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Multi-Omics analysis of CD38 expression in glioblastoma multiforme prognosis and treatment resistance

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ABSTRACT

This study employs a comprehensive multi-omics approach through genomics and transcriptomics particularly to investigate the role of CD38 expression in Glioblastoma Multiforme (GBM) prognosis, tumor development, and survival. Utilizing RStudio, we conducted an analysis comparing CD38 expression levels with GBM survival outcomes. Differential analysis using DESeq revealed a distinct gene expression profile, elucidating both upregulated and downregulated genes associated with high CD38 expression. Kaplan-Meier survival analysis demonstrated an unexpected and counterintuitive association between elevated CD38 expression and increased GBM survival, contradicting previous literature that CD38 posits shorter survival. To further understand the functional implications of the identified genes, we used PANTHER classification system to identify the molecular functions associated with the upregulated and downregulated genes from the DESeq analysis. Our findings challenge established literature, providing novel insights into the complex interplay between CD38 expression, molecular pathways, and GBM survival. More research may be needed to understand the intricacies of CD38's role in GBM prognosis and treatment resistance.

Keywords: glioblastoma, CD38, glioma, treatment, multi-omics

INTRODUCTION:

GLIOBLASTOMA EPIDEMIOLOGY

About 20,000 cases of gliomas are diagnosed annually within the United States, 60% of which are glioblastomas at approximately 12,000 cases. Out of all 80,000 yearly diagnosed cases of primary brain tumors, glioblastoma multiforme or glioblastoma tumors (GBM) account for 15%. GBM occurs in adults between the ages of 45 and 70 years, with poor prognosis. The median survival time is 10-12 months with 1% of patients surviving more than 10 years. 90% of adult GBM patients die within 24 months after diagnosis. Risk factors are generally unknown, but some established risk factors include genetics and ionizing radiation (Wrensch et al., 2002). Various recent studies have explored other risk factors including occupational exposures, medications, head trauma, blood transfusion, and lifestyle factors (Nelson et al., 2012). The incidence of GBM is increasing in recent years due to a combination of possible contributing risk factors including overdiagnosis, an increasingly older population, ionizing radiation exposure, environmental exposures, and other causes. From 2008 to 2017, within a well-defined global population, GBM incidence increased from 0.73 to 4.49 per 100,000, reflecting the increasing importance on research involving glioblastoma development (Grech et al., 2020).

PATHOPHYSIOLOGY REVIEW OF GLIOBLASTOMA

Gliomas describe a common type of brain cancer arising from glial cells, categorized into four grades from I, II, III, and IV depending on the aggressiveness of the disease. These types determine type and course of treatment. Gliomas are the most common type of brain tumor, accounting for about 33% of all brain tumors. Gliomas are further classified depending on the type of glial cells they arise from: astrocytes, oligodendrocytes, and microglial cells (Purves D,

Augustine GJ, Fitzpatrick D, et al., 2001). Regarding histology, Glioblastomas arise from astrocytes or oligodendroglia cells, non-electrical glial cells restricted to the brain and spinal cord which undergo various neuroprotective processes such as promoting synapse formation and stabilizing the blood-brain barrier (Wei & Morrison, 2023). Glioblastomas describe grade IV gliomas, the tumor characterized by infiltrative tendrils, the growth of which largely affects surrounding brain tissue by exerting pressure in the microvasculature, inducing edema. This pressure results in various symptoms depending on the location of the tumor. GBM tumor presence is more prominent in the frontal and temporal lobes but can spread through the brain and brainstem. Symptoms of GBM in the frontal lobe may present as difficulties in memory, movement, problem-solving, and other functions whereas presence of the tumor in the temporal lobe may impact auditory and visual perception, memory encoding, and emotional processing.

GLIOBLASTOMA TREATMENT:

Glioblastoma Multiforme (GBM), characterized by its aggressive nature and infiltrative growth within the brain, requires a robust treatment approach to address its complex pathology. Regarding GBM treatment, surgery is a primary intervention, albeit challenging due to the tumor's infiltrative behavior. Surgical resection is often complemented by radiation therapy, which targets residual cancer cells post-surgery.

Chemotherapy, particularly Temozolomide, is a standard adjuvant treatment administered concurrently with radiation and as maintenance therapy (Fernandes et al., 2017). The integration of targeted therapies, such as anti-angiogenic agents like Bevacizumab, aims to disrupt specific pathways implicated in tumor growth. Despite advancements in treatment development, the poor prognosis of GBM remains considering glioblastomas show resistance for even targeted

treatments due to the complex environment of the central nervous system (CNS). Resultant, ongoing research endeavors seek to enhance treatment efficacy, patient outcomes, and current understanding of the biological pathways and the tumor microenvironment (TME) involved in GBM development. Tumor-associated macrophages and microglia account for a significant amount of glioma tumor mass; these cells are the most common cells in the GBM TME (Wo et al., 2019).

CD38 BACKGROUND

CD38 or cyclic ADP ribose hydrolase is a robust transmembrane glycoprotein involved in a variety of cellular functions including cell adhesion, differentiation, proliferation, signal transduction in immune responses, and calcium signaling through the synthesis of cyclic-ADP ribose (cADPR). Calcium plays a crucial role in cell signaling as a versatile second messenger, regulating many cellular processes. Atypical calcium signaling can contribute to uncontrolled cell proliferation, invasion, and metastasis, thus its necessity in maintaining cellular homeostasis and preventing oncogenic development. Furthermore, CD38 has been observed to regulate the activation of the aforementioned tumor-associated macrophages through increasing calcium concentration (Mayo et al., 2008). Mammals have two proteins that exhibit cyclic ADP ribose hydrolase activity, CD38 and CD157, both understood to contribute to tumor microenvironments. However, this study will focus on CD38 due to CD38's particular role in immunosuppressive environment in a hypoxic tumor microenvironment (Wo et al., 2019), considering hypoxia is a hallmark of GBM through the inhibition of immune responses and treatment resistance (Park & Lee, 2022).

CD38 has implications in the progression of various autoimmune diseases and cancers, particularly B-cell chronic lymphocytic leukemia (CLL), nonsmall cell lung cancers, and multiple myeloma through CD38's altering of immune response pathways through receptor and enzymatic mechanisms. CD38 is upregulated by a variety of inflammatory mediators, thus its use in research as a cell activation biomarker. In CLL, CD38 has been observed to serve as an independent prognostic oncogenic marker with high CD38 expression (7% to 30% positive tumor cells, depending on the series) being associated with decreased survival (Byrd and Flynn, 2014). However, there is not as large a body of work detailing CD38 expression as a biomarker for survival in GBM patients, despite the notable immune suppression found in GBM patients.

TCGA-GBM DATASET

All histological diagnoses of GBM from The Cancer Genome Atlas (TCGA), specifically TCGA-GBM, a dataset encompassing comprehensive genomic and clinical data from a cohort of 509 patient barcode samples, making it a pivotal source for researchers investigating the mechanisms underlying GBM pathology. TCGA-GBM study has established four distinct molecular GBM subtypes: proneural, neural, classical, and mesenchymal; the subtypes involve different neural lineages and respond differently to different treatments (Verhaak et al., 2010).

This dataset includes information on genetic mutations, gene expression profiles, copy number alterations, and clinical outcomes for GBM patients. The dataset may be used to identify key molecular signatures, potential therapeutic targets, and prognostic factors associated with GBM. Currently, few molecular factors are indicative of prognosis, signatures including *MGMT* methylation, or mutations involving *TP53, RB1*, *PIK3R1, NF1*, *ERBB2, MDM2, CDK4*, and other genes. Furthermore, CD38 expression is known to play a role in the molecular landscape of

glioblastoma; CD38 deficiency significantly attenuates glioma expansion and prolongs survival time for gliomas (Levy et al., 2012). Exploring the association between CD38 expression levels and clinical outcomes within this dataset may provide vital information in understanding potential implications of CD38 in GBM progression and treatment response. As research expands into the TCGA-GBM dataset to better understand the complexities of glioblastoma pathophysiology and development, the integration of CD38 expression data adds a novel dimension, contributing to the broader understanding of GBM heterogeneity and offering potential routes for targeted therapeutic interventions. This study hypothesizes that aberrant CD38 expression and mutation may exacerbate glioblastoma tumorigenesis and reduce the overall survival time of patients that develop glioblastoma.

METHODS

Utilizing the R program, a free software environment for statistical computing, GBM patient RNA expression, mutation, and clinical data were extracted from TCGA to use for analysis. A boxplot was created to identify the cutoff between high and low CD38 expression, set at below 25% (Quantile 1) and above 75% (Quantile 3) respectively. Tumor samples from clinical data were separated into two cohorts, “high CD38 expression” and “low CD38 expression”. In total, there were 86 patients. After separation, the patient identifications of each cohort were applied to RNA data to segment out the same tumor samples. .

The two cohorts were inputted into code using the R packages DESeq2 and EnhancedVolcano for differential expression analysis, visualized with volcano plots. Genes were categorized as either upregulated, downregulated, or insignificant depending on whether CD38 expression was high or low. The RNA expression data frame was first filtered by p-value, using a

Boolean masking technique to remove all ensemble gene IDs with a p-value above a threshold of 0.05. To obtain the upregulated genes, genes with a log2FoldChange value < 1 in the RNA expression data frame were removed. To obtain the downregulated genes, genes with a log2FoldChange > –1 were removed. To identify the cellular pathways involved in the gene cohorts, the upregulated and downregulated ensemble gene IDs were input to the Protein ANalysis THrough Evolutionary Relationships Classification System (PANTHER), a comprehensive biological database used to identify the function of genes.

To evaluate survival between high CD38 and low CD38 cohorts, we utilized the "survival" package in R to perform survival analyses between high and low cd38 expression groups through Kaplan Meier (KM) Plotting, obtaining survival curves over days after diagnosis.

Mutation data was retrieved from TCGA, including information on mutation types and position along the gene between the two patient populations. To identify whether mutations should be a consideration for TCGA glioblastoma tumor samples, we generated a lollipop plot along the CD38 gene using the R package maftools.

RESULTS

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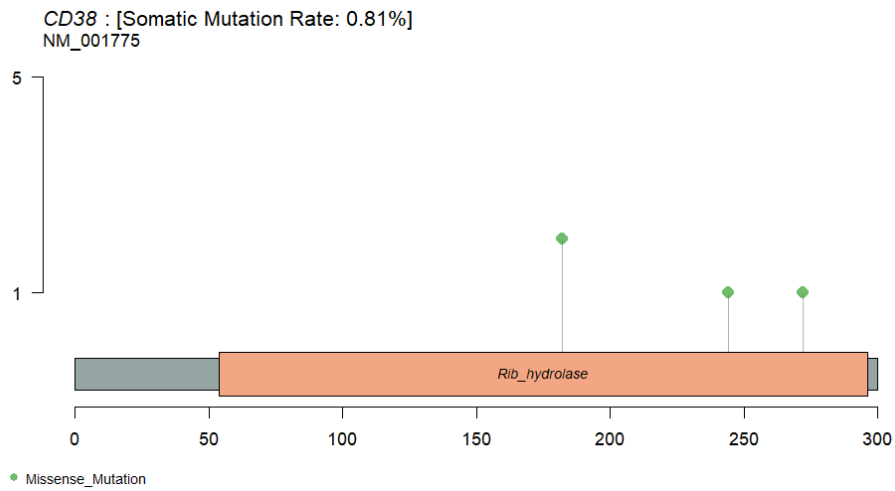

Figure 1. Lollipop plot of CD38. CD38 mutations occur as missense mutations, located approximately at amino acids 180, 245, and 270.

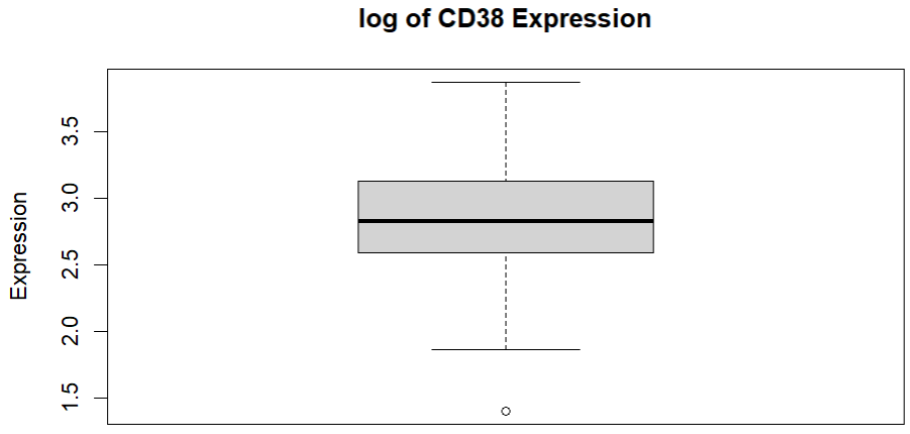

Figure 2: Box plot setting thresholds for high (>388) and low (<388) cd38 expression. Indicated above are the quartiles, of which 25% was used as the cutoff from low to high cd38 expression.

The cutoff at this point was based on quantiles since the data was normal. Furthermore, 25% was selected as a CD38 threshold based on literature. 0% was 25, 25% or Quantile 1 was 255, 50% or the mean was 677, 75% or Quantile 3 was 1348, and 100% was 7540.

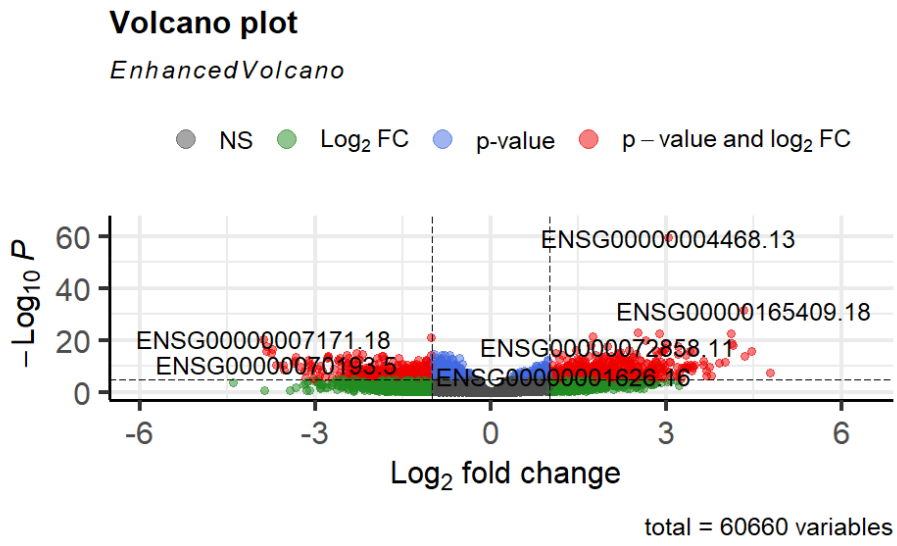

Figure 3: DESeq volcano plot identifying upregulated and downregulated genes based on high versus low CD38 expression.

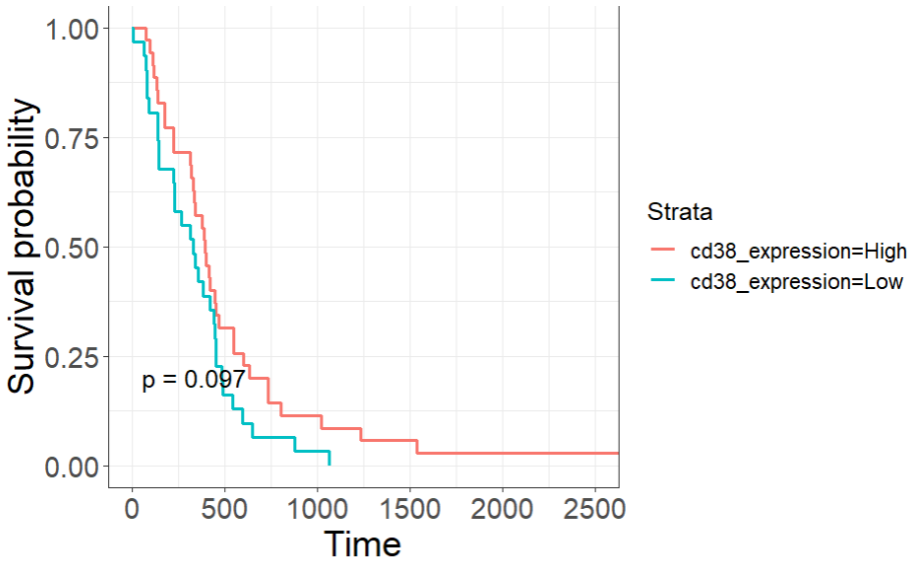

Figure 4. Kaplan-Meier survival plot shows that glioblastoma patients with high CD38 expression experience increased survival times in comparison to glioblastoma patients with lower CD38 expression. Time is expressed in units of days.

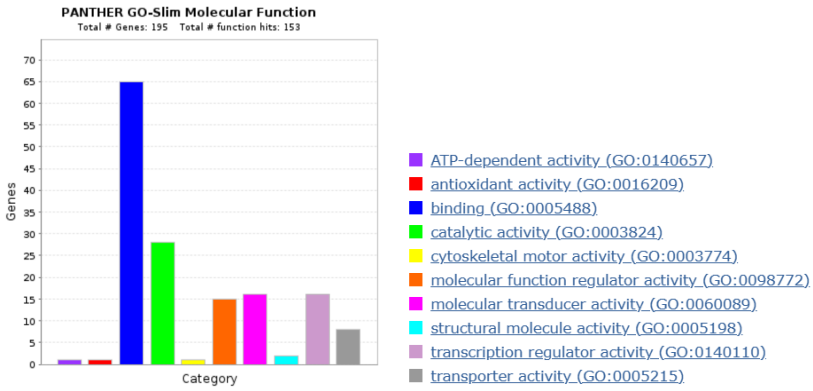

Figure 5. Bar graph showing the pathways for which downregulated genes identified by DESEq are most involved in. Included is a legend indicating the identify of each color. Aside from genes not assigned to any category, the most

prevalent pathways involve binding, most frequently heterocyclic compound, organic cyclic compound, and protein binding.

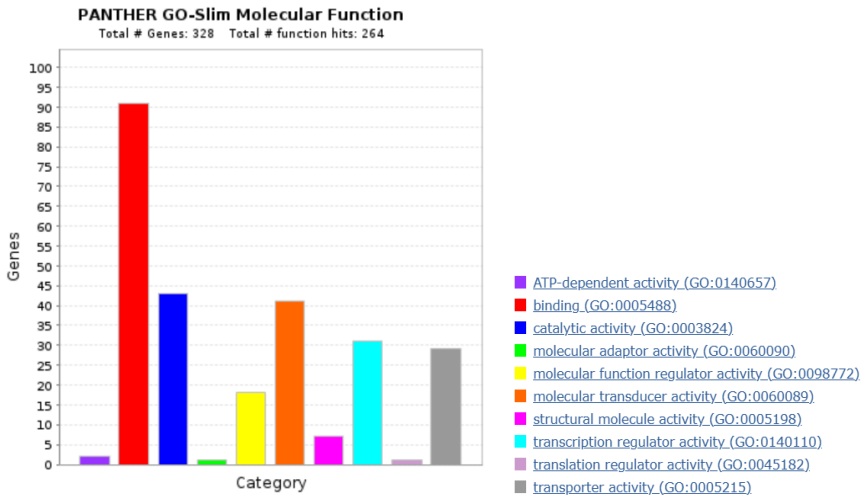

Figure 6. Bar graph showing the pathways for which upregulated genes identified by DESEq are most involved in. Included is a legend indicating the identify of each color. The most prevalent pathways involve binding, most frequently heterocyclic compound, organic cyclic compound, and protein binding, similar to downregulated genes.

DISCUSSION

Generally, it has been observed across current literature that high CD38 expression in the GBM TME is associated with poor patient prognosis. Our Kaplan Meier plot found that high CD38 is correlated with higher survival rates within our population sample; Our results do not support our hypothesis and contradict current understandings. However, the p-value of the Kaplan Meier plot was found to be 0.097, as shown in Figure 4, indicating that the survival trends shown there are not significant. Future studies could examine the survival between high and low CD38 glioblastoma patients in a different cohort, or larger patient population.

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Using the upregulated and downregulated genes identified from differential expression analysis, through the PANTHER database, we found gene expression to be different between the high CD38 and low CD38 groups. Although the two groups of genes performed similar functions, specifically in binding, catalytic activity, and molecular transducer activity, there are approximately twice as many downregulated genes as there are upregulated genes. (Figures 5, 6) However, the ratio of genes involved in biological pathways as identified by PANTHER does not reflect this trend. Although there are more downregulated genes, there are fewer partaking in each function; 65 downregulated genes are involved in binding, versus 91 upregulated genes involved in binding. This trend continues for nearly all other functions, bar the molecular adaptor activity, cytoskeletal motor activity, and translation regulator activity which all harbored below 5 genes. tLow CD38 expression may reduce the efficiency of certain biological processes, which could be a future avenue of research.

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From our lollipop plot, given n = 86, four missense mutations were identified along CD38, as illustrated in the lollipop plot (Figure 1). This, in tandem with the PANTHER plots for upregulated and downregulated genes eliminates mutations as a potential cause of the difference in survival within our population, leading the focus of our study to pivot towards transcription regulation, where the cell converts DNA to RNA as response to a variety of intracellular and extracellular signals.

Future directions of this area of study could potentially investigate CD38 expression and mutation on survival rates in glioma patients and where this role may diverge in the pathophysiology of other cancers such as hepatocellular carcinoma (HCC) and breast cancer, in which the presence of CD38 improves prognosis. This can be examined through conducting more thorough pathway analysis. Specifically, future studies can run differential expression

analysis between high and low CD38 cohorts of HCC and breast cancer patient populations in TCGA. Upregulated and downregulated genes may be extracted from the results, then compared in PANTHER to evaluate for correlations with glioblastoma pathways.

This is especially important given the blood brain barrier, one of the biggest limitations of glioblastoma treatment. Blood vessels in the central nervous system tightly regulate movement of ions and molecules between the semipermeable barrier, reducing the effectiveness of common treatments such as immunotherapy. Therefore, finding genes that may regulate CD38 expression can be a way to target CD38 in the TME of glioblastoma without interference of the blood brain barrier.

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