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ANXA2 is a Potential Biomarker Associated with Poor Prognosis in Glioma

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Abstract: *ANXA2, a calcium-dependent phospholipid-binding protein, regulates critical physiological processes such as membrane trafficking, signal transduction, and cell adhesion. While accumulating evidence implicates ANXA2 in tumor progression across multiple malignancies, its functional and prognostic significance in glioma remains poorly defined. In this study, we utilized The Cancer Genome Atlas (TCGA), the Genotypic Tissue Expression (GTEx) database, and the Chinese Glioma Genome Atlas (CGGA) to investigate the associations between ANXA2 and clinicopathological and molecular features of glioma patients, as well as the correlation of patient prognosis. Our study showed that high expression of ANXA2 was significantly associated with poor patient prognosis. Further analysis of the biological role of ANXA2 in gliomas revealed that ANXA2 is involved in a variety of biological processes involved in glioma development, including immune responses, cellular activation, and modulation of immune system defenses. Immuno-infiltration analysis of the TIMER database revealed a correlation between high ANXA2 expression and infiltration of various immune cells as well as immune checkpoints in glioma. In summary, ANXA2 represents a promising potential biomarker for clinical diagnosis and therapeutic targeting in glioma.*

Keywords: *glioma, ANXA2, prognosis, biomarker*

1. Introduction

Gliomas represent the most prevalent and lethal primary malignancies of the central nervous system (CNS), characterized by intricate pathogenesis, therapeutic resistance, and dismal clinical outcomes[1]. The World Health Organization (WHO) classification stratifies these tumors into grades I-IV based on histopathological features, with grade IV glioblastoma multiforme (GBM) constituting the most aggressive subtype[2,3]. In the United States, the incidence of gliomas is about 7/10 million, and about 49% of primary malignant brain tumors are glioblastomas[4].Therefore, in-depth study of the molecular mechanism of GBM occurrence and exploration of effective therapeutic targets are of great significance for improving the prognosis of patients.

ANXA2 (also known as Annexin A2), a key member of the annexin family, is a calcium-dependent phospholipid-binding protein composed of 339 amino acids [5]. It is widely expressed in various cell types and tissues and can form a heterotetrameric complex with S100A10 through its N-terminal domain, an interaction critical for its membrane localization and functional regulation [6,7].Recent studies have demonstrated that ANXA2 is significantly overexpressed in multiple malignancies, including breast cancer and GBM. Its pro-tumorigenic mechanisms primarily involve promoting tumor angiogenesis and inducing epithelial-mesenchymal transition (EMT), thereby accelerating tumor metastasis [8,9].

This study reveals overexpression of ANXA2 in glioma, with its expression levels significantly correlated with malignant tumor phenotypes and poor patient prognosis. Through differentially expressed gene (DEG) enrichment analysis and immune cell infiltration analysis, we demonstrated that high ANXA2 expression is closely associated with GBM progression. The integrated findings indicate that ANXA2 not only serves as a potential diagnostic biomarker for GBM, but may also represent a novel therapeutic target for intervention.

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2. Materials and Methods

2.1. Data Collection

Clinical data and RNA-sequencing (RNA-seq) profiles of glioma patients were obtained from two primary genomic repositories: The Cancer Genome Atlas (TCGA; http://cancergenome.nih.gov/) and The Chinese Glioma Genome Atlas (CGGA; http://www.cgga.org.cn/). Normal brain tissue gene expression data were acquired from The Genotype-Tissue Expression (GTEx) project (https://xenabrowser.net/datapages/)[10]. Three glioma-related RNA-seq datasets (GSE151352, GSE263588, GSE147352) were retrieved from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) .Furthermore, genes co-expressed with ANXA2 in gliomas were extracted from:The cBioPortal database (http://www.cbioportal.org/)[11].

2.2. Differential Expression Analysis

We evaluated the mRNA expression differences of ANXA2 across various malignancies using:The TIMER2.0 online analysis platform (http://timer.cistrome.org/)[12],The GEPIA2 tool (http://gepia2.cancer-pku.cn/)[13].Additionally, we compared ANXA2 protein expression levels across different tissues and obtained immunohistochemical (IHC) images of glioma versus normal brain tissues from:The Human Protein Atlas (HPA; https://www.proteinatlas.org)[14].

2.3. Clinical Prognostic Analysis

To evaluate the prognostic significance of ANXA2, we performed survival analysis using the following R packages:"limma" for differential expression analysis,"survival" and "survminer" to generate Kaplan-Meier survival curves,"timeROC" to plot receiver operating characteristic (ROC) curves for 1, 3 and 5 year survival rates.Additionally, we assessed the association between ANXA2 gene expression and clinicopathological characteristics using the "ggpubr" R package.

2.4. GO Enrichment and KEGG Pathway Analysis

We performed Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis using the "clusterProfiler" R package to identify biological pathways associated with ANXA2 expression in target genes. A false discovery rate (FDR)-adjusted $p < 0.05$ was considered statistically significant.

2.5. Immune Infiltration Analysis

We obtained glioma immune cell infiltration data from the CIBERSORT database (http://CIBERSORT.stanford.edu/)[15]. Differences in immune cell infiltration levels between ANXA2-high and ANXA2-low glioma samples were assessed using Wilcoxon rank-sum test. Spearman's correlation analysis was performed to calculate correlation coefficients between ANXA2 expression levels and various immune cell types, with statistical significance set at $P<0.05$. Furthermore, we employed Pearson correlation analysis to identify immune checkpoints significantly associated with ANXA2 expression, using a stringent threshold of $P<0.001$.

3. Results

3.1. ANXA2 is Overexpressed in Multiple Cancer Types

To investigate the differential expression profiles of ANXA2 across various malignancies and their matched normal tissues, the mRNA expression levels of ANXA2 were first analyzed using the TIMER2.0 database (Figure 1A). Subsequent validation was performed through integration of TCGA and GTEx datasets via the GEPIA2 web platform to systematically evaluate ANXA2 expression patterns in neoplastic tissues (Figure 1B). The integrated analysis revealed significant upregulation of ANXA2 in seven cancer types: cervical squamous cell carcinoma (CESC), colon adenocarcinoma (COAD), diffuse large B-cell lymphoma (DLBCL), glioblastoma multiforme (GBM), kidney renal papillary cell carcinoma (KIRP), hepatocellular carcinoma (HCC), and stomach adenocarcinoma (STAD).

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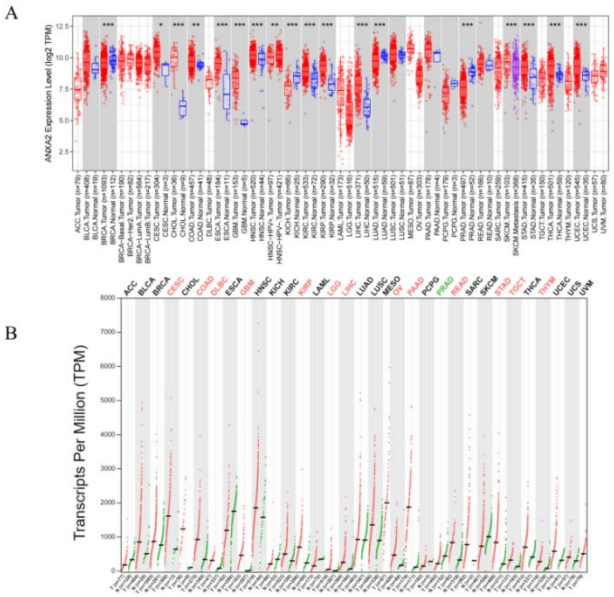
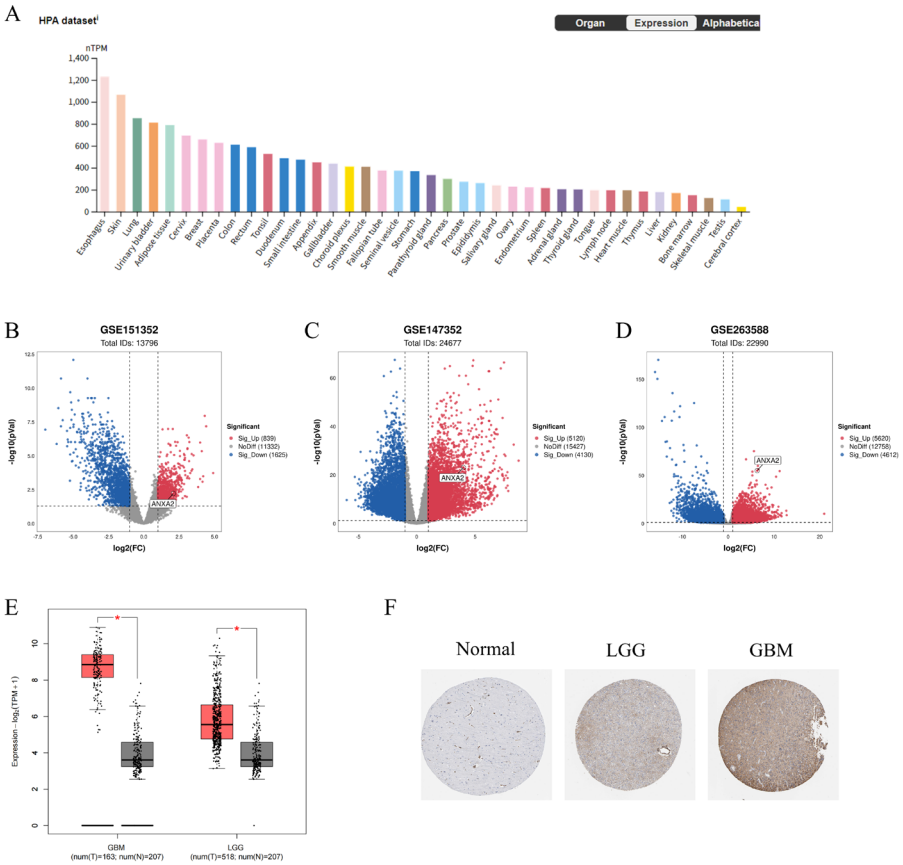


*A: mRNA expression levels of ANXA2 across pan-cancer analyses (TIMER2.0 database). B: ANXA2 expression in pan-cancer tissues from the TCGA/GTEx databases, analyzed using the GEPIA2 online platform. (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).*

Figure 1: Differential expression of ANXA2 between tumor and normal tissues.



A: ANXA2 mRNA expression across normal human tissues (Human Protein Atlas, HPA). B-D: Differential ANXA2 expression in glioma versus normal brain tissues from three GEO datasets (GSE151352, GSE263588, GSE147352). E: GEPIA2 analysis confirming elevated ANXA2 mRNA levels in glioblastoma (GBM) and lower-grade glioma (LGG) compared to normal brain.F: IHC images from HPA .

Figure 2. ANXA2 overexpression in gliomas

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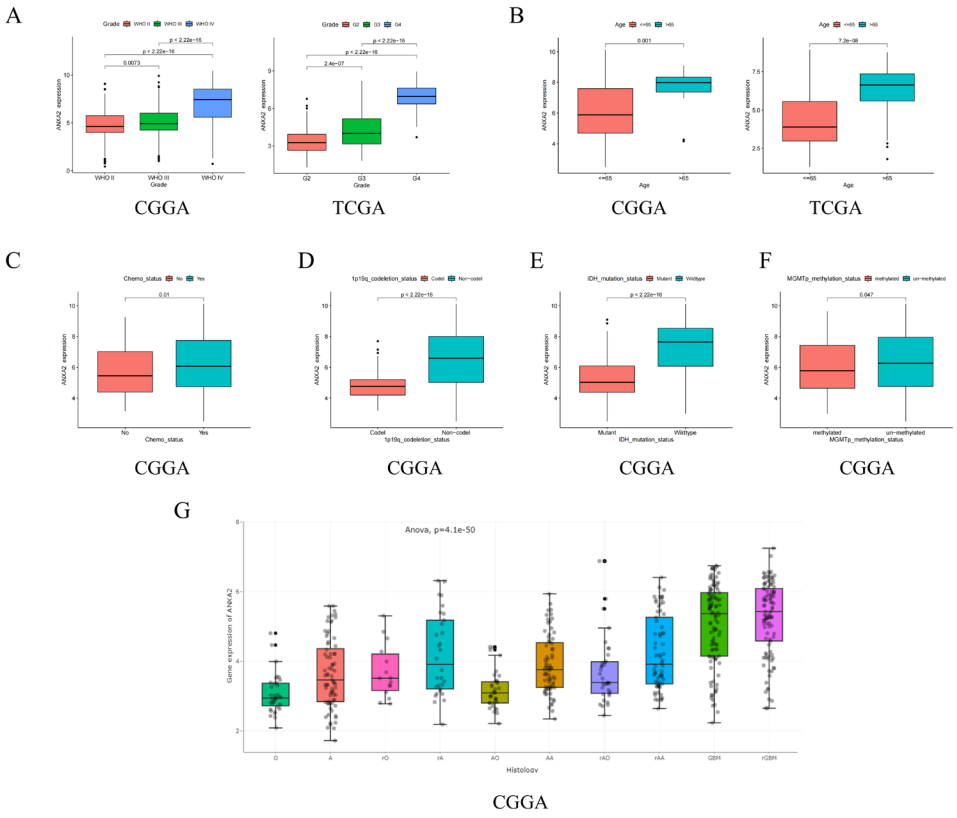
3.2. ANXNA2 is Highly Expressed in Glioma

To further investigate ANXA2 expression patterns, we analyzed its mRNA levels across human tissues using the Human Protein Atlas (HPA) database. The results showed that ANXA2 mRNA was highly expressed in tissues such as the esophagus, skin, and lung, while exhibiting the lowest expression in normal brain tissue (Figure 2A). This finding led us to hypothesize that ANXA2 may play a critical role in glioma pathogenesis.We then retrieved glioma-related RNA-seq datasets (GSE151352, GSE263588, and GSE147352) from the Gene Expression Omnibus (GEO) database, all of which included both normal brain and glioma tissue samples. Differential gene expression analysis revealed consistent upregulation of ANXA2 in gliomas across all three datasets, as demonstrated by volcano plots (Figures 2B-D).Furthermore, analysis via GEPIA confirmed that ANXA2 mRNA levels were significantly elevated in both glioblastoma (GBM) and lower-grade glioma (LGG) compared to normal brain tissue (Figure 2E). This observation was further supported by IHC staining data from the HPA database, which showed increased ANXA2 protein expression in glioma samples (Figure 2F).In summary, our integrated multi-dataset analysis consistently demonstrates that ANXA2 is overexpressed in gliomas, suggesting its potential involvement in tumor development and progression.

3.3. ANXA2 Overexpression is Significantly Associated with Clinicopathological and Molecular Features in Gliomas



A: Expression levels of ANXA2 in glioma patients in the TCGA and CCGA databases in relation to WHO classification.B-C: Expression levels of ANXA2 in relation to patient age and chemotherapy status.D-F: Expression levels of ANXA2 in relation to genetic molecular alterations in patients (1p/19q co-deletion, IDH mutation, and MGMT promoter methylation).F: ROC curves plotted based on the CGGA database. ROC curves drawn from the CGGA database.

Figure 3: Relationship between ANXA2 expression and clinical and genetic molecular features of gliomas

We analyzed the expression of ANXA2 in gliomas of different grades in the CGGA and TCGA databases and found that ANXA2 expression was positively correlated with the WHO grade of gliomas (Figure 3A). Specifically, the expression level of ANXA2 showed a significant up-regulation trend as the WHO grade of gliomas increased. In addition, the expression level of ANXA2 was also positively correlated with patient age and chemotherapy status (Figure 3B- C), suggesting that ANXA2 may be

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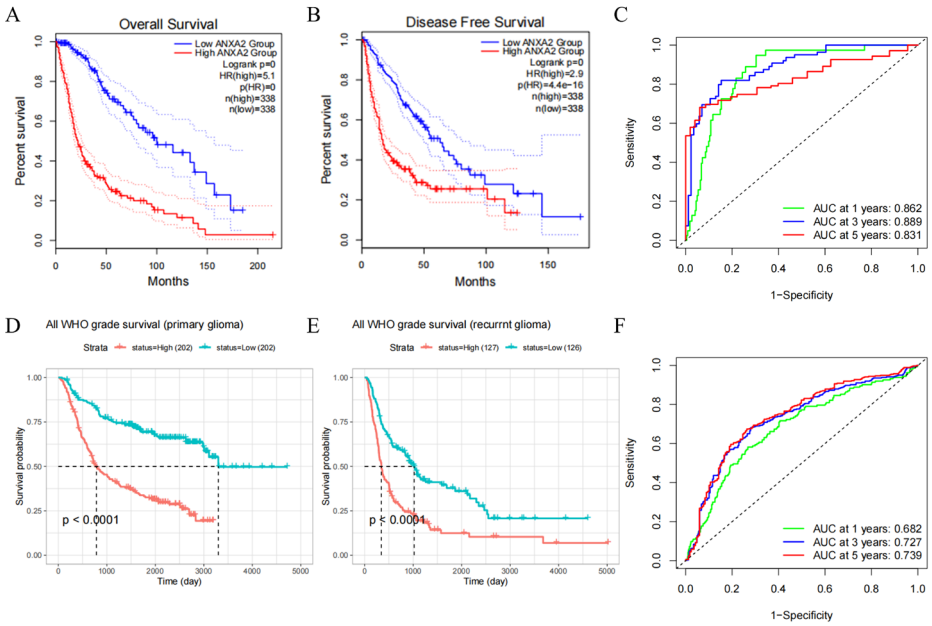
associated with treatment resistance and poor prognosis in glioma patients. Previous studies have shown that IDH mutations, 1p/19q co-deletion and MGMT promoter methylation are important molecular alterations in glioma development and are closely associated with patient prognosis[16]. We evaluated these genetic molecular alterations in glioma patients in the CGGA database and found that the expression levels of ANXA2 were significantly lower in the 1p/19q co-deletion, IDH mutation, and MGMT promoter methylation groups than in the wild-type group (Fig. 3D-F). In terms of histopathologic classification, recurrent glioblastoma had the highest ANXA2 expression level (Figure 3G). These results suggest that ANXA2 may act as an oncogene in gliomas and is closely associated with malignant progression of gliomas, treatment resistance, and poor patient prognosis.

3.4. Anxna2 Overexpression is Associated With Poor Prognosis in Glioma Patients

To clarify the impact of high ANXA2 expression in gliomas on patients' prognosis, we obtained clinical data from glioma patients in the TCGA and CGGA databases and categorized the patients into high- and low-expression groups according to the expression level of ANXA2. In the TCGA database, patients with high expression of ANXA2 had significantly lower overall survival (OS) and disease-free survival (DFS) than those in the low-expression group ($p < 0.001$) (Fig. 4A-B). Moreover, in the CGGA database, the survival of the low-expression ANXA2 group was significantly longer than that of the high-expression group in patients with primary gliomas and recurrent gliomas ($p < 0.001$) (Figure 4D-E). This suggests that upregulation of ANXA2 is associated with poor patient prognosis. To further assess the predictive value of ANXA2 expression levels on the prognosis of glioma patients, we plotted ROC curves at 1, 3, and 5 years and calculated the area under the curve (AUC). We found that in the CGGA dataset, the AUC values at 3 and 5 years were greater than 0.7, whereas in the TCGA dataset, the AUC values at 3 and 5 years were greater than 0.8 (Figure 4C-F). This shows that high expression of ANXA2 is significantly associated with poor prognosis of glioma patients, and its expression level has high accuracy in predicting the prognostic risk of patients.



A-C: Overall Survival (OS), Disease-Free Survival (DFS) Curves and ROC Curves for Glioma Patients Based on TCGA Database. D-E: The correlation between the expression level of ANXA2 and the survival of patients with primary and recurrent glioma in the CGGA database.F: ROC curves plotted based on the CGGA database.

Figure 4: The expression level of ANXA2 can predict the prognosis of glioma patients

3.5. Mutation and Potential Function Analysis of ANXNA2

To systematically investigate the functional implications of ANXA2 in glioma pathogenesis, we first characterized its genomic alteration patterns in glioma patients utilizing the cBioPortal database. The results of the analysis showed that the mutation rate of ANXA2 in glioma patients was low, and the most common type of molecular alteration was mutation with an incidence of no more than 3% (Figure

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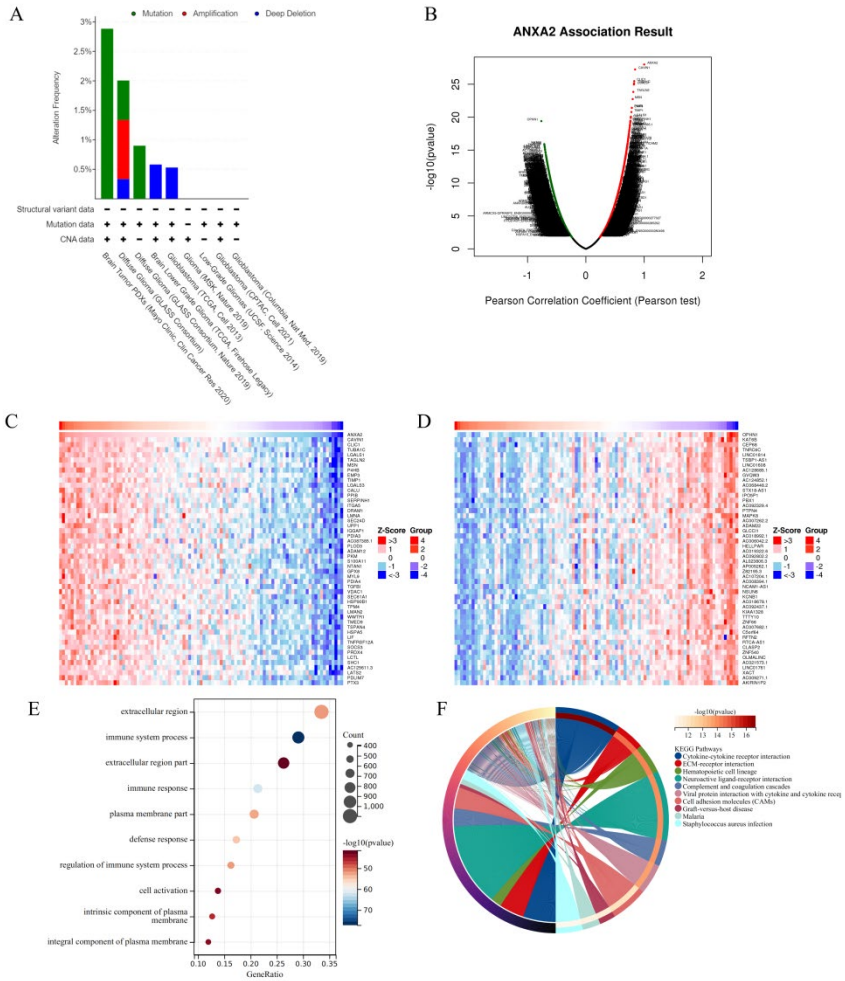
5A). We used Pearson correlation analysis to screen 50 important genes that were significantly associated with ANXA2 expression, which were demonstrated by heatmap. The results showed that CAVIN1, CLIC1, TUBA1C and METTL21A were significantly and positively correlated with ANXA2 expression, while CEP68, KAT6B and OPHN1 were significantly and negatively correlated with ANXA2 expression (Fig. 5B-D). Based on the expression levels of ANXA2 in gliomas, we divided the glioma samples in the TCGA database into high and low expression groups and analyzed the differentially expressed genes (DEGs) between the two groups using R language. To explore the biological pathways associated with these DEGs, we performed GO enrichment analysis. The results showed that DEGs were mainly enriched in biological processes such as immune response, cell activation, and regulation of immune system defense responses (Figure 5E). In addition, we performed KEGG pathway enrichment analysis of ANXA2-associated DEGs and found that these genes were significantly enriched in several biological pathways, including cytokine-ligand receptor interactions, extracellular matrix receptor interactions, and neuroactive ligand-receptor interactions (Figure 5F). The results of these analyses provide important molecular mechanistic clues to further understand the function of ANXA2 in gliomas.



A: Characterization of gene alterations of ANXA2 in cBioPortal database. b-d: LinkedOmics online database of genes co-expressed with ANXA2. e: causal GO analysis of differential bases. f: causal KEGG analysis of differential bases.

Figure 5: Gene alterations and potential biological functions of ANXA2

3.6. ANXNA2 Expression Level Affects Immune Cell Infiltration in Gliomas

Since the infiltration of immune cells in the tumor microenvironment has an important predictive value for the prognosis of tumors[17], we scored the stromal and immune cells of glioma samples in the TCGA database and obtained a composite score (Figure 6A). The analysis results showed that the stromal score, immune score and composite score of the ANXA2 high-expression group were significantly higher than those of the ANXA2 low-expression group, a finding that suggests that the

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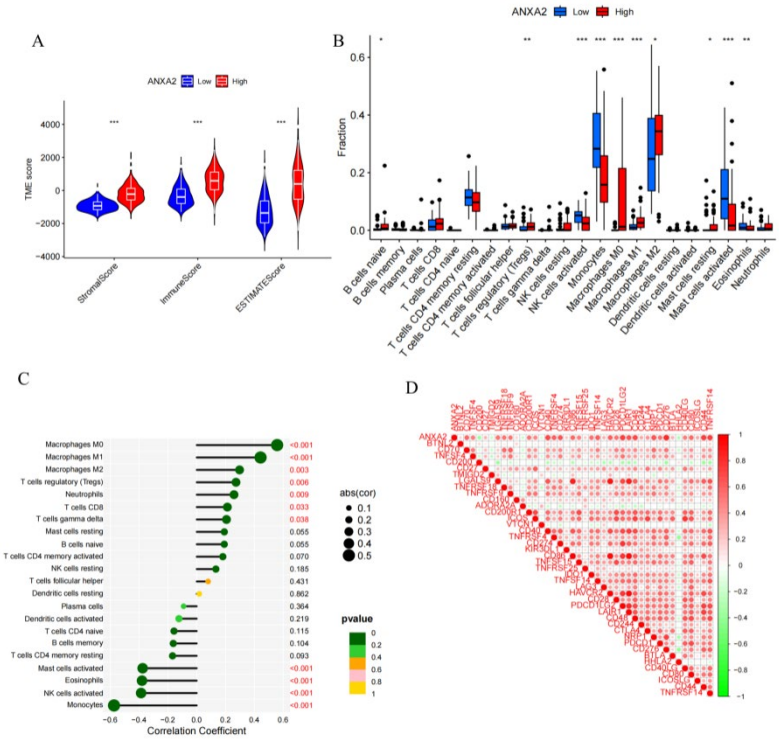
expression level of ANXA2 may have an important regulatory role in the composition and functional status of the tumor immune microenvironment. In addition, we further analyzed the differences in the distribution of 22 immune cells in gliomas between the high ANXA2 expression group and the low ANXA2 expression group(Figure 6B). The results showed that the infiltration levels of macrophages, regulatory T cells (Trg cells), neutrophils, $CD8^+$ T cells, and γδ T cells were significantly positively correlated with ANXA2 expression, whereas the infiltration levels of activated mast cells, activated natural killer (NK) cells, and monocytes were significantly negatively correlated with ANXA2 expression(Figure 6C). Considering that immune checkpoint molecules play a key role in regulating the immune response of immune cells to tumor cells, we further explored the correlation between ANXA2 expression levels and immune checkpoint molecules. We found that ANXA2 expression was significantly positively correlated with most immune checkpoint molecules (e.g., PD-1, CTLA-4, LAG-3, etc.)(Figure 6D). These results reveal the potential role of ANXA2 in regulating the infiltration of specific immune cell subpopulations, as well as influencing the sensitivity of tumors to immune checkpoint inhibitor therapy.



*A: Comparison of immune scores between ANXA2 high expression group and low expression group.B: Difference in infiltration of 22 TIICs between ANXA2 high expression group and low expression group.C: Bar graph of correlation between ANXA2 and immune cell infiltration.D: Correlation between ANXA2 and immune checkpoint genes. (Blue color indicates negative correlation, red color indicates positive correlation. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).*

Figure 6: Expression level of ANXA2 affects immune cell infiltration in gliomas

4. Discussion

Glioma represents the most aggressive primary malignant tumor of the central nervous system, characterized by extremely poor clinical prognosis with a 5-year survival rate of less than 5%[18]. The GBM microenvironment constitutes a complex immunosuppressive neuroinflammatory network, featuring enrichment of immunosuppressive cells but lacking T lymphocyte infiltration[19,20]. Therefore, there is an urgent need to intensively explore potential biomarkers that could help predict GBM patient prognosis or serve as targets for immunotherapy.

ANXA2 is a calcium-dependent phospholipid-binding protein widely involved in physiological processes such as membrane trafficking, signal transduction, and cell adhesion[21]. Our study reveals that ANXA2 is significantly upregulated in glioma tissues and is strongly associated with tumor stage, molecular genetic characteristics, and poor prognosis in glioma patients.Tumor immune evasion is a key mechanism by which tumors escape host immune surveillance[22]. We further explored the

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immunomodulatory function of ANXA2 in gliomas and found that ANXA2 is involved in multiple immune response pathways, showing significant correlations with immune cell infiltration and immune checkpoint expression in gliomas.

Although our current study provides insights into the relationship between ANXA2 and glioma, certain limitations remain. First, the lack of comprehensive clinical sample data limits a more robust interpretation of ANXA2's prognostic role in gliomas. Second, the precise mechanisms underlying ANXA2's immunomodulatory functions in gliomas require further investigation. Future studies will focus on elucidating these aspects to deepen our understanding of ANXA2's role in glioma progression, thereby providing stronger evidence for its potential as a therapeutic target or prognostic biomarker in glioma management.

5. Conclusions

Our study demonstrates that ANXA2 overexpression correlates with clinicopathological and molecular characteristics of glioma and predicts poor prognosis in glioma patients. Thus, ANXA2 may serve as a potential diagnostic biomarker and novel therapeutic target for glioma.

References

[1] Lim M, Xia Y, Bettegowda C, et al. Current state of immunotherapy for glioblastoma[J]. Nat Rev Clin Oncol, 2018, 15(7): 422-442.
[2] Chen R, Smith-Cohn M, Cohen A L, et al. Glioma Subclassifications and Their Clinical Significance[J]. Neurotherapeutics, 2017, 14(2): 284-297.
[3] Śledzińska P, Bebyn M G, Furtak J, et al. Prognostic and Predictive Biomarkers in Gliomas[J]. Int J Mol Sci, 2021, 22(19).
[4] Schaff L R, Mellinghoff I K. Glioblastoma and Other Primary Brain Malignancies in Adults: A Review[J]. Jama, 2023, 329(7): 574-587.
[5] Ma S, Lu C C, Yang L Y, et al. ANXA2 promotes esophageal cancer progression by activating MYC-HIF1A-VEGF axis[J]. J Exp Clin Cancer Res, 2018, 37(1): 183.
[6] Bharadwaj A G, Kempster E, Waisman D M. The ANXA2/S100A10 Complex-Regulation of the Oncogenic Plasminogen Receptor[J]. Biomolecules, 2021, 11(12).
[7] Hajjar K A, Jacovina A T, Chacko J. An endothelial cell receptor for plasminogen/tissue plasminogen activator. I. Identity with annexin II[J]. J Biol Chem, 1994, 269(33): 21191-7.
[8] Maule F, Bresolin S, Rampazzo E, et al. Annexin 2A sustains glioblastoma cell dissemination and proliferation[J]. Oncotarget, 2016, 7(34): 54632-54649.
[9] Wu B, Zhang F, Yu M, et al. Up-regulation of Anxa2 gene promotes proliferation and invasion of breast cancer MCF-7 cells[J]. Cell Prolif, 2012, 45(3): 189-98.
[10] GTEx Consortium. The Genotype-Tissue Expression (GTEx) project[J]. Nat Genet, 2013, 45(6): 580-5.
[11] Gao J, Aksoy B A, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal[J]. Sci Signal, 2013, 6(269): pl1.
[12] Li T, Fu J, Zeng Z, et al. TIMER2.0 for analysis of tumor-infiltrating immune cells[J]. Nucleic Acids Res, 2020, 48(W1): W509-w514.
[13] Tang Z, Kang B, Li C, et al. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis[J]. Nucleic Acids Res, 2019, 47(W1): W556-w560.
[14] Thul P J, Lindskog C. The human protein atlas: A spatial map of the human proteome[J]. Protein Sci, 2018, 27(1): 233-244.
[15] Chen B, Khodadoust M S, Liu C L, et al. Profiling Tumor Infiltrating Immune Cells with CIBERSORT[J]. Methods Mol Biol, 2018, 1711: 243-259.
[16] Appin C L, Brat D J. Biomarker-driven diagnosis of diffuse gliomas[J]. Mol Aspects Med, 2015, 45: 87-96.
[17] Ding X, Zhang L, Fan M, et al. TME-NET: an interpretable deep neural network for predicting pan-cancer immune checkpoint inhibitor responses[J]. Brief Bioinform, 2024, 25(5).
[18] Tykocki T, Eltayeb M. Ten-year survival in glioblastoma. A systematic review[J]. J Clin Neurosci, 2018, 54: 7-13.
[19] Jackson C M, Choi J, Lim M. Mechanisms of immunotherapy resistance: lessons from glioblastoma[J]. Nat Immunol, 2019, 20(9): 1100-1109.
[20] Bikfalvi A, Da Costa C A, Avril T, et al. Challenges in glioblastoma research: focus on the tumor

ISSN 2618-1584 Vol. 7, Issue 3: 8-16, DOI: 10.25236/FMSR.2025.070302

microenvironment[J]. Trends Cancer, 2023, 9(1): 9-27.
[21] Huang Y, Jia M, Yang X, et al. Annexin A2: The diversity of pathological effects in tumorigenesis and immune response[J]. Int J Cancer, 2022, 151(4): 497-509.
[22] Huang B, Zhang J, Zong W, et al. Myeloidcells in the immunosuppressive microenvironment in glioblastoma: The characteristics and therapeutic strategies[J]. Front Immunol, 2023, 14: 994698.