Role of an Alternatively Spliced *KCNMA1* Variant in Glioma Growth

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Abstract

Gliomas develop genetic traits to rapidly form aggressive phenotypes. Hence, management of gliomas is complicated and difficult. Besides genetic aberrations such as oncogenic copy number variation and mutations, alternative mRNA splicing triggers prooncogenic episodes in many cancers. In gliomas, we found alternative splicing at the *KCNMA* transcription process. *KCNMA1* encodes the pore forming α-subunit of large-conductance calcium-activated voltage-sensitive potassium (BK$_{Ca}$) channels. These channels play critical role in glioma invasion and proliferation. We identified a novel *KCNMA1* mRNA splice variant with a deletion of 108 base pairs (*KCNMA1v*) mostly overexpressed in high grade gliomas. We found that *KCNMA1* alternative pre-mRNA splicing enhanced glioma growth, progression and diffusion. The role of *KCNMA1* and its splicing as a critical posttranscriptional regulator of BK$_{Ca}$ channel expression is presented in this chapter. Our research implies that high grade gliomas express *KCNMA1v* and BK$_{Ca}$ channel isoform to accelerate growth and transformation to glioblastoma multiforme (GBM). We demonstrated that tumors hardly develop in mice injected with *KCNMA1v* transected cell line expressing short-hairpin RNA (shRNA) compared to mice injected with *KCNMA1v* transected glioma cells. We conclude that targeting the *KCNMA1* variants may be a clinically beneficial strategy to prevent or at least slow down glioma transformation to GBM.

Keywords: *KCNMA1*, BK$_{Ca}$ channels, gliomas, tumorigenicity, potassium channels
1. Introduction

1.1. KCNMA1-encoded BKCa channels in glioma

Brain tumors are the most common type of solid tumors. In the United States, an estimated 20,000 new primary brain tumor cases are reported [1]. The most common form of malignant glioma is glioblastoma multiforme (GBM). The treatment of brain tumors is highly complicated due to their highly aggressive phenotypic and genotypic changes [2]. The median survival among GBM patients is only 15 months or less [3]. GBM contains heterogeneous subpopulations of glioma and other mixed supporting cells that are cancerous cells. They have the intrinsic ability that adapt in the brain tumor microenvironment and invade the normal brain. Gene expression profiling studies have identified many genes that have distinct expression patterns among different histological types and grades of gliomas [4]. The response of “normal cells” to malignant transformation involves changes in gene expression and is thought to be regulated by transcription [5]. The potassium ion channels are implicated in the malignant transformation to a higher grade in several cancers [5–7]. For example, we reported that low-grade gliomas might undergo certain epigenetic changes to develop into a GBM [8].

The physiological features of BKCa channels also known as maxi K or BK channels are well described [6–9]. These channels are unique since its activity is triggered by depolarization and enhanced by an increase in μM range of cytosolic calcium (Figure 1). The BKCa channels provide a crucial link between the metabolic and electrical states of cells. The BKCa channel overexpression was observed in biopsies of patients with malignant gliomas compared with nonmalignant human cortical tissues and the level of expression correlated positively with increased malignancy [7]. Studies have shown the importance of BKCa channels in brain tumor biology [5]. Lastly, BKCa currents in glioma cells are more sensitive to intracellular [Ca2+] compared to BKCa channels in healthy glial cells [9, 10].

Figure 1. BKCa channel is a tetramer of four monomeric pore-forming alpha-subunits encoded by KCNMA1. The seven transmembrane channels S0 to S6 are voltage-sensing domains, S1 to S4 channels form pore domain, S5 is a selectivity filter, and S6 is an extracellular N-terminal segment. The cytoplasmic C-terminal domain has RCK1 and RCK2 (with calcium bowl) segments.
2. Diverse role of KCNMA1 in glioma

KCNMA1-encoded BK\textsubscript{Ca} channel plays a pivotal role in cancer cell proliferation. Amplification of KCNMA1 was observed in breast, ovarian, and endometrial cancer with the highest prevalence in invasive ductal breast cancers and serous carcinoma of ovary and endometrium (3–7%) and gliomas. KCNMA1 amplification was significantly associated with high tumor stage, high-grade, high tumor cell proliferation, and poor prognosis. Due to the large number of protein interactions and activating factors influencing BK\textsubscript{Ca} channel function, intracellular Ca\textsuperscript{2+}, membrane voltage, pH, shear stress, carbon monoxide, phosphorylation states, and steroid hormones, it is generally difficult to predict its direct role in a given tissue. However, in many diseases including cancers, defective regulation and/or expression of BK\textsubscript{Ca} channels have repeatedly been associated with altered cell cycle progression [11], cell proliferation [11], and cell migration [11]. These altered cell functions are implicated in development of malignancy [11].

3. KCNMA1: STRING analysis

In order to understand the possible interactions of KCNMA1 with other genes and molecules, we used the tool STRING 9.1. It is a database consisting of known and possible protein–protein interactions with a gene of interest. The gene may have a direct (physical) or indirect (functional) association with other molecules. With this tool we can easily identify possible interaction of KCNMA1 with other associated molecules. We can derive detailed information

![Figure 2. STRING 9.1 software-derived possible association of KCNMA1 with top 20 most interacting genes.](http://dx.doi.org/10.5772/intechopen.74509)
of the protein being investigated as well as its associated molecules, crystal structure of the proteins with its PDB ID, and combined score [confidence score, neighborhood score, fusion score, homology score] on the basis of some parameters like experimental results, text-mining, co expression, databases, and co-occurrence (Figure 2).

4. Possible KEGG pathway following activation and suppression of KCNMA1 in glioma cells

Glioma cell line U-87 MG was obtained from the American Type Culture Collection (Manassas, VA) and cultured in MEM supplemented with 10% FBS and 0.1 mM nonessential amino acids. Cells were maintained at 37°C in 5% CO₂. In order to study the biological significance of up- and down-regulation of KCNMA1 on associated genes, we performed microarray using the Affymetrix Human Genome U133 Plus 2.0. Array analyses of U-87 MG cell lines where KCNMA1 was either overexpressed or suppressed showed significant changes in genes involved in cell proliferation, angiogenesis, cell cycle, and invasion (Figure 3). Class comparison tests indicated significant changes in global expression patterns. Twenty genes highly downregulated by suppression but upregulated by overexpression of KCNMA1 or vice versa are shown in Figure 3. This data support our rationale that KCNMA1 plays a critical role in the above cellular processes.

Array analyses of U-87 MG cell lines where KCNMA1 was either overexpressed or suppressed showed significant changes in genes involved in cell proliferation, angiogenesis, cell cycle,
and invasion (Figure 4; see arrows). Cluster analysis of 476 transcripts that are altered in opposite directions by expressing KCNMA1 gene up and down in U-87 cells. These transcripts were identified from the significantly altered genes by ANOVA at p < 0.05, and the fold-change thresholds such that one of KCNMA1 up or down altered the gene expression by twofold and the other altered it at least by 1.5-fold in the opposite direction.

5. KCNMA1 splicing in glioma

The KCNMA1 encodes the pore-forming α-subunits of large-conductance Ca\(^{2+}\)-activated K\(^+\) (BKCa) channels. More than 20 variants of this gene are associated with alternative splicing at ten or more different sites [12, 13], while majority of the splice sites are located in the large cytoplasmic domain. This domain is called the C-terminal half of the channel that contains multiple Ca\(^{2+}\) binding sites [14–16]. Gating properties and kinetics with regard to the voltage and Ca\(^{2+}\) dependence of gating are altered by alternative splicing in these regions [17–19]. Expressions of different BK\(_{Ca}\) isoforms have been implicated in auditory processing [20] and alter the sensitivity of BK\(_{Ca}\) to modulation by phosphorylation [21] and other processes [22]. However, the role of BK\(_{Ca}\) isoforms in cancer is now being investigated [23]. More specifically, KCNMA1 is altered in a wide variety of cancers, and their overexpression linked to increased malignancy in gliomas [4–7]. The BK\(_{Ca}\) protein isoform transcribed by its alternatively spliced mRNA in cancer cells is known as likely to respond differently to changes in intracellular
calcium ([Ca\(^{2+}\)]_i) and membrane potential. We and others have demonstrated that BK\(_{Ca}\) channels are overexpressed in gliomas [4–9] and play an important role in glioma invasion and migration [24, 25].

BK\(_{Ca}\) channels show a variety of electrophysiological properties due to alternative splicing of their \(\alpha\)-subunits. In glioma cells, Liu et al. [6] reported that BK\(_{Ca}\) channels exhibit distinct electrophysiological properties due to alternate splicing of its \(\alpha\)-subunits. These BK\(_{Ca}\) variants showed higher Ca\(^{2+}\) sensitivity in glioma cells compared to BK\(_{Ca}\) channels present in normal glial cells. The amplified sensitivity to intracellular [Ca\(^{2+}\)]_i was shown in a novel splice isoform (gBK) of hSlo, the gene that encodes the \(\alpha\)-subunits, specifically expressed in glioma [6]. We have recently shown (submitted for review) that KCNMA1 that encodes \(\alpha\)-subunit (pore forming) of BK\(_{Ca}\) channel undergoes specific splicing at mRNA to form a variant (KCNMA1v) that encodes for a novel BK\(_{Ca}\) channel isoform only in glioblastoma multiforme (GBM). Other types of Ca\(^{2+}\)-activated K\(^+\) channels such as intermediate (IKCa) and small (SKCa) [10] have been characterized in human glioma cells, but their roles in brain tumor biology are yet to be explored.

The alternative RNA splicing might increase protein expression levels and functions. In cancer, it was shown that abnormal mRNA splicing often leads to tumor-promoting splice variants that are translated into activated oncogenes or inactivated tumor suppressors [26, 27]. Interestingly, the brain appears to have maximum alternative splicing of exons [28]. The present knowledge suggests that alternative or aberrant pre-mRNA splicing results in oncoproteins with diverse functions in the development, progression, and dispersal of glioma cells [29, 30]. Further, genomic studies have shown that gliomas often have splice isoforms than in normal brain [30]. For instance, KCNMA1 was shown to undergo alternative pre-mRNA splicing at several sites in humans and mice [31, 32] to generate physiologically diverse BK\(_{Ca}\) channels. These altered BK\(_{Ca}\) channels respond differently to calcium/voltage changes. Often, these channels show abnormal regulation of cellular signaling pathways in glioma cells [13, 19]. Hence, the cause–effect of KCNMA1 splicing in functional modification of BK\(_{Ca}\) channels in brain tumors is a matter of great interest.

We have described an unknown KCNMA1 mRNA splice variant with a deletion of 108 base pairs of exon 22 (KCNMA1v) between the S9 and S10 protein subunits (C-terminus) overexpressed in high-grade gliomas. This serendipitous finding prompted to study the role of KCNMA1v as a critical posttranscriptional regulator of BK\(_{Ca}\) channel isoform expression and altered channel function in gliomas (submitted for review). The complex interaction between various ions and their respective ion channels at the invadopodia of the malignant gliomas is speculated to explain some of the invasive properties of gliomas [24, 25]. The role of various ions and their respective ion channels in glioma is recently well documented [33]. Among many ion channels, BK\(_{Ca}\) channels have many known spliced variants. Liu et al. have initially described a spliced variant, glioma BK (gBK), channel in human glioma cells [6]. Inherited and acquired changes in pre-mRNA splicing have been shown to play a significant role in human disease development (pre-mRNA splicing and human disease [29]. Venables et al. [34] showed that alternative splicing of pre-mRNA increases the diversity of protein functions in ovarian and breast cancer samples. Specifically, they found that expression of FOX2 was downregulated in ovarian cancer and its splicing is altered in breast cancer samples affecting cell proliferation.
However, studies on the association of changes in gene splicing pattern and malignancy are rare. However, few studies have shown the presence of BK\textsubscript{Ca} channels at the invadopodia of the malignant gliomas that lead to speculation that these channels may help the invasive properties of gliomas. A recent study found a clinical relevance where the investigators found T cells derived from GBM patients who were sensitized to the gBK peptide could also kill target cells expressing gBK. This study shows that peptides derived from cancer-associated ion channels maybe useful targets for T-cell-mediated immunotherapy \cite{23}. Several sites of alternative pre-mRNA splicing of KCNMA1 have been described, and majority of them are located within the intracellular C-terminal domain of the channel \cite{19}. In the past a novel splice variant of KCNMA1 (gBK) with an additional 34-amino-acid exon at splice site 2 in the C-terminal has already been described in gliomas \cite{6}.

In addition to the above studies, we present herein the cloning, functional characterization, and splicing of a novel KCNMA1 splice variant. KCNMA1 encodes the alpha-subunit of human BK\textsubscript{Ca} channels and is known to form BK\textsubscript{Ca} channel isoforms. Here, we report hitherto unknown KCNMA1 splice variant, which has a 108-base-pair deletion at the splice site on one of its exons, which we termed as KCNMA1\textsubscript{v}. More importantly, KCNMA1\textsubscript{v} expression correlates positively with the relative degree of malignancy of the glioma cell lines (under publication). Moreover, we found that KCNMA1\textsubscript{v} was expressed only in high-grade glioma samples and not in normal brain tissues as evidenced by examination of human biopsy specimens (under publication). Expression of KCNMA1\textsubscript{v} in HEK (null type) revealed that the pharmacological and biophysical properties of the variant were consistent with the properties of wild-type KCNMA1 gene in glioma cells suggesting that KCNMA1\textsubscript{v} is likely to encode the principal wild-type BK\textsubscript{Ca} channels (under publication). Although we have not separated wild-type and splice variant isoform for sequence and structure analysis, the biological properties of both wild-type and isoform protein appear to be similar. However, when overexpressed in glioma cell line (under publication), the variant showed distinct biological properties such as enhanced Ca\textsuperscript{2+} sensitivity at physiologically relevant [Ca\textsuperscript{2+}]i levels (under publication).

Progression of brain tumor from localized, slow-growing tumors to more aggressive brain tumors capable of invading the surrounding brain most likely involves a series of stepwise biological events \cite{35}. For example, miR-182 was found to be a valuable marker of glioma progression and that high miR-182 expression is associated with poor prognosis \cite{36}. Such a multistep process of tumorigenesis has been proposed to involve a series of mutational events which ultimately lead to development and progression of neoplasia \cite{35}. Aberrant pre-mRNA splicing is an important factor in tumor progression and has been proposed to result in the loss of a normal pathway of differentiation, which could lead to tumor progression. Several studies have implicated BK\textsubscript{Ca} channel expression to oncogenic cell transformation \cite{37,38}. Increased activity of BK\textsubscript{Ca} channels appeared to be required for the mitogenic stimulation of non-transformed cells and may play a role in cell proliferation \cite{39}. Consistent with the above studies, we show that KCNMA1\textsubscript{v}-induced effects promote proliferation in glioma cell lines when the variant was overexpressed. The upregulation of KCNMA1\textsubscript{v} in glioma cell lines provides an opportunity to determine variant-specific changes that enhance gliomagenesis in vivo. The overexpression of KCNMA1\textsubscript{v} resulted in increased proliferation in glioma cell lines. It has also been suggested that cell invasion into narrow brain spaces may
require tumor cells to shrink and squeeze through tight interstitial space [40]. Cell shrinkage requires the efflux of K\(^+\) and Cl\(^-\) ions [41], and BK\(_{Ca}\) channels may serve as pathway for regulated K\(^+\) efflux [42]. Consistent with these findings, the overexpression of KCNMA1v increased the invasion potential of glioma cells (under publication). The role of BK\(_{Ca}\) channels in cell migration was already described [43]. The changes in proliferation and migration of cells over-expressing KCNMA1v were mostly attributed to increased levels of KCNMA1 and BK\(_{Ca}\) channel protein expression in transfected cells. Additionally, overexpression of KCNMA1v in glioma cells may assist them to diffusely invade the normal brain. Due to this phenomenon, GBM patients typically show high propensity to recur as the cancer cells expressing KCNMA1v might survive surgical and therapeutic treatment. The xenograft tumors in mice likewise demonstrated increased growth, which correlated well with Ki-67 expression (under publication). The overexpression of KCNMA1v resulted in increased angiogenesis in the tumor xenografts, supporting the angiogenic role of KCNMA1v. The observation that the overexpression of KCNMA1v in human gliomas correlates with increased angiogenesis in high-grade gliomas further supports that KCNMA1 splicing event is an important biological process for glioma progression. Consistent with this observation, we found that glioma cells over-expressing KCNMA1v secreted significantly the high level of angiogenic factor VEGF (under publication).

6. Conclusion

Further investigation into the mechanisms and cellular events caused by KCNMA1 splicing may lead to the development of future therapies for this highly deadly disease. Splice variants that are found in high-grade gliomas have clear diagnostic and prognostic values besides providing potential targets for anticancer drug development. Clinical outcome of KCNMA1v expression in high-grade glioma is expected to reveal the variants’ clinical importance. This analysis is being performed in our laboratory. In conclusion, the results presented here might suggest that quantifying the levels of KCNMA1v could be useful to identify biological process that increases the malignancy and affect prognosis of high-grade glioma patients.

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