Journal of Neurosurgery Imaging and Techniques
JNSIT, 4(2): 205-212
www.scitcentral.com
ISSN: 2473-1943
Review Article: Open Access

Primary and Metastatic Brain Tumors Dissipate - Understanding and Intervention

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Received March 30, 2019; Accepted April 04, 2019; Published July 21, 2019

ABSTRACT

We researched and reviewed peer-reviewed articles to provide an overview of the primary and metastatic brain tumor growth, dissemination mechanisms in their microenvironment. We studied the role of alternative pre-mRNA splicing events of KCNMA1, which encodes the pore forming α-subunit of calcium-activated voltage-sensitive potassium (BK_{Ca}) channels, in migration, invasion, proliferation, dispersal of brain tumor. It is conceivable that by targeting epigenetic events and gene variants that contribute to brain tumor growth, we might attenuate tumor diffusion, distant metastasis and angiogenesis. We reviewed literature on the alternative splicing events of KCNMA1, specific to brain tumor, its microenvironment and the biological activity of known alternatively spliced isoforms. The blood-brain tumor barrier (BTB) prevents delivery of anticancer drugs to micro-macro metastases requiring novel strategies to enhance drug delivery across the BTB. We also revealed the interaction between the BK_{Ca} channel isoform expression and VEGF secretion in brain tumors that can be exacerbated under hypoxia with significant implications on neoangiogenesis, vascular permeability and anticancer drug delivery.

Keywords: KCNMA1, BKCa channels, Splice variants, Gliomas, Metasttic brain tumors, Drug delivery, Blood brain-tumor barrier

INTRODUCTION

Primary and metastatic cancers

Primary brain tumors start in the brain or brain's contiguous structures. Most common types of primary brain tumors are gliomas, which begin in the glial tissue. Major types of brain tumors are gliomas that also include, astrocytoma arising from astrocytes and oligodendrogliomas arising from oligodendrocytes or from a glial precursor cell. In children, medulloblastoma is common arising from cerebellar primitive neuroectodermal tumor (PNET), originating from the posterior fossa and can spread to other parts of the brain and to the spinal cord. In fact, there are 16 types of brain tumors according to WHO report. The 2016 CNS WHO presents major restructuring of the diffuse gliomas, medulloblastomas and other embryonal tumors and incorporates new entities that are defined by both histology and molecular features, including glioblastoma [1]. This report addresses the challenges of primary brain tumor diagnosis, prognosis and targeted treatment based on the molecular basis of the tumor. Secondary brain tumors or metastatic brain tumors are those that spread to the brain from somewhere else in the body. For example, cancers of the lung, breast, kidney, stomach, colon and melanoma skin cancer have the potential to travel through the bloodstream and lodge themselves in the brain. Then they will begin to grow into new tumors in the new microenvironment supported by increased vascularization by avoiding host immune response **(Figure 1)**. With more-sensitive and accurate detection of distant metastases by improved imaging modalities should increase incidence of brain metastases and prolong survival of patients. Innovative preclinical models that more accurately represent clinical

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Citation: Khaitan D & Ningaraj N. (2019) Primary and Metastatic Brain Tumors Dissipate - Understanding and Intervention. J Neurosurg Imaging Techniques, 4(2): 205-212.

brain metastases and imaging techniques will pave way to developing anticancer drugs to manage brain metastases.

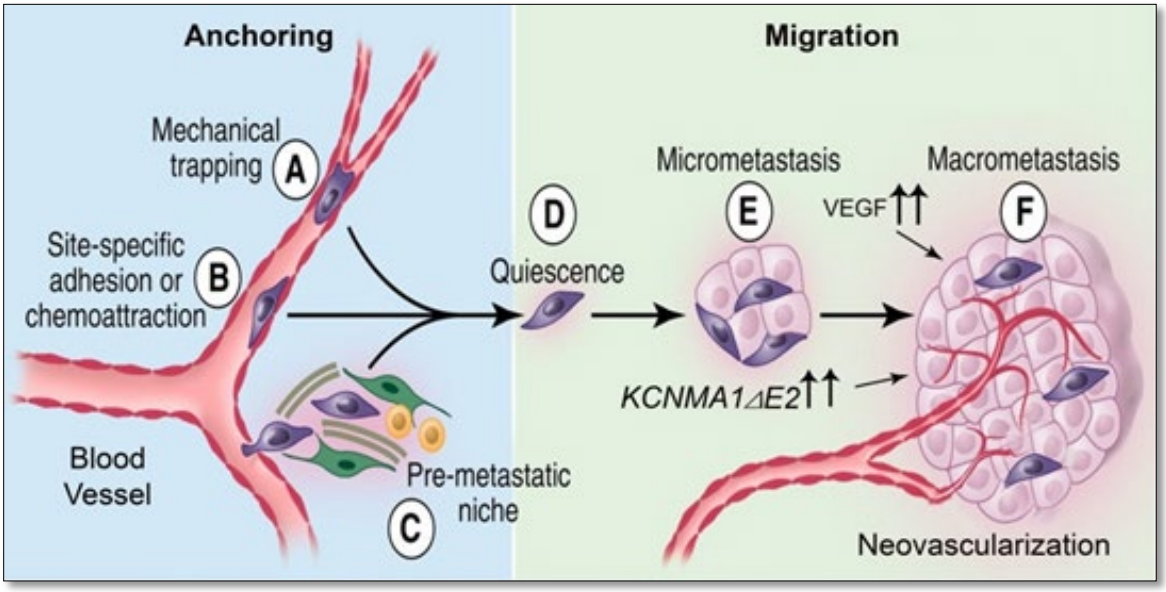

Figure 1. A complex but simplified metastatic process is depicted in this cartoon. Aberrant cancer cells from the lung, breast, kidney, stomach, colon and skin escape through the bloodstream and colonize in the brain. The local microenvironment in the brain allows the colonized cancer cells to avoid immune response and develop distant metastases, which are extremely difficult to treat. The increased expression of BK_{Ca} channels and its variants might support metastatic cancer cells to secrete more VEGF, triggering neovascularization and growth of tumors.

Brain tumor dissipation

New generation sequencing (NGS) technologies have contributed to the giant leap in understanding the genomic and epigenomic analyses of both primary and metastatic brain tumor tissues and tumor cells. Mutations identified at the genome or transcriptome levels helped us understand the differences in gene regulation of cellular functions in identical brain tumors as related but functionally different tumor entities [2-6]. Novel mutations detected in brain metastases suggested potential drivers tumor progression [7], strengthening the argument that brain tumors are highly heterogeneous with complex microenvironments. Epigenetics play an important role in cancer initiation, growth and progression.

Understanding the precise mechanism helps us in developing diagnosis, prognosis and treatment strategies for affected cancer patients. For example, overexpression of Ezh2 plays a role in many cancers, including breast cancer and brain tumors. H3K27M serves as an oncohistone and, if mutated it contributes to tumor development as Ezh2 is no longer able to methylate the histone and gene expression is aberrantly upregulated. We studied the methylation status of the promoter region using low grade and high-grade glioma cell lines and showed that there is a significant difference in the expression of genes between low and high-grade glioma cells, when treated with 5-aza plus TSA. Our study suggested that studying the methylation status of ADFP, CDCP1 and ZFP42 in brain tumor biopsies may indicate the potential aggressive nature of gliomas [8]. More functional genomics and cell-based molecular analyses are required to qualify mutated or amplified genes as clinical and therapeutic markers. A case in point is the data available from The Cancer Genome Atlas (TCGA) and The Human Protein Atlas that refers to testing of KCNMA1 and BK_{Ca} channels as a pathological marker in cancer [9].

The complication in understanding metastatic process and metastatic spread in the brain calls for rationalized tumor model systems. Researchers are using both *in vitro* and *ex vivo* primary brain tumor model systems to better understand the molecular mechanisms of brain tumors and neovascularization for designing targeted novel anti-metastatic therapies [10]. Metastatic brain tumors dissipate and colonize in distant parts of brain metastases grow by drafting existing blood vessels and/or by forming new blood vessels. The unique brain microenvironment such as hypoxia promotes tumor cell survival, tumor growth and resistance to therapy. The ion channels are shown to be involved in hypoxia-induced aggressiveness of glioblastomas [11]. We also found that KCNMA1 and the alternate splicing of KCNMA1, which encode for BK_{Ca} channels, under hypoxia impact vascular endothelial growth factor (VEGF) secretion in glioma cells **(Figure 2)**. Hence, there is an opportunity to control cancer growth by developing safe and effective VEGF and BK_{Ca} channel inhibitors.

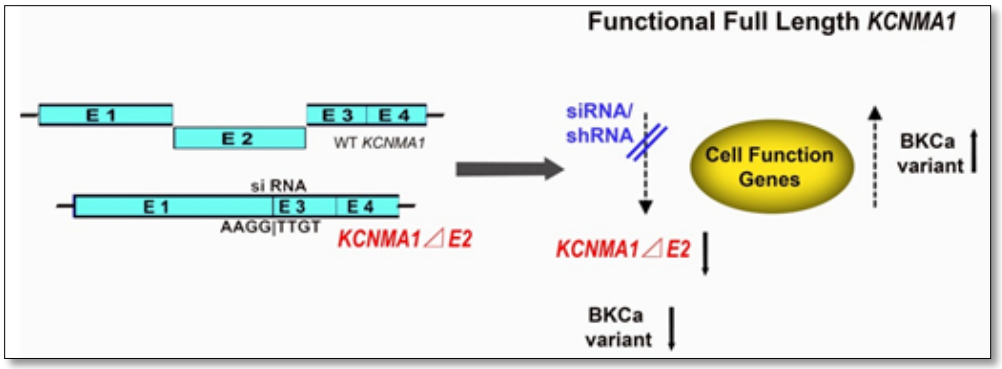

Figure 2. Alternative splicing of KCNMA1 in gliomas. Potential location KCNMA1ΔE2 (deletion of entire Exon 2) mRNA of BK_{Ca} channels, site of deletion and siRNA designing strategy**.** KCNMA1ΔE2 was detected in high grade glioma cell line (U87 MG) and metastatic breast cancer cell line (MDA-MB361). It is proposed that by developing siRNA/shRNA or specific inhibitors, tumor promoting KCNMA1 and its constituent genes may be down-regulated to attenuate primary and metastases tumor growth.

Biomarkers of brain tumors: Due to the multi-factorial and heterogeneous nature of brain tumors (primary and metastatic), it is extremely difficult to identify the most optimal and novel biomarker for effective management of brain tumor patients. The risk loci for glioma susceptibility, 5p15.33 (TERT), 8q24.21 (CCDC26), 9p21.3 (CDKN2A-CDKN2B), 20q13.33 (RTEL1) and 11q23.3 (PHLDB1) are identified by a large genome-wide association study. Biomarker research had relied heavily on quantifying increase or decrease in gene expression in tumors, but these changes may not always result in altered protein expression. The epigenetic silencing of the MGMT (O6-methylguanine-DNA methyltransferase) DNA-repair gene by promoter methylation is an independent prognostic factor in GBM [12] and in metastatic brain tumor patients [13]. A targeted drug, temozolomide has been effective in prolonging the survival in patients with silenced MGMT but ineffective in patients with active MGMT. Epidermal growth factor receptor (EGFR) amplification and presence of EGFR variant (EGFRvIII) overexpression are observed in GBM [14] and metastatic brain tumors [15].

Mutations of the isocitrate dehydrogenase genes (IDH1 and IDH2) predict prolonged progression-free and overall survival of glioma patients [16]. Therefore, molecular markers of brain tumors can predict survival and will become increasingly important in the diagnosis, prognosis and treatment of brain tumors. We showed that KCNMA1 and BK_{Ca} channel are overexpressed in high-grade gliomas [17]. Perhaps the brain tumors are likely to exploit tumor micro environmental factors in brain by overexpressing potassium ion channels such as BK_{Ca} and KATP channels to gain functional advantages over normal cells **(Figure 3)**.

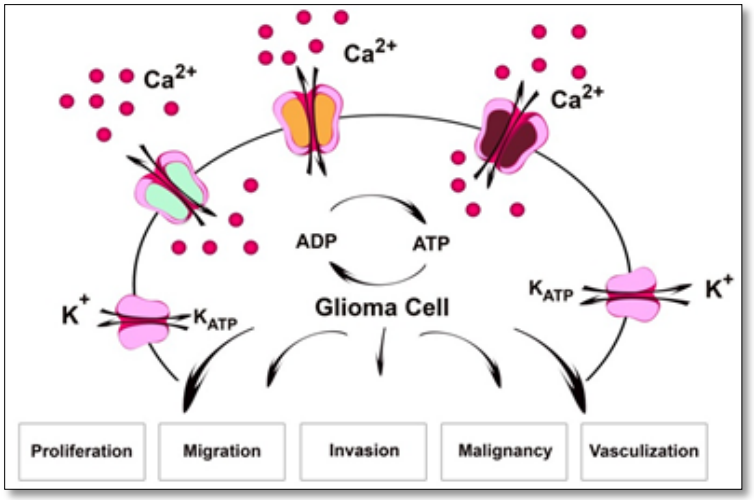

Figure 3. Glioma cells gain functional advantage over the normal cells in order to proliferate, migrate, invade, vascularize for transformation into highly malignant GBM. We and others have shown that brain tumor cells overexpress BK_{Ca} and K_{ATP} channels, which are highly sensitive to intracellular Ca^{2+} and ATP, respectively to squeeze through the tight spaces in the brain parenchyma.

KCNMA1/BKCa channels in brain tumor biology

Ion channels are implicated in the development of several cancers. We and others have demonstrated that BK_{Ca} channel is overexpressed in gliomas and plays a regulatory role in glioma invasion and migration [18-28]. There is now evidence that KCa–Ca^{2+} channel complexes found in cancer cells and contribute to cancer-associated functions such as cell proliferation, cell migration and the capacity to develop metastases [29]. Studies including our work indicate that BK_{Ca} channels contributed to the high proliferative or invasive potential in a number of malignant cell lines, such as non-metastatic (MCF-7) breast cancer cells [18], brain-specific metastatic (MDA-MB-361) breast cancer cells [29-32], human prostate cancer [33], colorectal carcinogenesis [34] and glioma [16].

The metastatic brain tumor cells colonize and develop distant metastases and subvert the microenvironment to avoid host immune response **(Figure 4)**. The BK_{Ca} channel regulates proliferation of the human neuroblastoma cells through PKC and PKA protein kinases [35]. However, a few studies concluded that BK_{Ca} channels are not required for the proliferation in glioma [36] or breast cancer cells [37].

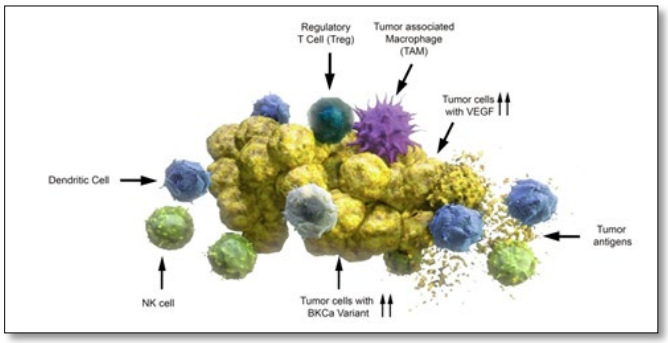

Figure 4. The metastatic brain tumor cells subvert the microenvironment to avoid host immune response. We showed that brain tumor cells that express BK_{Ca} variants increase VEGF secretion and recruit brain endothelial cells to develop new blood vessels.

KCNM1-endoded BK_{Ca} channels in brain tumors as therapeutic targets: Several potassium channels, including BK_{Ca} channels with defined molecular identities have been proposed as candidates for therapeutic intervention in cancer [38]. Treatment strategies include classical small molecules as inhibitors as well as gene therapy approaches targeting potassium channels as direct targets for adjuvant cancer therapy are proposed [39]. A recent review discussed the important role of BK_{Ca} channels in glioma cell biology, including cell division, invasion and migration [40]. Proteomic and array studies have shown BK channel's role in cancer and its interaction with various other proteins [18]. Therefore, some of these protein-protein interactions in cancers, especially in brain tumors can be exploited for developing new class of targeted therapies.

Alternative splicing in brain tumors

In humans, most pre-mRNAs undergo alternative splicing and disruption of normal splicing patterns can cause diseases [41]. A vast majority of human genes contains introns. Most pre-mRNAs undergo alternative splicing. Several excellent reviews provide detailed information on splicing and the regulation of splicing [42]. The discovery of alternative RNA splicing can greatly influence protein levels and functions. In cancer, abnormal splicing often leads to tumor-promoting splice variants that are translated into activated oncogenes or inactivated tumor suppressors [42,43]. Of all tissues, the brain shows maximum alternative splicing of exons [16]. Growing evidence indicates that alternative or aberrant pre-mRNA splicing resulting in protein isoforms with diverse functions occurs during the development, progression and dispersal of glioma cells [44].

Genome-wide studies have identified genes expressing splice isoforms more frequently in glioma than in normal brain [45]. For example, KCNMA1 was shown to undergo alternative pre-mRNA splicing at several sites in humans and mice [46,47] to generate physiological diversity in BK_{Ca} channels. We have identified two new KCNMA1 variants (KCNMA1E22 and KCNMA1ΔE2) as shown in **Figure 5**. These isoforms showed differences in calcium/voltage sensitivity and regulation of cellular signalling pathways [48,49]. However, the cause-effect of KCNMA1 splicing in functional modification of BK_{Ca} channels in gliomas is still unclear. We recently reported that the high-grade gliomas express KCNMA1v and BK_{Ca} channel isoform accelerate growth and transformation of low grade-glioma to GBM [17].

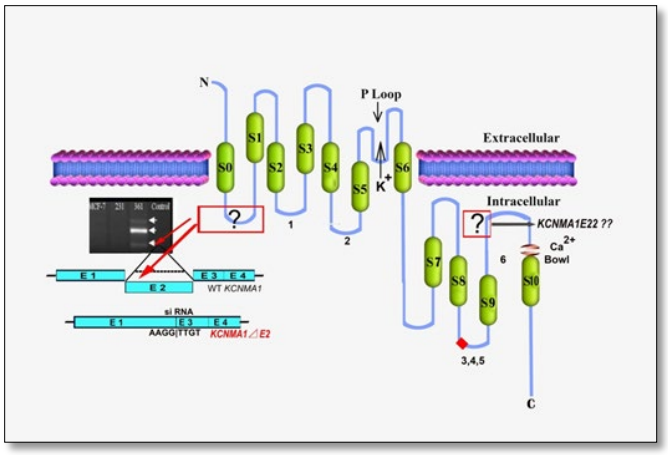

Figure 5. KCNMA1vE22 land KCNMA1ΔE2 locations in the BK_{Ca} channel protein, site of deletion and siRNA designing strategy. Representative result of agarose gel electrophoresis of KCNMA1vE22 RT-PCR products from breast cancer cell lines (MCF-7, MDA-MB231, MDA-MB361 and control.

BBB/BTB: Challenges of imaging and delivery of anticancer drugs to brain tumors

A significant number of primary and metastatic brain tumor cases are reported each year in the US and around the world. Estimates show that about fifty percent of patients receiving brain radiation and/or surgical resection have recurrences in the brain within a year, severely shortening life expectancy [19]. Targeting brain tumors is extremely difficult because brain provides a "safe haven" for tumor cells **(Figure 3)**.

The cerebral microvessels/capillaries that form the blood-brain barrier (BBB) not only protect the brain from toxic agents in the blood but also pose a significant hindrance to the delivery of 98% of CNS small and large therapeutic molecules. Different strategies are being employed to circumvent the physiological barrier posed by blood-brain tumor barrier (BTB). Research now is focusing on targeted cancer therapy by supplementing conventional chemotherapy and radiotherapy with monoclonal antibodies (MAbs). The purpose of antibody treatment of cancer is to induce the direct or indirect destruction of cancer cells, either by specifically targeting either the tumor or the tumor vasculature [50]. The EGFR is often amplified and mutated in human gliomas, but its expression is low or undetectable in normal brain and hence targeted by cetuximab (Erbitux®). New therapeutic MAbs such as Herceptin, ABX-EGF, EMD 720000 and h-R3 are routinely used in the clinic. These promising MAbs, however, have poor penetration across BTB, rendering them ineffective against brain tumors.

Targeting tumor and tumor blood vessel specific marker(s) is a good strategy to control tumor growth [26]. It is however, critical to study whether tumor-specific drug delivery has the potential to minimize toxicity to normal tissues, and improve bioavailability of cytotoxic agents to neoplasms. The established blood vessels feeding the proliferating brain tumor edges as well as the brain tissue surrounding the tumor are similar to intact BBB [51]. Therefore, understanding the biochemical regulation of the BBB permeability in its normal and abnormal states (BTB) is crucial as new anticancer drugs targeting brain tumors are being developed.

The dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) is primarily used for human brain tumor detection. However, detection of tumor microsatellites (smaller than 1 mm) remains challenging. In addition, BTB collapse is visualized as gadopentate (Gd-DTPA)-contrast enhancing lesions on brain MRI. There is an opportunity to increase Gd-DTPA delivery to diffused brain tumors for better detection. Research, presently should be focused towards delivering contrast enhancing agents and therapeutic drugs selectively to diffused brain tumors and distant metastases for accurate detection and proper management of disease in patients.

We recently reviewed and reported on the advance made in drug delivery research focused on several innovative methods, including nanoparticles, microparticles as carriers of anticancer agents, PEG technology, encapsulating anticancer drugs in liposomes and MAbs for the delivery of anticancer payloads [38,52]. We also reported on the significant differences between normal human brain and brain tumor capillaries, including differential expression of ion channels including BK_{Ca} [51] and KATP channels [53]. Based on these studies, there are number of researchers who are studying the molecular targeting by tumor-specific antigens and specific agents to circumvent delivery challenges posed by BBB/BTB.

CONCLUSION

We know that primary and metastatic brain tumors are distinct in their etiology, biology, response and resistance to anticancer drugs. Hence innovative preclinical and clinical study designs are required to develop effective diagnosis,

prognosis and treatment. We should embrace the new DNA, RNA, protein sequencing and imaging technologies to understand each tumor type and customize treatment strategies. Furthermore, studies are essential to understand the host microenvironment including molecular aberrations such as gene mutations and alternative splicing. Specifically, in hypoxic microenvironment metastatic brain tumor cells adapt very well and thrive by forming new blood vessels. Targeting VEGF and VEGFR that are involved in angiogenesis with Bevacizumab like molecules should take a priority. In addition, BBB/ BTB pose hurdles to anti-cancer drug and imaging agents' delivery. These challenges are different in primary and metastatic brain tumors. Molecules involved in extravasation, metabolism, cell adhesion and cellular signalling in brain-specific metastatic clones should be identified for targeted therapies.

More effective anticancer drugs and specific biologics similar to clinically used inhibitors of EGFR, HER2, PI3K and BRAF, should be developed to get an upper hand on unique challenges posed by both primary and metastatic brain tumors. Finally, our work suggests that validating KCNMA1/BK_{Ca} channel variants in clinically relevant tumor samples will be useful in identifying biological process that promote malignancy and affect prognosis and survival of brain tumor patients.

ACKNOWLEDGEMENT

The authors thank the Scintilla Group, Bangalore, India; Anderson Cancer Institute and Mercer University Medical Center, Savannah, GA, USA; Vanderbilt-Ingram Cancer Center, Nashville, TN, USA; Cedars-Sinai Medical Center, Los Angeles, CA, USA; American Cancer Society, USA; Georgia Cancer Coalition, Atlanta, GA, USA; and NIH for providing the opportunity and research grant support.

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