the DESeq2 package in R (v4.5.0), and only genes above a certain expression threshold were kept. These benchmarks were set to maintain significant expression across the samples in question.

2.2. Data validation and assessment

The supplementary files provided by the original study listed 98 samples; however, only 96 samples were available and processable in the NCBI-generated raw counts matrix. This discrepancy arises because the GEO count matrix (BETA version) is generated exclusively from FASTQ files successfully deposited and processed via the NCBI Sequence Read Archive (SRA), whereas author-supplied supplementary files may include QC-failed, incomplete, or non-SRA samples [23]. To maintain analytical consistency, only the 96 samples present in the NCBI-generated matrix were used for downstream analysis, as these represent validated, complete, and uniformly processed sequencing data. Data integrity was verified by confirming that all 96 samples matched the GEO metadata and that sample identifiers aligned correctly with the metadata table constructed for grouping (GBM, GNC, NSC). The structure and dimensions of the raw count matrix were inspected in R (v4.5.0) to ensure correct formatting and sample naming consistency. Genes with zero or extremely low counts were filtered out using the criterion rowSums(counts) > 10, thereby removing transcripts with insufficient read support and improving statistical reliability. Additional validation and quality-control procedures were performed to ensure dataset robustness. Library size distributions across samples were visualized using boxplots (Supplementary Figure S1), and Principal Component Analysis (PCA) was used to assess sample clustering according to biological groups and detect potential outliers (Supplementary Figure S2). A sample-to-sample distance heatmap was generated to quantify global transcriptomic similarity, confirming the homogeneity and integrity of the dataset (Supplementary Figure S3). DESeq2 normalization and variance-stabilizing transformation (VST) were applied to detect irregular count distributions or anomalous samples, and sample clustering patterns were reviewed to verify correct biological grouping. Batch effects were assessed through principal component analysis (PCA) and sample clustering approaches. No significant batch-driven separation was observed; therefore, no additional batch correction was applied.

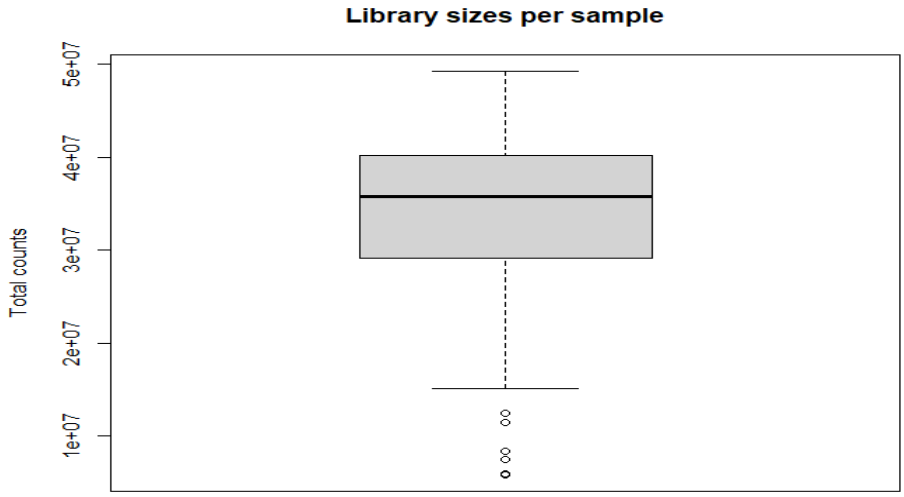


Fig. S1: Distribution of Total Read Counts Across Samples (Library Size Boxplot).

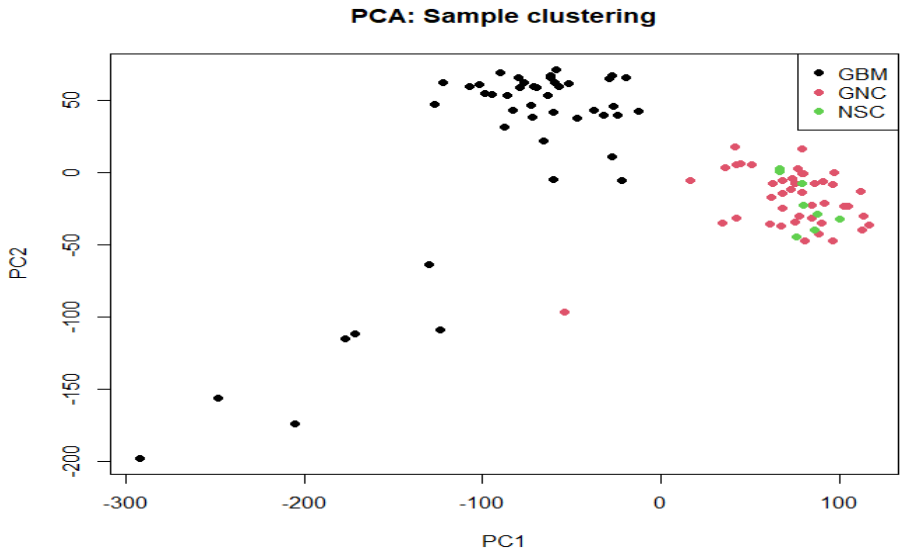


Fig. S2: Principal Component Analysis Revealing Sample Clustering by Group (PCA Plot).

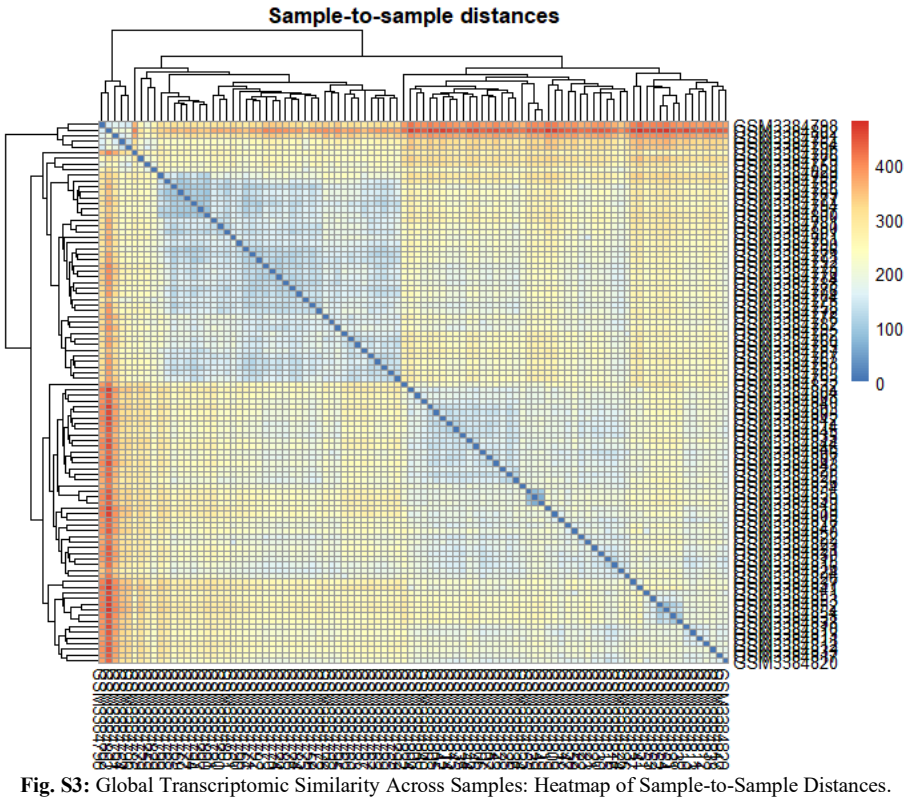


Fig. S3: Global Transcriptomic Similarity Across Samples: Heatmap of Sample-to-Sample Distances.

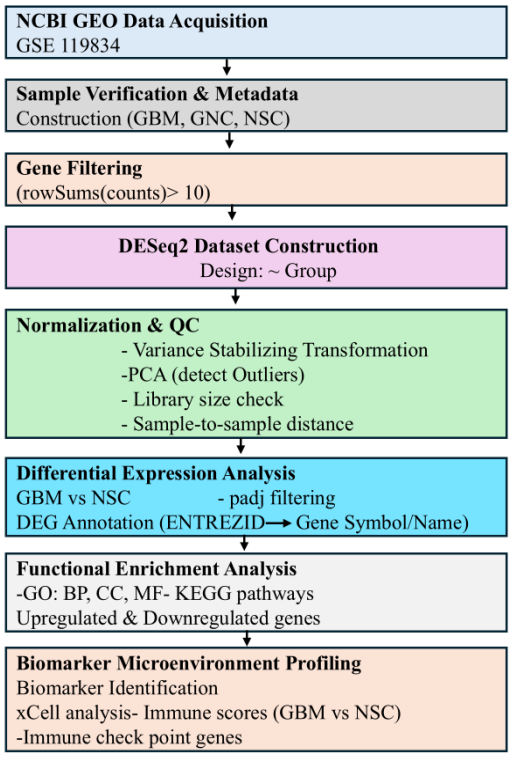


Fig. S4: Analysis Workflow.

2.3. Differential gene expression analysis (DEG)

Raw gene-level count data were imported into DESeq2 (v1.42.0) and combined with the manually created metadata table. A DESeqDataSet object was constructed using the model design = ~ Group. After low-count filtering, differential expression analysis was performed using the DESeq() pipeline, which applies normalization, dispersion estimation, and negative binomial modelling. Pairwise comparison between GBM and NSC was performed using the contrast argument in DESeq2. Log2 fold-change estimates were further refined using apeglm shrinkage to improve effect-size accuracy. Genes were considered significantly differentially expressed based on padj < 0.05 and |log2FoldChange| > 1. The threshold of |log2FoldChange| > 1 was selected to ensure biological relevance by focusing on genes with at least a two-fold change in expression, a commonly used cutoff in transcriptomic studies. Significant genes were separated into upregulated and down-regulated categories for further analyses [17].

2.4. Gene annotation

Differentially expressed genes (DEGs) were annotated using the org. Hs.eg.db database. Entrez IDs were mapped to gene symbols and official gene names using the select () function from AnnotationDbi. Annotated DEGs were merged with statistical outputs to generate a biologically interpretable result set suitable for functional profiling.

2.5. Gene ontology (GO) and KEGG pathway enrichment analysis

Gene Ontology (GO) enrichment analysis was performed using clusterProfiler (v4.10.0). Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) categories were assessed independently for both upregulated and downregulated DEGs. Enrichment significance was determined using the Benjamini-Hochberg adjusted p-value (p-adj < 0.05). Pathway enrichment analysis was conducted using KEGG (Kyoto Encyclopedia of Genes and Genomes) through the enrichKEGG () function in clusterProfiler, specifying hsa as the organism code. The top enriched GO terms and KEGG pathways were visualized using bar plots.

2.6. Immune microenvironment profiling

To assess immune cell infiltration patterns, normalized expression data were transformed using a variance-stabilizing transformation (VST). Immune cell enrichment scores were computed using the xCell algorithm, which infers relative abundances of multiple immune and stromal cell populations from bulk RNA-seq data. Gene symbols were matched with expression matrix identifiers before applying xCellAnalysis (). Immune scores were transposed and combined with sample group information, and differences between GBM and NSC were visualized using grouped boxplots. Specific immune populations of interest, including CD8+ T cells, macrophages, dendritic cells, NK cells, and M2 macrophages, were compared statistically using t-tests. Additionally, expression levels of key immune checkpoint genes (CD274, CTLA4, LAG3, CD8A, GZMB, CD68, and CD163) were extracted and visualized to characterize immunosuppressive features of the GBM micro-environment.

2.7. Data visualization

Plots were generated using ggplot2 in R. GO and KEGG enrichment results were visualized as bar plots. Immune cell scores and gene expression comparisons were presented using boxplots with statistical annotations.

2.8. Statistical analysis

All statistical analyses were conducted in R (v4.5.0). For two-group comparisons, due to potential non-normal distribution of immune cell scores, non-parametric Wilcoxon rank-sum tests were used for group comparisons. Multiple testing corrections were applied using the Benjamini-Hochberg procedure where appropriate.

2.9. Data quality and validation

RNA-seq data from 96 GBM, GNC, and NSC samples were subjected to comprehensive quality control. PCA of variance-stabilized counts revealed clear clustering by biological group, although seven GBM samples and one GNC sample appeared as outliers (Supplementary Figure S2). Sample-to-sample distance analysis corroborated these findings, indicating strong intra-group similarity for the remaining samples. Library size evaluation and variance-stabilizing transformation (VST) confirmed consistent sequencing depth and expression distribution across the dataset. Density distributions and hierarchical clustering dendrograms further supported overall dataset integrity.

2.10. Screening of DEGs in GBM and neural stem cell (normal)

A total of 45 GBM and 8 NSC from NCBI were used in this study to identify the DEGs between GBM patient brain tissues and normal neural stem cells. We detected a total of 15,405 differentially expressed genes (2,044 up-regulated and 13,361 down-regulated genes) based on selection criteria. In addition, AGBL5, ZDHHC16, FDPS, NONO, SLC25A3, and NUTF2 were identified because these six genes are presented as highly up-regulated (Table 1). GBM and normal samples were screened for DEGs through the construction of volcano plots (Figure 1).

3. Results

Table 1: DEGs of Interest. Expression Level of 12 Selected Genes Significantly Up- Or Down-Regulated in GBM Primary Tumors Based on Analysis of NCBI GEO -GBM Samples

Gene ID (ENTREZID)	Gene Name	Log2FoldChange
60509	AGBL5	1.423797
84287	ZDHHC16	1.816341
2224	FDPS	2.041001
4841	NONO	1.570249
5250	SLC25A3	1.515132
10204	NUTF2	1.746994
84966	IGSF21	-13.4799
140689	CBLN4	-11.6385
8857	FCGBP	-10.2511
4583	MUC2	-12.063
2857	GPR34	-6.88241
1436	CSF1R	-6.26999

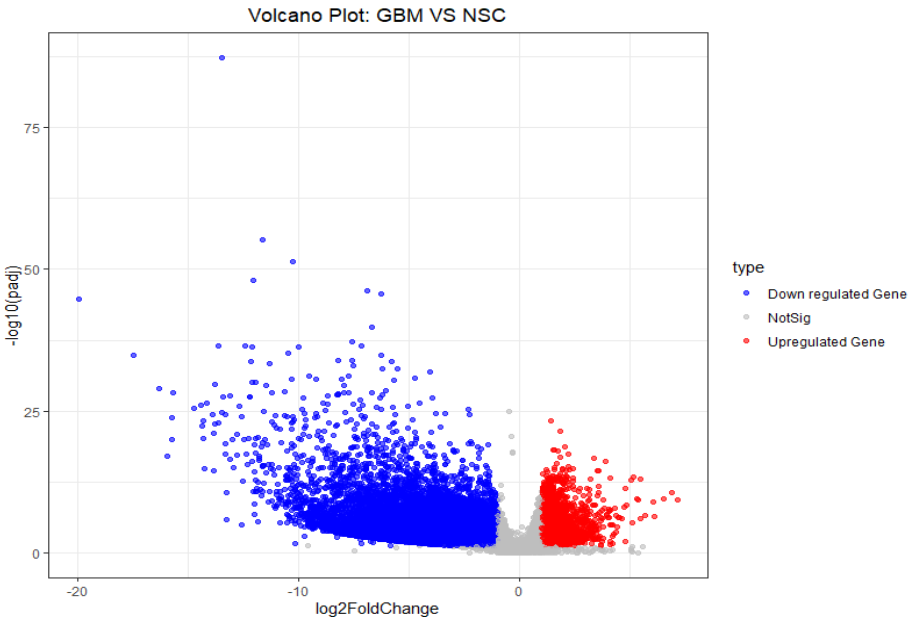


Fig. 1: Volcano Plots Showing the Log2 Fold Change of DEGs. The Red Nodes Represent the Up-Regulated DEGs, and the Blue Nodes Represent the Down-Regulated DEGs. The Grey Region Refers to DEGs that are Considered Insignificant.

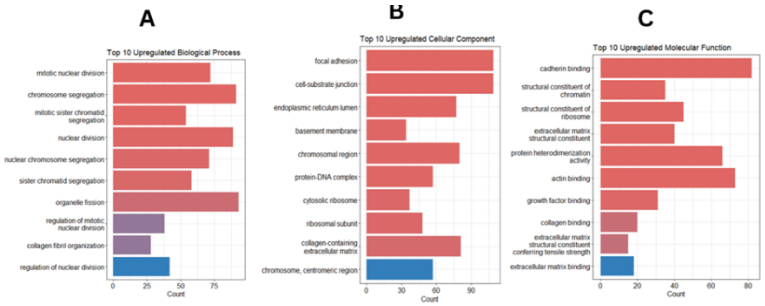


Fig. 2: Enrichment GO Analysis Results of DEGs Associated with Glioblastoma TUMOR Tissue (GBM) And Normal Stem Cell (NSC). A) Enriched GO Terms of Up-Regulated Genes, Biological Process. B) Enriched GO Terms of Up-Regulated Genes, Cellular Component. C) Enriched GO Terms of Up-Regulated Genes. Molecular Function. C) Most Significantly Enriched KEGG Pathway of the Up-Regulated DEGs. GO Refers to Gene Ontology; BP for Biological Process; MF for Molecular Function; CC for Cell Component; KEGG for Kyoto Encyclopedia of Genes and Genomes.

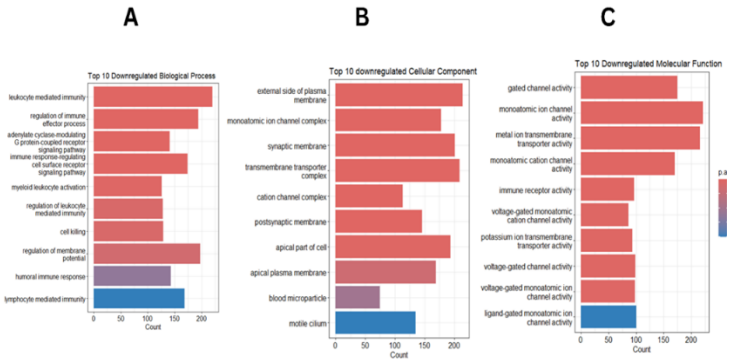


Fig. 3: Enrichment GO Analysis Results of DEGs Associated with Glioblastoma TUMOR Tissue (GBM) and Normal Stem Cell (NSC). A) Enriched GO Terms of Down-Regulated Genes, Biological Process. B) Enriched GO Terms of Down-Regulated Genes, Cellular Component. C) Enriched GO Terms of Down-Regulated Genes, Molecular Function.

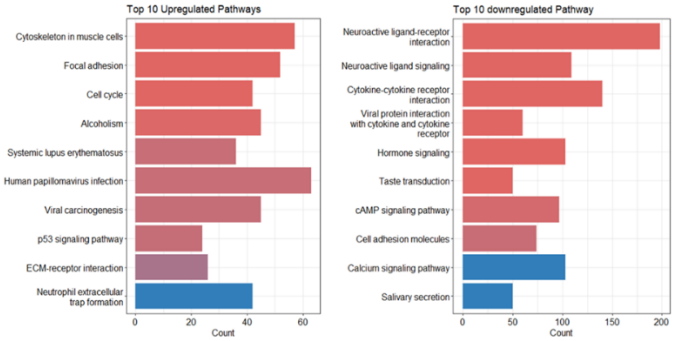


Fig. 4: Most Significantly Enriched KEGG Pathways of the Up-Regulated and Down-Regulated DEGs. KEGG For Kyoto Encyclopedia of Genes and Genomes.

3.1. GO and KEGG pathway enrichment analysis of differentially expressed genes

As shown in Figure 2 (Panel A-C, top row), upregulated DEGs were significantly enriched in biological processes (BP) related to cell division and mitosis, including mitotic nuclear division, chromosome segregation, sister chromatid segregation, and organelle fission. This shows an enhanced proliferative activity within the tumor microenvironment. In terms of cellular component (CC), upregulated genes were localized primarily to focal adhesions, cell-substrate junctions, endoplasmic reticulum lumen, and the extracellular matrix, suggesting structural remodeling and increased adhesion capacity. For molecular function (MF), genes were involved in cadherin binding, actin binding, extracellular matrix structural components, and heterodimerization activity, reflecting dynamic cell-cell and cell-matrix interactions. In contrast, downregulated DEGs (Figure 3) were highly enriched in immune-related biological processes, which include leukocyte-mediated immunity, lymphocyte-mediated immunity, myeloid leukocyte activation, and cell killing, indicating widespread suppression of anti-tumor immune functions. GO cellular component terms were predominantly associated with plasma membrane, ion channel complexes, synaptic membranes, and transporter complexes, suggesting impaired signaling and communication. Corresponding molecular functions showed significant reductions in gated channel activity, ion transport, immune receptor activity, and ligand-gated ion channel function, pointing to diminished immune and sensory responses in GBM. KEGG analysis further supported the GO results. As shown in Figure 4 (Left Panel), upregulated DEGs were enriched in pathways related to structural dynamics, immune signaling, and cell proliferation, which include the cytoskeleton in muscle cells, focal adhesion, ECM-receptor interaction, cell cycle, and the p53 signaling pathway. Notably, the neutrophil extracellular trap formation pathway was also enriched, indicating possible immune system dysregulation or inflammation. Additional enrichment in Systemic lupus erythematosus and Human papillomavirus infection pathways suggests overlap with known inflammatory and viral response mechanisms. Conversely, downregulated DEGs (Figure 4, Right Panel) were primarily associated with neuroimmune and receptor signaling pathways, which included neuroactive ligand-receptor interaction (involving ~200 genes), hormone signaling, cytokine-cytokine receptor interaction, and calcium signaling. These results underscore a substantial suppression of signaling pathways critical for immune and neural communication. Enrichment of salivary secretion and taste transduction pathways further points to systemic sensory and secretory dysfunctions associated with GBM progression.

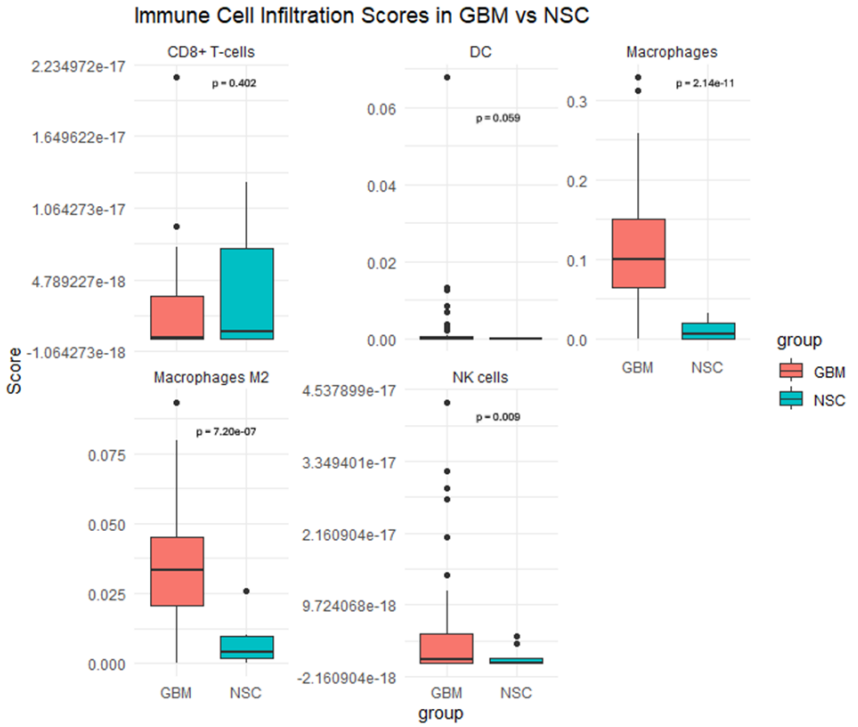


Fig. 5: Immune Cell Infiltration Score in GBM and NSC.

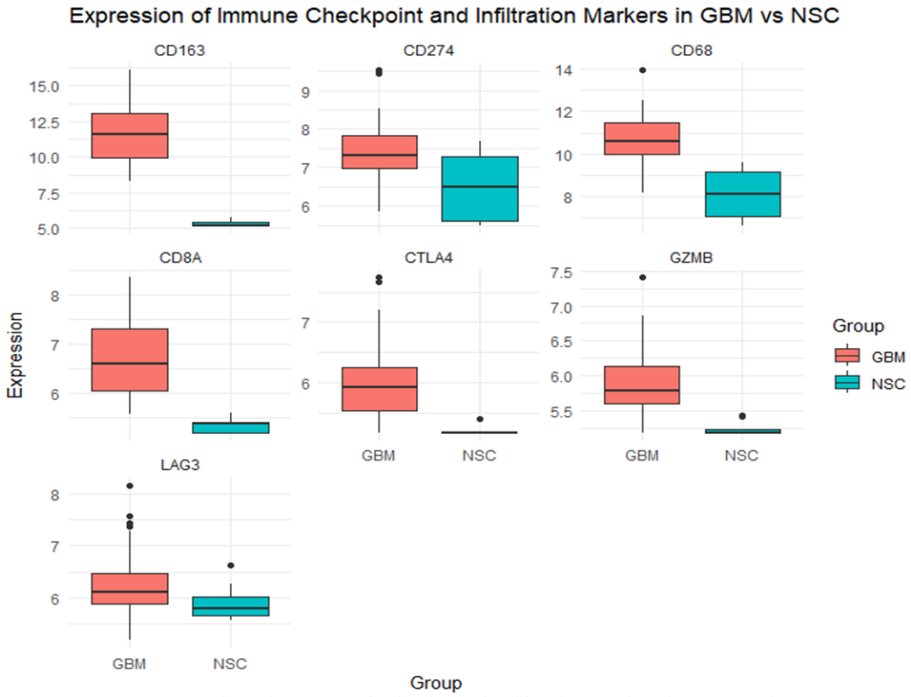


Fig. 6: Expression of Immune Checkpoint and Infiltration Markers in GBM and NSC.

3.2. Characterization of the immunosuppressive landscape in glioblastoma

To further characterize the immune landscape of glioblastoma (GBM), immune cell infiltration scores and immune-related gene expression compared to normal stem cells (NSC) were analysed. Figure 5 shows the infiltration analysis, which revealed a significantly elevated presence of macrophages in GBM ($p = 2.14 \times 10^{-11}$), particularly of the M2 subtype ($p = 7.20 \times 10^{-7}$), suggesting the dominance of immunosuppressive, tumour-supportive myeloid cells within the tumour microenvironment. Natural killer (NK) cell infiltration was significantly reduced in GBM ($p = 0.009$), indicating potential evasion of innate immune surveillance. Although dendritic cell scores trended lower in GBM ($p = 0.059$), this difference was not statistically significant. $CD8^+$ T cell infiltration did not differ significantly between GBM and NSC ($p = 0.402$), suggesting a similar level of T cell presence, though this does not preclude functional impairment. In Figure 6, gene expression analysis supported these observations. Expression of CD68 and CD163, markers for macrophages and M2 macrophages, respectively, was elevated in GBM, consistent with increased macrophage infiltration. Immune checkpoint genes, including CD274 (PD-L1), CTLA4, and LAG3, were also upregulated in GBM, reflecting a tumour environment favouring immune evasion through T cell inhibition or exhaustion. CD8A and GZMB were modestly elevated, indicating some cytotoxic T cell presence and activity, although likely constrained by the immunosuppressive environment. Collectively, these findings highlight that GBM establishes a strongly immunosuppressive tumour microenvironment, driven by macrophage enrichment and immune checkpoint upregulation, which likely impairs effective antitumor immune responses and presents a major barrier to immunotherapy.

4. Discussion

This study provides a comprehensive immunogenomic analysis of glioblastoma (GBM) by comparing transcriptomic profiles between 45 GBM patient brain tissue samples and 8 normal neural stem cell (NSC) samples obtained from the NCBI Gene Expression Omnibus. Our differential gene expression analysis identified a total of 15,405 differentially expressed genes (DEGs), with 2,044 genes upregulated and 13,361 downregulated in GBM. This pronounced transcriptional repression in the tumour samples is consistent with global immune dysregulation and reduced expression of neuroimmune signalling genes, as also observed in other transcriptome-based studies of GBM [15]. Among the top upregulated genes, AGBL5, ZDHHC16, FDPS, NONO, SLC25A3, and NUTF2 stood out for their significantly elevated expression levels. Many of these genes are implicated in metabolic reprogramming, RNA splicing, or transport processes, hallmarks of cancer cell survival and proliferation. For instance, FDPS (farnesyl diphosphate synthase) is involved in the mevalonate pathway and has been shown to promote tumour growth via cholesterol biosynthesis and membrane integrity in rapidly dividing cancer cells [18]. SLC25A3, a mitochondrial phosphate transporter, is often overexpressed in metabolically active tumours and supports energy production critical for GBM progression [19]. Similarly, NONO has been identified as a multifunctional nuclear protein involved in RNA processing and DNA repair, processes frequently hijacked in cancer cells to support their survival under genotoxic stress [11]. These transcriptional patterns were visualized using volcano plots, which clearly demonstrated the distinct separation between up- and downregulated genes (Figure 1). The extent of transcriptional downregulation in immune-related and synaptic genes likely reflects a systemic suppression of immune surveillance and neural communication in the GBM tumour microenvironment. Supporting this, Gene Ontology and KEGG pathway analyses showed that downregulated DEGs were heavily enriched in pathways involved in immune receptor signalling, cytokine-to-cytokine interactions, and neuroactive ligand-receptor signalling. These changes align with the known immune evasion strategies employed by GBM, including reduced antigen presentation, T cell exclusion, and suppression of interferon signalling [12], [20]. Notably, the tumour immune microenvironment (TME) in GBM is dominated by immunosuppressive macrophages, with low infiltration of effector T cells and dendritic cells, patterns that were evident in our immune cell scoring analysis. In line with this, key immune checkpoint genes such as PD-L1 (CD274), CTLA4, and LAG3 were significantly overexpressed in GBM samples, suggesting that exhausted T cell phenotypes may be present despite modest expression of CD8A and GZMB. These findings are consistent with recent reports that identify PD-L1+ tumour-associated macrophages and dysfunctional T cells as central mediators of immune resistance in GBM [10], [21], [24]. Taken together, our findings demonstrate that GBM is associated with widespread transcriptional suppression of immune signalling and a coordinated upregulation of tumour-supportive metabolic and immune evasion genes. This dual mechanism of immune exclusion and checkpoint activation underscores the difficulty in eliciting effective antitumor immune responses in GBM and highlights the urgent need for combinatorial therapeutic strategies that can overcome these layered barriers to immune activation [22]. The highly expressed DEGs identified in this study, particularly AGBL5, FDPS, and SLC25A3, may represent promising candidates for further experimental validation as biomarkers or therapeutic targets for GBM progression and immune modulation. However, these findings should be interpreted in light of several limitations, including the relatively small number of control samples, potential dataset-specific biases, and the lack of experimental validation, which may affect the generalizability and translational applicability of the results.

5. Conclusion

Our immunogenomic analysis revealed that glioblastoma exhibits widespread transcriptional suppression of immune-related pathways alongside upregulation of tumour-promoting genes such as AGBL5, FDPS, and SLC25A3. The tumour microenvironment was characterized by high infiltration of immunosuppressive macrophages and elevated expression of immune checkpoints, including PD-L1, CTLA4, and LAG3, suggesting a dual mechanism of immune evasion and tumour progression. These findings highlight key genes and pathways that may serve as potential targets pending further experimental validation for future therapeutic interventions aimed at overcoming GBM's immunosuppressive landscape.

6. Significance of the Study

This study provides additional insight into the immunogenomic architecture of glioblastoma, a cancer that remains one of the most treatment-resistant tumours in medicine. The comparison of cancerous brain tissue with healthy brain cells makes us identify the important changes that happen inside glioblastoma tumours. These changes include certain genes that become unusually active, as well as signs that the tumour creates an environment that weakens the body's natural immune defences. Understanding these unique patterns is important because it can help in the discovery and development of better ways to detect glioblastoma early, predict how it may behave, and design treatments that are more effective and personalized. The findings also point to new targets for therapy that could make future treatment options stronger and more successful. Hence, this study serves as a strong basis for developing next-generation diagnostic and immunotherapeutic agents aimed at treating glioblastoma and overcoming the cancer immune evasion mechanisms.

7. Limitations

This study relied solely on publicly available data, which limited control over sample quality and clinical details. The number of glioblastoma samples was much larger than the number of normal samples, which may affect the strength of comparisons. The analysis was entirely computational, and none of the findings were confirmed in laboratory experiments. The bulk RNA data that is utilized also prevents pinpointing which specific cell types are responsible for the observed changes. Finally, the dataset lacked patient information such as survival, treatment history, or tumour subtype, restricting the ability to link the findings to real clinical outcomes.

8. Recommendation

Future studies to validate these findings with laboratory experiments are recommended, especially using larger, well-annotated datasets that include clinical details to examine the genes identified in this study to clarify their roles differentially in glioblastoma growth and immune suppression.

Acknowledgement

The authors express their sincere gratitude to the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) for providing open access to the transcriptomic dataset used in this study. We also acknowledge the developers of the bioinformatics tools and R packages employed in the analysis for their valuable contributions to open science. The constructive feedback and guidance received from colleagues and mentors during the course of this research are deeply appreciated.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Data Availability

All data used and analyzed during the present study are available in the NCBI Gene Expression Omnibus databases [16] (Accession No. GSE119834).

Ethics Approval and Consent to Participate

This study did not involve human participants or animal experiments. All analyses were performed using publicly available, de-identified data obtained from the NCBI Gene Expression Omnibus (GEO) database (Accession No. GSE119834). Therefore, ethical approval and informed consent were not required. The study was conducted in accordance with the principles of the Declaration of Helsinki and institutional research policies for secondary data analysis.

Abbreviations

GBM: Glioblastoma
TME: Tumor environment
TAM: Tumor-associated macrophage
Tregs: Regulatory T cells
MDSC: Myeloid-derived suppressor cell
NSC: Normal stem cell
GO: Gene ontology
DEG: Differentially expressed genes
BP: Biological process
CC: Cellular component
MF: Molecular function
NK: Natural killer
FDPS: Farnesyl diphosphate synthase

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