Voltage-gated and ATP-sensitive K^+ channels are associated with cell proliferation and tumorigenesis of human glioma

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Abstract. Increasing evidence indicates that potassium (K^+) channels play important roles in the growth and development of human cancer. In the present study, we investigated the contribution of and the mechanism by which K^+ channels control the proliferation and tumor development of U87-MG human glioma cells. A variety of K^+ channel blockers and openers were used to differentiate the critical subtype of K^+ channels involved. The *in vitro* data demonstrated that selective blockers of voltage-gated K^+ (K_V) channels or ATP-sensitive K^+ (K_{ATP}) channels significantly inhibited the proliferation of U87-MG cells, blocked the cell cycle at the G_0/G_1 phase and induced apoptosis. In the U87-MG xenograft model in nude mice, K_V or K_{ATP} channel blockers markedly suppressed tumor growth *in vivo*. Furthermore, electrophysiological results showed that K_V or K_{ATP} channel blockers inhibited K_V/K_{ATP} channel currents as well as cell proliferation and tumor growth over the same concentration range. In contrast, iberiotoxin, a selective blocker of calcium-activated K^+ channels, had no apparent effect on the cell proliferation, cell cycle or apoptosis of U87-MG cells. In addition, the results of fluorescence assays indicated that blockers of K_V or K_{ATP} channels attenuated intracellular Ca^{2+} signaling by blocking Ca^{2+} influx in U87-MG cells. Taken together, these data suggest that K_V and K_{ATP} channels play important roles in the proliferation of U87-MG cells and that the influence of K_V and K_{ATP} channels may be mediated by a Ca^{2+}-dependent mechanism.

Introduction

Gliomas are the most common malignant brain tumors and are characterized by relentless growth and aggressive invasion into the brain parenchyma (1). Under the current standard of care, the life expectancy of patients with glioma is ~14 months after diagnosis despite aggressive surgery, radiation and chemotherapies (2). Therefore, a better understanding of the mechanism through which glioma develops is necessary for efficient and specific inhibition of the progression of this form of cancer.

Potassium (K^+) channels are the most diverse ion channels in the plasma membrane (3). Over the last 10 years, accumulating evidence has indicated that diverse types of K^+ channels, including voltage-gated, calcium-activated, two-pore domain and inward rectifier K^+ channels, are overexpressed in a number of tumor types, such as prostate, colon and breast (4-6). Most previous studies have demonstrated that, in addition to controlling physiological parameters, K^+ channels also play important roles in the onset, proliferation and malignant progression of various types of cancer (7). However, mammalian cells constitutively express an array of various types of K^+ channels. Although most previous studies have described the expression of a particular type of K^+ channel in a cancer cell line and have correlated the expression and functional activity of the channel with proliferation, it is not clear to what degree these individual K^+ channels contribute to proliferation or whether a specific association exists between particular K^+ channel subtypes and proliferation. Moreover, the mechanisms by which these K^+ channels facilitate cell proliferation remain obscure.

Increasing evidence has demonstrated that a variety of K^+ channels, such as voltage-gated K^+ channels (K_V), calcium-activated K^+ channels (K_{Ca}) and ATP-sensitive K^+ channels (K_{ATP}) (8-10), are overexpressed in glioma biopsies and cultured cells. A previous study found a correlation between the activity of K_{ATP} channels and proliferation of glioma cells and xenografted tumors (8). However, it remains uncertain whether other K^+ channels are also proliferative molecules in glioma cells. Identification of K^+ channels that play a role in glioma growth may provide novel therapeutic targets. In the present study, we attempted to identify K^+ channels that affect the growth of human glioma U87-MG cells *in vitro* and *in vivo*. Using a variety of K^+ channel blockers and openers, we found that K_V and K_{ATP} channels play roles in controlling cell proliferation and tumorigenesis, whereas other K^+ channel subtypes do not. We also analyzed the mechanism of action of K^+ channels in cell proliferation by studying the relationship between K^+ channel activities and Ca^{2+} entry with the use of

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a fluorescent cytosolic Ca^{2+} assay. Our results demonstrated that K_V and K_{ATP} channels may affect cell proliferation by modulating Ca^{2+} influx.

Materials and methods

Chemicals and drug preparations. Tetraethylammonium (TEA), 4-aminopyridine (4-AP), glibenclamide, phloretin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), diazoxide, iberiotoxin, tetrodotoxin (TTX) and propidium iodide (PI) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). RPMI-1640 medium and fetal bovine serum (FBS) were obtained from Invitrogen Life Technologies (Carlsbad, CA, USA). The Annexin V-fluorescein isothiocyanate/propidium iodide (Annexin V-FITC/PI) apoptosis detection kit was procured from Antgene Biotech (Wuhan, China). RNase A was purchased from Fermentas International Inc. (Burlington, Ontario, Canada). All other chemicals were of standard analytical grade.

Cell culture. The human glioma cell line U87-MG was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and grown in RPMI-1640 supplemented with 10% FBS and 100 units of penicillin/streptomycin in 5% CO_2 at 37˚C. The cells were passaged every 3 days and maintained in exponential growth to ~80% confluency for later experiments.

MTT proliferation assay. A modified MTT assay was used to examine the effect on cell proliferation. Briefly, cells were seeded in a 96-well plate at 5,000 cells/well and incubated overnight. After drug treatment for 48 h, 20 μl of MTT solution (5 mg/ml) was added to each well, and the samples were incubated for an additional 4 h. Subsequently, the supernatant was removed, and the cells were dissolved in 150 μl of DMSO. Finally, absorbance was measured at 570 nm using a 96-well microplate reader (Thermo Scientific, USA).

Annexin V-FITC/PI apoptosis assay. Cells were double stained using an Annexin V-FITC/PI apoptosis detection kit. Briefly, after exposure to different drugs for 48 h, the cells were harvested, washed twice with cold PBS and resuspended in Annexin V binding buffer. Then, 5 μl of FITC-labeled Annexin V and 5 μl of PI were added. The cells were incubated for 15 min at room temperature in the dark with gentle oscillation. After the addition of 200 μl of binding buffer to each tube, the cells were analyzed within 1 h using a flow cytometer (Becton-Dickinson, USA).

Cell cycle assay. Cells were detached by trypsinization, seeded at $5x10^5$ cells/well in a 6-well plate and incubated overnight. The cells were then treated with various drugs at different concentrations. Subsequently, the cells were harvested into cold PBS at different time points, fixed in ice-cold 70% ethanol and stored at 4˚C overnight for subsequent cell cycle analysis. Fixed cells were washed twice with PBS and resuspended in 1 ml of the staining reagents (100 μg/ml RNase A and 50 μg/ml PI). The samples were incubated in the dark for 30 min, and the distribution of cells in the various phases of the cell cycle was measured by flow cytometry.

$[Ca^{2+}]_i$ measurements. Cells were grown overnight, washed twice with Hank's balanced salt solution (HBSS) and loaded with 1 μmol/l Fluo3-AM for 30 min in the dark at room temperature. Then cells were washed twice with Ca^{2+}-free HBSS, re-suspended in Ca^{2+}-free HBSS and incubated at room temperature for 20 min in the dark. When appropriate, the cells were pretreated with K^+ channel blockers or openers for 5 min before the initiation of Ca^{2+} influx. Ca^{2+} influx was measured as changes in fluorescent signals, which were recorded using a fluorescence microscope (Olympus, Japan) and analyzed using Matlab software.

In vivo therapy experiments. To further verify the antiproliferative efficacies of K^+ channel blockers, an *in vivo* experiment was performed using 6- to 8-week old nude mice. The nude mice were randomly divided into control and treated groups. The treated groups were subcutaneously injected with a suspension of $5x10^6$ U87-MG cells with 4-AP (5 mmol/l), TEA (20 mmol/l) or glibenclamide (200 μmol/l), and mice in the control group were injected with a suspension of $5x10^6$ cells with 0.01 mol/l PBS (8). Tumor size was measured every 3 days and tumor volume was determined by calculating $(\text{length x width})^2/2$. At the end of the experiment, tumors were excised and weighed. A tumor growth curve was plotted according to the tumor volume, and the inhibitory rates of tumor growth were calculated according to the tumor weight. All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Electrophysiological assay. Membrane currents were recorded using a whole-cell voltage clamp and borosilicate glass pipettes (outer diameter, 1.5 mm; direct current resistance, 3-6 MΩ) constructed using a two-step puller (P-97; Sutter, USA). To investigate the K_V currents, the pipette solution consisted of (in mmol/l) KCl 140, $MgCl_2$ 2.5, HEPES 10, EGTA 11 and ATP 5 and the pH was adjusted to 7.2 (11). To investigate the K_{ATP} currents, the micropipettes were filled with (in mmol/l) KCl 140, $MgCl_2$ 1, ATP 0.01, EGTA-KOH 5 and HEPES-KOH 5 and the pH was adjusted to 7.3 (12). The cells were bathed in an extracellular solution containing (in mmol/l) NaCl 135, KCl 5.4, $MgCl_2$ 1.0, NaH_2PO_4 0.33, $CaCl_2$ 1.8, HEPES 10 and D-glucose 10 with 1 μmol/l TTX. The osmolarity was adjusted to 330 mOsm/l with sucrose and the pH was adjusted to 7.4. Whole-cell patch clamp recordings were performed at room temperature using a patch clamp amplifier (Axon-200B) (13). Adjustments of capacitance compensation and series resistance compensation were performed before the membrane currents were recorded. The membrane currents were filtered at 10 kHz (-3 dB) and the data were processed using Clampfit (Axon, USA).

Statistical analysis. The data are expressed as the means ± standard error (± SE). Statistical significance was assessed using analysis of variance (ANOVA). P-values of <0.05 were considered to indicate statistically significant results.

Results

Effects of K^+ channel blockers and openers on cell proliferation. To identify the K^+ channels that affect U87-MG cell prolifera-

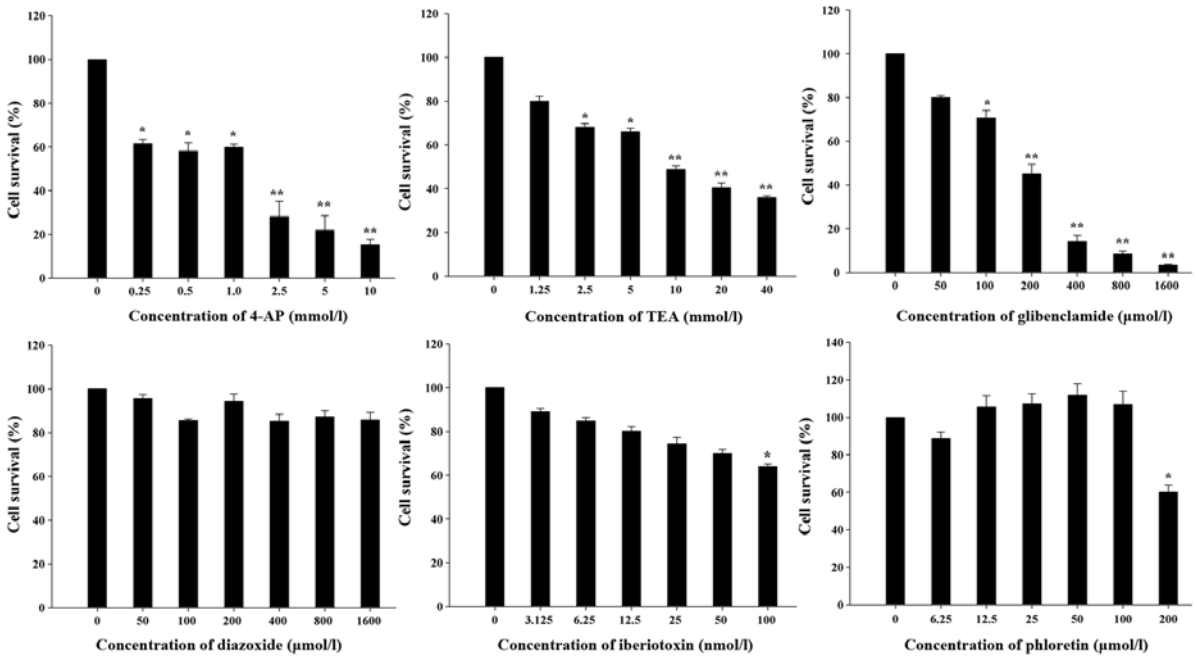

Figure 1. Effects of K^+ channel blockers and openers on the proliferation of U87-MG cells. U87-MG cells were plated at 5,000 cells/well and treated with the indicated concentrations of the tested K^+ channel blockers and openers for 48 h. $^*P<0.05$ and $^{**}P<0.01$ when compared to the group treated with a solvent control. K^+ channel, potassium channel.

tion, we used a variety of K^+ channel blockers and openers and an MTT assay was used to determine the number of live cells remaining after the drug treatment. Among the blockers, 4-AP and TEA are transient outward and delayed rectifier K_V channel blockers, respectively (14), whereas glibenclamide is a specific blocker of K_{ATP} channels, and iberiotoxin is a specific K_{Ca} channel blocker. As shown in Fig. 1, cell proliferation was significantly inhibited by 4-AP and TEA in a dose-dependent manner and glibenclamide also significantly decreased the U87-MG cell number. However, 100 nmol/l iberiotoxin, a concentration that completely blocks K_{Ca} channels (15), only moderately inhibited U87-MG cell proliferation. Meanwhile, diazoxide, an opener of K_{ATP} channels and phloretin, an opener of K_{Ca} channels, had no significant effects on cell proliferation. Taken together, these data reveal that K_V and K_{ATP} channels but not K_{Ca} channels have important roles in the proliferation of U87-MG cells.

Effects of K^+ channel blockers and openers on the cell cycle distribution. To elucidate the mechanisms through which K^+ channels influence the proliferation of glioma cells, flow cytometry was performed to investigate whether the U87-MG cell cycle may be influenced by various K^+ channel blockers or openers. As shown in Fig. 2A and B, the percentage of U87-MG cells in the G_0/G_1 phase was significantly enhanced after exposure to 4-AP, TEA and glibenclamide for 48 h, whereas the percentage of cells in the S phase was markedly reduced. Iberiotoxin had no obvious effect on the U87-MG cell cycle at a concentration of 25 nmol/l. Meanwhile, activating K_{ATP} channels with diazoxide and activating K_{Ca} channels with phloretin increased the percentage of U87-MG cells in the S phase. These results were consistent with previous reports (16) that K^+ channels are believed to facilitate progression through G_1/S checkpoint.

Effects of K^+ channel blockers and openers on cell apoptosis. To determine whether the reduction in cell viability caused by K^+ channel blockers was related to apoptotic cell death, the effects of different K^+ channel blockers and openers on U87-MG cell apoptosis were studied using Annexin V-FITC/PI staining. As illustrated in Fig. 2C, the percentage of Annexin V-FITC-positive apoptotic cells was increased by adding 4-AP or glibenclamide to the U87-MG cells (5.1±0.8% of control, 44.7±3.8% of 5 mmol/l 4-AP and 18.3±1.32% of 200 μmol/l glibenclamide). However, 4-AP only slightly increased necrosis, whereas glibenclamide induced noticeable necrosis (1.8±0.26% of control, 3.2±0.26% of 5 mmol/l 4-AP and 24.8±3.08% of 200 μmol/l glibenclamide). In contrast, at some of the tested concentrations, treatment with TEA, iberiotoxin, phloretin or diazoxide for 48 h had no apparent effect on cell apoptosis.

Effects of K^+ channel blockers and openers on Ca^{2+} influx. To further explore the mechanism of K^+ channel involvement in U87-MG cell proliferation, we examined the effects of different K^+ channel blockers and openers on Ca^{2+} influx. Ca^{2+} influx was induced by the addition of 0.5 mmol/l $CaCl_2$ to the bathing medium (Ca^{2+}-free HBSS), which caused a rapid increase in cytosolic Ca^{2+} to a level that was sustained for several minutes. As shown in Fig. 3, 4-AP (5 mmol/l) and glibenclamide (200 μmol/l) nearly abolished the increase in $[Ca^{2+}]_i$ induced by exogenously applied Ca^{2+} ($P<0.01$), and TEA (20 mmol/l) reduced the peak Ca^{2+} response by 8.5±0.5% ($P<0.05$), indicating that K_V and K_{ATP} channels may modulate Ca^{2+} influx into glioma cells and subsequently modulate the proliferation and apoptosis of these cells. Iberiotoxin (25 nmol/l) decreased the peak Ca^{2+} response by 2.1±0.1%, which was not significant when compared with the control cells ($P>0.05$).

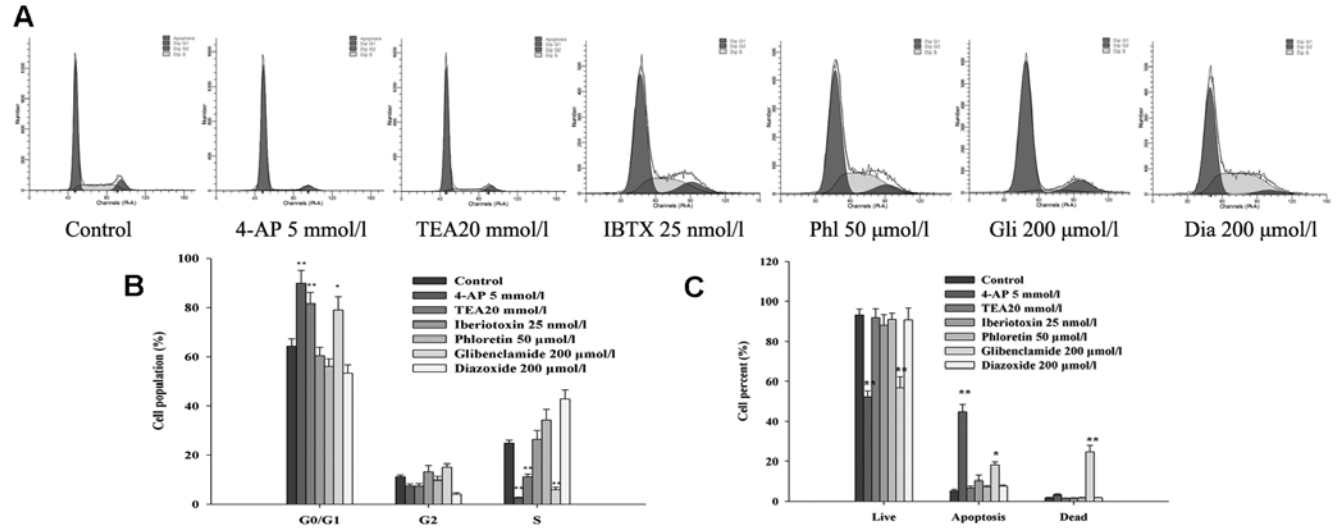

Figure 2. Cell cycle distribution and analysis of apoptosis in U87-MG cells treated with K^+ channel blockers and openers. (A) U87-MG cells were treated with K^+ channel blockers or openers for 48 h at the indicated concentrations, and the cell cycle distribution was determined by flow cytometry after PI staining. (B) Quantitative analysis of the cell population in the different phases of the cell cycle. (C) U87-MG cells were treated with K^+ channel blockers or openers for 48 h at the indicated concentrations, labeled with both Annexin V-FITC and PI and subsequently assessed for apoptosis by flow cytometry. 4-AP, 4-aminopyridine; TEA, tetraethylammonium; IBTX, iberiotoxin; Phl, phloretin; Gli, glibenclamide; Dia, diazoxide. *P<0.05 and **P<0.01 when compared to the control. K^+ channel, potassium channel.

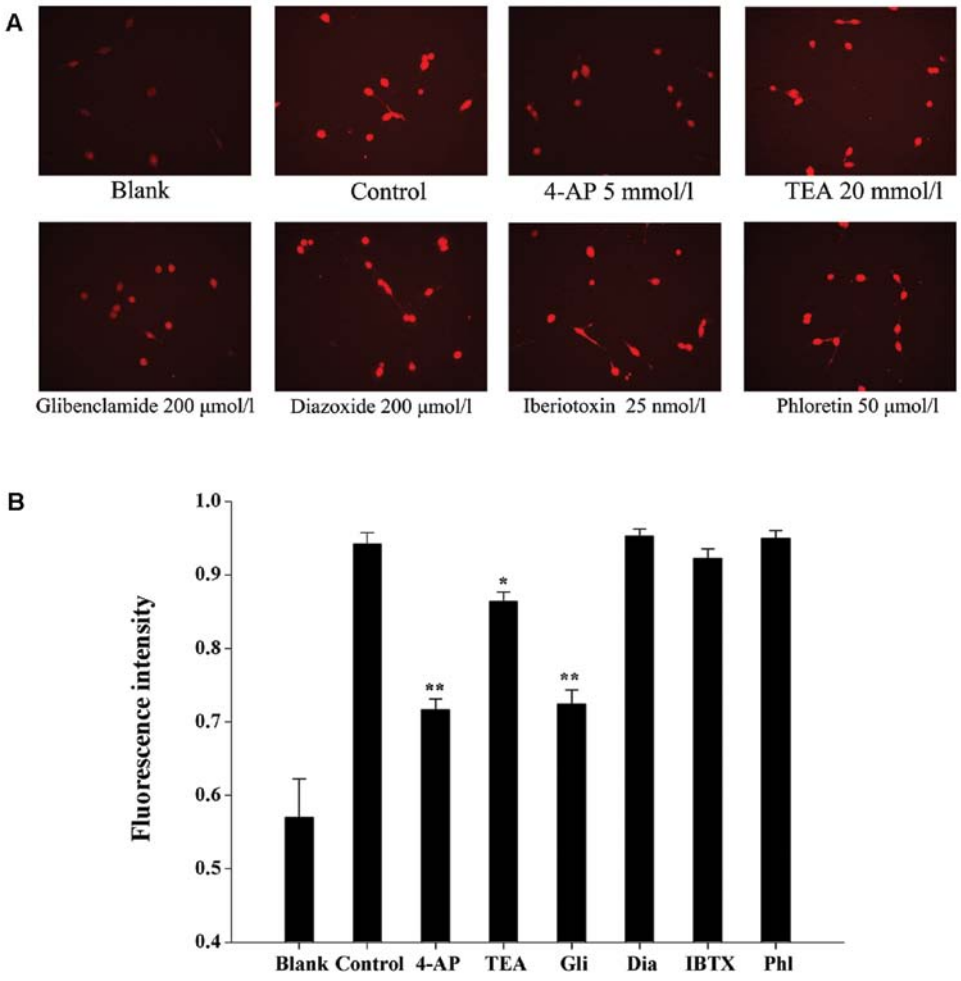

Figure 3. Effects of K^+ channel blockers and openers on the increase in $[Ca^{2+}]_i$ induced by exogenously applied Ca^{2+}. (A) Cells were placed in Ca^{2+}-free HBSS with or without blockers. Blank, cells were incubated with Ca^{2+}-free HBSS. Control, Ca^{2+} influx was induced by adding 0.5 mmol/l $CaCl_2$ to Ca^{2+}-free HBSS. (B) The results of Ca^{2+} influx were analyzed using Matlab software and $[Ca^{2+}]$i was determined based on the fluorescence intensity. 4-AP, 4-aminopyridine; TEA, tetraethylammonium; Gli, glibenclamide; Dia, diazoxide; IBTX, iberiotoxin; Phl, phloretin. *P<0.05 and **P<0.01 when compared to the control. K^+ channel, potassium channel; HBSS, Hank's balanced salt solution.

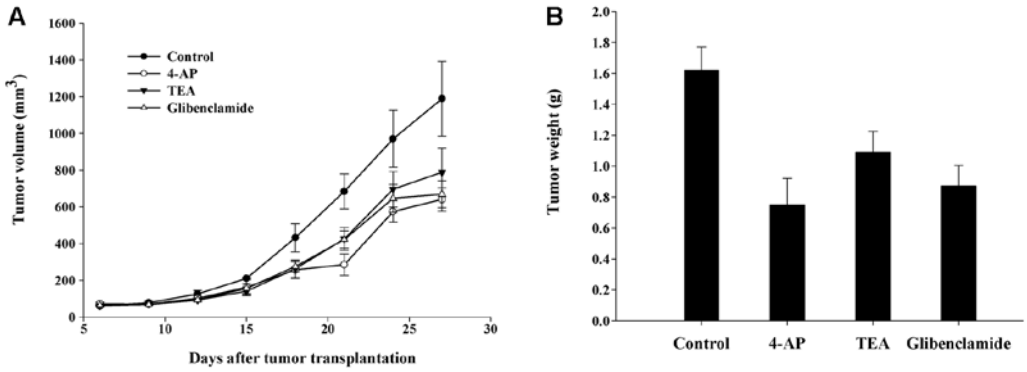

Figure 4. K_V and K_{ATP} channels influence tumor development in nude mice. (A) Suspensions of U87-MG cells with 4-AP (5 mmol/l), TEA (20 mmol/l) or glibenclamide (200 μmol/l) were injected into the flanks of nude mice. Tumor volume was measured every 3 days, and a growth curve was plotted according to tumor volume. (B) Actual tumor weights at the time of sacrifice were measured. No obvious toxic effects were observed in any of the groups during the experiment. *P<0.05 and **P<0.01 when compared to the control. K_V, voltage-gated K^+, K_{ATP}, ATP-sensitive K^+; 4-AP, 4-aminopyridine; TEA, tetraethylammonium.

Therapeutic efficacy of K^+ channel blockers. To provide direct evidence that K_V or K_{ATP} channels are responsible for tumor development, the antitumor activities of K_V or K_{ATP} channel blockers were investigated in nude mice with established human glioma U87-MG xenografts. As shown in Fig. 4A, U87 xenografts grew rapidly in mice, and the tumor size in the control group reached 1189.2±203.3 mm^3 over the duration of the experiment (27 days). Statistically, there were significant differences in both tumor volume and weight between the control and the treated groups. Mice treated with 4-AP at 5 mmol/l and glibenclamide at 200 μmol/l showed inhibition rates of 46.2 and 43.8%, respectively, which were significant (P<0.01) when compared with the control. TEA at 20 mmol/l suppressed tumor growth by 33.7% (P<0.05). The actual tumor weights at the time of sacrifice are shown in Fig. 4B. No obvious toxic effects were observed in any of the groups during the experiment. These results suggest that K_V and K_{ATP} channels play important roles in glioma development *in vivo*.

Effects of K^+ channel blockers on K^+ currents. To determine whether K^+ channel blockers inhibits cell proliferation and tumorigenesis via blockage of whole-cell K^+ currents, we next studied the dose-dependent effects of these three blockers on K_V/K_{ATP} currents in U87-MG cells. Representative recordings of the K_V currents (transient and persistent K^+ currents) were evoked with a step-up depolarization protocol. Briefly, the membrane potential was pre-hyperpolarized from -50 mV to -110 mV for 100 msec, depolarized from -50 to +60 mV (10 mV increment/step, duration 200 msec) and subsequently restored to the original depolarizing potential of -50 mV (Fig. 5A). Depolarizing the voltage from -110 to -50 mV and maintaining the level of -50 mV for 100 msec inactivated transient K^+ currents and a further depolarization voltage step from -50 to +60 mV evoked persistent K^+ currents (Fig. 5B). The transient component (Fig. 5C) was then visualized in isolation using point-by-point subtraction of the persistent component (as shown in Fig. 5B) from the total outward current (as shown in Fig. 5A). As shown in Fig. 5D, 5 mmol/l TEA remarkably inhibited the persistent outward currents and the persistent component was reduced completely after the application of TEA (40 mmol/l). Meanwhile, 4-AP (1 mmol/l) markedly suppressed the transient outward current and 4-AP (10 mmol/l) completely and reversibly blocked the transient components in each cell examined (Fig. 5E).

To examine the effect of glibenclamide on K_{ATP} currents, K_{ATP} currents were activated by using standard whole cell recording with a pipette solution containing only 10 mmol/l ATP (12). The membrane potential was normally held at -40 mV and the currents were evoked by a series of 400 msec depolarizing and hyperpolarizing current steps (-100 mV to +80 mV in 20 mV steps, Fig. 5F). As shown in Fig. 5G, the whole-cell K^+ currents observed with low intracellular ATP were inhibited by extracellular glibenclamide (300 μmol/l). These data suggest that these three K^+ channel blockers have the same working concentration range for inhibiting K_V/K_{ATP} currents, cell proliferation and tumor growth, indicating that the effects of 4-AP, TEA and glibenclamide are indeed mediated by blockage of K^+ channels.

Discussion

As a crucial cellular function, cell growth is strictly controlled by several independent mechanisms. Since the pioneering study in lymphocytes by DeCoursey *et al* (17), accumulating evidence has indicated that K^+ channels are relevant players in the control of this process. Diverse types of K^+ channels have been shown to be overexpressed in tumorous tissues and the roles of K^+ channels in tumor cell growth have been demonstrated (9,15). However, the types of K^+ channels involved in this process vary (18,19). For example, K_{Ca} channels were found to be involved in prostate (5), gastric (20) and colorectal cancer (4). In other tumors, proliferation was supported by K_{ATP} channels or two-pore (2P)-domain channels (21,22). Nevertheless, in the majority of cancer cells, K_V channels were correlated with proliferation (7,16,23). It remains unknown why different types of K^+ channels induce proliferation in different cancer models.

In the present study, we showed that 4-AP, TEA and glibenclamide significantly suppressed cell proliferation *in vitro*. The percentage of U87-MG cells in the G_0/G_1 phase was also significantly enhanced after exposure to 4-AP, TEA or glibenclamide for 48 h. On the other hand, diazoxide and phloretin increased the percentage of cells in the S phase. These findings, together with previous reports (8,24), demonstrate that

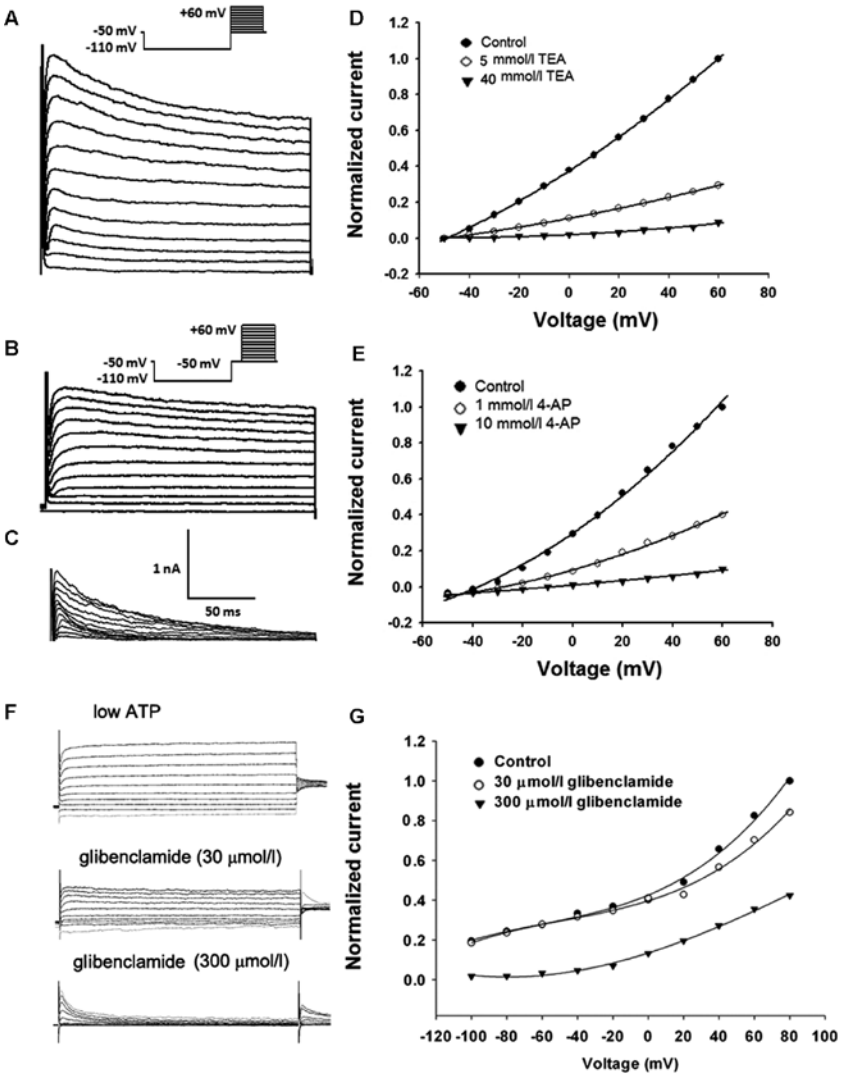

Figure 5. Effects of K^+ channel blockers and openers on K_V/K_{ATP} currents. (A) Representative traces of total K_V currents evoked by a voltage depolarizing step. The voltage protocol is shown at the top. (B) The slow persistent currents recorded from the same cell evoked by the depolarization protocol are shown at the top. (C) The fast transient currents obtained by subtracting the persistent currents from the total K_V currents. (D) TEA suppressed the I-V curve of the slow persistent K^+ currents. (E) 4-AP suppressed the I-V curve of the fast transient K^+ currents. (F) K_{ATP} currents in U87-MG cells in the absence (low ATP) and presence of glibenclamide (300 μmol/l) were evoked by a series of 400 msec depolarizing and hyperpolarizing current steps (-100 mV to +80 mV in 20 mV steps). (G) Glibenclamide suppressed the I-V curve of the K_{ATP} currents. K^+ channel, potassium channel; K_V, voltage-gated K^+, K_{ATP}, ATP-sensitive K^+; TEA, tetraethylammonium; 4-AP, 4-aminopyridine.

K^+ currents are the key determinant of progression through the G_1/S checkpoint of the cell cycle. In addition, we also found that 4-AP and glibenclamide both significantly increased the percentage of apoptotic cells. In the U87-MG xenograft model in nude mice, 4-AP, TEA and glibenclamide markedly suppressed tumor growth *in vivo*. Taken together, these results suggest that K_V and K_{ATP} channels are the critical K^+ channel subtypes for U87-MG glioma growth.

Given the important role of K^+ channels in tumors, it is necessary to clarify their role in proliferation. One hypothesis is that K^+ channels keep the resting membrane potential sufficiently polarized to allow the influx of Ca^{2+} via membrane ion channels (25), implying that blocking K^+ channels will directly modulate Ca^{2+} entry in malignant cells. This may be a partial reason for the induction of tumor growth via the abnormal expression of K^+ channel subtypes since Ca^{2+} acts as an activator involved in many cellular signal transduction pathways, including the cell growth and mitosis pathways (26). For example, early reports indicate that growth of the colorectal adenocarcinoma cell line DLD-1 and the ovarian cancer cell line A2780 was associated with the regulatory effect of K_V channels on Ca^{2+} influx (24,25). Nevertheless, to date, no direct evidence has ever been presented to support the relationship between K^+ channel activity and Ca^{2+} ion entry in glioma cells. Here, we found that 4-AP and glibenclamide nearly abolished the increase in $[Ca^{2+}]_i$ induced by exogenously added Ca^{2+}. Sine the change in $[Ca^{2+}]_i$ may be due to a change in Ca^{2+} influx from the extracellular medium or an alteration in Ca^{2+} release from an internal store, we further examined the potential effects of K^+ channels on the release of Ca^{2+} from intracellular stores, and we found that $[Ca^{2+}]_i$ in U87-MG cells was not affected by K^+ channel blockers or openers (data not shown). Through these experiments, we established a link between K_V and K_{ATP} channel activities and Ca^{2+} influx, indicating that modulation of Ca^{2+} influx may influence the proliferation of U87-MG cells.

The expression of K_{Ca} channels is correlated with glioma malignancy, with higher levels of K_{Ca} protein observed in more malignant glioma biopsy samples (27). However, the relationship between K_{Ca} channels and glioma cells is controversial. Some recent publications have proposed the idea that K_{Ca} channels may have antiproliferative and antitumorigenic properties (28). Other results implicating K_{Ca} channels in the control of glioma cell growth have been collected using specific cell growth conditions, such as an elevated extracellular K^+ concentration (29) or serum deprivation (30). Our data showed that the inhibitory effect of iberiotoxin was only observable at concentrations greatly exceeding those necessary for complete channel blockage (15), and increases in apoptotic cells and cells in the G_0/G_1 phase were not clearly observed, suggesting that K_{Ca} channels may not play a role in the growth of U87-MG glioma cells *in vitro*.

Although all the blockers of K_V and K_{ATP} channels tested in this study suppressed glioma cell proliferation and tumor development, it remains unclear whether their effects occur at the concentrations required to block K^+ channel activities. Electrophysiological results showed that 4-AP, TEA and glibenclamide inhibited cell proliferation and tumor growth in the concentration range required to block K_V/K_{ATP} channel currents, indicating that these K^+ channel blockers suppress glioma cell proliferation by blocking K^+ channel activities and providing further support for the roles of K_V/K_{ATP} channels in mediating cell proliferation and tumor growth.

In summary, the present findings provide strong evidence that K_V and K_{ATP} channel blockers inhibit proliferation and tumorigenesis of U87-MG glioma cells. It is likely that K^+ channel activities modulate Ca^{2+} influx into U87-MG cells and therefore affect the proliferation, cell cycle progression and apoptosis of these cells. Further study is needed to define the precise mechanisms responsible for the antiproliferative effects of pharmacological blockers of K_V and K_{ATP} channels. In any case, it is worthwhile to further explore the possibility of using K_V and K_{ATP} channels as new anti-glioma targets in the coming years.

Acknowledgements

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