Interaction of ASIC and BK Channels in Human Glioma and its Role in cell Migration and Proliferation

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ABSTRACT

Acid sensing ion channels (ASICs) and large conductance calcium and voltage-activated potassium (BK) channels are expressed throughout the nervous system. Studies have showed that ASICs can act as endogenous regulating BK channels in a pH dependent manner. At physiological pH 7.4, ASIC blocks BK channels, when pH is reduced, ASIC channels are activated and the inhibition of BK is relieved (Petroff et al., 2008). Our previous studies suggest that pH-dependent relief of BK inhibition by ASICs increases cell proliferation in glial cells. Extracellular acidosis has been associated with cerebral trauma and brain cancer known as glioma (Andersen et al. 1988 and Wike-Hooley 1984). We hypothesized that the activation of BK channels by a decrease in pH may promote cell migration and invasion in gliomas. Different stages of human glioma cell lines that express both ASIC and BK channels were used. The cells were cultured at physiological pH 7.4 and reduced pH (7.0), in the presence and in the absence of 200 nM of charybdotoxin, a BK channel blocker, and assessed for cell migration and invasion. Our data suggests a complex mechanism for migration and invasion of gliomas. There was evidence supporting the null hypothesis for migration of astrocytoma stage III and IV. Invasion decreased in glioblastoma stage IV with the BK inhibitor. Intratumoral heterogeneity is a hallmark of glioma tumors as evident by our results. Better understanding of this heterogeneity will be essential to design effective therapies against this devastating disease to avoid tumor growth, migration and invasion. This work is supported by the R15 NIH grant to E.P.
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Carola M. Springer

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INTRODUCTION

Ion channels are membrane proteins that mediate flux of charged ions (sodium, potassium, calcium and chloride) through an otherwise impermeant lipid membrane. They are present both at the plasma membrane level and in the membrane of intracellular organelles. Ion channels in the plasma membrane engage in fundamental functions such as establishing a resting membrane potential, shaping action potentials, muscle contraction, cell excitability, hormone secretion, mechanosensitivity. Ion channels can also change the concentrations of second messengers such as calcium, and are important in the ionic homeostasis of the cytoplasm, of intracellular organelles and of extracellular compartments (Jentsch, T. J., et al., 2004). Thus, the understanding of their physiology and pathophysiology remains an important task for science.

Potassium channels constitute the most abundant family of ion channels. They are categorized based on the number of transmembrane segments in their pore-forming alpha-subunit. There are four main classes of K⁺ channels, Calcium-activated, Inwardly rectifying, tandem pore domain and Voltage-gated. The K⁺ channel subunits are coded by at least 75 genes. Conserved sequence of five amino acids (TYGYG; threonine, valine, glycine, tyrosine, glycine) located in the a re-entrant loop between two transmembrane α-helices form the potassium selectivity filter that allows only K⁺ ions to pass through the membrane (Sandhiya, S, et al., 2009).

The Big Potassium (BK) channels, also known as BKca/MaxiK/Slo1/Kca1.1 channels, are encoded by the KCNMA1 gene and characterized by a large conductance to potassium (up to 300 pS), their sensitivity to calcium and membrane depolarization, and ubiquitous expression (Toro, L., et al., 2014). A BK channel consists of four subunits of
the calcium and voltage sensing α subunit that comes together to form the pore (Fig 1) (Zhang, J., & Yan, J. 2014). BKα is structurally distinct from most other K⁺ channels because it possesses an extra N-terminal transmembrane segment (S0) and a large Ca²⁺ sensing cytosolic C-terminus (Zhang, J., & Yan, J. 2014). Splicing of the Slo1 mRNA as well as modulation by tissue specific β subunits allows for functional diversity of BK channels (Zhang, J., & Yan, J. 2014). Four different beta subunits (β 1- β 4) have been cloned and identified in mammals. BK channels are synergistically activated by both, Ca²⁺/Mg²⁺ metal ions and by membrane voltage (Hermann, A., et al., 2015). Specific blockers of BK channels are tetraethylammonium (TEA), iberiotoxin and charybdotoxin (ChTX) from scorpion venom and paxilline from Penicillium paxilli. The activity of BK channels can also be regulated by the binding of heme (Tang, X. D., et al., 2003) and by the interaction with a different kind of ion channel, the acid sensing ion channel (ASIC) (Petroff, E. Y., et al., 2008).

Figure 1. Structure and membrane topology of BK channel alpha α and β subunits (Zhang, J., & Yan, J. 2014).
ASICs are a family of proton-gated cation channels expressed in both the central and peripheral nervous system (Zha, X. M. 2013). They are involved in neurotransmission in the central nervous system, in nociception, neuroplasticity, mechanotransduction and fear (Bässler, E. L., et al., 2001). ASICs belong to the epithelial sodium channels (ENaC) and degenerin (DEG) super family with the N- and C-termini located inside the cell and a large well-organized, cysteine-rich extracellular domain (Zha, X. M. 2013) (Noël, J., Salinas, et al., 2010) (Sluka, K. A, et al., 2009). These channels display a large extracellular domain projecting substantially above the membrane where the acid pocket, which plays a role in pH-dependent gating, is located (Fig 2) (Sherwood, T. W., et al., 2012). The extracellular domain of each subunit resembles a clenched hand, and sub-regions in the extracellular domain include the wrist, palm, finger, knuckle, thumb and B-ball domain (Sherwood, T. W., et al., 2012).

Figure 2. Overview of ASIC1 structure (Sherwood, T. W., et al., 2012)
Four genes (\textit{ASIC1-ASIC4}) encode at least six different isoforms cloned to date. Both ASIC1 and ASIC2 have splice variants ASIC1a and ASIC1b, ASIC2a and ASIC2b respectively. The composition of the ASIC subunits determines the channel’s properties including pH sensitivity, ion selectivity, activation kinetics and desensitization kinetics (Sluka, K. A., et al., 2009). Homomeric channels of ASIC1a and ASIC3 are activated at a pH \(\leq 7\), whereas ASIC2a homomeric channels are not activated until the pH decreases to \(< 6\) (Sluka, K. A., et al., 2009). Heteromeric channels composed of different subunits can produce channels with properties that are different than those of homomeric channels (Sluka, K. A., et al., 2009). For example, the desensitization rate of ASIC1a/ASIC2a heteromeric channels is more rapid than that of homomeric channels composed of either ASIC1a or ASIC2a (Askwith, C. C., et al., 2004). ASICs conduct mostly Na\(^+\) with low permeability to Ca\(^{2+}\) seen in homomeric ASIC1a and human ASIC1b, as well as ASIC1a/2b heteromers (Waldmann, R., et al., 1997) (Hoagland, E. N., 2010) (Bässler, E. L., et al., 2001) (Sherwood, T. W., et al., 2011).

ASIC activity can be pharmacologically inhibited by amiloride and by two high-affinity peptides, psalmotoxin from spider venom and APETx2 from sea anemone that block ASIC1a and ASIC3 channels, respectively (Diochot, S., et al., 2004) (Diochot, S., et al., 2007). Increased activity of ASICs has been implicated in both epilepsy and Parkinson’s disease. Studies in mice have shown a role of ASIC1 in synaptic plasticity, learning, and memory formation. One of the major pathologies for which a potential role of ASICs is currently emerging is cancer. Multiple studies show an amiloride-sensitive inward Na\(^+\) current in malignant gliomas suggesting activation of ASICs in brain cancer.
Gliomas are primary brain tumors with complex biology characterized by extensive genomic heterogeneity at the cellular and molecular levels (Vila-Carriles, W. H., et al., 2006) (Bonavia, R., et al., 2011). They are the most common type of brain tumors accounting for 90% of brain neoplasms (Wen, Patrick Y., et al., 2008). Patients with malignant gliomas may present with a variety of symptoms, including headaches, seizures, focal neurologic deficits, confusion, memory loss, and personality changes (1). Gliomas are classified according to their hypothesized line of differentiation, whether they display features of astrocytic, oligodendroglial or ependymal cells (Maher, E. A., et al., 2001). They are then graded on a scale of I to IV according to their degree of malignancy as judged by various histological features (Maher, E. A., et al., 2001). Grade I tumors are biologically benign and can be surgically cured; grade II tumors are low-grade malignancies that may follow long clinical courses but are not curable by surgery; grade III tumors are malignant and lead to death within a few years; grade IV tumors are highly malignant, usually resistant to chemotherapy, and lethal within 9-12 months (Maher, E. A., et al., 2001). According to the American Brain Tumor Association, the statistics for the median survival of optimally treated adults with the most aggressive of these tumors, glioblastoma multiforme, has not varied for more than 30 years. What makes these tumors difficult to treat is the lack of fundamental understanding on how they grow and how they modify their environment to support their increase energy demands and to proliferate (Berdiev, Bakhrom K., et al. 2003). The understanding of the
molecular alterations and signaling pathways in gliomas is crucial for the development of new therapeutic options.

In the past two decades, advances in genome sequencing and microarray analysis have provided a comprehensive view of the complex genetic alterations seen in gliomas, particularly glioblastomas, that helps fuel cell proliferation and enhance cell survival in these tumors (Chen, Jian, et al., 2012). One of the pathways that are frequently affected in glioblastoma includes channels involved in transport of sodium, potassium and calcium ions. Glioblastoma cells display up-regulation of Cl⁻ and K⁺ channels not found in normal glia. It has been suggested that Cl⁻ channels in glioma membrane are instrumental in controlling cellular volume and the rapid changes in cell size and shape that allows for tumor invasion throughout brain tissue (Olsen, M. L., et al., 2003).

Evaluation of biopsy tissues from patients with revealed significant expression of BK channel protein and studies on established cell lines have confirmed that BK channels are the predominant K⁺ channels type in these cells (Liu, X., et al. 2002) (Ningaraj, N. S., et al. 2002) (Ransom, C. B., et al., 2002). Molecular cloning of identified the glioma BK channel as a novel splice variant of the hSlo gene with enhanced Ca²⁺ sensitivity that was named gBK (Liu, X., et al., 2002) (Ransom, C. B., et al., 2002). gBK channels have been suggested to play a role in the extensive migrating behavior of glioblastoma cells. Treatment with paxilline and tetraethylammonium, both gBK channel inhibitors, reduced in-vitro cellular migration (Wondergem, R., et al., 2008) (Wondergem, R., & Bartley, J. W 2009).
The role of gBK in glioma proliferation still remains unclear. Some studies have implicated this channel in the glioma proliferation after observing growth inhibition and tumor shrinkage in cells treated with pharmacological inhibitors (Weaver, A. K., et al., 2006) (Basrai, D., et al., 2002) (Weaver, A. K., et al., 2004). In contrast, more recent literature contradicts these findings and suggests that gBK channels are not required for glioma proliferation or even have antitumorigenic properties. Pharmacological inhibitors of gBK did not reduce cell growth in-vitro, and downregulation of gBK channels by siRNA reduced K\(^+\) current densities but caused no changes in cell proliferation (Abdullaev, I. F., et al., 2010).

Gliomas, like many other types of malignant cancers show an increase flux of carbons through fermentative glycolysis, and display acidification of the surrounding interstitium with pH values as low as 6.5 (Estrella, Veronica, et al., 2013). Mounting evidence show that acidosis surrounding a malignant tumor increases activation of lysosomal enzymes with acidic optimal pH, and activates genes involved in pro-metastatic factors (Furnari, Frank B., et al., 2007). Thus acidic pH correlates with tumor spread by promoting extracellular matrix degradation and angiogenesis through acid-induced release of vascular endothelial growth factor and interleukin (6). Proton-activated neuronal currents have been identified in different brain regions. ASICs generate excitatory currents in response to decreased extracellular pH. The function of ASIC in glia is not well understood, however, previous studies suggest a possible role in glial cell proliferation resulting from the interaction of ASIC1a with BK channels (Guercio, J. C., et al., 2011).
A highly conserved sequence of amino acids found in ASIC extracellular domain resembles part of the sequence in scorpion toxin that inhibits K+ channels. The R/KY/MGKC sequence in the thumb domain of ASIC is thought to interact with the pore region of BK in response to changes in extracellular pH (Fig 3) (Petroff, E. Y., et al., 2008). An increased proliferation of glia cells resulting from this interaction has been reported (Guercio, J. C., et al., 2011). A study where glia cells were treated with a synthetically made ASIC1a wild type peptide containing the sequence RYGKS that closely resembles that found in vivo (RYGKC), and a mutant peptide with two amino acid substitutions (AYGAS) showed increased cell proliferation with the mutant peptide (Guercio, J. C., et al., 2011). These results suggest that the mutant peptide was able to displace endogenous ASIC from BK but was unable to block the channel, thus increasing proliferation.

Figure 3. ASIC inhibits BK channel under physiological pH 7.4. BK channel inhibition is released with increased in extracellular protons (Petroff, E. Y., et al., 2008).
Under normal physiological pH, ASIC1a inhibits BK channels (Fig 3). In the excess of protons (acidosis), BK channel inhibition is released possibly as a result of a conformational change produced by the activation of ASIC1a (Fig 3) (Petroff, E., et al., 2012). Therefore, an increase in acidosis, could lead to an increase in the activity of these two different types of channels correlating with what has been observed in high-grade gliomas. This study proposes that ASICs may function as endogenous inhibitors of glial cell growth through inhibition of BK channels, and that a disruption of this interaction in the acidic environment of a malignant brain tumor may lead to glioma cell proliferation and invasion. Furthermore, it is hypothesized that under normal physiological pH (7.4), ASIC would inhibit BK channels and decrease invasion and migration of glioma cells. An extracellular environment with a lower pH (7.0) should relieve ASIC blockade and activate BK channels leading to an increased in migration and invasion of glioma cells. In contrast, blocking BK channel with charybdotoxin should decrease glioma cell invasion and proliferation in a pH independent manner. Lastly, it is hypothesized that the degree of migration and invasion resulting from the interaction of ASIC and BK channels correlates with the stage of the glioma tumor. The more aggressive grade IV tumors should progress faster than the lower grade tumors when BK blockade by ASIC is relieved.

MATERIALS AND METHODS

Cell Culture

Astrocytoma (SW1783, SW1088) and Glioblastoma (U87MG) cell lines were purchased from American Type Culture Collections, Manassas, VA (ATCC No. HTB-13, HTB-12...
and HTB-14 respectively). Cells were cultured in 75 cm² flasks with DMEM (Gibco Cat No. 70011-069), 10% fetal bovine serum (FBS) (Gibco Cat. No. 10438), incubated at 37°C, 95% humidity, ambient O₂ and 5% CO₂ (pH 7.4) or 10% CO₂ (pH 7.0) (Fig. 4). Cells were grown to about 70% confluency prior to experiment.

![Cell images](Image)

**Figure 4.** Cultured glioma cell lines

**Scorpion toxin**

Charybdotoxin (ChTX) was purchased from Sigma (Cat No. C7802). Cells were treated with 200nM concentration for 24 hours.

**Boyden chamber assay**

To assess ASIC-BK channel involvement in glioma migration and invasion, we performed an in-vitro model, the Boyden chamber assay (EMD Millipore Cat. No. ECM 509 and ECM 554) (Fig. 5). The day prior to the experiment, 70% confluent flask glioma cells were rinsed with PBS and supplied with serum-free media overnight. A suspension of 1.0x10⁶ cell/mL cells in 300 µl of growth factor-free medium was seeded in the upper chamber of the Boyden device (BD). An uncoated membrane divides the chambers. The insert consists of a filter with 8 µm pores that cells must navigate to cross to the bottom.
side of the transwell filter. The insert in the invasion assay contains a thin coating layer of ECMatrix™, through which the cells migrate. The lower chamber was loaded with 500 µl DMEM supplemented with 10% FBS as chemoattractant. Experimental cells were treated with 200nM ChTX. Plates were incubated for 24 h in pH 7.4 (5% CO₂) or pH 7.0 (10% CO₂). After 24 h incubation, cells/media from the top side of the insert was removed by pipetting out the remaining cell suspension. Placed the migration insert into a clean well containing 225 µL of prewarmed 0.05% trypsin and incubated for 30 minutes at 37°C. Diluted the CyQUANT® GR Dye 1:75 with 4X Lysis Buffer (4 µL dye in 300 µL of 4X Lysis Buffer) and added 75 µL of this Lysis Buffer/Dye Solution to each well containing 225 µL of 0.05% trypsin with the cells that migrated through the membrane. Incubated 15 minutes at room temperature. Transferred 200 µL of the mixture to a 96-well plate suitable for fluorescence measurement. Read with a fluorescence plate reader using 480/520 nm filter set.

Figure 5. Boyden chamber assay
Plate reader

Fluorescence was read with a Hitachi Fluorescence Spectrophotometer F-7000 using 480/520 nm filter set.

Statistical Analysis

Statistical data are presented as means of n cells ± SEM. To test for significant differences, a non-pair t test was used with Excel worksheet and R software.

RESULTS

To test the hypothesis that BK channel currents are involved in the progression of glioma tumors, ChTX, a BK channel inhibitor, was added to glioma cells of various etiologies and stages and performed migration and invasion assays using a Boyden chamber as described previously. For the experiments, three cell lines were used, astrocytoma, grades III and IV and glioblastoma, grade IV. Experimental samples were cultured in the presence or absence (control) of 200nM ChTX and placed in pH 7.4 or pH 7.0 for 24h. Fluorimetric quantification of cells that migrated through the Boyden membrane insert was obtained.

The results show increased migration with ChTX at low pH in astrocytoma grade III (SW1783) and no change in migration at physiological pH 7.4 (Fig. 6). Interestingly, the inhibition of BK channels by ChTX failed to decrease migration of astrocytoma grade III under low pH 7.0.
Figure 6. No change in migration of astrocytoma, grade III with ChTX at physiological pH 7.4. Increased migration with ChTX was seen at low pH 7.0.

An increased migration of astrocytoma grade IV (SW 1088) with ChTX was observed at both physiological and low pH (Fig. 7). Migration in this more advanced stage of glioma seemed independent of pH condition.
Figure 7. No change in migration of astrocytoma, grade IV with ChTX. Cell migration increased in response to ChTX in both pH conditions.

Lastly, migration of glioblastoma grade IV (U87MG) with ChTX at pH 7.4 was observed (Fig. 8). Migration remained unchanged under both pH conditions.
Figure 8. Decreased migration with ChTX was observed at pH 7.4 in glioblastoma grade IV (U87MG). Migration remained in the more acidic condition, pH 7.0.

To summarize, inhibition of BK channels showed statistically significant effects in all three cell lines (Fig. 9). The effect of ChTX seen both astrocytoma cell lines stages III and IV was an increase cell migration in pH 7.0 with astrocytoma stage IV showing an increase in migration in physiological pH as well. In the contrary, glioblastoma stage IV showed decrease migration in the presence of ChTX at pH 7.4 and no effect under lower pH.
Figure 9. Effect of ChTX on glioma migration of astrocytoma grade III (SW1783), Astrocytoma grade IV (SW1088) and glioblastoma grade IV (U87MG) cell lines.

Invasion of glioma tumors was assessed using the same Boyden chamber technique as in the migration assays but with an insert with 8 μm pores. The small pore size provides special constraint that the cells will have to overcome in order to cross to the other side that has been coated ECMatrix™. The cells were incubated for 24 h in the presence or absence (control) of 200nM ChTX and analyzed through a fluorescent plate reader.

The results show unchanged invasiveness with ChTX in astrocytoma grade III (SW1783) (Fig. 10). Invasion remained constant in the presence and absence of the BK channel inhibitor and this was independent of pH condition.
Figure 10. Invasiveness remained constant in astrocytoma grade III (SW1783) the presence and in the absence of the BK channel inhibitor, and this was independent of pH condition.

A slight increase in invasion of astrocytoma grade VI (SW1783) was observed in the presence of ChTX in pH 7.0 compared to the control and to physiological pH; however, this increase was not statistically significant (Fig. 11). Cells cultured at physiological pH of 7.4 showed no decrease in migration when exposed to BK channel inhibitor.
Figure 11. Invasiveness in astrocytoma grade VI (SW1783) remained constant in the presence and in the absence of the BK channel inhibitor, and this was independent of pH condition.

The most aggressive cell line tested, glioblastoma grade IV showed decreased invasion in the presence of BK inhibitor under both pH conditions (pH 7.4 and pH 7.0) (Fig. 12).
Figure 12. Decreased invasiveness with ChTX seen glioblastoma grade VI (U87MG).

Summarizing the results of the invasion assay for the three cell lines, it can be seen that inhibition of BK channels by charybdotoxin had a significant effect in glioblastoma stage IV (Fig. 13). Decrease in invasion of glioblastoma was observed under both pH conditions. Invasion remained unchanged in both astrocytoma cell lines.
Figure 13. Effect of ChTX on glioma invasion of astrocytoma grade III (SW1783), astrocytoma grade IV (SW1088) and glioblastoma grade IV (U87MG) cell lines.

DISCUSSION

Studies have showed that ASICs can act as endogenous regulators of BK channels (Petroff, E. Y., et al., 2008). ASIC has a toxin-like extracellular domain that inhibits BK currents at physiological pH, and this inhibition is relieved with the activation of ASIC when the pH decreases (Petroff, E. Y., et al., 2008). Relieve of the BK blockade by ASIC has been correlated with an increase in normal glia proliferation (Guercio, J. C., et al., 2011). Conditions characterized by low extracellular pH such as stroke, ischemia and cancer may provide the mechanism for glia proliferation by the activation of ASIC currents and the subsequent relieve of BK channel inhibition.
The ubiquitous expression of BK channels in gliomas of all malignancy grades and the amiloride-sensitive currents observed in aggressive glioblastomas suggest that these channels play an important role in one or more cellular functions including migration and invasion of tumorigenic cells. This study proposed that the acidic extracellular environment of gliomas causes a disruption in the ASIC-BK interaction and leads to higher BK activity and consequently to increased glial proliferation. To test this hypothesis, ChTX, a BK channel inhibitor, was added to glioma cells of various stages. Astrocytoma, grades III and IV and glioblastoma, grade IV were cultured in 75 cm$^3$ to about 70% confluency. A day prior to the experiment cells were rinsed with PBS and cultured in serum-free media for 24 h. Cells were then seeded in a Boyden chamber as described previously. Experimental samples were cultured in the presence or absence (control) of 200 nM ChTX and incubated in pH 7.4 or pH 7.0 for 24 h. Fluorimetric quantification of cells that migrated through the Boyden membrane insert was obtained.

Previous experiments to determine the role of BK channels in gliomas have shown contradictory results; our study suggests a very complex biology for migration and invasion of these tumors. Inhibition of BK channels with ChTX showed statistically significant effects that supported our null hypothesis for both astrocytoma cell lines, stages III and IV. An increase in migration with ChTX in low pH condition (pH 7.0) was observed for these two cell lines. Migration of glioblastoma stage IV remained unchanged. A possible explanation for what we observed is that that glioma cells may have a large functional BK channel reserve which was not completely and constantly blocked by the channel inhibitor. If this is the case, only a small fraction of BK channels
need to be active to maintain cell function, therefore, despite the addition of ChTX, enough channels remained opened for the cells to maintain regular function.

Glioma cells show an unusual ability to invade the normal brain diffusely. It has been suggested that cell invasion into narrow brain spaces may require cells to shrink their volume (Mcferrin, M. B., et al., 2006) (Weaver, A. K., et. al., 2006). Cell shrinkage requires the efflux of KCL, and BK channels may serve as the pathway for K⁺ efflux during cell invasion (Mcferrin, M. B., et al., 2006). Consistent with this hypothesis, we found a decrease in invasion of glioblastoma stage IV with the BK channel inhibitor. A greater decrease in invasion with ChTX was seen in the low pH conditions correlating with our hypothesis that activation of ASIC channels relieve BK channel inhibition. Surprisingly, however, ChTX had no effect in the astrocytoma cell lines (stages III and IV). It is possible that our results reflect a higher expression of BK channels in glioblastoma versus the less aggressive astrocytoma. Previous studies have found that deregulation of BK channel expression is linked to tumor malignancy and patient prognosis (Wang, R., et al., 2015) (Lastraioli, E., et al., 2015).

Our data suggests that there are two different complex mechanisms underlying migration and invasion of gliomas. Considering the great heterogeneity among astrocytoma and glioblastoma cells, there might be tumor cells which can migrate/invade without depending on ASIC/BK channels. Heterogeneity is a hallmark of glioma tumors as evidenced by early studies that found markedly different karyotypes among cells freshly isolated from clinical specimens (Furnari, F B., et al., 2007) or even within an established cell lines, and variable expression of antigenic markers (Kato, Yasumasa, et al., 2003). Consequences of this heterogeneity were reflected in the in vitro phenotype of
those cells: in their morphologies, growth rates, and, most importantly, their drug responses (Berdiev, B. K., et al., 2002). Future studies to determine the levels of expression of ASIC and BK channels in the different glioma cell lines used in this study might shed some light in our findings.

Variability in the Ca$^{2+}$ sensitivity between different glioma cell lines could be an indication of multiple isoforms of gBK channels that may not be fully inhibited by ChTX. These gBK channel isoforms may provide the electrochemical driving force to facilitate migration despite the presence of an inhibitor. Electrophysiological measurements to determine if there is a partial or complete blockage of gBK current with ChTX in the cell lines used, as well as mRNA isolation and RT-PCR to determine the gBK isoforms may provide definitive results. Finally, cloning of the gBK channel to study its modulation by ASIC in transfected cells may be important in determining the role of this interaction in gliomas.

REFERENCES


(2010). Identification of a calcium permeable human acid-sensing ion channel 1

unravelled by dysfunction. Nature cell biology, 6(11), 1039-1047.

Benos, D. J. (2009). Knockdown of ASIC1 and epithelial sodium channel subunits
inhibits glioblastoma whole cell current and cell migration. Journal of Biological
Chemistry, 284(36), 24526-24541.


cancer biomarker. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1848(10),
2685-2702.

characterization of glioma BK, a novel BK channel isoform highly expressed in


Regulation of blood-brain tumor barrier permeability by calcium-activated potassium

Current perspectives on acid-sensing ion channels: new advances and therapeutic

Expression of voltage-gated chloride channels in human glioma cells. The Journal of
neuroscience, 23(13), 5572-5582.


