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Research Article

Glioblastoma Multiforme Prognostic Role of the GNAI3 Gene Family Inhibitors

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Abstract:

One of the most aggressive malignancies with a very poor overall prognosis is glioblastoma. Because the BBB obstructs medicine delivery and invasive techniques alone are ineffective in removing it entirely, GBM is extremely difficult to treat. To treat GBM, it is critical to identify the key pathways and biomarkers. We carried out this investigation with the objective to determine the pathways linked to GBM. To find the DEGs, we compared the GEO database to the TCGA GBM genomic data set. Using the CGGA dataset, we also looked at the predictive significance of GNAI family genes in GBM. G-protein alpha inhibiting subunit 3 (GNAI3) gene expression was strongly linked to a poor prognosis in the tumour microenvironment across the sample. The function of the GNAI3 gene was examined using MetaCore and GO analysis in conjunction with transcript analysis, co-expressed genes, and related signalling pathways like the "Immune response B cell antigen receptor (BCR) pathway" and "Cytoskeleton remodelling Regulation of actin cytoskeleton organisation by the kinase effectors of Rho GTPases." According to the results, the carcinogenesis of GBM patients was highly correlated with the GNAI family genes, particularly GNAI3. In conclusion, it was proposed that the GNAI3 genes could be a predictive biomarker for GBM.

Keywords: glioblastoma; genome; malignancy; bcr signaling pathway; molecular biomarkers; prognosis; signaling pathway; gpcrs

Introduction

Glial cells, which are supporting cells in the brain, give rise to glioblastoma, also known as glioblastoma multiforme (GBM), a very aggressive and lethal kind of brain tumour. This kind of tumour, called a glioma, arises from the glial cells that envelop and sustain nerve cells. GBM can develop in any part of the brain and is extremely invasive and fast-growing. Nonetheless, the cerebral hemispheres—the biggest region of the brain in charge of speech, memory, and movement—are where it most frequently arises. Rarely, it may also extend to other organs including parts of the central nervous system (CNS)[1]. Research suggests that certain genetic abnormalities and abnormal changes in the DNA of glial cells contribute to the growth of GBM, even if the exact aetiology of the disease is still unclear. While GBM may affect people of any age, older people are more likely to experience it. Depending on the tumor's size and location, GBM can present with a variety of symptoms, including recurring headaches, seizures, cognitive decline, behavioural or personality changes, limb weakness or numbness, difficulties speaking or seeing, and nausea or vomiting episodes. The tumor's pressure on the surrounding brain tissue or interference with its regular processes causes these symptoms [2]. A thorough review of the patient's medical history, a neurological examination, imaging tests such as computed tomography (CT) or magnetic resonance imaging (MRI), and a biopsy procedure—in which a sample of the tumour tissue is taken for additional analysis—are all part of the diagnostic process for GBM. Chemotherapy, radiation therapy, and surgical resection (removal) of the tumour are commonly used in the treatment of GBM. Maximising tumour removal while minimising damage to nearby healthy brain tissueis the primary goal of surgery. High-energy beams are used in radiation treatment after surgery to target and eliminate any tumour cells that may still be present. Medications are used in chemotherapy to either kill or stop the growth of cancer cells. Because of its infiltrative nature and high recurrence rate, GBM is still difficult to fully cure even with rigorous treatment techniques [3]. With a typical survival time of 12 to 15 months after diagnosis, the prognosis for GBM is dire. However, there is hope for better results and a higher quality of life for those with GBM because to continuous research and treatment improvements.

The roles of certain proteins within cellular signalling networks and their effects on various illnesses have drawn increasing attention from researchers in recent years. The GNAI family of proteins, in particular the G-protein alpha inhibiting subunit 3 (GNAI3), is one such protein family of interest [4]. GNAI proteins are essential members of the larger family of G-proteins, which are responsible for directing a variety of cellular functions by sending signals from cell surface receptors to the inside of cells. GNAI1, GNAI2, and GNAI3 are the three main members of the GNAI family. By reducing the activity of adenylcyclases, the enzymes responsible for producing the second messenger molecule cyclic adenosine monophosphate (cAMP), these proteins mostly block cellular signalling pathways. GNAI proteins reduce

cAMP levels by blocking adenylcyclases, which has an impact on cellular processes. Neurotransmission, hormone signalling, cell development, differentiation, and motility are just a few of the many functions that GNAI proteins perform in many organs and cell types. They can control downstream signalling pathways and physiological reactions by modulating cAMP levels through adenylcyclase activity reduction. Numerous illnesses and conditions have been linked to GNAI protein mutations or dysregulation. For example, changes in GNAI3 have been connected to growth hormone excess issues and specific types of pituitary tumours. Certain somatic overgrowth disorders have been shown to contain GNAI2 mutations. Additionally, it has been shown that GNAI protein dysregulation affects tumour development and metastasis in a number of cancers, including colorectal and breast cancer. The adaptability of the GNAI protein family—which includes GNAI1, GNAI2, and GNAI3—highlights its significance in cellular signalling and regulation, supporting a variety of physiological and pathological processes.

Specifically focussing on GNAI3, it is a gene that codes for the GNAI3 protein, a member of the GNAI family. The brain, heart, and skeletal muscle are among the tissues where GNAI3 exhibits action. Numerous studies have clarified the functions and consequences of GNAI3 in signalling networks and cellular functions. G-protein coupled receptor (GPCR) signalling is one of GNAI3's most important functions. When ligands activate particular GPCRs, GNAI3 is activated, separates from the receptor, and takes part in subsequent signalling cascades. GNAI3 reduces cAMP levels by blocking adenyl cyclase, an enzyme that produces cAMP, which in turn affects a number of cellular reactions. The effect that GNAI3 has on cardiac function within the cardiovascular system has been thoroughly investigated. It helps control the contractility and rhythm of the heart. Research on mice lacking GNAI3 has revealed changes in cardiac function, such as increased heart rate and contractility. These results highlight how important GNAI3 is for preserving healthy cardiovascular function. Furthermore, GNAI3 was implicated in the contraction of vascular smooth muscle cells, where its activation inhibits calcium signalling pathways, resulting in the relaxation and dilatation of blood vessels. These findings demonstrate the role that GNAI3 plays in blood vessel function and vascular health in general. GNAI3 was implicated in the regulation of neurotransmitter release inside the central nervous system. GNAI3 regulates synaptic transmission by lowering cAMP levels and adenylcyclase activity in neurones. The function of GNAI3 in regulating neurotransmission and preserving appropriate neuronal signalling is highlighted by this method. Additionally, several cancers have been linked to GNAI3 dysregulation. Several cancer forms, including lung adenocarcinoma, colorectal cancer, and breast cancer, have been found to contain genetic abnormalities or aberrant expression of GNAI3. In these situations, tumour development, metastasis, and treatment response may be impacted by GNAI3 dysregulation. Gaining insight into GNAI3's function in carcinogenesis might help with the creation of tailored treatments and personalised medicine strategies.

Finally, the intricate relationship between GBM and the GNAI3 protein family offers an intriguing study topic. The necessity for more research into their interactions and possible treatment strategies is highlighted by the aggressive nature of GBM and the many roles of GNAI3. Clarifying GNAI3's function in cellular functions and its consequences for illnesses like GBM can lead to the development of novel therapeutic approaches and, eventually, better patient outcomes. In order to identify a new potential biomarker for immunotherapy, we have tried to establish a connection between the GNAI3 and pathways that it regulates and immune infiltration in GBM. To determine the expression level, pancancer analysis, overall survival, molecular functions, biological processes, cellular activities, and immune infiltration, we conducted bioinformatics analysis. It was discovered that GNAI3's function included vital biological mechanisms that can encourage the growth and motility of cancer. We provide an overview of the procedure for a thorough survey of various members. GBM is one of the cancer possibilities that has genes encoding the GNAI family of proteins. The following are the interpretations of immunostaining, enrichment analysis, expression levels, and molecular processes and function (Figure 1).

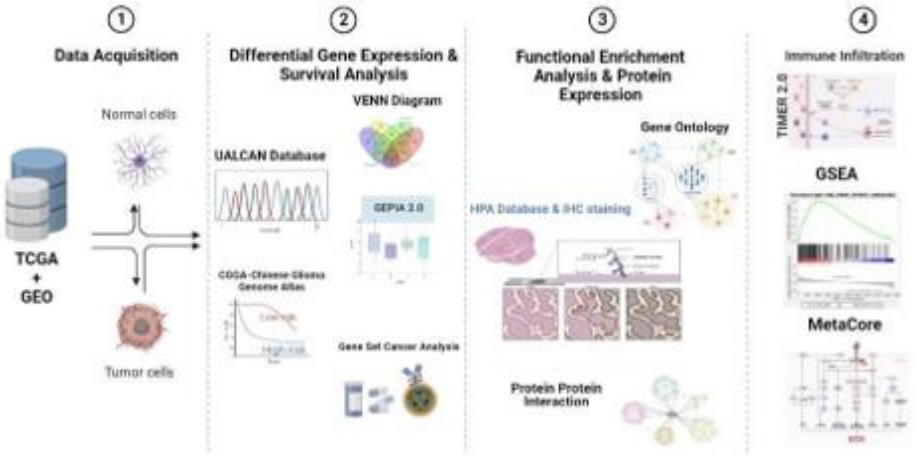


Figure 1: Work Flow summary. TCGA data was retrieved to find the DEGs to find the expression level, overall survival, immune infiltration, protein expression, GO functions, and pathway analysis.

Materials and Methods

DEGs and expressive genes and volcano plot

The DEGs for Glioblastoma Multiform were determined using data from The Cancer Genome Atlas (TCGA). The Glioblastoma TCGA data for DEGs between primary tumour tissues (602 samples) and solid normal tissues (12 samples) were found using data from the Xena Browser. Limma-Voom and Z normalisation were used to exclude variables that were unrelated or might have an impact on the research. GSE 182670 (15 samples), GSE 74187 (60 samples), and GSE 83300 (50 samples) in the Gene Expression Omnibus (GEO) database provided the microarray and overall survival data for 125 GBM samples. The shared DEGs between these datasets were discovered by combining them. Later, we employed the Volcano plot with statistical significance and log10-fold change = 1.5 to obtain better gene expression results.

UALCAN for Pan-Cancer Analysis

With the help of CSS and Javascript, UALCAN (http://ualcan.path.uab.edu) is an easy-to-use online data platform that provides clinical data for over 30 distinct cancer types. The cancer OMICS is publicly accessible. The

expression levels of the RNA sequence analysis of the genes in this database were obtained using the RSEM technique. To determine the gene relevance across various cancer types, transcripts per million (TPM) was employed. In Glioblastoma Multiform, this data has obtained the TCGA data with the GNAI family genes from tumour samples (n = 156) and normal samples (n = 5).

GEPIA box plots

The expression of GNAI1, GNAI2, and GNAI3 and their importance in GBM were examined using patient data from TCGA and GTEx that included solid normal tissues (n = 207 samples) and tumour tissues (n = 163 samples) using the online GEPIA tool (http://gepia2.cancer-pku.cn/). Log2FC=1 and a statistically significant log-rank P value <0.05 were employed.

CGGA data set for survival analysis

More than 2000 brain tumour samples have been clinically and sequenced by the open-access CGGA Chinese Glioma Genome Atlas database (http://www.cgga.org.cn/). The distribution of GNAI family gene mRNA expression levels in WHO grades I, II, and III as well as the expression of GNAI 2 and 3 in tumour samples with and without IDH mutations were obtained by accessing the CGGA data set. For both primary and recurrent gliomas, the Overall Survival analysis was conducted using a significant P value <0.05. The mRNA seq325 platform, which includes 325 samples (pLGG=144, rLGG=38, pGBM=85, rGBM=24, sGBM=30) of mRNA sequencing data utilising the Illumina High throughput sequencing platform, was used to conduct survival analysis for GNAI2 and GNAI3 [31].

Drug sensitivity

We utilised GSCA (Genome Set for Cancer Analysis) (http://bioinfo.life.hust.edu.cn/GSCA/), an integrated web program that incorporates TCGA data set for 30 cancer types for pharmacogenomic and imunogenomic cancer analysis, to comprehend the drug sensitivity for our particular genes. Two categories of drug data sets are available from GSCA: (1) GDSC (Genomics of Drug Senstivity in Cancers), which lists medications and uses IC50 values to correlate their sensitivity with a particular gene expression, and (2) CTRP (The Cancer Therapeutics Response Portal), which includes information on small molecules that target a particular gene or pathway.

Functional Enrichment Analysis GO

An online resource called cBioportal (https://www.cbioportal.org) contains datasets of various kinds that use mRNA expression to statistically describe gene correlation and co-expression. The TCGA dataset for Glioblastoma Multiforme (n=592 samples) was obtained by accessing this database. Using Spearman's correlation, the co-expression gene data was utilised for Gene Ontology enrichment analysis, including Biological Process (BP), Molecular Function (MF), and Cellular Function (CF). The KEGG pathway, which illustrates the connections and abundance of the pathways, is also included in the enrichment analysis. In the dot plots for the routes, the x-axis represents fold enrichment using -10 log (P-value) [5], [6], [7]. Shiny Go (http://bioinformatics.sdstate.edu/go/), an online bioinformatics tool for BP, MF, CF, and KEGG, was used to conduct the enrichment analysis [8]. The Metacore analysis (https://portal.genego.com/) for cell signalling pathway analysis was also carried out using it. Gene set enrichment analysis (http://software.broadinstitute.org/gsea) was also performed on this data set to determine the gene activities determined by normalised enrichment analysis (NES) and q value false discovery rate (FDR). The threshold was defined as the notional P value <0.05, q value<0.25, and NES value>1.5.

Protein-protein interaction

String analysis, an online tool that provides data for over ten different types of creatures and people, was used to do the protein-protein interaction (https://string- db.org/). It has 19303 protein linkage linkages. We analysed the genes belonging to the GNAI family in humans. An example of protein interactions is provided by the investigation that was done [9].

Timer 2.0 for immune infiltration

With TIMER 2.0 (http://timer.comp-genomics.org/), we investigated immune cell infiltration across 31 cancer types using TCGA datasets. Statistical significance and Spearman's correlation coefficient were employed. In GBM, we investigated the relationship between the highly expressive GNAI family genes and the infiltration of inflammatory cells and immunological infiltration in default immune cells, including CD4+ T cells, CD8+ T cells, B cells, macrophages, dendritic cells, and neutrophils.

Statsitics Analysis

To gather patient information and investigate how the GNAI family gene affects overall survival (OS), the online CGGA data was utilised. A cBioPortal (https://www.cbioportal.org/dataset) called the TCGA Pan-cancer Atlas was accessed. Spearman correlation and statistical significance were used to ascertain the relationship between tumour immune cells and the expression of GNAI family genes. The default parameters were used to continue the survival research. Using a log-rank p-value of less than 0.05 was considered statistically significant.

Results

Expression of GNAI-3 in pancancer analysis by DEGs using TCGA data

We collected the TCGA data set using the Xena browser to determine the DEGs between the primary tumour and normal tissue samples. Then, using the Venn diagram, we compared it with the GSE 74187, GSE 83300, and GSE 182670 from the GEO database to determine the expression of the GNAI protein family in various cancers (Figure 2). Out of all the data sets, it revealed that 47.5% of genes are present. We created the volcano plot among the data set by setting a criterion of log2FC> ±1.3 in GBM over control (P<0.05). This demonstrates that 6254 DEGs with GNAI-3 are markedly elevated in GBM.

Using the UALCAN database, the pan-cancer study displayed the expression of GNAI-2 and GNAI-3 across 20 distinct cancer types. Glioblastoma Multiforme and Sarcoma were found to have higher levels of GNAI-2 expression, whereas Breast Invasive Carcinoma, Cervical Squamous Cell Carcinoma, Esophageal Carcinoma, Head & Neck Squamous Cell Carcinoma, Glioblastoma, Sarcoma, and Skin cutaneous carcinoma had higher levels of GNAI-3 pan-cancer mRNA expression.

The box plots utilised in this work to determine the expression of GNAI family genes and their importance in GBM were obtained using the GEPIA 2.0. Of all the family members, the GBM tumour and normal tissue samples expressed GNAI-2 and GNAI- 3. In contrast to normal ones, this demonstrated that GNAI-2 and GNAI-3 exhibited elevated mRNA expression in GBM (figure 3). We typically concentrated on GBM, and GNAI-3 was more expressed in GBM than in the other cancer types.

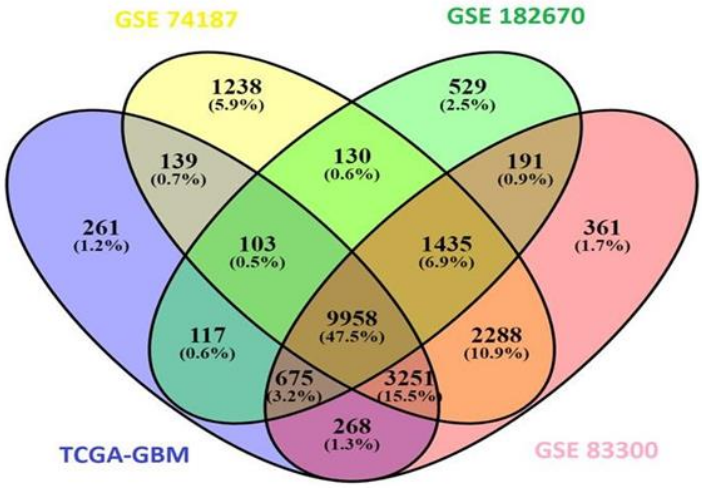


Figure 2: Venn Diagram: (A) TCGA-GBM data and GEO data for GBM samples were used for comparingv DEGs, which shows 47.5% data is common among all the 4

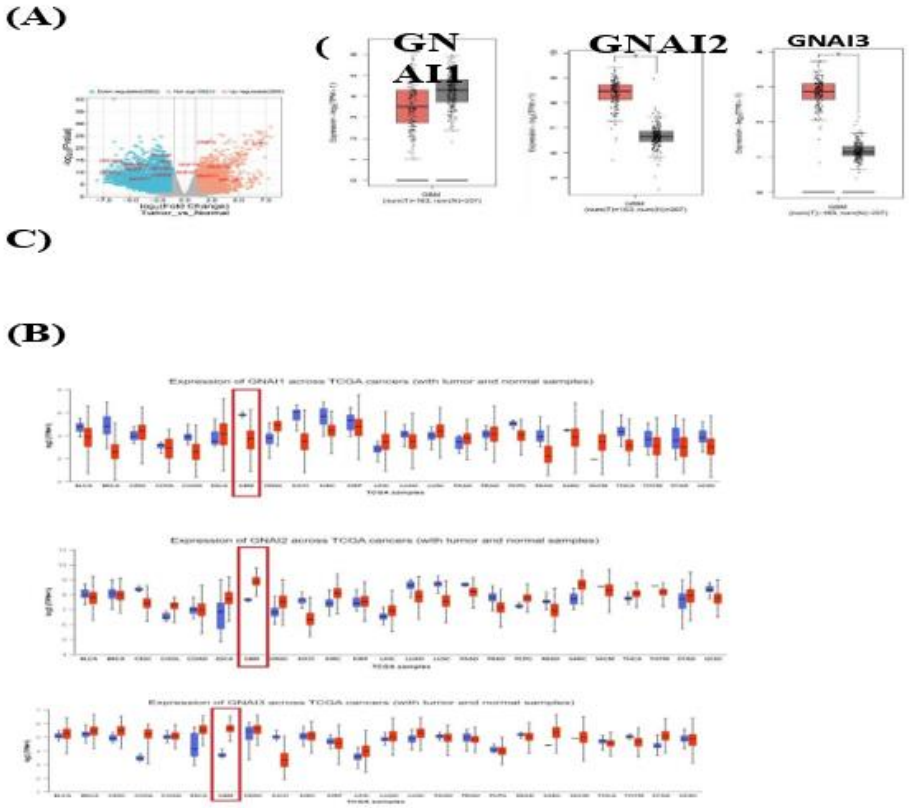


Figure 3: DEGs Expression and Pancancer Analysis: (A) Volcano Plot showing the up and down- regulated genes using Log2FC >1.3. (B) Expression of GNAI 1-3 across TCGA Pan-cancers showing the expression between primary tumor and Normal tissues, (C) Box plot using GEPIA 2.0 showing GNAI1- noncancerous, GNAI2 & GNAI3 both cancerous and significant P<0.05.

Upregulation of GNAI3 expression in IDH wild types in HWO grade II. III, and IV glioblastoma

We performed the study to ascertain the expression of GNAI family genes in WHO grade II, III, and IV GBM using the mRNA-325 dataset from the CGGA database. We found a highly significant (P=7.7-15, P=1.1-12) consistent increase in the expression level from grade II to IV in both GNAI-2 and GNAI-3.

We next employed a t-test and defined P<0.05 as a significant value to investigate the effect of IDH mutation status on GNAI2 and GNAI3 expression levels. It revealed that the mild type had somewhat higher mRNA expression compared to the mutant IDH samples. GNAI2 expression levels steadily rose among the IDH wild types, reaching a highly significant level in WHO grade IV. Furthermore, GNAI3's expression level steadily rose in grade III and IV wild-type IDH; however, WHO II's expression of mutant IDH was notable and showed the influence of IDH (figure 4). [41]. We performed the expression of GNAI family members for overall survival rate using the mRNA-325 dataset for primary and recurrent gliomas and the CGGA database. The results showed that both primary and recurrent gliomas have an overall poor prognosis, and the GNAI-2 is statistically significant

(P<0.0001) in primary gliomas. Furthermore, GNAI-3 has a poor prognosis and disease-free survival and is statistically significant for both primary (P<0.0001) and recurrent (P=0.0017) gliomas. The expression profile of the GNAI family demonstrated that as gene expression increased, patients' disease-free survival declined (Figure 5). Since GNAI-3 has demonstrated statistical significance in primary and recurrent gliomas, we will investigate its role as a predictive biomarker for GBM in more detail for the following reasons: First, it was very significant and had a high mRNA expression; second, as the tumour grew, so did its expression; and third, a higher expression of this gene is associated with a worse prognosis and a lower survival rate.

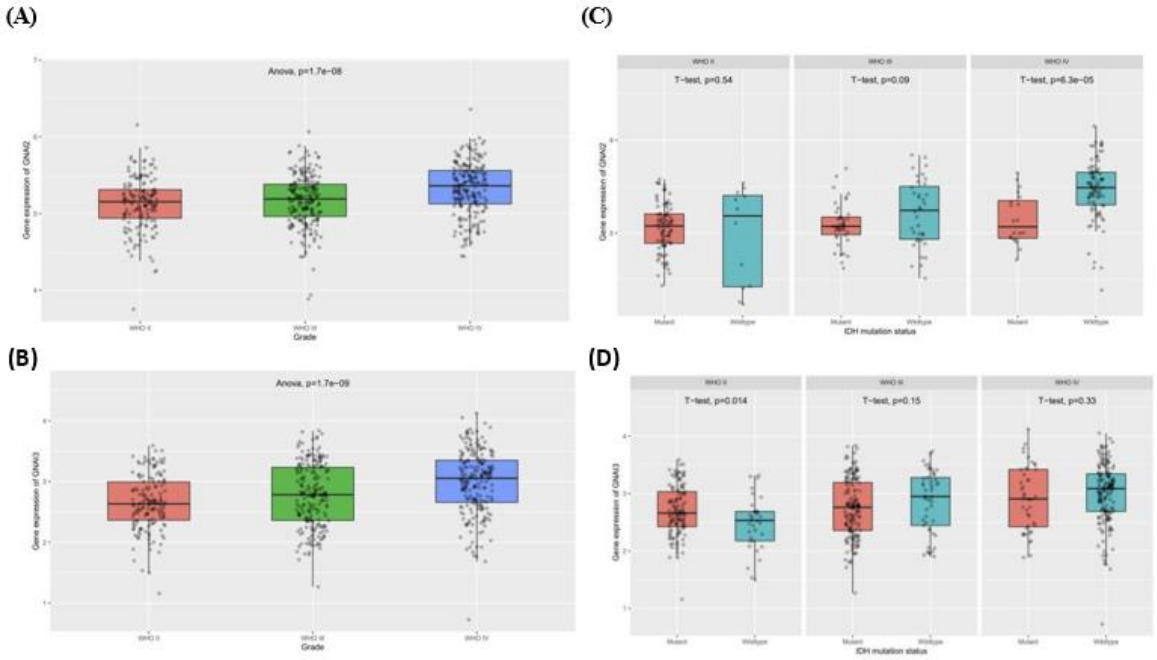


Figure 4: Gene expression in WHO Grade II, III & IV GBM: (A, B) CGGA data set is used for the expression GNAI2 & GNAI3 in WHO grade II, III, & IV. (C, D) Expression of GNAI2 & GNAI3 using IGH mutant and wild type in WHO grade II, III & IV GBM.

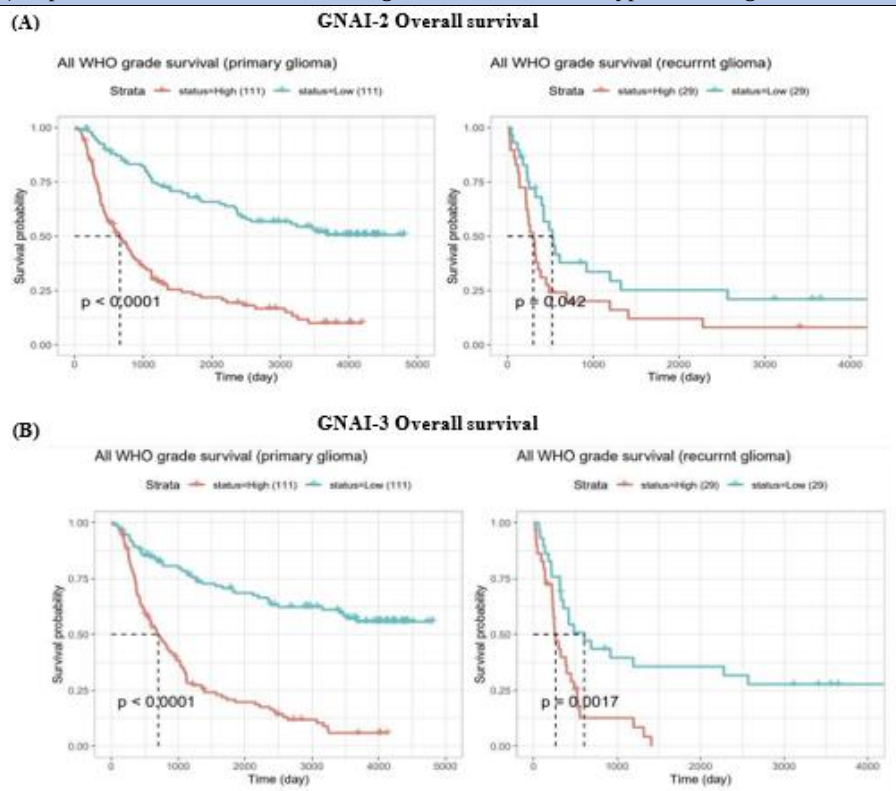


Figure 5: Overall Survival analysis of GNAI2 & GNAI3 in Primary & recurrent GBM: (A, B) GNAI2 & GNAI3 overall survival analysis shows the poor prognosis in both the primary & recurrent Glioma having significant p- value <0.05.

Drug analysis and their role in GBM treatment

Using GDSC data set, GNAI2 showed sensitivity and correlation with the Afatinib, while GNAI3 showed more sensitivity with the debrafenib. They both are tyrosine kinase inhibitors and target the EGFR and ERRB2 pathways that plays important role in tumor progression and metastasis. While CTRP data showed that Lapatinib, Austocystin D and Afatinib were effective for GNAI2 while Linsitinib was shown to be effective for GNAI3, all these drugs are Tyrosine kinase inhibitors except Austocytin D which is inducer for DNA damage and target nuclear receptor subfamily 1 while other molecules are tyrosine kinase inhibitors and target EGFR and ERRB2 pathway (Figure 6). It was obvious from the GDSC and CTRP data set that tyrosine kinase inhibitors stunt the cell progression and metastasis. Hence it is pretty obvious from the previous studies that tyrosine kinase inhibitors play important role for tumor progression control. A study showed that tyrosine kinase inhibitors induce the autophagy in the NB cells that leads to the significant increase in the cell death [10].

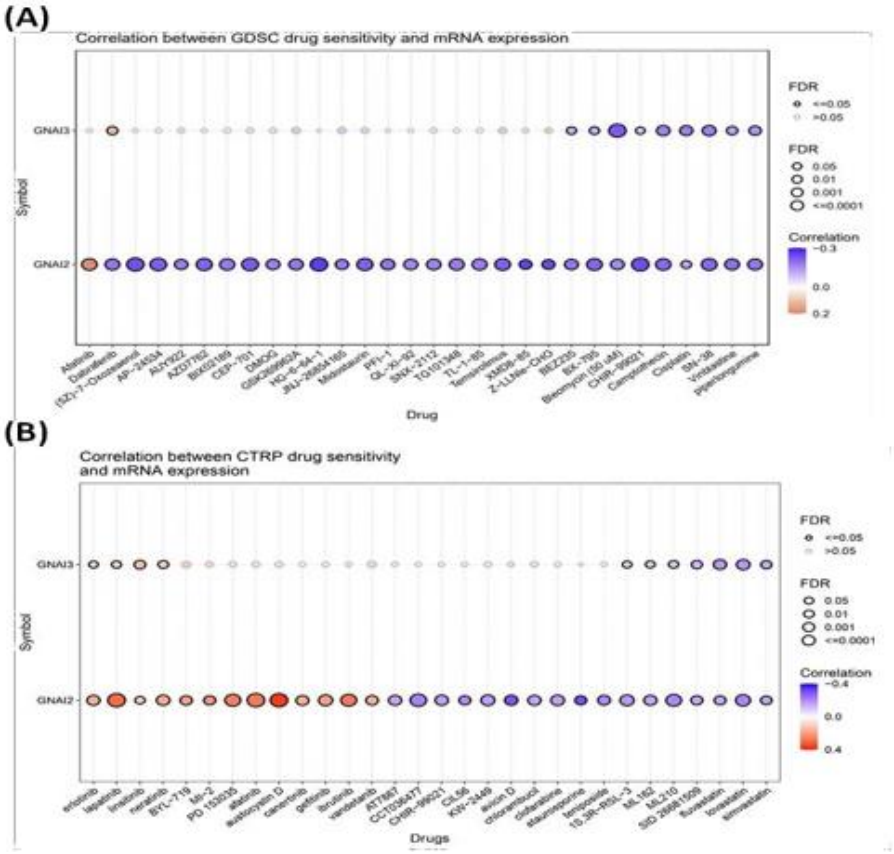


Figure 6: Drug Analysis using GSVA. (A) It shows the GDSC drugs and sensitivity for GNAI2 and GNAI3, (B) CTRP drugs sensitivity for GNAI2 and GNAI3. Pathway interaction using the Gene ontology tools for the GNAI family genes

To fully understand the role of GNAI2 & GNAI3 we extracted the pancancer TCGA data to perform the Gene ontology results in MF, CF, BP and KEGG. The GO was performed by R software using cluster profiler for the pathway analysis using a co-expression dataset from the cBioportal. For Molecular function, GNAI2 was involved in GTPase regulator activity, Nucleoside triphosphatase regulator activity, Actin binding while for Cellular components shows involvement in secretory granule lumen, Cytoplasmic granule lumen, vesicle lumen and in BP the pathways are leukocyte mediated immunity, T cell activation, and leukocyte migration. Eventually in KEEG pathways it is involved in Tuberculosis, MAPK signaling pathway, and cytokine- cytokine receptor interaction.

In case of GNAI3 the Molecular Function includes Catalytic activity-acting on RNA, Ribonucleoprotein Complex binding. While CC shows involvement in Nuclear Speck, Spliceosomal Complex and in BP the pathways are Ribocucleoprotein complex biogenesis, Ribosome biogenesis, ncRNA processing, and RNA splicing. Finally, in KEGG the pathways associated are spliceosome, nucleocytoplasmic transport, and Ribosome biogenesis in eukaryotes (Figure 7). It showed that the GNAI-3 was more involved in the cellular responses and cell signaling processes. GNAI2 was more involved in immune response while GNAI3 has major role in cellular pathways and tumor progression.

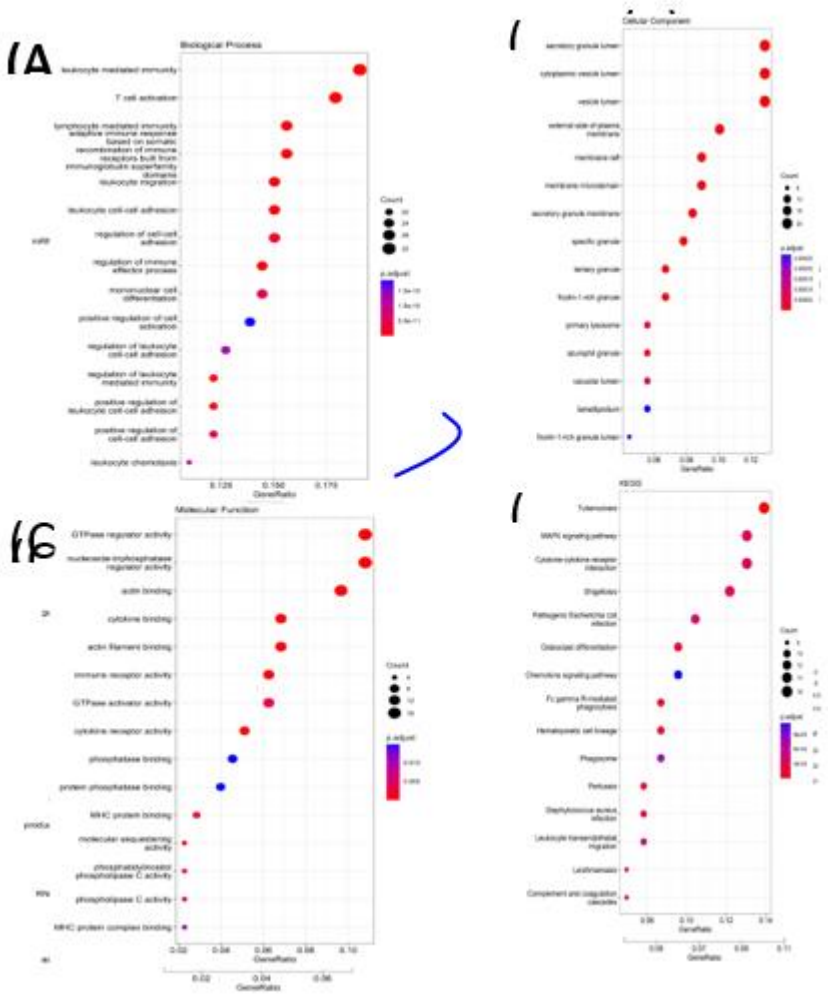
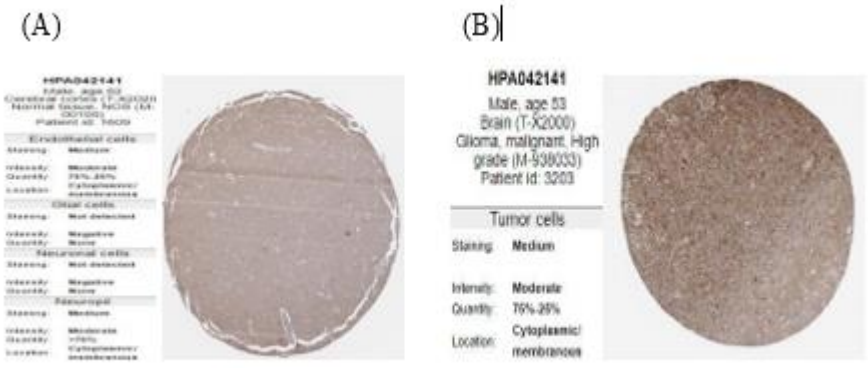


Figure 7: Gene Ontology (GO). Enrichment results using TCGA GBM Cancer Differential Gene Expression analysis. (i) GNAI2 (ii) GNAI3 GO like MF, CF, BF and KEGG using upregulated genes having Spearman's Correlation >0.45.

HPA analysis results

After performaing GO analysis, we found that the GNAI3 is In the Human Protein Atlas, we investigated the protein expression among GNAI-3 in GBM using the IHC score. The Immuno- histochemistry images include the clinic-pathological information such as the patient's age, gender, ID, normal and tumors sample. It was obvious from the previous results that higher protein expression was observed in the GBM patients. To show the effect of staining by the antibody bar graph was prepared. We also observed the expression of GNAI3 among different GBM cancer cell lines using the CCLE database. Further analysis showed that the expression of GNAI3 was higher and more significant among DOAY, U87MG ATCC, and SF172 cell lines as compared to the other cell lines (n=65) (Figure 8).



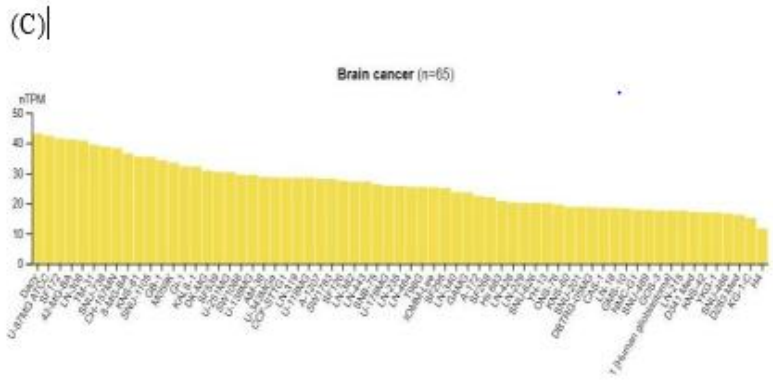


Figure 8: Representation of Protein Expression in GBM. (A-B) IHC staining images show the expression of GNAI3 protein in Normal and GBM tissue. (C) The bar graph shows the expression of the GNAI3 protein in different cell lines.

TIMER analysis for Immune Infiltration

We investigated the TIMER database to find the immune infiltration association with the GNAI-3 in GBM. The TME is changing and emergent entity and it varies among different cancer types and is still under continuous research. It has shown the important role of TME in cancer growth and progression [11-14]. To understand the effect of immune cells we performed the TIMER analysis for GNAI3. It shows positive correlation with the Myeloid Dendritic cells (Rho=0.283, P=8.00e-04) only and negatively correlated with the B Cells (Rho=0.055, P=5.23e-01), CD4+ T cells (Rho= -0.01, P=9.12e-01), Macrophages (Rho=0.091, P=2.93e-01), Neutrophils (Rho=0.145, P=9.14e-02) and CD8+ T cells (Rho=0.029, P=7.33e-01) (Figure 9). The role of B cells varies in each cancer type and the growth is enhanced by the B cells and can induce the immunosuppressive response by activating inhibiting the fc receptors on myeloid cells [15, 16].

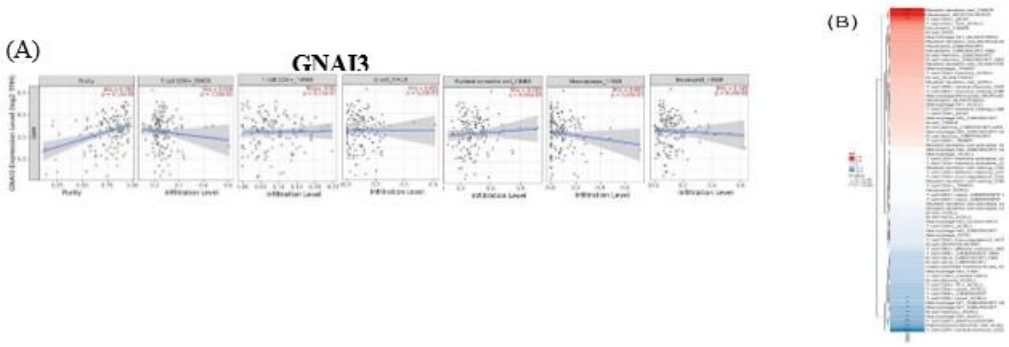


Figure-9: Relationship between expression of GNAI3 and Immune infiltration cells in GBM. (A) GNAI-3 shows a response with the DC and a negative correlation with B-cell and Cytotoxic T-cell immune response, (B) Heat map of GNAI3 shows significant & high correlation with the dendritic cells and macrophages.

Expression of GNAI3 linkage with MYC V1 pathway

For a better understanding of GNAI-3 involvement in the pathways with the Co-expressed genes, we performed the Gene Set Enrichment Analysis (GSEA) to access the (MSigDB-Hallmark) Molecular signature database for gene set in GBM with high GNAI-3 expression. The MYC proteins are known as proto-oncogenes and are known to promote cell proliferation in normal and cancer cells as well as having the highest enrichment score (NES=3.9447074). MYC V1 and V2 targets are associated with tumor aggressiveness, poor prognosis and metastasis in ER breast cancer patients [17]. MYC has three family members and their activity depends on the cell types such as MYCL, MYCN, and c-MYC. c- MYC is very crucial in brain cells [18, 19] and they are involved in normal brain development as well as in cellular functions by its transcription factor activity and regulating other genes [20]. The MYC expression levels are usually high in brain tumors and they are also involved in brain development [21]. MYC is related to tumorgenicity, especially n glioblastomas and its expression is relatively high in primary and recurrent GBM [22]. It is also linked with the aggressive nature and overall poor survival in different cancers as well as in GBM. MYC is involved in the mRNA splicing although the exact pathway is unclear so far [23]. Due to the complexity of MYC targeting is very difficult and it is possible to target the co-expressive genes and their pathways for the tumor treatment. Besides GNAI-3 has shown high expression and involvement in cancer factors such as E2F Targets (NES=3.3097093, p-value=0.0), G2m Checkpoint (NES=2.8216267, p-value=0.0), Unfolded Protein Response (NES=2.7011473, p-value=0.0), MTORC1 Signaling (NES=2.6889768, p-value=0.0), Oxidative Phosphorylation (NES=2.5031726, p-value=0.0), DNA Repair (NES=2.4667199, p-value=0.0), TNF Signaling via NFKB (NES=2.3867846, p-value=0.0), MYC Targets V2 (NES=2.3836884, p- value=0.0), Protein Secretion (NES=2.19933, p-value=0.0), Glycolysis (NES=2.1113517, p- value=0.0), Epithelial-Mesenchymal Transition (NES=1.9598433, p-value=0.0), Hypoxia (NES=1.9154502, p-value=0.0), Androgen Response (NES=1.7960849, p-value=0.0), PI3K AKT MTOR signaling (NES=1.671638, p-value=0.0), TGF Beta Signaling (NES=1.6660402, p-value=0.0007) (Figure 10).

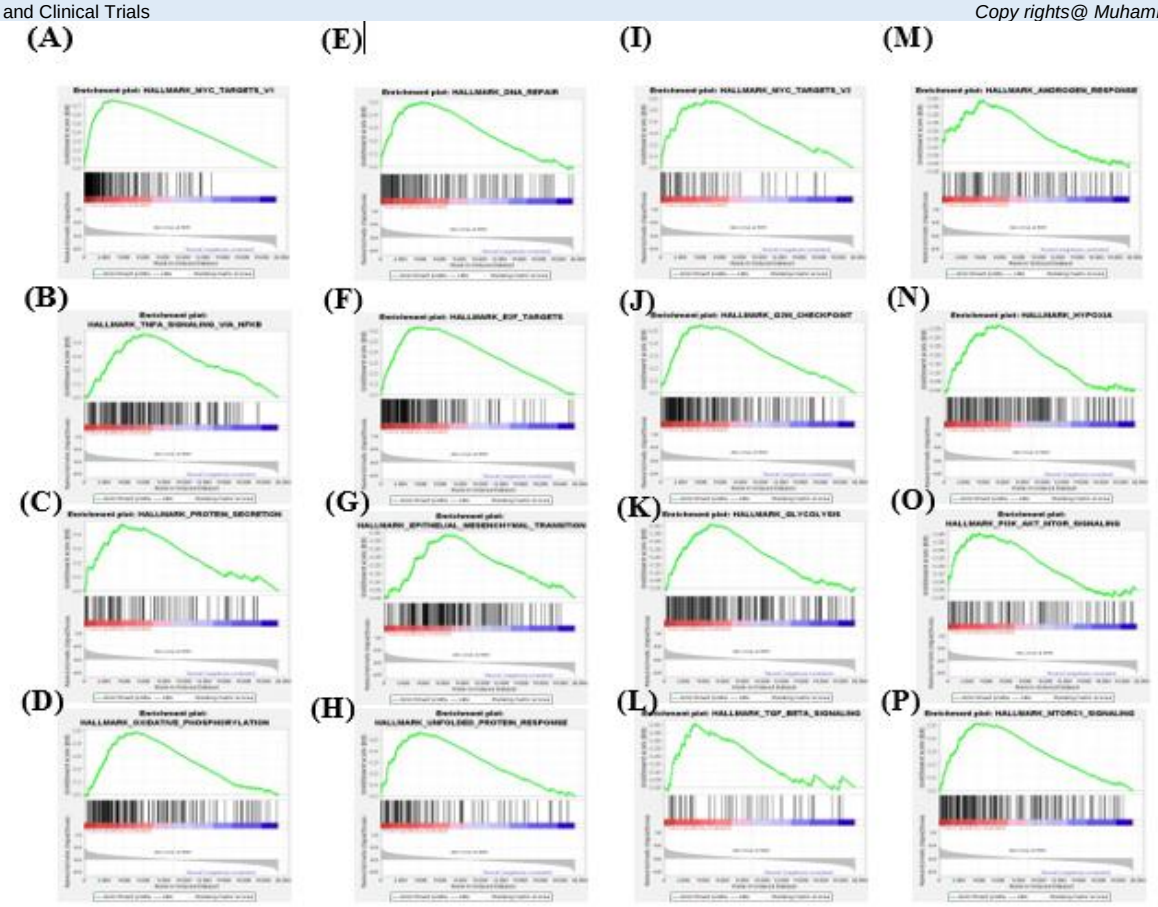


Figure-10: Results of Gene set enrichment analysis GSEA for GNAI3 expression in GBM. (A-O) Patients were separated into two groups based on their GNAI3 mRNA expression levels in the TCGA Pan-Cancer dataset; afterwards, a gene ranking list was produced and entered into the GSEA. As indicated by the GSEA database, statistical significance was evaluated using a false detection rate (FDR) value of 0.25, a normalized enrichment score (NES) of more than 1.3, and a nominal p-value of 0.05. Enrichment at the top of the list is indicated by a positive NES value, which indicates the enrichment pathway.

PPI & Pathways analysis

We investigated the Protein-Protein Interaction using String online web tool and the network consists of 13 nodes and 51 edges. It's revealed that the GNAI family genes interact with each other and other members of the GNB family which were involved in cell cycle responses and signaling pathways (Figure 11). While pathways exploration indicated that it was more involved in cellular Homeostasis, Protein Folding, Trans-synaptic signaling, G-Protein coupled signaling pathways, Coagulation, Cyclase modulating G protein-coupled receptor signaling pathway and negative regulation of protein tyrosine phosphate activity, adenylate cyclase-activating adrenergic receptor signaling pathway. Pathway's analysis showed the interaction of GNAI-3 in upregulating and downregulating several cellular pathways.

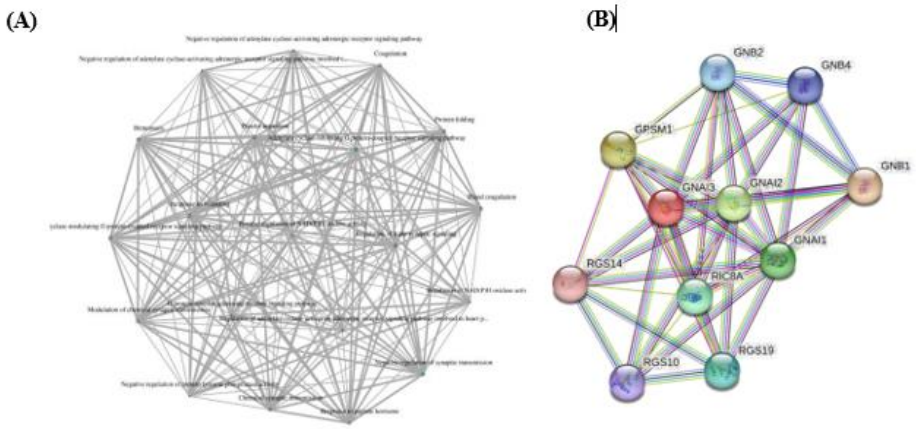


Figure 11: Pathway and Protein-Protein Interaction. (A) Pathway's interaction shows the involvement of GNAI3 in upregulated & downregulated pathways. (B) Protein-Protein Interaction shows the interaction of GNAI family with the GNB protein family in cellular responses.

Expression of GNAI3 with cytoskeleton remodelling Regulation of actin cytoskeleton organization by the Kinase effectors of Rho GTPases

MetaCore platform was used to identify the BPs pathways stimulated by the GNAI-3, by the co- expressive genes TCGA data set (spearman's correlation =>0.45). It revealed that the GNAI-3 was involved in various significantly important pathways that regulate the Biological Processes such as "Cytoskeleton remodeling Regulation of actin cytoskeleton organization by the kinase effectors of Rho GTPases" (Figure 12), "DNA damage Double-strand break repair via homologous recombination", "Immune response B cell antigen receptor (BCR) pathway", "G-protein signaling Ras family GTPases in kinase cascades", "Development &Regulation of telomere length and cellular immortalization", "Cytoskeleton remodeling Regulation of actin cytoskeleton nucleation and polymerization by Rho GTPases", "DNA damage ATM/ATR regulation of G2/M checkpoint: cytoplasmic signaling", "Development Regulation of cytoskeleton proteins in oligodendrocyte differentiation and myelination", "Cell cycle DNA replication initiation", "Immune response ETV3 effect on CSF1-promoted macrophage differentiation". This finding gives us a better understanding of cancer development in GBM (Figure. S1, Table S1-2).

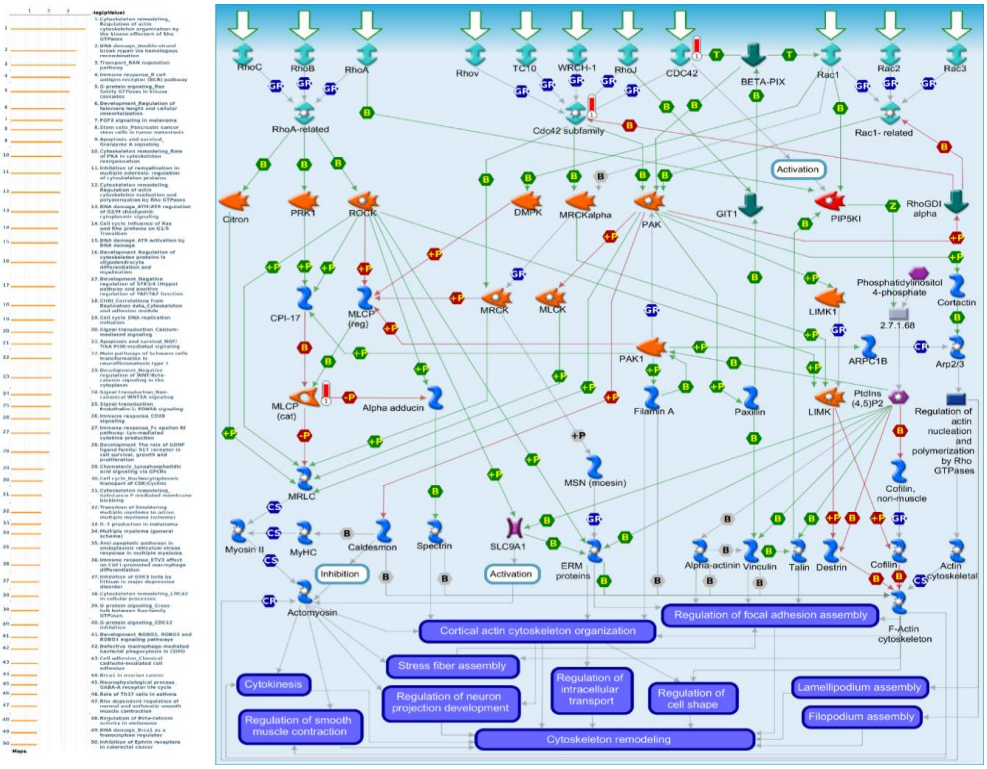


Figure 12: Expression of GNAI3 signaling pathways in GBM using (MetaCore). Using MetaCore to analyze the compression of genes with GNAI3 using the TCGA dataset, we found that the Role of GNAI-3 in "Cytoskeleton remodeling Regulation of actin cytoskeleton organization by the kinase effectors of Rho GTPases" was linked with GBM progression (having using $p<0.05$).

Discussion

Glioblastoma multiforme (GBM) is the most common and most serious malignant primary brain tumor in adults, accounting for 54% of all gliomas and 16% of all primary brain tumors. Despite intensive treatment with very safe surgical excision, radiotherapy and chemotherapy, the median survival is about 14.6 months, and the 5-year survival rate is less than 10%. To date, the standard treatment for GBM remains the 'Stupp regimen', which includes surgery, when possible, followed by simultaneous radiotherapy and chemotherapy with the drug temozolomide (TMZ), followed by TMZ [24]. Other therapies such as checkpoint inhibitors, CAR-T cell therapy and vaccines are all under investigation. However, the brain's immune system is unique and can be a challenging area for immunotherapy. The most recent focus of GBM research is glioblastoma stem cells (GSCs) [25]. These cells are known to be resistant to conventional therapies and may be responsible for tumor recurrence. A variety of targeted therapies are being tested in clinical trials. These include drugs designed to suppress growth signals (such as EGFR inhibitors) or inhibitors of angiogenesis to cut off the blood supply to a tumor [26, 27]. However, we can provide an overview of how GNAI family proteins, including GNAI-3, influence tumor progression, immune response and progression, and may serve as cancer biomarkers. Gα12/13 proteins are part of the G protein family involved in cell signal transduction. Alterations in these signaling pathways can disrupt cellular processes such as cell proliferation, differentiation, migration, and apoptosis that contribute to tumorigenesis. GNAI3 protein, may play a role in these cancer-related processes.

In the context of tumor progression, GNAI-3 may affect cell migration and invasion, two major processes involved in tumor metastasis. For immune responses, protein-coupled receptor (GPCR) signaling involving GNAI-3 is known to play an important role in the regulation of immune cells. Any disruption in this pathway can affect the immune system's ability to respond effectively to tumors [23]. In terms of growth, G proteins are involved in transducing signals from growth factor receptors to downstream effectors that promote cell proliferation. Abnormalities in these signals can lead to uncontrolled cell growth, which is the hallmark of cancer. Finally, the role of GNAI-3 as a cancer biomarker depends largely on whether its expression or level of activity is consistently associated with specific aspects of cancer, such as its presence, stage, or response to treatment.

Using the TCGA database, we explored GNAI-3 expression in different types of cancers and its association with overall survival, poor prognosis, and immune infiltration in GBM. This is the behavior of bioinformatics research

that used GNAI-3 as a prognostic biomarker for GBM for the first time and showed that GNAI expression gradually increased in GBM WHO grade II-IV and that IDH mutations were associated with GNAI-3 indicating that GNAI-3 expression was not affected. GNAI-3 is associated with poor prognosis, and GO pathway analysis revealed key roles for GNAI-3 in cell signaling, ribosomal synthesis, and mRNA splicing. Role of GNAI3 in cellular processes and tumor progression leads to focus on GNAI3 for further Bioinformatics analysis. We performed GSEA analysis and observed that GNAI-3 expression correlated with the expression of the target MYC pathway V1. The MYC oncogene is a transcription factor that regulates many aspects of cell biology, including proliferation, growth, and apoptosis. When dysregulated, MYC may contribute to the development of several cancers, including glioblastoma multiforme (GBM).

In the context of GBM, MYC is overexpressed and associated with a poor prognosis. This may contribute to the aggressive behavior of the tumor and its resistance to treatment. In particular, MYC can promote glioma stem cell proliferation and self-renewal, which are thought to contribute to tumor recurrence. The relationship between MYC and GNAI-3 is less clear than between MYC and its normative targets. However, it is conceivable that GNAI-3, which is involved in G protein signaling, may interact with pathways downstream of MYC or participate in processes affected by MYC overexpression [28]. GNAI proteins are involved in EGFR signaling by recruiting Gab1 and further PI3K, that in turn involves cell migration and proliferation [29].

Now, regarding the involvement of GNAI-3 in the specific pathways mentioned above, Rho GTPases play an important role in cytoskeleton remodeling and actin regulation. Actin is a protein (along with myosin) that forms contractile filaments of muscle cells and is involved in a variety of cellular movements, including cell motility, cell structure, integrity, and intracellular trafficking. Changes in the cytoskeleton and organization of actin frequently accompany cellular transformation, including the formation and development of cancer cells. Therefore, if GNAI-3 significantly affects this pathway, it may play a role in the initiation or progression of glioblastoma multiforme by altering cellular structure and behavior [30, 31]. Using GNAI-3 as a biomarker requires consideration of its expression levels in GBM samples compared to healthy tissues. Significantly increased or decreased expression levels in GBM may indicate a link between the gene and cancer and could be used to detect or monitor disease. In addition, the WHO score analysis will include consideration of how GNAI-3 expression and involvement pathway change with GBM progression from lower (less severe) WHO scores (more severe) to higher (more severe) WHO scores. This may provide insight into how GNAI-3 promotes GBM progression and may influence treatment efficacy.

The positive association between GNAI-3 and dendritic cells in the context of infiltrating immune cells of glioblastoma (GBM) may indicate that increased GNAI-3 signaling may enhance dendritic cell recruitment or activity. Dendritic cells are antigen-presenting cells that play an important role in initiating the adaptive immune response. They may enhance antitumor responses by presenting tumor antigens to and activating T cells. On the other hand, the negative correlation between GNAI-3 and B cells, CD4+ T cells, CD8+ T cells, macrophages, and neutrophils may indicate that GNAI-3 signaling may somehow inhibit the recruitment or function of these cells[32]. GNAI-3 also plays an important role in signal transduction. Involved in several intracellular signaling pathways, the B cell antigen receptor (BCR) pathway is important for B cell activation and function. When an antigen binds to the BCR, it triggers a cascade of intracellular signaling events that lead to B cell activation, proliferation, differentiation, and antibody production. The BCR pathway and the GPCR pathway are separate, but they may overlap. Several studies have suggested that G proteins may be involved in regulating BCR signaling, influencing B-cell activation and function. Moreover, little is known about the role of GNAI- 3 and B cell function in the context of glioblastoma multiforme (GBM). B cells are part of a complex immune response against GBM, but their specific roles and interactions in the GBM tumor microenvironment remain to be explored. More research is needed to understand how GNAI-3 affects B-cell function and immune responses in GBM [33]. APCs participate in IL1, 6, 8, and 10 signaling, macrophages and dendritic cells as part of innate immunity, and tumor cells as part of senescence activity. They detect tumor antigens by detecting MHC class I deficiency on tumor cells or by uptake of the antigen by dying tumor cells, which in turn activate CD4+ T cells through TCR, CD28, and B cell responses, and thus specifically activate Th2 cells. direct or indirect. Methods of activating Th1 cells. Th2 cells release cytokines such as IFN-γ to suppress tumors. Th1 cells activate B cell immunity, natural killer cells. IFN-γ also regulates M1 macrophages, which in turn kill cancer cells[34-38]

For example, G proteins including GNAI-3 can influence cell proliferation and survival, which are key factors in tumorigenesis and tumor progression. They also play a role in cell migration and invasion, which are critical for metastasis[39] [40].

In the context of immune responses, G protein-coupled receptors (GPCRs), including GNAI-3, are known to play a role in regulating immune cells, so alterations in this pathway may influence immune responses to GBM. Our constraint is that we have not undertaken more thorough verification by experimental assays in vitro, and in vivo, but we anticipate that the findings of bioinformatics analysis will serve as the foundation for future research. GNAI3 has the potential to be a predictive biomarker for immune infiltration in GBM cancer development.

Conclusions

Our findings showed that overexpression of GNAI family genes may be a possible biomarker for GBM malignancy and a sign of a bad prognosis. The bioinformatics study suggests that this marker may be the focus of more thorough experimental validations in the future.

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