Calcium Channels and Associated Receptors in Malignant Brain Tumor Therapy

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ABSTRACT
Malignant brain tumors are highly lethal and aggressive. Despite recent advances in the current therapies, which include the combination of surgery and radio/chemotherapy, the average survival rate remains poor. Altered regulation of ion channels is part of the neoplastic transformation, which suggests that ion channels are involved in cancer. Distinct classes of calcium-permeable channels are abnormally expressed in cancer and are likely involved in the alterations underlying malignant growth. Specifically, cytosolic Ca$^{2+}$ activity plays an important role in the regulation of cell proliferation, and Ca$^{2+}$ signaling is altered in proliferating tumor cells. A series of previous studies emphasized the importance of the T-type low-voltage-gated calcium channels (VGCC) in different cancer types, including gliomas, and remarkably, pharmacologic inhibition of T-type VGCC caused antiproliferative effects and triggered apoptosis of human glioma cells. Other calcium permeable channels, such as transient receptor potential (TRP) channels, contribute to changes in Ca$^{2+}$ by modulating the driving force for Ca$^{2+}$ entry, and some TRP channels are required for proliferation and migration in gliomas. Furthermore, recent evidence shows that TRP channels contribute to the progression and survival of the glioblastoma patients. Likewise, the purinergic P2X7 receptor acts as a direct conduit for Ca$^{2+}$ influx and an indirect activator of voltage-gated Ca$^{2+}$-channel. Evidence also shows that P2X7 receptor activation is linked to elevated expression of inflammation promoting factors, tumor cell migration, an increase in intracellular mobilization of Ca$^{2+}$, and membrane depolarization in gliomas. Therefore, this review summarizes the recent findings on calcium channels and associated receptors as potential targets to treat malignant gliomas.

INTRODUCTION
Gliomas represent the most common type of malignant tumors of the central nervous system (Ganau et al., 2015), and they are the most aggressive and lethal (Robins et al., 2009; Van Meir et al., 2010; Wei et al., 2010) among the primary brain tumors (Noch and Khalili, 2009). Despite recent therapeutic advances in multimodality therapies, including surgery and radio/chemotherapy, the treatment of these malignant gliomas remains palliative, with an average survival of about 1 year (Omuro and DeAngelis, 2013). The pathologic features of glioblastoma (GBM), the most aggressive of malignant gliomas, are exemplified by uncontrolled cell proliferation, diffuse infiltration, intense resistance to apoptosis, genomic instability, giant cells, and cellular and nuclear pleomorphism (Wen and Kesari, 2008; Yamanaka and Saya, 2009). Gliomas are composed of a heterogeneous population of tumordifferentiated cells and a subpopulation with stem cell properties. Cancer stem cells are very aggressive as they are highly invasive, mobile, resistant to radiation and chemotherapy, and have the capacity to self-renew (Oh et al., 2012). Recent studies have shown that calcium (Ca$^{2+}$) channel signaling controls a variety of stem cells and cancer cell line functions, such as proliferation and migration (Wee et al., 2014). Moreover, Ca$^{2+}$ channel interference was able to drive liver tumor-initiating cells into apoptosis (Zhao et al., 2013) and glioma stem-like cells were more sensitive to Ca$^{2+}$ disturbances compared with more mature differentiated glioma cells (Wee et al., 2014).

Evidence for the role of ion channels in cancer includes the altered regulation of ion channels during neoplastic transformation (Prevarskaya et al., 2011; Rao et al., 2015). Ion channels mediate the transport of ions across the cell membrane, and the results of the transmembrane ion flux participate in the regulation of tumor cell survival, death, and migration, especially in gliomas (Cuddapah and Sontheimer, 2016).
Cytosolic Ca\(^{2+}\) activity in particular plays a decisive role in the regulation of cell proliferation, and intracellular Ca\(^{2+}\) signaling is altered in proliferating tumor cells (Becchetti, 2011). Several studies have revealed that voltage-gated ion channels can contribute significantly to cell mitotic biochemical signaling, cell cycle progression, and cell volume regulation, processes that are critical for cancer cell proliferation (Rao et al., 2015). Distinct classes of Ca\(^{2+}\)-permeable channels are abnormally expressed in cancer and are likely involved in the alterations underlying malignant growth (Lang and Stournaras, 2014). The main calcium channels described in cancer cells are voltage-gated Ca\(^{2+}\) channels (VGCC; L-type: Ca\(^{1.1}\)-1.4; N-type: Ca\(^{2.2}\); T-type: Ca\(^{3.1}\)-3.3; R-type: Ca\(^{2.3}\); and P/Q-type: Ca\(^{2.1}\)), purinergic receptors, and the Ca\(^{2+}\)-permeable ion channels of the transient receptor potential (TRP) family (Leanza et al., 2016). Moreover, a wide variety of distinct Ca\(^{2+}\)-permeable channels, such as TRP channels, have been linked to tumor proliferation and metastasis (Lang and Stournaras, 2014). Experimental modification of TRP channels activity impacts tumor cell function and motility, which suggests that the channels are a potential molecular target for tumor neovascularization control (Fiorio Pla et al., 2012).

Various studies have suggested that the ionotropic purinergic receptor P2X7 receptor (P2X7R) plays a role in GBM (Morrone et al., 2003, 2005, 2006; Gehring et al., 2012, 2015), raising the possibility that calcium channels are involved in glioma progression. Thus, this review summarizes the recent findings on these calcium channels and associated receptors as potential targets in the treatment of malignant brain tumors.

**Voltage-Gated Calcium Channels**

VGCC are a class of calcium-permeable channels divided into two different groups: low-voltage activated (T-type) and high-voltage activated (L, N, P/Q, and R-types) (Catterall and Swanson, 2015). The VGCC are found in the membrane of excitable cells, where they exert crucial physiologic processes by converting the electrical signal in the cell surface to an intracellular key response (Dolphin, 2006). Different cell types evoke different VGCC subtypes that might mediate transient currents and membrane depolarization, which results in several regulatory properties for the channels. Calcium (Ca\(^{2+}\)) is a second messenger participating in the regulation of fundamental biologic events, and the Ca\(^{2+}\) influx through VGCC has been implicated in cell growth and proliferation, migration, and apoptosis (Chen et al., 2013; Prevarskaya et al., 2013). Many recent studies have also focused on the relevance of VGCC in the maintenance of several biologic processes of malignant cells; the transformation of a normal cell into a tumor cell has been related to the Ca\(^{2+}\) oscillations, and the homeostasis imbalance can define the malignant phenotype. As in other kinds of cancer, brain tumors often present genetic mutations in tumor suppressors and oncogenes; surprisingly, alterations in ion channel/transporter activity are linked to up- or down-regulation of these encoding genes in most brain tumor cases (Ransom et al., 2001; Masselli et al., 2012). Furthermore, dysregulation in Ca\(^{2+}\) channel activity in the central nervous system is related to many types of neurologic disorders, including epilepsy (Zamponi et al., 2010), Alzheimer’s disease (Amenta et al., 2009), and chronic pain (Rigo et al., 2013a).

In the brain, the P/Q-, N- (Ca\(^{2.2}\)), and T-type (Ca\(^{2.3}\)) Ca\(^{2+}\) channels are being widely explored as targets for treating neuronal disease (Nimmrich and Gross, 2012). In neuronal cells, VGCC are linked to electrical signals by modulating vesicular release of neurotransmitters and activation of several key enzymes and Ca\(^{2+}\)-dependent ion channels (Dolphin, 2006; Prevarskaya et al., 2013). Among brain tumors, GBM is highly lethal and aggressive (Omuro and DeAngelis, 2013). In GBM cells, as in other cancer cells, Ca\(^{2+}\) channels are involved in uncontrolled proliferation, enhanced migration and invasion, sustained angiogenesis, and abnormal cell death. These GBM-related Ca\(^{2+}\) channels comprise the VGCC family, especially the P/Q-type, N-type, and T-type channels that are abundant in the central nervous system; they are considered attractive therapeutic targets for several neurologic disorders (Nimmrich and Gross, 2012).

Recently, studies have suggested that oscillations in intracellular calcium concentrations are linked with GBM cell migration, which is positively correlated to glioma aggressiveness and malignancy (Montana and Ronshheimer, 2011; Watkins and Sontheimer, 2012). It is tempting to suggest that the imbalance in Ca\(^{2+}\) signaling through altered VGCC might be involved in the mechanisms implicated in cancer progression.

Evidence also suggests that N- and P/Q-type Ca\(^{2+}\) currents are involved in the pathology of Alzheimer’s disease and epilepsy. N- and P/Q-type channels blockers are suggested to lead to a clinical improvement of cognitive decline in Alzheimer’s patients (Amenta et al., 2009) and to an absence of seizures in epilepsy (Zamponi et al., 2010). Additionally, peptide neurotoxins found in animal venoms have received great interest and have been related to neuropathic pain control (Souza et al., 2008; Rigo et al., 2013a) and the management of cancer-associated pain (Rigo et al., 2013b) in animal models during P/Q- and N-type modulation. Pinheiro et al. (2006, 2009) showed a neuroprotective role for a P/Q-type blocker in an in vitro model of hippocampal ischemia induced by oxygen and glucose deprivation; the neurotoxins prevented neuronal death by inhibition of glutamate release. Accordingly, these peptides also decreased cell neuronal death in retina slices subjected to ischemic injury (Agostini et al., 2011). Taken together, these data support the idea that the development of P/Q- and N-type blockers could be a successful therapeutic strategy for multiple central nervous system diseases, including some forms of cancer.

Recent studies involving T-type channels have focused on primary brain tumors. Zhang et al. (2012) demonstrated that T-type low-VGCC blockade decreases cell proliferation and migration in U87 human GBM cells (Zhang et al., 2012). Another study using U-251MG and U87 human cell lines showed that T-type channel inhibition, induced by the anti-hypertensive drug mibebradil or small-interfering RNA down-regulation expression, disrupted protein kinase B (Akt) signaling and led to apoptotic death in GBM cells (Valerie et al., 2013). In a murine xenograft model, mibebradil also inhibited human GBM growth and potentiated the effect of the cytotoxic agent temozolomide in resistant cells (Keir et al., 2013). These studies, which suggest that inhibition of VGCC T-type channels has an antitumoral effect, provide new insights regarding other VGCC channels as effective therapeutic targets for GBM.
It is known that the blood–brain barrier is a limitation for chemotherapy and contributes to ineffective drug delivery in brain tumor therapy, but a temporary disruption of the blood–brain barrier can be achieved by ion channel modulation. Peptide neurotoxins act on Na\(^{+}\), K\(^{+}\), and Ca\(^{2+}\) channels and induce blood–brain barrier breakdown by stimulating glyco-protein P efflux and phosphorylation of functional proteins (Raposo et al., 2012) or by inducing changes in vascular endothelial growth factor expression (Mendonca et al., 2014). Therefore, effective ion channel modulation via derived neurotoxins is arising as a new strategy for brain tumor drug delivery.

**Calcium Channel–Associated Transient Receptor Potential**

Other calcium channels, such as the calcium channel–associated TRP, provide Ca\(^{2+}\) entry pathways and modulate the driving force for calcium entry (Lang and Stournaras, 2014). These channels have continuously emerged as important factors in several highly prevalent pathologies, cancer included (Holzer and Izzo, 2014), which increases the potential of TRP therapies.

It is well described how malignant transformation is often accompanied by changes in ion channel expression, including the altered expression of numerous members of the TRP family Ca\(^{2+}\)- and Na\(^{+}\)-permeable channels in cancer cells (Arcangeli et al., 2009). However, it is unknown whether these changes in TRP expression are central to the success of the cancer or are a secondary step to other cellular modifications (Mistretta et al., 2014). The nature of cancer cells, tumor progression, and metastatic spreading might be implicated in mutations and in the altered expression of numerous key signaling proteins such as TRP channels (Prevarskaya et al., 2007).

There is accumulating evidence for the expression of TRP channels in cancer cells and tissues and for the channels’ role in malignant cell processes during cancer progression (Holzer and Izzo, 2014). The Ca\(^{2+}\)-permeable transient receptor potential cation channel 1 (TRPC1) is required for cytokinesis in proliferation and migration in gliomas (Bomben and Sontheimer, 2008; Cuddapah et al., 2013), and TRPC1 also regulates endogenous glioma Cl\(^{-}\) channels (Cuddapah et al., 2013). Additionally, the overexpression of TRPC6 and its inhibition in gliomas lead to human glioma cell alternation (Ding et al., 2010; Simon et al., 2015), and TRPC6 regulates endogenous glioma Cl\(^{-}\) channels (Cuddapah et al., 2013). Other calcium channels, such as the calcium channel–associated TRP, provide Ca\(^{2+}\) entry pathways and modulate the driving force for calcium entry (Lang and Stournaras, 2014). These channels have continuously emerged as important factors in several highly prevalent pathologies, cancer included (Holzer and Izzo, 2014), which increases the potential of TRP therapies.

In a recent study, the expression of TRP channel genes was investigated in 33 patients with GBM. The TRPC1, TRPC6, TRPM2, TRPM3, TRPM7, TRPM8, TRPV1, and TRPV2 channels were significantly higher in GBM patients, and there was a positive association between TRP genes overexpression and the enhanced survival of these patients (Alptekin et al., 2015). Several recent studies have also suggested the potential of TRP channels as pharmacologic targets for cancer treatment. The coadministration of cannabidiol, a TRPV2 agonist, potentiated the activity of cytotoxic drugs temozolomide, carbustine, or doxorubicin, and increased drug uptake in human GBM cells (Nabissi et al., 2013). Interestingly, in malignant human gliomas, the chronic application of the TRPC inhibitor SKF 96365 (1-[2-[4-methoxy-3-[3-(4-methoxyphenyl)propoxy]phenyl](ethyl)imidazole) caused near total growth arrest (Bomben and Sontheimer, 2008).

Other proteins, such as the store-operated calcium channels stromal interaction molecule 1 and Orai, play a minor role in gliomas, yet they have been suggested to participate in migration and proliferation in different cancer cells (Zhu et al., 2014; Leanza et al., 2016).

**The Ionotropic ATP-Gated P2X7 Receptor**

Purinergic signaling was first proposed in 1972 (Burnstock and Di Virgilio, 2013). Extracellular ATP is one of the main ligands of the P2 purinergic receptor class, which were later subdivided into ionotropic P2X and metabotropic P2Y subtypes (White and Burnstock, 2006).

P2X7R, an ATP-gated cation permeable (Na\(^{+}\), Ca\(^{2+}\), and K\(^{+}\)) channel and member of the purinergic ionotropic receptors family (Bianco et al., 2009; Costa-Junior et al., 2011; Volonte et al., 2012), has attracted considerable attention during recent years in the context of cancer (Gartland et al., 2001; White and Burnstock, 2006). The primary intracellular signal triggered by ATP acting at P2X7R consists of a fast influx of Ca\(^{2+}\) and Na\(^{+}\) and an efflux of K\(^{-}\). This leads to intracellular signaling pathways that are associated with numerous physiologic processes correlated to inflammatory cascade induction and cell survival and proliferation (Bianco et al., 2009; Roger et al., 2015). In contrast, upon repeated and/or prolonged ATP stimulation, P2X7R induces the opening of nonspecific larger pores that allow permeation of molecules up to 900 Da generally associated with cell death, such as etidium bromide and Yo-Pro-1 (Bianco et al., 2009; Volonte et al., 2012; Roger et al., 2015).

Recently, it has been suggested that pore formation does not depend on the recruitment and clustering of P2X7R subunits, but rather involves the opening of a distinct membrane protein, pannexin-1, which can form hemichannels (Pelegrin and Surprenant, 2006; Bianco et al., 2009). The P2X7 receptor C terminus has been implicated in the regulation of receptor functions involving cellular localization, protein–protein interactions, signaling pathways, and posttranslational modifications (Costa-Junior et al., 2011).

At least in humans, the P2X7R gene is highly polymorphic, and P2X7R genetic differences affect receptor pore formation and channel function (Di Virgilio and Wiley, 2002; Saunders et al., 2003; Sluyter et al., 2004; Fuller et al., 2009; Volonte et al., 2012). Typical carboxyl tail features are suggested to allow for the formation of the large pores, but a naturally occurring truncated P2X7R splice variant, isoform B (P2X7B),
also has been identified. Because it lacks the carboxy terminus, this isoform is deficient in pore formation but maintains the ability to respond to ATP with cation movement (Cheewatrakoolpong et al., 2005; Adinolfi et al., 2010). The P2X7B isoform is highly expressed in several human tissues and participates in the cell growth induction (Adinolfi et al., 2010).

In most cells, pharmacologic activation of P2X7R is associated with membrane permeabilization, blebbing, cell swelling, an increase in Ca\textsuperscript{2+} intracellular levels, and mitochondrial damage (Roger and Pelegrin, 2011). P2X7R expression and activity, which has been reported in several cancers, has been suggested as a potential cancer cell biomarker (Baricordi et al., 1999; Adinolfi et al., 2012; Amoroso et al., 2015). However, the role of P2X7R in oncology is still unclear, and two opposite hypothesis have been proposed. One hypothesis suggests that P2X7R is an antitumor protein that induces cancer cell death, and the other proposes that P2X7R is an aggressive protein that promotes cancer cell survival and growth or invasiveness (Roger and Pelegrin, 2011). P2X7R is expressed at both the mRNA and protein level in human and in mouse glioma cells (Roger et al., 2015).

In the tumor microenvironment, ATP acts as a trophic factor, a danger signal, and the main source of the immunosuppressant adenosine (Amoroso et al., 2015). High levels of extracellular ATP can inhibit proliferation and induce apoptosis/necrosis in mouse GL261 cells and in human M059J glioma cells (Tamajusuku et al., 2010; Gehring et al., 2012, 2015; Bian et al., 2013). It is important to note that tumor cells that respond to ATP-stimulate P2X7-mediated cytotoxicity express higher levels of P2X7R when compared with a subpopulation that is less sensitive to ATP-mediated cytotoxicity (Tamajusuku et al., 2010; Gehring et al., 2012). Accordingly, P2X7R silencing drastically reduced ATP-induced cell death,

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**Fig. 1.** Altered regulation of calcium channels in brain tumors is part of neoplastic transformation. In the brain, the transformation of a normal cell into a tumor cell might be related to Ca\textsuperscript{2+} oscillations, and the homeostasis misbalance can define the malignant phenotype, which includes uncontrolled proliferation, enhanced migration and invasion, and abnormal cell death. The activation of P2X7R leads to extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), phosphatidylinositol 3-kinase (PI3K), and mitogen-activated protein kinase kinase ½ (MEK1/2) activation. High P2X7R functionality and pore activity are linked to apoptosis/necrosis in glioma cells and better progression-free survival.
suggesting that the receptor is necessary for an ATP effect (Tamajusuku et al., 2010; Gehring et al., 2015). Other glioma cell lines (U-87 MG, U-373 MG, U-138MG, U-251MG, and C6) are resistant to ATP-P2X7R-induced cell death (Morrone et al., 2003; Jacques-Silva et al., 2004). It is known that high concentrations of ATP (>100 μM) are required to activate P2X7R (Wiley et al., 2011).

Interestingly, in human glioma (Gehring et al., 2015), lung (Boldrini et al., 2015), and breast (Ghirighelli et al., 2009) biopsies, high P2X7R expression has been correlated with progression-free survival and overall survival. Patients harboring the P2X7R gene polymorphism associated with the P2RX7 loss-of-function allele have a significantly greater risk of metastatic disease progression (Ghirighelli et al., 2009). Cells that express a functional P2X7R (P2X7A) are sensitive to death induced by the receptor (Tamajusuku et al., 2010; Gehring et al., 2012), whereas cells that do not respond to ATP-P2X7R-induced cytotoxicity may express the P2X7R isofrom B correlated to cell growth (Adinolfi et al., 2010). The expression of accessory proteins that are required for mediating ATP toxic effects, such as pannexin, are also lacking, another cause for the different responses observed after P2X7R stimulation (Pelegrin and Surprenant, 2006). Furthermore, a study showed that GL261 P2X7R silenced-bearing mice presented a negligible response to radiotherapy, whereas GL261 WT-bearing mice that constitutively express P2X7R presented a pronounced response after radiotherapy with a significant reduction in tumor volume, showing that functional P2X7R expression is essential for an efficient radiotherapy response in gliomas (Gehring et al., 2015).

P2X7R activation is also linked with inflammatory factors; chronic exposure of C6 rat glial cells to 3'-O-(4-benzoylbenzoyl)adenosine 5'-triphosphate led to increased mobilization of [Ca2+]i, large pore induction, and increased expression of proinflammatory factors such as monocyte chemoattractant-protein-1, interleukin-8, and vascular endothelial growth factor (Wei et al., 2008). Similar data were observed upon P2X7R activation on human glioma cells, which caused monocyte chemoattractant protein-1 and interleukin-8/chemokine (C-X-C motif) ligand 8 secretion in a P2X7-dependent manner (Braganhol et al., 2015), and P2X7R regulates C6 glioma cell mobility and tumor cell migration (Wei et al., 2008; Ryu et al., 2011; Braganhol et al., 2015). Regarding signal pathways activated in malignant brain tumors, P2X7R mediated the activation of extracellular signal-regulated protein kinases 1 and 2 in human 1321N1 astrocytoma cells via an increase in [Ca2+]i. This was linked to the phosphorylation of the proline-rich/Ca2+-activated tyrosine kinase Pyk2, c-Src, phosphatidylinositol 3'-kinase, protein kinase C5 activities, and was dependent on extracellular Ca2+ (Gendron et al., 2003). Figure 1 summarizes the main calcium

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**TABLE 1**

Summary of calcium channels and their possible mechanisms/effectors in malignant brain tumors.

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Artifacts: Akt, protein kinase B; ATP3, activation of transcription factor-3; EGFR, epidermal growth factor receptor; ERK1/2, extracellular signal-regulated protein kinases 1 and 2; HIF-1α, hypoxia-inducible factor 1-α; IL-8, interleukin-8; MCP-1, monocyte chemoattractant protein-1; MEK1/2, mitogen-activated protein kinase kinase 1/2; PI3K, protein kinase C5; PI3K, phosphatidylinositol 3-kinase; VEGF, vascular endothelial growth factor.
channels and their potential mechanisms in malignant brain tumors.

**Conclusion**

An important issue to consider is the difference between various GBM subtypes that are classified by their histopathologic and molecular profile (Verhaak et al., 2010). In fact, the Cancer Genome Atlas has established the existence of four subtypes of GBM: proneural, neural, classic, and mesenchymal (Tomczak et al., 2015). Although many of the results in this review do not discuss this apparent diversity, it is important to mention that tumor cell lines often have phenotypic and genetic alterations (Ledur et al., 2015) that may limit their translation to patient tumors.

Recent evidence indicates that modulating ion channels or ion channel regulators impacts the growth of some tumors. In Table 1, we summarize the main calcium channels and the possible mechanisms/effectsors involved in malignant brain tumor growth. In fact, the potential role of high- and low-VGCC modulation is emerging as a feasible and attractive approach in pharmacologic and clinical application of malignant brain tumors. Additionally, emerging evidence attributes the role of TRP channels in the regulation of homeostasis, growth control, cell survival, and describes their promising implications in GBM therapy. Furthermore, P2X7R activation is linked with elevated expression of inflammation promoting factors, tumor cell migration (Wei et al., 2008; Ryu et al., 2011; Braganhol et al., 2015), an increase in intracellular mobilization of Ca2+, and membrane depolarization in malignant gliomas. Further studies are required though to assess which other calcium channels are associated with the development and progression of malignant brain tumors, and the roles that these channels play in the process.

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