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High expression of N-type calcium channel indicates a favorable prognosis in gliomas

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Abstract

For the diagnosis and prognosis of glioma, the development of prognostic biomarkers is critical. The N-type calcium channel, whose predominant subunit is encoded by calcium voltage-gated channel subunit alpha1 B (*CACNA1B*), is mostly found in the nervous system and is closely associated with neurosensory functions. However, the link between the expression of *CACNA1B* and glioma remains unknown. We used ONCOMINE to explore the differences in *CACNA1B* expression among different cancers. We then conducted survival analysis and COX analysis using TCGA_LGG and TCGA_GBM datasets, which were divided into $CACNA1B^{high}$ and $CACNA1B^{low}$ based on the median. We examined the differences in other favorable prognostic markers or clinical characteristics between $CACNA1B^{high}$ and $CACNA1B^{low}$ using *t* tests. Differentially expressed genes were identified, and KEGG pathway enrichment was performed. We compared the expression of methyltransferases and analyzed the differentially methylated regions. Immunohistochemistry results were retrieved from the Human Protein Atlas database for validation purposes. *CACNA1B* was expressed at lower levels in gliomas, and, for the first time, we found that high expression of *CACNA1B* in gliomas predicts a good prognosis. Other favorable prognostic markers, such as isocitrate dehydrogenase mutation, 1p/19q codeletion, and O6-methylguanine-DNA methyltransferase promoter methylation, were increased in tandem with high expression of *CACNA1B*. Differentially expressed genes were enriched in multiple pathways related to cancer progression and aberrant epigenetic alterations were significantly associated with *CACNA1B*. High expression of N-type calcium channels indicates a favorable prognosis for gliomas. This study provides a better understanding of the link between gliomas and N-type calcium channels and may offer guidance for the future treatment of gliomas.

Abbreviations: CACNA1B = calcium voltage-gated channel subunit alpha1 B, CNS = central nervous system, DEG = differentially expressed genes, DNMT = DNA methyltransferase, GBM = glioblastoma, HPA = Human Protein Atlas, IDH = isocitrate dehydrogenase, KEGG = Kyoto Encyclopedia of Genes and Genomes, LGG = low-grade gliomas, MGMT = O6-methylguanine-DNA methyltransferase, TCGA = The Cancer Genome Atlas.

Keywords: calcium channel, calcium voltage-gated channel subunit alpha1 B, glioma, N-type, prognostic biomarker

1. Introduction

Glioma is the most frequent type of cancer of the central nervous system (CNS), accounting for approximately a quarter of all cases.[1] Based on their histology, the World Health Organization has categorized them into 4 grades (grades I–IV). The most prevalent form of glioma is glioblastoma (GBM; grade IV), which has an incidence rate ranging from 0.59 to 3.69 every 100,000 people, according to the reporting organization or country.[2] Patients with GBM have the lowest overall survival rate, with <5% surviving for 5 years. Low-grade glioma (grade II) is a lethal disease that affects young individuals with an average survival time of approximately 7 years.

In 2016, molecular features consisting of 1p/19q codeletion and isocitrate dehydrogenase (IDH) mutation were included in the revised classification of gliomas. Gliomas with 1p/19q codeletion or IDH mutations have an extended survival time.[3] In all grades of glioma, O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation is a prognostic factor.[4] *EGFR* amplification was demonstrated to be an independent, substantial, and negative predictor of overall survival by Shinojima et al.[5]

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All data were obtained from TCGA, ONCOMINE, and HPA databases. The informed consent was obtained from all patients before our study.

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With the advancement of next-generation sequencing, a growing number of biomarkers have been discovered. Yang et al[6] found that high expression of *TNFRSF1A* was related to poor prognosis. Elevated *PIEZO1* expression is associated with shorter survival time and can be used as a valid index for the poor prognosis of glioma.[7] *MEOX2* was found to be one of the transcription factors most closely connected to overall survival in glioma patients by Tachon et al.[8]

These molecular indicators improve the diagnostic ability, diagnostic results, and prognostic evaluation of patients with glioma. Furthermore, research on the molecular subtypes and indicators of glioma remains a major scientific subject.

Voltage-gated calcium channels (VGCCs) are a flock of ion channels located in the cell membrane that can be penetrated by calcium ions. L-type,[9] R-type,[10] N-type,[11] P/Q-type,[12] and T-type[13] are 5 subtypes of VGCCs, each of which is encoded by a single subunit gene. These calcium channels have also been associated with mitogenesis, proliferation, differentiation, apoptosis, metastasis, and other cellular processes. Ion channels have been associated with the progression of various malignancies. *CACNA1A*, *CACNA1C*, and *CACNA1D* were revealed to be strongly expressed in the majority of types of cancer, while *CACNA1F* was identified to be significantly expressed in testicular cancer in a previous study.[14] Ion channel transcript expression has been discovered as a possible biomarker for several types of cancer, including breast and prostate cancer.[15–17] Therefore, studying the functional significance of ion channels in cancer growth could lead to the development of novel approaches for tumor prognosis.

N-type calcium channels, also called Cav2.2 channel, are most often found in the brain and peripheral nervous system. It is composed of several subunits, including the major subunit 1 B (encoded by calcium voltage-gated channel subunit alpha1 B [*CACNA1B*]) and auxiliary subunits. N-type calcium channels are crucial for neurotransmitter release and pain pathways in the adult nervous system.[18,19] Both N-type calcium channels and gliomas are highly associated with the nervous system; therefore, it is important to study this relationship. However, the relationship between N-type calcium channels and gliomas, particularly in terms of prognosis, remains unknown. Nevertheless, no research has been conducted on this topic.

In the present study, the prognostic value of *CACNA1B* expression in gliomas was determined. High expression of *CACNA1B* indicated excellent prognosis in patients with glioma. Concurrently, it was also found that the high expression of *CACNA1B* is related to certain other molecular or clinical characteristics of the patient. Furthermore, the distinctive transcriptomics and epigenomic patterns associated with *CACNA1B* expression were discussed. The results of the present study can provide references and horizons for further research on N-type calcium channels, glioma, and the relationship between the 2.

2. Materials and methods

2.1. Data preparation and transform

The ONCOMINE database was utilized to compare *CACNA1B* levels between tumor and normal tissues of different types of cancer based on $P < 1e{-}04$, a gene rank percentile of <10%, and a fold change of >2.0.

RNA-seq data (both Fragments Per Kilobase of transcript per Million mapped reads [FPKM] and counts format) and other associated data were then downloaded from The Cancer Genome Atlas_low-grade gliomas (TCGA_LGG) and TCGA_GBM using the Genomic Data Commons data portal API by R package TCGAbiolinks.[20] The TCGA_LGG project consisted of 529 patients, whereas the TCGA_GBM project contained 174 samples. The gene ID of the RNA-seq data from the Ensembl gene ID was converted to the HUGO Gene Nomenclature Committee symbol. To integrate the transcriptome data, the RNA-seq data were transferred from FPKM to the transcripts per kilobase million format.

2.2. Survival analysis

To explore the relationship between *CACNA1B* expression and glioma prognosis, the survival information of the aforementioned samples, including survival status and survival time, was extracted, and TCGA glioma patients were divided into a high-expression group $CACNA1B^{high}$ and a low-expression group $CACNA1B^{low}$ according to the median value of the expression of *CACNA1B*. Survival analysis was then performed using this stratum.

After survival analysis, COX regression analysis was conducted among different factors, including age, sex, race, and *CACNA1B* expression. These analyses and response plots were conducted using the R package survminer and survival.[21]

2.3. Association of CACNA1B expression and other features

To investigate the relationship between *CACNA1B* expression and patient characteristics, other molecular or clinical characteristics of patients were extracted from the aforementioned samples, including age, sex, MGMT methylation, IDH mutation, 1p/19q_codel, and genomic mutation counts. The difference between $CACNA1B^{high}$ and $CACNA1B^{low}$ was examined by Student *t* test, and $P < .05$ was considered to indicate a statistically significant difference, using R package ggplot2[22] for plotting.

2.4. Association of CACNA1B expression and other genes

To determine the relationship between high and low expression of *CACNA1B* and other genes, the R package limma[23] was used to obtain differentially expressed genes (DEGs) between $CACNA1B^{high}$ and $CACNA1B^{low}$ with a filter condition of $|\log2FC| \geq 1.5$ and $P < .01$. After obtaining the DEGs, cluster Profiler[24] was used for the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis.

2.5. Association of CACNA1B expression and methylation

To explore methylation differences, the expression levels of 3 methylation transferases (*DNMT1*, *DNMT3A*, and *DNMT3B*) were compared between $CACNA1B^{high}$ and $CACNA1B^{low}$. The R package TCGAbiolinks function TCGAanalyze_DMC (diffmean.cut = 0.2, p.cut = 0.01) was used to conduct the differentially methylated region analysis.

2.6. The validation of expression of CACNA1B in tissues

Immunohistochemistry images were downloaded from The Human Protein Atlas (HPA) database (https://www.proteinatlas.org/)[25] and the expression level of *CACNA1B* in tissues was verified by comparing the results of immunohistochemistry in normal tissue and cancer tissue of gliomas.

3. Results

3.1. CACNA1B expression and survival analysis

First, the expression of *CACNA1B* was compared between different cancer tissues and the corresponding normal tissues in the ONCOMINE database and it was revealed that *CACNA1B* was decreased in 2 types of malignancies, brain and CNS cancer (5 datasets supported) and breast cancer (only 1 dataset supported; Fig. 1A).

Research was then conducted using TCGA database to compare the expression of *CACNA1B* between glioma tumors and normal tissues. It could be observed that the expression of *CACNA1B* in normal tissues was markedly higher than that in tumor tissues, which was consistent with the result from ONCOMINE ($P = .01453$; Fig. 1C). Survival analysis revealed

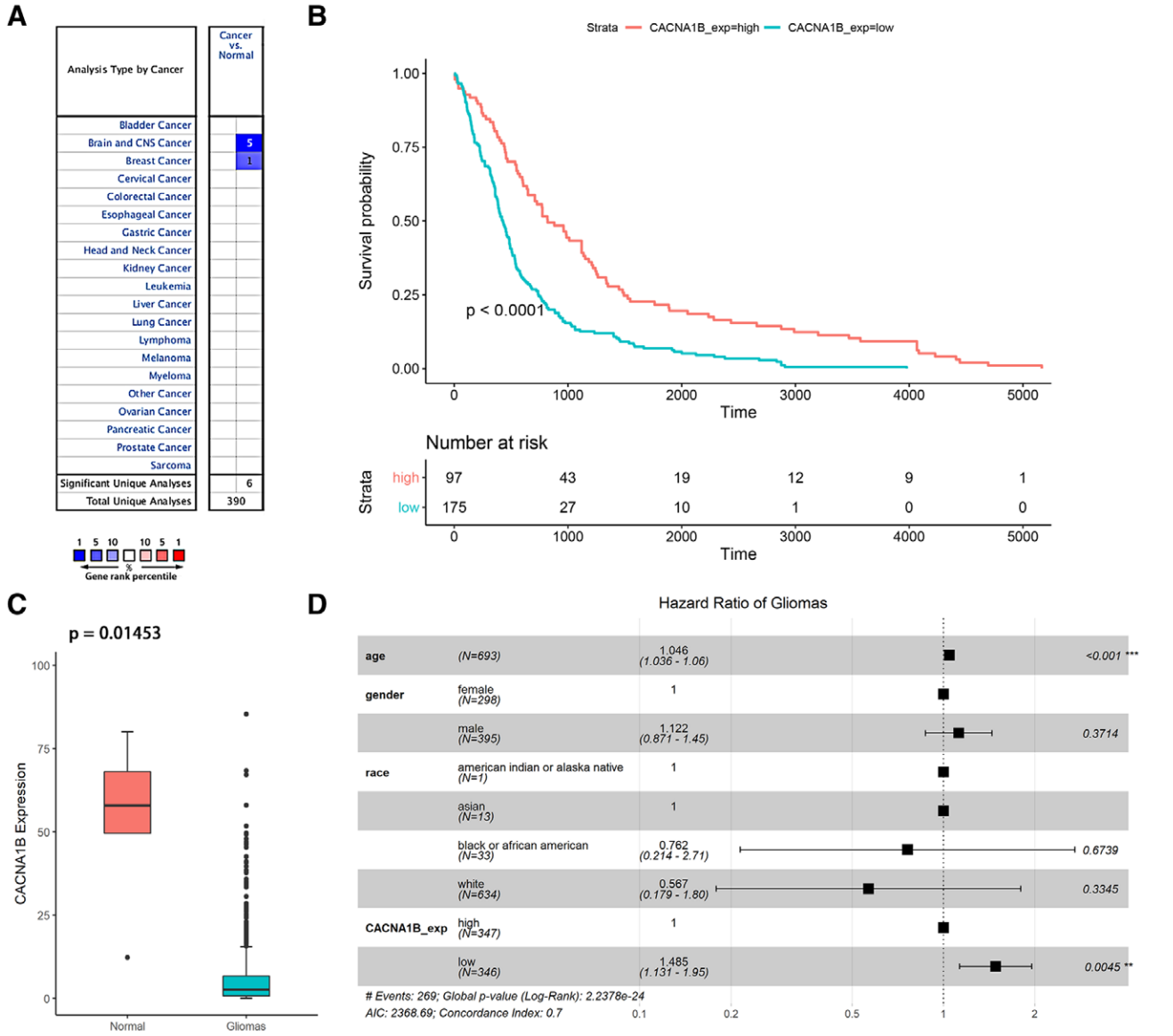

Figure 1. *CACNA1B* expression and survival analysis. (A) Expression of *CACNA1B* in different types of cancer compared with numerous normal tissues according to ONCOMINE. (B) Survival analysis of *CACNA1B* in gliomas. (C) Boxplot of *CACNA1B* expression in gliomas compared with normal tissues according to The Cancer Genome Atlas. (D) COX proportional hazards regression analysis. *CACNA1B* = calcium voltage-gated channel subunit alpha1 B.

that the *CACNA1B*^{high} group had an improved prognosis (P < .0001; Fig. 1B), which indicated that high expression of *CACNA1B* indicated a favorable prognosis in gliomas.

For the data used in the present study, COX proportional hazards regression was used for modeling, and the results were displayed using forest plots (Fig. 1D). It was observed that age (HR = 1.046, P < .001) and *CACNA1B* (HR=1.485, P = .0045) increased risk. Regarding sex, although the risk in men was slightly higher than that in women, it was not statistically significant (HR ratio = 1.122, P = .3714).

3.2. Association of CACNA1B expression and other features

The patients in the *CACNA1B*^{high} group tended to be younger. The average age of *CACNA1B*^{high} group was 42.68, while the *CACNA1B*^{low} group has an average age of 50.63 (P = 1.96e–12; Fig. 2A. The relationship between the male-to-female ratio and *CACNA1B* expression was evaluated and it was observed that there was no difference in the male-to-female ratio between the *CACNA1B*^{low} and *CACNA1B*^{high} groups (1.386 vs 1.268, P = .6142; Fig. 2B). It was also found that the average number of mutations in the *CACNA1B*^{high} group was 28.649, while the average number of mutations in the *CACNA1B*^{low} group was 39.621, which was statistically higher (P = 2.09e–16; Fig. 2D).

In the *CACNA1B*^{high} group, the ratio of MGMT methylation and IDH mutation was significantly higher than that in the *CACNA1B*^{low} group (0.824 vs 0.67, P = 1.32e–05; 0.821 vs 0.469, P = 1.18e–20, respectively; Fig. 2C, E). It was also identified that the proportion of 1p/19q_codel cases in the *CACNA1B*^{high} group was higher (0.279 vs 0.232), although the difference was not statistically significant (P = .1964; Fig. 2F). MGMT methylation, IDH mutation, and 1p/19q_codel, all 3 biomarkers, indicated an improved prognosis in gliomas.

3.3. Association of CACNA1B expression and genome-wide expression profiles

To further study and explore the relationship between *CACNA1B* expression and other genes, through the comparison of transcriptome data (|log2FC| ≥ 1.5 and FDR-adjusted

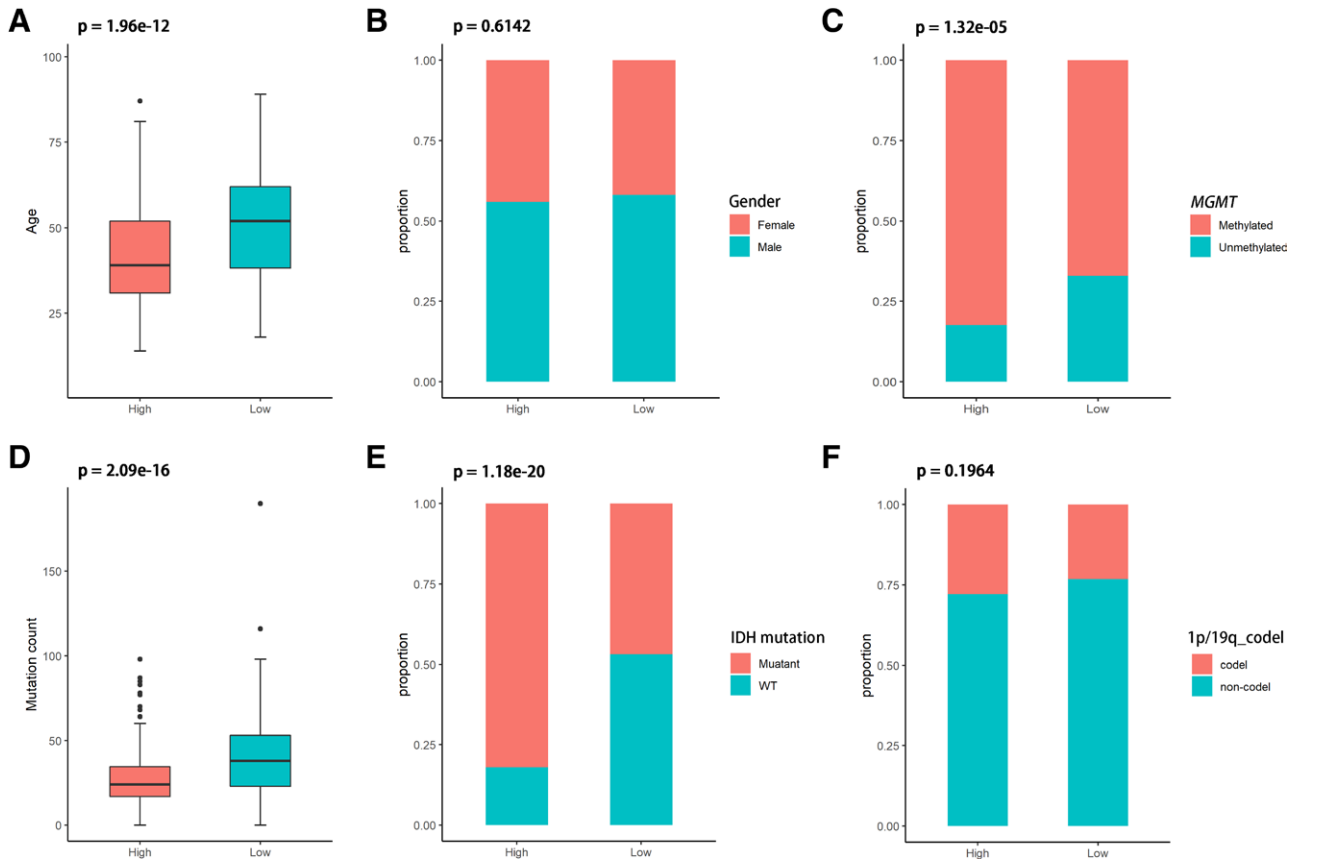

Figure 2. Association of *CACNA1B* expression and other characteristics. (A) Boxplot of age between *CACNA1B*^{high} and *CACNA1B*^{low}. (B) Comparation of sex proportion. (C) Comparation of O6-methylguanine-DNA methyltransferase methylation proportion. (D) Boxplot of genome mutation counts between *CACNA1B*^{high} and *CACNA1B*^{low}. (E) Comparation of IDH mutation proportion. (f) Comparation of 1p/19q codeletion proportion. *CACNA1B* = calcium voltage-gated channel subunit alpha1 B, IDH = isocitrate dehydrogenase.

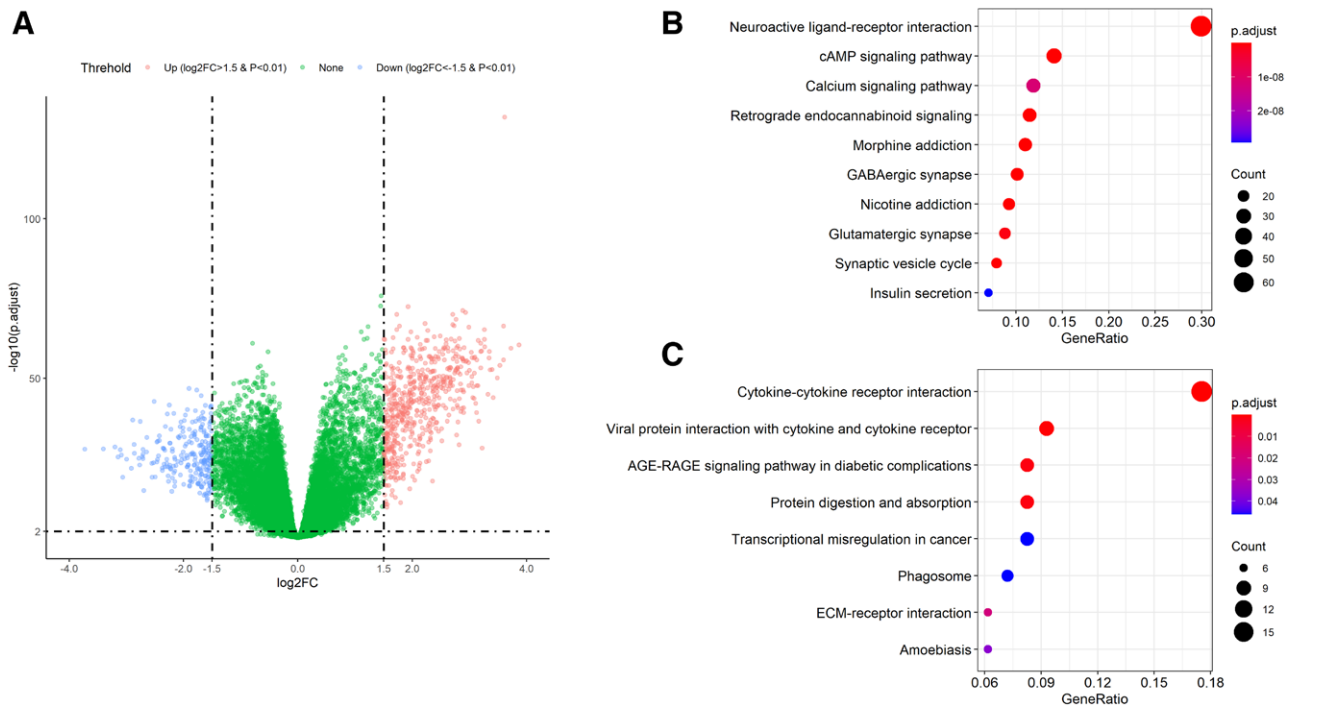

Figure 3. Association of *CACNA1B* expression and genome-wide expression profiles. (A) Volcano plot of differentially expressed genes between *CACNA1B*^{high} and *CACNA1B*^{low} ($|\log_2 FC| \geq 1.5$ and FDR-adjusted $P < .01$). (B) KEGG pathway enrichment of upregulated genes between *CACNA1B*^{high} and *CACNA1B*^{low}. (C) KEGG pathway enrichment of downregulated genes between *CACNA1B*^{high} and *CACNA1B*^{low}. *CACNA1B* = calcium voltage-gated channel subunit alpha1 B, cAMP = cyclic adenosine monophosphate, ECM = extracellular matrix, FDR = false discovery rate, KEGG = Kyoto Encyclopedia of Genes and Genomes.

P < .01), it was found that 837 genes were differentially expressed between *CACNA1B*^{high} and *CACNA1B*^{low}. A total of 588 genes were upregulated, whereas 249 were downregulated (for details see Table S1, Supplemental Digital Content, http://links.lww.com/MD/G851; Fig. 3A).

KEGG pathways that upregulated genes enriched in included neuroactive ligand–receptor interaction, cyclic adenosine monophosphate (cAMP) signaling pathway, calcium signaling pathway, retrograde endocannabinoid signaling, morphine addiction, etc (for details see Table S2, Supplemental Digital Content, http://links.lww.com/MD/G852; Fig. 3B).

KEGG pathways that downregulated genes enriched in contains cytokine–cytokine receptor interactions, viral protein interactions with cytokines and cytokine receptors, protein digestion and absorption, AGE-RAGE signaling pathway in diabetic complications, transcriptional misregulation in cancer, and others (for details see Table S3, Supplemental Digital Content, http://links.lww.com/MD/G853; Fig. 3C).

3.4. Association of CACNA1B expression and methylation

DNA methylation is a basic epigenetic mechanism that affects cells by regulating gene expression through 3 DNA methyltransferases: *DMNT1*, *DMNT3A*, and *DMNT3B*. The expression of these 3 DNA methyltransferases was compared between the *CACNA1B*^{high} and *CACNA1B*^{low} groups and it was found that *DMNT1* was highly expressed in the *CACNA1B*^{low} group; however, the difference was not statistically significant (P = .1453; Fig. 4A). *DMNT3B* was expressed more highly in the *CACNA1B*^{low} group (P = .0235; Fig. 4C), whereas *DMNT3A* was expressed at a lower level in the *CACNA1B*^{low} group (P = .02534; Fig. 4B). The methylation differences between the 2 groups (*CACNA1B*^{high} and *CACNA1B*^{low}) were compared; 11,294 differentially methylated sites were identified, of which 10,645 were hypermethylated and 649 were hypomethylated in the *CACNA1B*^{high} group (for details see Table S4, Supplemental Digital Content, http://links.lww.com/MD/G854; Fig. 4D).

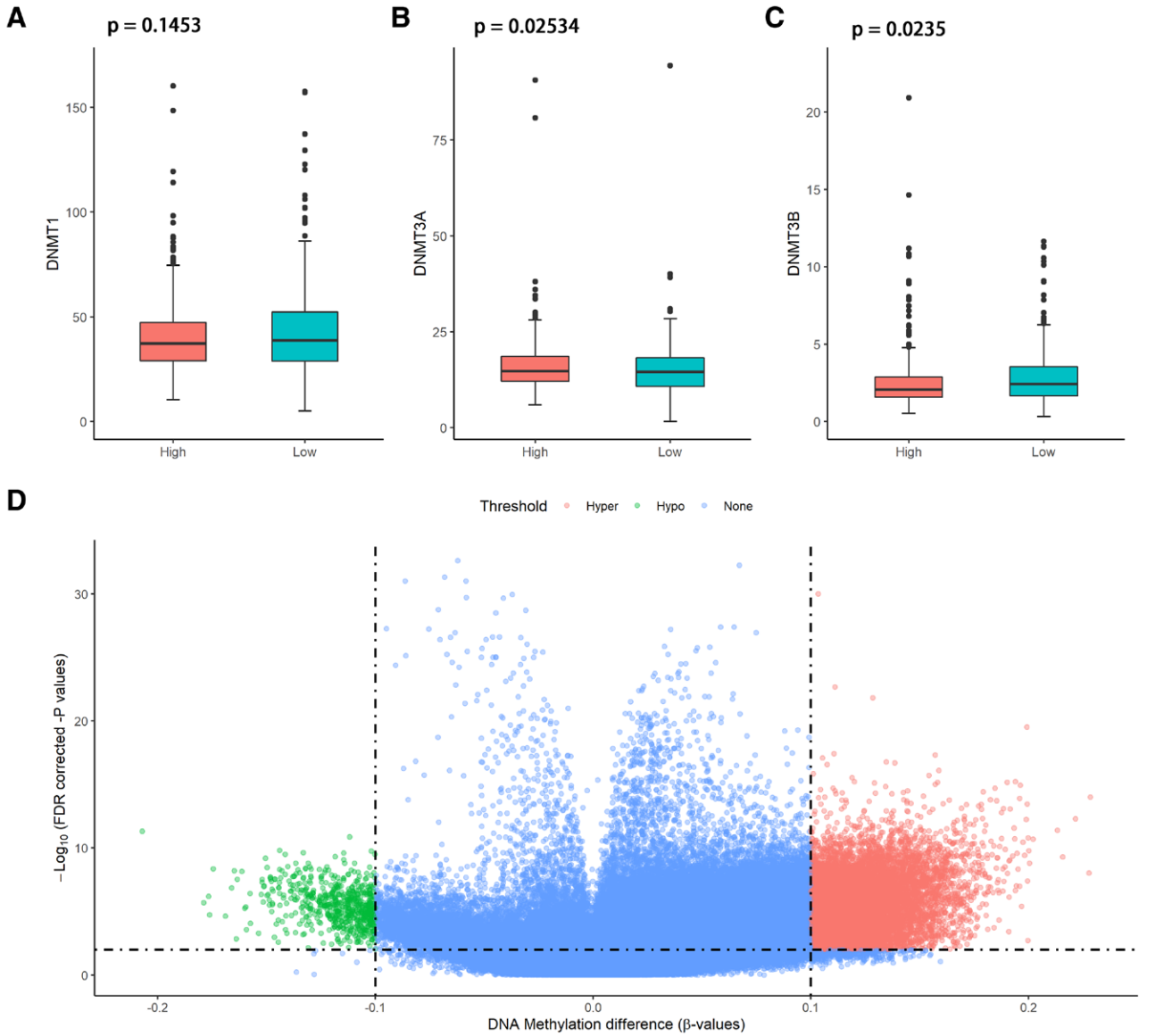

Figure 4. Association of *CACNA1B* expression and methylation. (A) Differential expression of *DNMT1* between *CACNA1B*^{high} and *CACNA1Blow*. (B) Differential expression of *DNMT3A* between *CACNA1B*^{high} and *CACNA1B*^{low}. (C) Differential expression of *DNMT3B* between *CACNA1B*^{high} and *CACNA1B*^{low}. (D) Volcano plot of differentially methylated sites. *CACNA1B* = calcium voltage-gated channel subunit alpha1 B, *DNMT* = DNA methyltransferase.

3.5. The validation of expression of CACNA1B in tissues

To confirm the expression of *CACNA1B* in tissues, immunohistochemical results from the HPA database were retrieved.[26] The antibody (HPA044347) was labeled with 3,3'-diaminobenzidine, and the resulting brown staining indicated where the antibody was combined with CACNA1B protein. According to the immunohistochemical results, it was found that in normal brain tissues (patient ID: 1609, male, age 62), *CACNA1B* was highly expressed (Fig. 5A), while in brain tissues of patients with gliomas (patient ID: 3023, female, age 1), CACNA1B protein could not be detected by immunohistochemical methods (Fig. 5B).

4. Discussion

Calcium ions are key cell messengers that regulate a variety of cellular functions, including cell growth and inhibition and activation of intracellular enzymes. Different calcium ion channels, pumps, and exchangers regulate changes in calcium ion levels in cells. These changes are decoded by a carefully designed calcium ion sensor toolkit, which converts calcium ion signals into operational cellular machinery to regulate numerous calcium ion-dependent physiological processes. Calcium channel transcription levels may have various domino effects on cell function, proliferation, motility, and even apoptosis.[27]

In the present study, the expression of *CACNA1B* was first examined in different tumors and it was revealed that it was low in brain and CNS cancer. The expression levels between tumors and normal tissues were then compared and it was identified that *CACNA1B* was underexpressed in tumor tissues. At the same time, the present study revealed that low expression of *CACNA1B* had poor prognostic significance in gliomas. Finally, this was verified using the HPA database.[26] These findings consistently indicated the prognostic value of *CACNA1B* in gliomas.

The relationship between *CACNA1B* and other clinical or molecular characteristics of the patients was investigated and it was found that the *CACNA1B*^{high} group was younger and the ratio of MGMT methylation, IDH mutation, and 1q/19_codel was higher. These are well-documented biomarkers that predict good prognosis. Concurrently, the mutation counts of the genome, which are unfavorable for prognosis, decreased. These results indicated a positive correlation between *CACNA1B* and other prognostic markers.

In the adult nervous system, N-type calcium ion channels are closely associated with neurotransmitter release and pain pathways. Common clinical features of brain cancer include headaches, cramps, personality changes, paralysis, dizziness, and other neurological problems. Our study shows that N-type calcium channels are underexpressed in glioma tissues (including protein levels), which provides clues to the relationship between the 2. It is very likely that the reduction in N-type calcium channels in gliomas causes the patient to develop such neurological symptoms. The degree of decrease in N-type calcium ion channels can reflect the degree of deterioration in the patient's condition, which may explain the relationship between N-type calcium ion channels and the prognosis of gliomas.

We compared the DEGs between *CACNA1B*^{high} and *CACNA1B*^{low} and then examined the pathways in which the upregulated genes were enriched. Among the upregulated pathways, the cAMP signaling pathway has clear significance for inhibiting the progression of cancer. cAMP plays a crucial role in cell signaling and regulates the transcription of a variety of target genes, mostly through protein kinase A (PKA) and its downstream effectors, including cAMP-responsive element-binding protein. PKA has been shown to decrease tumor growth in the GBM cell line A-172 in previous research. PKA activation can promote differentiation, inhibit A-172 cell proliferation, and induce apoptosis by increasing cAMP levels or by introducing cAMP analogs (dcAMP and 8-Br-cAMP).[28] Increased intracellular cAMP levels cause upregulation of p21 and p27 as well as activation of PKA and Epac1-Rap1 signaling, culminating in A-172 cell growth arrest and death.[29,30]

Among the downregulated pathways, cytokine–cytokine receptor interactions and transcriptional misregulation in cancer are related to tumors. Transcriptional dysregulation in cancer is a pathway in which changes in transcription factors affect the expression of target genes, leading to changes in a variety of cell characteristics that are conducive to tumorigenesis. Cytokine–cytokine receptor interactions also have an important impact on the development of cancer. Cytokines, a type of protein released in response to infection, inflammation, and immunology, have been shown to slow down the progression of cancer.[31] Cancer cells can also respond to cytokines produced by the host, which boosts proliferation, inhibits apoptosis, and aids invasion and metastasis.[32]

DNA methylation is controlled by DNA methylases (*DNMT1*, *DNMT3A*, *and DNMT3B*) in animals. DNA methylation has a significant impact on gene expression and cancer.[33,34] Rajendran et al[35] showed that compared with normal tissues, *DNMT1* and *DNMT3B* are overexpressed in gliomas, but *DNMT3A* is not overexpressed. Similarly, our study showed that in glioma

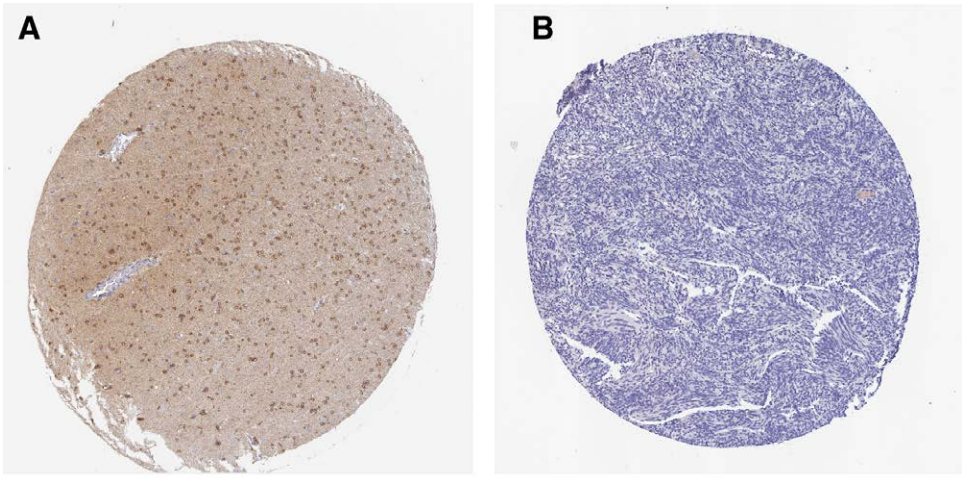

Figure 5. Validation of expression of *CACNA1B* in tissues. The brown staining indicates where CACNA1B protein was detected. (A) The immunohistochemical result of normal brain tissue. (B) The immunohistochemical result of gliomas brain tissue. *CACNA1B* = calcium voltage-gated channel subunit alpha1 B.

patients with low expression of *CACNA1B*, the expression of *DNMT1* and *DNMT3B* increased, and the low expression of *CACNA1B* indicated a poor prognosis. Overexpression of DNA methylases can lead to hypermethylation and tumor-suppressed gene inactivation. Overexpression of *DNMT1* can lead to lymph node metastasis and a poor prognosis.[36,37] *DNMT3B* is necessary for colon cancer growth.[38] Further investigation of these phenomena will increase the understanding of epigenetics and glioma generation.

In summary, high *CACNA1B* expression in gliomas was revealed to predict a favorable prognosis. Other positive prognostic markers, such as IDH mutation, 1p/19q codeletion, and MGMT promoter methylation, were also enhanced along with high *CACNA1B* expression. The findings of the present study serve as a foundation for future research on N-type calcium channels, gliomas, and the link between the 2. Understanding the underlying mechanism behind the role of N-type calcium channels in cancer progression may help determine therapeutic targets for brain cancer treatment.

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Author contributions

The datasets generated during and/or analyzed during the current study are publicly available.

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