Original Research Article

CALHM5 Expression in Glioblastoma: An Exploratory Bioinformatics Analysis

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ABSTRACT

CALHM5 (Calcium Homeostasis Modulator Family Member 5) encodes a protein predicted to form an ion channel involved in calcium-related signaling. Disruption of calcium signaling pathways has been shown to play a crucial role in the development of tumors within the central nervous system. This study examines *CALHM5* RNA expression and its clinical associations in glioblastoma, together with its predicted protein structure. We hypothesized that *CALHM5* RNA expression is associated with glioblastoma-related clinical features. We utilized publicly available datasets from the Human Protein Atlas, NCBI Gene Expression Omnibus, and Ensembl to analyze *CALHM5* RNA expression across cancer types, its association with glioblastoma patient survival, its expression in CD133-positive and CD133-negative glioblastoma cell lines, and its predicted protein structure. Patients with high CALHM5 RNA expression showed lower observed survival than patients with low *CALHM5* RNA expression, with a p score of 0.065. Because *CALHM5* is predicted to encode a pore-forming ion channel, its structure may be relevant to calcium-related signaling. However, this study did not measure genomic amplification, protein abundance, calcium influx, or ion-channel activity. These findings support further investigation of *CALHM5* RNA expression as a possible biomarker in glioblastoma.

Keywords: glioblastoma; *CALHM5*; calcium signaling; ion channel; bioinformatics

INTRODUCTION

Over a million Americans are currently living with a brain tumor, while an estimated 94,390 will be diagnosed in the following year. Due to their location, limited treatment options, and resistance to standard therapies, brain tumors remain one of the most difficult cancers to treat, with a five-year survival rate for malignant brain tumors (tumors that originate in brain tissue) of 35.7%, highlighting the need for better diagnosis and treatment (1). Brain tumors are classified based on their cell type origin and whether they are benign (non-cancerous) or malignant (cancerous). These tumors are graded by the World Health Organization (WHO) on a scale of I to IV derived from histological features, invasiveness, and mitotic activity (2). Glioblastoma is the most prevalent of these brain cancers, accounting for around 49% of all malignant brain tumors, whereas lower-grade gliomas make up roughly 30% (1, 2). As the most aggressive subtype of astrocytoma, glioblastoma multiforme (GBM) is categorized by the WHO as a grade IV tumor. Its poor prognosis, roughly 15 months or under, is driven by key genetic alterations: amplification of *EGFR* occurs in approximately 50–60% of cases, increasing the rate

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of tumor growth, while mutations in *TP53* and loss of *PTEN* further disrupt cell regulation, allowing cancer cells to bypass apoptosis and continuously divide. Although *IDH1* mutations are often related to lower-grade gliomas, they are not often found in glioblastoma. These molecular characteristics contribute to the tumor's resistance to treatment, reinforcing the importance of developing more precise, targeted therapies (2, 3).

Glioblastomas are primarily driven by genetic mutations in oncogenes and tumor suppressors. However, recent studies have shown that genes related to calcium signaling may also contribute to tumor development (4). One such gene is *CALHM5* (Calcium Homeostasis Modulator 5), which encodes a protein predicted to form an ion channel involved in calcium-related signaling.

In cell signaling, calcium ions serve as an essential secondary messenger, controlling cell growth and programmed cell death (4, 5). Calcium flow is meticulously managed in healthy brain tissue, preventing unchecked growth. *CALHM5* is predicted to encode a non-selective ion-channel protein that may contribute to calcium-related signaling and ionic homeostasis. When intracellular calcium levels are increased in glioblastoma cells, the activation of pro-tumorigenic signaling pathways occurs, which are known to promote angiogenesis and proliferation (4, 5, 6). Moreover, high levels of calcium can result in mitochondrial malfunction, which glioblastoma cells utilize to their advantage, improving their resilience and adaptability (5). Glioblastoma cells may undergo epithelial-to-mesenchymal transition-like changes, which have been associated with increased invasiveness and treatment resistance (7).

Current glioblastoma treatments include surgery followed by radiation and chemotherapy, most commonly utilizing the alkylating agent temozolomide (TMZ). TMZ is an oral chemotherapy drug that kills cancer by adding alkyl groups to its DNA, leading to cell death. However, TMZ comes with numerous side effects, including bruising, internal bleeding, fatigue, and dizziness. Even with these treatments, tumor progression is often only temporarily delayed, with the median survival only being at around 12 to 15 months, with a 5-year survival rate of 6.8% (8). Second-line therapies often have little benefit; for example, surgical resection is nearly impossible due to the tumors' highly aggressive growth pattern, allowing the tumor to spread throughout the brain. Furthermore, glioblastoma cells are extremely resistant to apoptosis due to alterations in genetic material, such as loss of *TP53* or *PTEN*. The blood-brain barrier (BBB) is a selective membrane that restricts access of therapeutic drugs into the brain, preventing many chemotherapy agents from reaching the tumor site. Due to the current treatment's low success rate and harsh side effects, new solutions and strategies are vital. CALHM5 RNA expression was higher in glioblastoma than in other cancer types shown in the descriptive dataset. Elevated CALHM5 RNA expression was also associated with lower patient survival and was detected in CD133-positive glioblastoma cell lines. If validated in independent studies, *CALHM5* RNA expression may have potential as a glioblastoma biomarker. This paper aims to characterize *CALHM5* RNA expression and its clinical associations in glioblastoma using publicly available bioinformatics datasets.

METHODS AND MATERIALS

Study design

This study was conducted as a secondary bioinformatics analysis of publicly available datasets. No new experimental data, patient samples, or clinical records were collected.

Data sources

Publicly available databases were used to evaluate *CALHM5* predicted structure, RNA expression, and clinical associations in glioblastoma. *CALHM5* RNA expression across cancer types was examined using the Human Protein Atlas. NCBI Gene Expression Omnibus profiles were used to evaluate *CALHM5* RNA expression in glioblastoma-related samples, including CD133-positive and CD133-negative glioblastoma cell lines. Ensembl was used to visualize the predicted *CALHM5* protein structure.

Protein structure analysis

The predicted structure of *CALHM5* was visualized using Ensembl's protein structure viewer for the human *CALHM5* protein, Ensembl protein identifier ENSP00000357588. The model was examined to describe the overall transmembrane channel shape, including the ring-like arrangement and central pore.

Expression analysis

CALHM5 RNA expression across cancer types was evaluated using the Human Protein Atlas cancer expression dataset. Expression values were reported in protein-coding transcripts per million, or pTPM. CALHM5 RNA expression in glioblastoma was

compared with RNA expression levels across the other cancer types shown in the Human Protein Atlas dataset.

Survival analysis

The association between *CALHM5* RNA expression and glioblastoma patient survival was evaluated using Kaplan-Meier survival analysis from the Human Protein Atlas. Patients were grouped into high and low *CALHM5* RNA expression cohorts according to the cutoff provided by the Human Protein Atlas. Survival between groups was compared using the log-rank test. The Human Protein Atlas reported a *CALHM5* RNA expression cutoff of 1.8 pTPM and a p score of 0.065. Three-year survival was 19% in the high-expression group and 58% in the low-expression group.

CD133 expression analysis

CALHM5 RNA expression in CD133-positive and CD133-negative glioblastoma cell lines was evaluated using NCBI Gene Expression Omnibus profile data from dataset GDS2728. The *CALHM5* probe shown in the GEO profile was 230254_at. Expression patterns were compared between CD133-negative and CD133-positive glioblastoma cell lines.

Ethics statement

This study used only publicly available, de-identified secondary data. Because no direct human participant interaction, private health information, biospecimen collection, or animal experimentation was involved, institutional review board approval was not required.

RESULTS

CALHM5 Protein Structure

The predicted *CALHM5* protein model showed a ring-like structure composed of multiple alpha-helical regions surrounding a central cavity. This structural model was examined descriptively and did not provide measurements of *CALHM5* protein abundance, calcium conductance, or ion-channel activity (Figure 1).

CALHM5 Expression Across Cancer Types

Among the cancer types displayed in the Human Protein Atlas dataset, glioblastoma had the highest observed median *CALHM5* RNA expression, at approximately 3.3 pTPM, with values extending to approximately 8.25 pTPM. The graph descriptively compared *CALHM5* RNA expression across the cancer types included in the Human Protein Atlas dataset. The majority of other cancers shown in the Human Protein Atlas dataset had lower *CALHM5* RNA expression than glioblastoma. This analysis was descriptive and did not include statistical comparisons, multiple-testing correction, or independent validation (Figure 2).

CALHM5 Expression and Patient Survival

Patients with high *CALHM5* RNA expression (n=46) showed lower survival than patients with low *CALHM5* RNA expression (n=12). The Human Protein Atlas survival analysis reported a *CALHM5* RNA expression cutoff of 1.8 pTPM, with 3-year survival of 19% in the high-expression group and 58% in the low-expression group. This difference showed a trend toward poorer survival in the high-expression group but did not meet conventional statistical significance, p score = 0.065 (Figure 3).

CALHM5 Expression in CD133-positive Cells

CALHM5 RNA expression was detected in CD133-positive glioblastoma cell lines in GEO dataset GDS2728 using probe 230254_at. CD133-negative cell lines showed lower or absent *CALHM5* RNA expression in the same dataset. These results describe *CALHM5* RNA expression patterns across CD133-positive and CD133-negative glioblastoma cell lines (Figure 4).

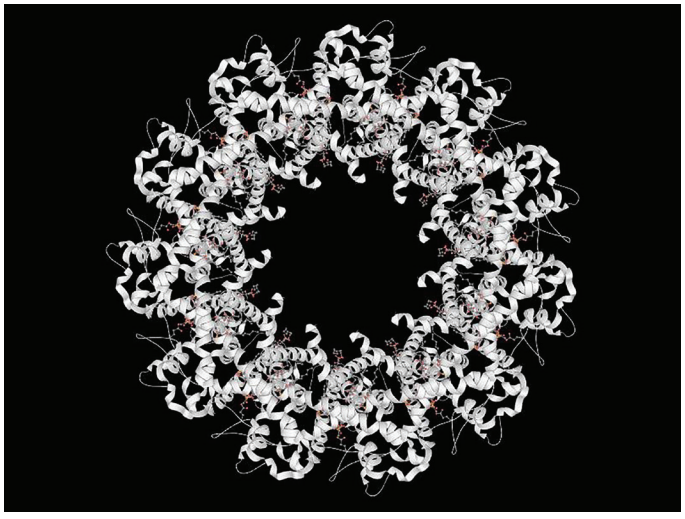

Figure 1. Predicted CALHM5 protein structure. *The predicted human CALHM5 protein structure was visualized using Ensembl's protein structure viewer, Ensembl protein identifier ENSP00000357588. The model shows a transmembrane channel-like structure with multiple alpha-helical regions arranged around a central pore.*

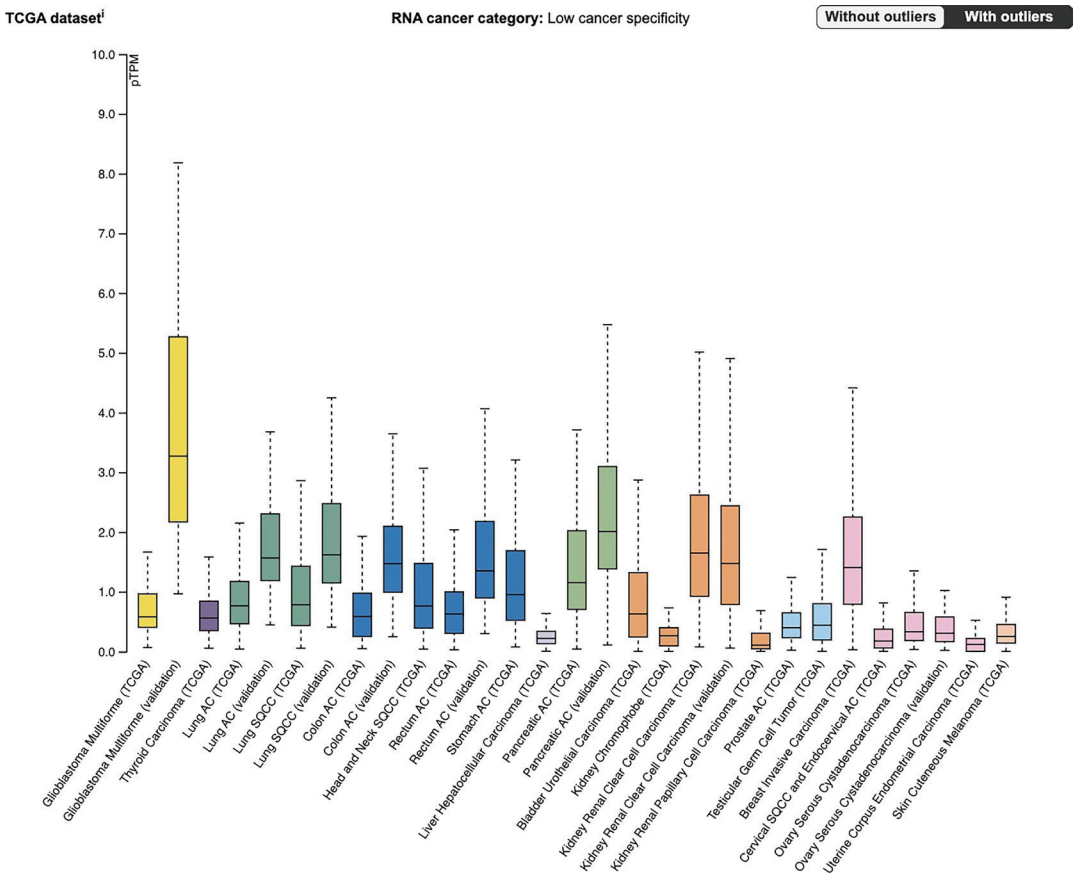

Figure 2. ***CALHM5 RNA expression across cancer types.*** *This box-and-whisker plot shows CALHM5 RNA expression across cancer types using The Cancer Genome Atlas, or TCGA, data as processed by the Human Protein Atlas. Expression values are reported in protein-coding transcripts per million, or pTPM. Glioblastoma showed higher CALHM5 RNA expression than the other cancer types shown.*

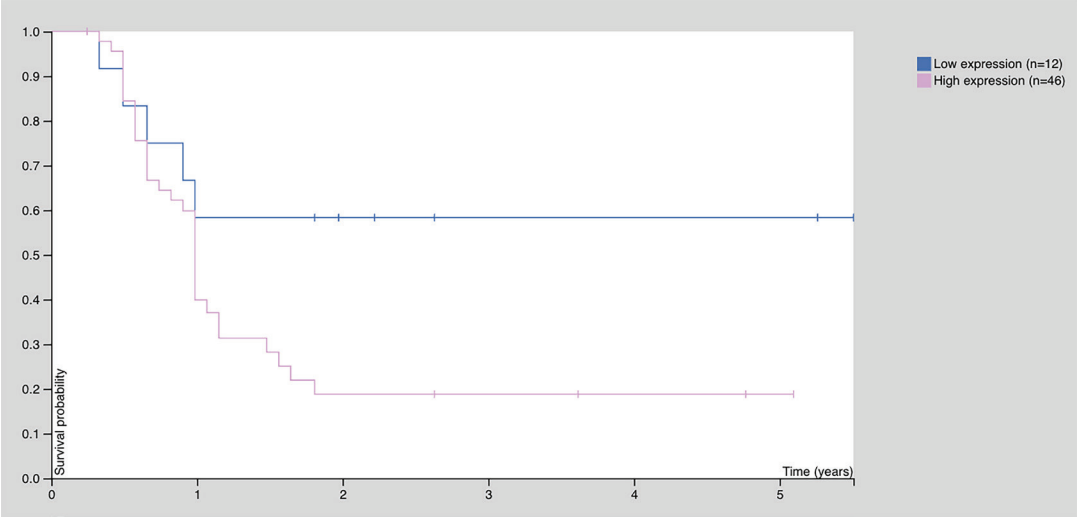

Figure 3. ***Kaplan-Meier survival analysis of CALHM5 RNA expression in glioblastoma.*** *Patients were divided into low CALHM5 RNA expression (n = 12) and high CALHM5 RNA expression (n = 46) groups using the Human Protein Atlas cutoff of 1.8 pTPM. The high-expression group had 19% 3-year survival, while the low-expression group had 58% 3-year survival. The Human Protein Atlas survival analysis reported a p score of 0.065.*

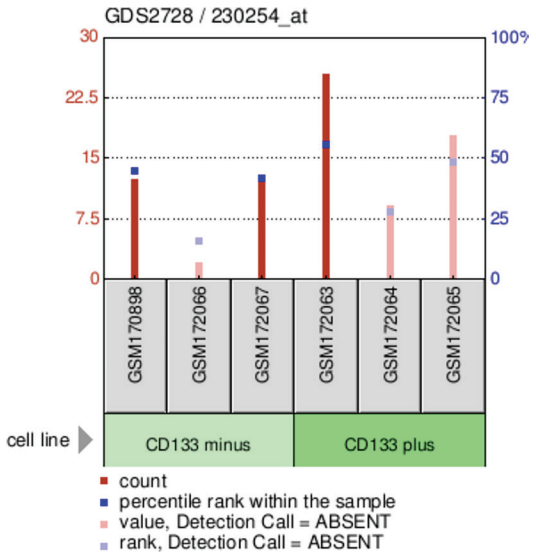

Figure 4. CALHM5 RNA expression in CD133-negative and CD133-positive glioblastoma cell lines. *Data were obtained from NCBI GEO dataset GDS2728 using probe 230254_at. Red bars represent expression count values, and blue markers represent percentile rank within each sample. CALHM5 RNA expression was detected in CD133-positive cell lines and was lower or absent in CD133-negative cell lines.*

DISCUSSION

This study found that *CALHM5* RNA expression was elevated in glioblastoma compared with several other cancer types and that higher *CALHM5* RNA expression was associated with lower 3-year survival in the Human Protein Atlas glioblastoma cohort. *CALHM5* RNA expression was also detected in CD133-positive glioblastoma cell lines in the GEO dataset analyzed. Together, these findings suggest that *CALHM5* may be associated with more aggressive glioblastoma features, although this study cannot determine whether *CALHM5* directly causes tumor progression. The findings reported here concern *CALHM5* RNA expression. They do not demonstrate *CALHM5* genomic amplification, increased protein abundance, or increased ion-channel activity.

The possible relevance of *CALHM5* in glioblastoma may relate to its predicted function as a pore-forming ion channel. Calcium signaling regulates multiple cancer-related processes, including cell proliferation, apoptosis, migration, and survival (4, 5). Prior studies have shown that altered calcium signaling can contribute to cancer progression, and calcium-permeable channels have been implicated in glioblastoma cell migration and apoptosis (4, 6). The predicted channel-like structure provides structural context but does not establish functional calcium conductance. Protein-level and functional studies are needed to determine whether elevated *CALHM5* RNA expression corresponds to increased protein abundance or altered ion-channel activity in glioblastoma cells.

The detection of *CALHM5* RNA expression in CD133-positive glioblastoma cell lines may also be relevant because CD133 has been used as a marker of glioblastoma stem-like cells (9). These cells have been associated with tumor initiation, therapeutic resistance, and recurrence (9). However, the present study only shows an expression pattern in a public dataset. It does not prove that *CALHM5* regulates CD133-positive cells or causes treatment resistance.

These findings should also be interpreted in the context of prior research on calcium signaling and glioblastoma. Previous studies have shown that calcium-permeable AMPA receptors can promote glioblastoma cell migration and survival, while broader calcium signaling pathways have been linked to apoptosis, autophagy, and cancer cell proliferation (5, 6). Compared with these better-studied calcium-related mechanisms, *CALHM5* remains relatively underexplored in glioblastoma. This makes *CALHM5* a candidate for further investigation rather than a confirmed oncogenic driver.

This study has several limitations. All analyses were based on publicly available secondary datasets, and the results are correlational. The survival analysis showed lower 3-year survival in the high *CALHM5* RNA expression group, but the p score was 0.065 and did not meet the conventional threshold for statistical significance. The survival analysis was also limited by the small and unequal group sizes, with 46 patients in the high-expression group and 12 in the low-expression group. No multivariable analysis was performed to adjust for age, treatment, molecular subtype, MGMT promoter methylation, IDH status, or other prognostic factors. Therefore, the observed survival difference may reflect confounding and should not be interpreted as an independent prognostic effect of *CALHM5* RNA expression. In addition, this study did not include laboratory experiments, functional assays, or independent clinical validation. Therefore, the results should be interpreted as hypothesis-generating.

Future studies should test *CALHM5* function directly in glioblastoma models. Useful experiments would include *CALHM5* knockdown or overexpression in

glioblastoma cell lines, calcium influx assays, apoptosis assays, proliferation assays, and evaluation of *CALHM5* RNA expression in independent patient cohorts. These studies would help determine whether *CALHM5* has a functional role in glioblastoma biology or whether its expression is mainly a correlated biomarker.

CONCLUSION

This exploratory bioinformatics analysis examined *CALHM5* RNA expression, predicted protein structure, and clinical associations in glioblastoma. *CALHM5* RNA expression was higher in glioblastoma than in the other cancer types shown in the descriptive Human Protein Atlas dataset. Higher *CALHM5* RNA expression was also associated with lower survival in the analyzed cohort and was detected in CD133-positive glioblastoma cell lines.

These findings are correlational and do not establish *CALHM5* genomic amplification, increased protein abundance, increased ion-channel activity, or a causal role in glioblastoma progression. The survival analysis was limited by small and unequal groups, lack of multivariable adjustment, and a p score of 0.065.

Further studies should assess *CALHM5* protein abundance, channel function, calcium signaling, and biological effects in experimental glioblastoma models. Independent clinical cohorts will also be needed to determine whether *CALHM5* RNA expression has value as a biomarker.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this work.

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