

Increased KCNN4 Expression Is Correlated With Poor Survival in Lower Grade Glioma

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Abstract. *Background/Aim:* Networks of glioma cells are linked to small groups of pacemaker cells in which levels of calcium ions pulse periodically, driving a signal through the network that causes tumor growth. Using inhibitors, one study blocked the activity of the Ca²⁺ activated potassium-channel protein KCa3.1 in *in vitro* models and mice, preventing the proliferation of glioma cells and tumor expansion. Marked reduction of tumor cell viability occurred within the entire network, as well as reduced tumor growth in mice and extended animal survival. *Materials and Methods:* KCa3.1 is encoded by the gene potassium calcium-activated channel subfamily N member 4 (KCNN4) on the chromosomal location 19q13.31. We used the Cancer Genome Atlas (TCGA) to evaluate the effect of KCNN4 on human glioma survival in the TCGA Lower Grade Glioma (LGG) dataset. *Results:* In humans, KCNN4 is prognostic in glioma; high expression is unfavorable. In addition, KCNN4 copy number variations are prognostic. Increased masked copy number segments are unfavorable in lower grade glioma. KCNN4 is lost in gliomas with the 1p 19q co-deletion, which may explain in part the comparatively favorable prognosis of 1p 19q co-deletion tumors. *Conclusion:* Our finding of increased KCNN4 expression related to poor survival in human lower grade glioma suggests that developing novel therapies, such as KCa3.1-inhibiting drugs, might be worthwhile.

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Glioma is a fatal disease that affects young people; death occurs after roughly 7 years. Patients with low-grade gliomas fare better than those with high-grade (WHO grade III/IV) gliomas, but most gliomas progress to high-grade gliomas that are deadly (1). The National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) program reports that the grim outlook for glioma patients has not changed in the past 30 years (1, 2).

Hausmann *et al.* (3) report that networks of glioma cells are linked to small groups of pacemaker cells in which levels of calcium ions pulse periodically, driving a signal through the network that causes tumor growth (3, 4). Using inhibitors, Hausmann *et al.* (3) blocked the activity of the Ca²⁺ activated potassium-channel protein KCa3.1 in *in vitro* models and mice, preventing proliferation of glioma cells and tumor expansion. They found marked reduction of tumor cell viability within the entire network, reduced tumor growth in mice and extended animal survival.

KCa3.1 is encoded by the potassium calcium-activated channel subfamily N member 4 (KCNN4) gene, at the chromosomal location 19q13.31 (5). We used the Cancer Genome Atlas (TCGA) to evaluate the effect of KCNN4 on human glioma survival in the TCGA Lower Grade Glioma (LGG) dataset.

Materials and Methods

TCGA contains the analysis of over 11,000 tumors from 33 of the most prevalent forms of cancer (6). TCGA molecular profiling has already revealed biologically discrete subsets and pathways of progression in diffuse glioma (7). The TCGA WHO tumor classifications are the revised 4th edition, not the current 5th edition (8).

To access and analyze the data we used the Genomic Data Commons Data Portal (<https://portal.gdc.cancer.gov>) and the UCSC Xena browser (<https://xenabrowser.net>). UCSC Xena is a web-based visual integration and exploration tool for TCGA data, including clinical and phenotypic annotations (7). In addition, we used the Glioma Bio Discovery Portal tool (<https://glioma-biodp.nci.nih.gov/>).

For analysis and the creation of Kaplan-Meier curves for overall mortality, survival data from the glioma subgroup was extracted.

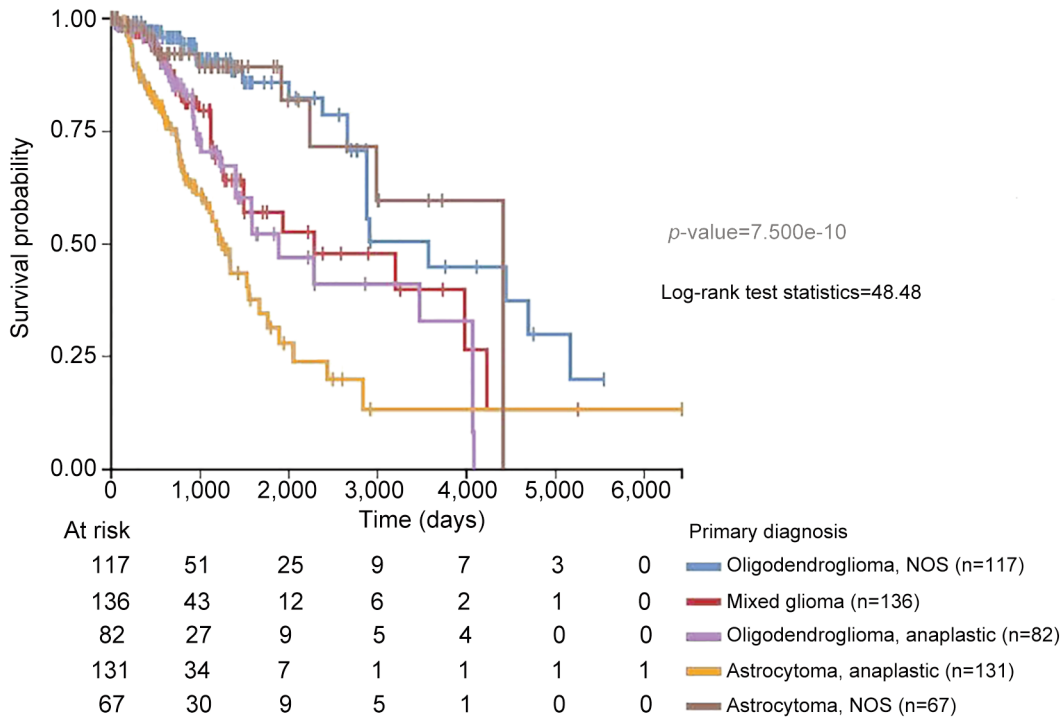


Figure 1. Overall survival of glioma patients stratified by primary diagnosis. Note that anaplastic astrocytoma has the worst survival rate, while oligodendroglioma has the best survival.

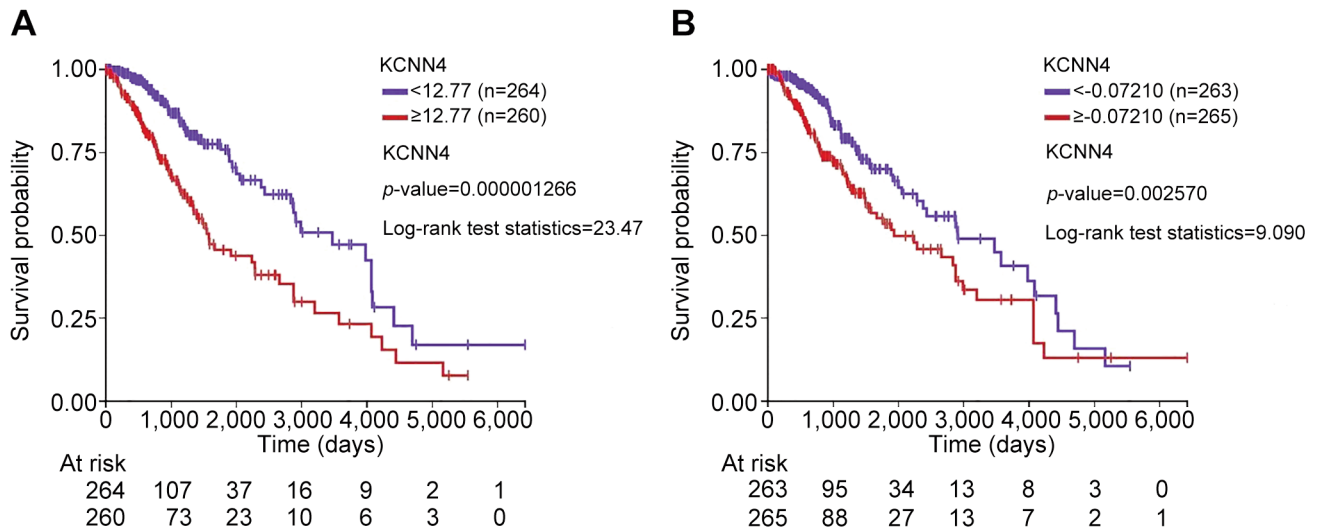


Figure 2. (A) Effect of KCNN4 expression on glioma overall survival. High expression (red curve) is unfavorable. (B) Effect of KCNN4 masked copy number segments on glioma overall survival. Increased masked copy number segments (red curve) are unfavorable.

The interval from the date of surgery to the date of death was referred to as the survival time. The last follow-up date was used for KM right censoring if date of death was not accessible. Using the log-rank (Mantel-Cox) test, differences between Kaplan-Meier survival curves were determined. Statistical analyses were performed with SPSS v26 (IBM, Armonk, NY, USA).

Results

We analyzed data from 506 patients with gliomas. The patients' mean age was 43±14 (mean±SD). 55% of the patients were male and 45% female. 92.1 % were white, 4.1%

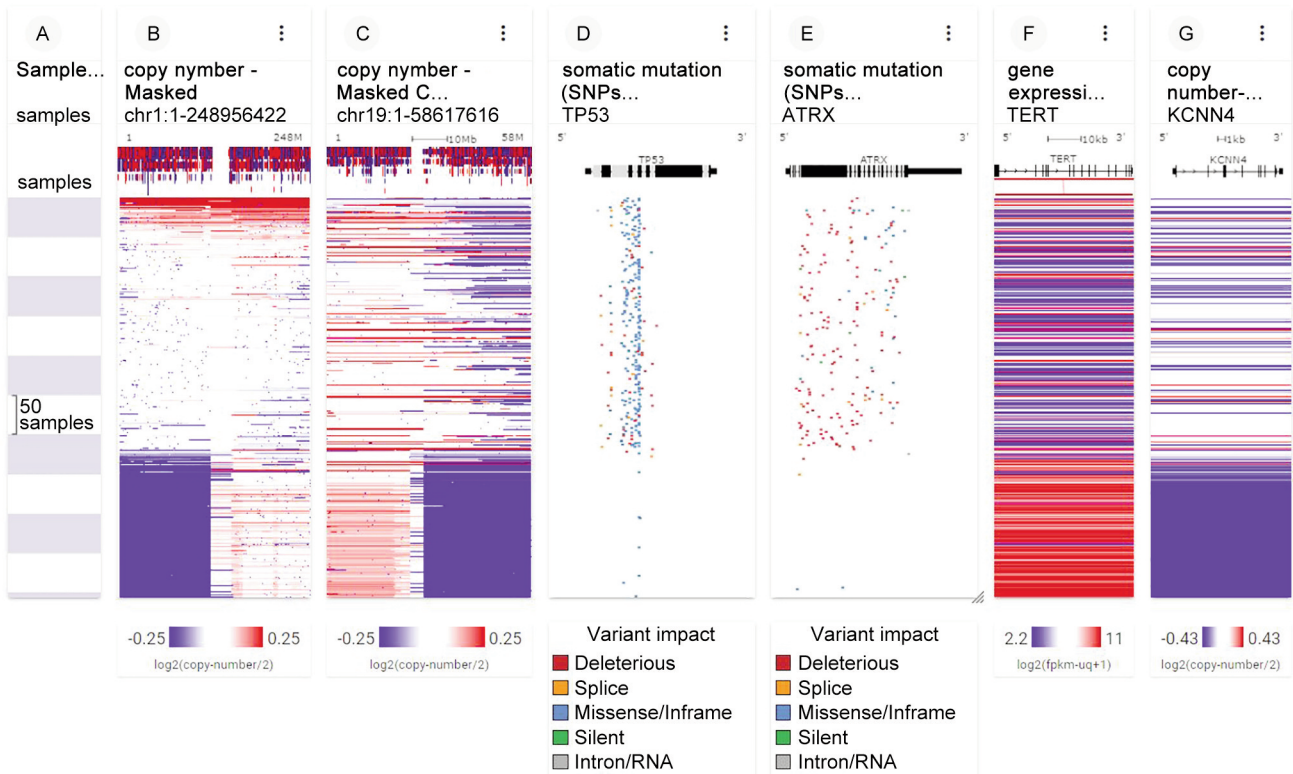


Figure 3. (A) Genetic separation of gliomas into two disease groups in 506 patients. Group 1 is characterized by co-deletion of chromosome arms 1p and 19q (blue blocks, columns B and C) and TERT over-expression (red block column F); group 2 by TP53 and ATRX mutations (upper section columns D and E). Each row contains data from a single sample. Row order is determined by sorting the rows by their column values. Note that KCNN4 is lost in gliomas with the 1p 19q co-deletion (blue block, column G) which may explain in part the relatively favorable prognosis of 1p 19q co-deletion tumors in group 1. Data are derived from UCSC Xena.

African American, 1.6% Asian, 0.2 % American Indian or Alaska native, and 1.9% unclassified. Mean survival was 7.8 years. Overall survival of subjects stratified by primary diagnosis is shown in Figure 1. Glioma prognosis is related to tumor histologic grade. Patients with Grade 3 tumors had significantly worse survival than patients with grade 2 tumors ($p < 0.001$). We found that in humans KCNN4 is prognostic in glioma: high expression is unfavorable (Figure 2A).

In addition, KCNN4 copy number variations were found to be prognostic. Increased masked copy number segments are unfavorable in lower grade glioma (Figure 2B). Genomic changes known as copy number variations (CNVs) cause an excessive number of duplicates of one or more genes. CNVs can result from structural genetic rearrangements: duplications, deletions, translocations and inversions. Copy number segments are derived from partitioning a genome into segments of constant total copy numbers based on DNA microarray data. Masked copy number segments carry the same information as copy number segments except that segments identified with probes known to contain germline mutations are removed.

Gliomas may be genetically divided into two disease entities. One entity with better prognosis is characterized by

co-deletion of chromosome arms 1p and 19q and TERT gene over-expression. The other entity with worse prognosis is characterized by TP53 and ATRX mutations (9). Figure 3 illustrates this genetic separation in 506 patients. Group 1 has co-deletion of chromosome arms 1p and 19q and TERT over-expression; group 2 has TP53 and ATRX mutations. Note that KCNN4 is lost in gliomas with the 1p 19q co-deletion, which may partly explain the relatively favorable prognosis of 1p 19q co-deletion tumors in group 1.

In addition, KCNN4 expression seems to affect survival in astrocytoma (and not in oligoastrocytoma or oligodendroglioma), based on data from the Glioma Bio Discovery Portal using patient stratification by histologic type (Astrocytoma/Oligoastrocytoma/Oligodendroglioma). Significantly different KCNN4 expression is related to IDH mutation status using patient stratification by IDH mutation status (IDHwt/IDHmut) (data not shown).

Discussion

KCNN4 is a crucial molecule that remodels several components in the tumor microenvironment and may serve

as a biomarker for predicting cancer prognosis in many tumor types, among them oligodendrogliomas (10, 11). KCNN4 channels participate in the epithelial–mesenchymal transition induced by phosphatase of regenerating liver-3 (PRL-3) in colorectal cancer (12). KCNN4 has been reported to enhance the stemness potential of liver cancer stem cells through glucose metabolism (13).

In addition, 1p 19q co-deletion defines a unique entity characteristic of oligodendrogliomas. Both the short arm of chromosome 1 and the long arm of chromosome 19 are completely deleted. The incidence of 1p 19q-co-deleted oligodendrogliomas peaks between the fourth and sixth decade of life, and they are typically adult-onset tumors (14). They often affect the frontal lobe, are associated with seizures, and some tumors have calcifications that can be seen in brain imaging. In both diffuse low-grade and anaplastic gliomas, the presence of 1p 19q co-deletion represents a potent independent prognostic indicator linked with increased survival (15).

Temozolomide, an oral alkylating agent, administered with radiotherapy increases survival in gliomas (16). Calcium channel blockers can enhance the activity of temozolomide in the treatment of gliomas and glioblastoma multiforme. Timed sequential therapy of the selective T-type calcium channel blocker mibefradil and temozolomide may benefit patients with recurrent high-grade gliomas (17). Nifedipine, a dihydropyridine calcium channel antagonist, can improve the therapeutic effect of temozolomide against glioblastoma multiforme (18). Senicapoc, an orally bioavailable inhibitor of the calcium-activated potassium channel SK4, might be repurposed as a glioma treatment (19). In addition, it would be worthwhile to examine the role of other small conductance calcium-activated potassium channels such as SK1, SK2 and SK3 (20).

Conclusion

Based on their study of cell lines and mice, Hausmann *et al.* (3) suggest that dependency of glioblastoma networks on periodic Ca^{2+} activity generates a vulnerability that can be exploited for the development of novel therapies, such as KCa3.1-inhibiting drugs. Our finding of increased KCNN4 expression related to poor survival in human lower grade glioma corroborates this suggestion and indicates that further studies would be worthwhile. Senicapoc, an orally bioavailable inhibitor of the calcium-activated potassium channel SK4, might be repurposed as a glioma treatment. In addition, it would be worthwhile to examine the role of other small conductance calcium-activated potassium channels such as SK1, SK2 and SK3.

Conflicts of Interest

None to be declared.

Authors' Contributions

Drs Lehrer and Rheinstein contributed equally to this study. Dr. Lehrer did calculations and writing, while Dr. Rheinstein reviewed results and outcomes.

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