ATP-sensitive potassium channels control glioma cells proliferation by regulating ERK activity

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Ion channels are found in a variety of cancer cells and necessary for cell cycle and cell proliferation. The roles of K+ channels in the process are, however, poorly understood. In the present study, we report that adenosine triphosphate (ATP)-sensitive potassium channel activity plays a critical role in the proliferation of glioma cells. The expression of KATP channels in glioma tissues was greatly increased than that in normal tissues. Treatment of glioma cells with tolbutamide, KATP channels inhibitor, suppressed the proliferation of glioma cells and blocked glioma cell cycle in G0/G1 phase. Similarly, downregulation of KATP channels by small interfering RNA (siRNA) inhibited glioma cell proliferation. On the other hand, KATP channels agonist diazoxide and overexpression of KATP channels promoted the proliferation of glioma cells. Moreover, inhibiting KATP channels slowed the formation of tumor in nude mice generated by injection of glioma cells. Whereas activating KATP channels promoted development of tumor in vivo. The effect of KATP channels activity on glioma cells proliferation is mediated by extracellular signal-regulated kinase (ERK) activation. We found that activating KATP channel triggered ERK activation and inhibiting KATP channel depressed ERK activation. U-0126, the mitogen-activated protein kinase kinase (MAPK kinase) inhibitors blocked ERK activation and cell proliferation induced by diazoxide. Furthermore, constitutively activated MEK plasmids transfection reversed the inhibitory effects of tolbutamide on glioma proliferation, lending further support for a role of ERK in mediating this process. Our results suggest that KATP channels control glioma cell proliferation via regulating ERK pathway. We concluded that KATP channels are important in pathological cell proliferation and open a promising pathway for novel targeted therapies.

Introduction

Human malignant gliomas are aggressive tumors that are most common malignancy in brain tumor. Many patients with gliomas respond poorly to traditional radiation and chemotherapy, and recurrence rate of human malignant gliomas is 100% (1). Therefore, to understand the mechanism by which glioma develops is necessary for an efficient and specific inhibition of the progression of this form of cancer.

It is commonly accepted that cells require K+ channels to proliferate (2). In any case, it is intuitively acceptable that K+ channels, as key players in controlling membrane potential, are critical in proliferation processes (2,3). In addition, K+ channels also control cell volume to modulate cell proliferation (4,5).

Abbreviations: ATP; adenosine triphosphate; ERK; extracellular signal-regulated kinase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; MAPK, mitogen-activated protein kinase kinase; MEK, mitogen-activated kinase kinase; MTT, Methyl-thiazolyl-tetrazolium; PBS, phosphate-buffered saline; siRNA, small interfering RNA; SUR, sulfonylurea receptor.

1These authors contributed equally to this work.

Adenosine triphosphate (ATP)-sensitive potassium channel (KATP channel) was first discovered by Akinori Noma in cardiac myocytes (6) and was subsequently found to be expressed in many other cell types, e.g. in cardiac and smooth muscle, pancreatic beta cells and various brain regions. KATP channels are octameric proteins consisting of two different types of subunits: members of the Kir6 inwardly rectifying potassium channel family and sulfonylurea receptor (SUR) subunits, which are members of the ATP-binding cassette transporter superfamily. In functional channels, four pore-forming Kir6 subunits are joined together with four regulatory SUR subunits (7). In central nervous system, KATP channels are composed of Kir6.2 and SUR1 subunits (7). Evidence showed that in both primary rat hepatocytes and human liver cell lines, KATP channels regulate mitogenically induced proliferation (8), but the effects of KATP channels on glioma cells are poorly understood.

Mitogen-activated protein kinases (MAPKs) are a family of serine/threonine protein kinases that regulate a wide array of cellular processes. Three major classes of MAPKs have been identified: extracellular signal-regulated kinases (ERKs), Jun N-terminal kinases and p38 MAPKs. Jun N-terminal kinase and p38 MAPKs participate in the cellular response to environmental stress and are cumulatively known as stress-activated protein kinases (9–11). In contrast, ERK activation is associated with cell survival and cell proliferation in response to growth factors such as platelet-derived growth factor and epidermal growth factor (11), which makes ERK the principal candidate in the studies of cell proliferation. The classic pathway of ERK activation is characterized by sequential phosphorylation of upstream kinases, namely, Raf-1 and mitogen-activated protein kinase kinase (MAPK Kinase) (9–13).

The aim of this study is to investigate the regulation mechanisms by which KATP channels control glioma cells proliferation. Our results demonstrate that KATP channels augment glioma cell proliferation via activating ERK and suggest that KATP channels play a critical role in the development of human malignant gliomas.

Materials and methods

Materials

Antibodies against Kir6.2 (sc-20809), SUR1 (sc-25683) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (sc-47724) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA); β-actin (BM0627) were purchased from BOSTER (Wuhan, China); anti-phosphorylated ERK (#9101) and anti-total ERK (#9102) were purchased from Cell Signaling (Danvers, MA); diazoxide, tolbutamide and U-0126 were from Sigma (St Louis, MO); final concentration of diazoxide and tolbutamide was 100 μM; propidium iodide was from Molecular Probes (Eugene, OR); RNase A was from Roche Diagnostics (Mannheim, Germany). All other reagents were from Sigma.

Cell culture

Stock cultures of human glioma cell lines U87 were obtained from American Type Culture Collection (Rockville, MD). Eagle’s minimum essential medium supplemented with 10% fetal bovine serum (GibcoBRL, Gaithersburg, MD) and glutamine (2 mM) was used to culture U87 cells. The human glioma cell lines U251 were provided by the Committee on Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). U251 cell line was grown in RPMI medium 1640 (Life Technologies, Rockville, MD) containing glutamute (5 mM) and 10% fetal bovine serum. The two cell lines were cultured in the same conditions and maintained at 37°C in a humidified incubator with 5% CO2. For proliferation assays, cells were counted and seeded into 96-well plates (1 × 104 cells per well). Drug or vehicle was added to each well, medium was added for a total volume of 100 μl.

Biological samples

Twenty glioma biopsies were obtained with the patients’ consent from the patients registered at the Zhijiang Hospital of Southern Medical University.
following the local ethical consideration. The biopsies from patients who had not received any anticancer therapy (chemotherapy, radiotherapy, hormone therapy or surgery) were studied. The patients with stages I–IV accounted for 20, 20, 30 and 30%, respectively. Ten normal astrocyte biopsies were obtained with the patients’ consent by surgery diagnosed as benign tissue from patients registered at the hospitals. The tissue fragment ~0.5 cm² was obtained for total protein extraction.

**Cell transfection**

The sequences of siRNA-1 against Kir6.2: 5’-CCAAGAAAGGCACACUG-CAATT-3’ (sense) and 5’-UUCCUGAUUCGUUUUUCGTTT-3’ (antisense); the sequences of siRNA-2 against Kir6.2: 5’-CAUUGAGUACGUGGAG-GATT-3’ (sense) and 5’-UCCUCUCAGCUCAAAUGGTT-3’ (antisense); the sequences of non-silencing RNA are 5’-UUCUCUGAACCAGUUCAC-GUTT-3’ (sense) and 5’-ACGUGACACGUUGAGAATT-3’ (antisense). All the siRNAs were synthesized by GenePharma Co. Ltd (Shanghai, China). Kir6.2 and SUR1 plasmids are gifts from Prof. Susumu Seino (Kobe University, Japan). Constitutively activated MEK plasmids (MEK CA) are gifts from Prof. Tian-Ming Gao (Southern Medical University, China). The U87 cells at ~1 × 10⁵ cells per dish were transfected with siRNA or plasmids using Lipofectamine2000 (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. The efficiency of siRNA to knockdown the target protein was determined by western blot analysis and the efficacy of siRNA transfection was evaluated by fluorescence microscope, using fluorescein isothiocyanate-siRNA as a control.

**Immunocytochemistry**

Cells were cultured on coverslips (Macalaster Bicknell, New Haven, CT) for 1–3 days. Cells were subsequently washed twice in phosphate-buffered saline (PBS). After washing, cells were fixed in 4% paraformaldehyde for 30 min at room temperature then washed twice in PBS and blocked and permeabilized in PBS containing 0.3% triton and 5% normal goat serum for 30 min. Cells were washed in PBS and incubated overnight with primary antibodies at 4°C. Cells were washed four times in PBS and labeled with secondary antibodies for 1 h in the dark. After labeling, cells were washed with PBS and incubated with Hoechst 33342 (10 µM) for 15 min. Cells were washed twice more and mounted onto slides with Gel/Mount (Biomedia Corp., Forest City, CA). Images were acquired with Olympus 200M (Japan) microscope system. Hoechst 33342-stained cells were illuminated with an argon laser (353–365 nm) and fluorescence was detected at 480 nm.

**Cell cycle analysis**

Cell cycle analysis was conducted using the standard method as reported previously by Wang et al. (18). Briefly, ~4 × 10⁵ cells after trypsinization were suspended in PBS and centrifuged for 2 min at 1500g. The cells were resuspended and fixed in 1 ml cold 70% ethanol for 12 h. The cell pellets were mixed 1:1 with 2× sample buffer (20% glycerol, 4% sodium dodecyl sulfate, 10% β-mercaptoethanol, 0.05% bromophenol blue and 1.25 M Tris–HCl, pH 6.8; all from Sigma). Equal amount of proteins were loaded onto a 12% sodium dodecyl sulfate–polyacrylamide gel. Cell proteins were transferred to polyvinylidene difluoride membranes. The polyvinylidene difluoride membranes were then blocked with 5% milk in Tris-buffered saline with 0.1% Tween 20 and then incubated with the primary antibody (antibodies to phospho-ERK, ERK, Kir6.2, SUR, GAPDH or β-actin) overnight at 4°C. The blots were washed and incubated for 1 h with horseradish peroxidase-conjugated anti-IgG antibody (Jackson Laboratories, West Grove, PA). Immunoreactive bands were developed using a chemiluminescent substrate (enhanced chemiluminescence Plus, Amersham Biosciences, Piscataway, NJ).

**Mice tumor model**

All nude mice were kept under specific pathogen-free conditions. Five- to seven-week-old nude mice were housed three per cage, fed with sterile food and water. Animals were housed according to the standards set by the Southern Medical University Animal Care and Use Committee. The nude mice were

**MTT assay**

Methyl-thiazolyl-tetrazolium (MTT) assay was performed according to the method described previously (14). In brief, cells were plated in a 96-well plate. MTT reagent was added to a final concentration of 100 µM and incubated until purple precipitate was visible (~2 to 4 h). Absorbance was recorded (570 nm).

**Western analysis**

Western analysis for the presence of specific proteins or for phosphorylated forms of proteins was performed on whole-cell sonicates and lysates from U87 cells (15–17). Protein (30–100 µg) was mixed 1:1 with 2× sample buffer (20% glycerol, 4% sodium dodecyl sulfate, 10% β-mercaptoethanol, 0.05% bromophenol blue and 1.25 M Tris–HCl, pH 6.8; all from Sigma). Equal amount of proteins were loaded onto a 12% sodium dodecyl sulfate–polyacrylamide gel. Cell proteins were transferred to polyvinylidene difluoride membranes. The polyvinylidene difluoride membranes were then blocked with 5% milk in Tris-buffered saline with 0.1% Tween 20 and then incubated with the primary antibody (antibodies to phospho-ERK, ERK, Kir6.2, SUR, GAPDH or β-actin) overnight at 4°C. The blots were washed and incubated for 1 h with horseradish peroxidase-conjugated anti- IgG antibody (Jackson Laboratories, West Grove, PA). Immunoreactive bands were developed using a chemiluminescent substrate (enhanced chemiluminescence Plus, Amersham Biosciences, Piscataway, NJ).

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randomly divided into control and treated groups. Treated groups were injected with 8 × 10⁶ U87 or U251 cells suspension with tolbutamide (100 µM) or diazoxide (100 µM) into the right flank, and the control group were injected 8 × 10⁶ U87 or U251 cells suspension with dimethyl sulfoxide (100 µM) (18–20). The mice were killed and the tumors were taken out 18 days after injection. The tumor size was measured in mass with a scale.

**Statistical analysis**

Multiple comparisons were assessed by one-way analysis of variance; otherwise, Student’s unpaired t-test was used for statistical analysis. Results were considered significant difference at *P < 0.05; **P < 0.01.

**Results**

**The expression of KATP channels in glioma cells**

To explore the role of KATP channels in glioma cell development, we first examined the expression of KATP channels in both human glioma tissue and human glioma cell lines. As shown in Figure 1a and b, the expression of KATP channel was detectable in U87 and U251 cells. The protein levels for KATP channels were further tested in normal human astrocyte, human glioma tissue (grade I and grade IV) and human glioma cell lines (U87 and U251) by western blot (Figure 1c), and the protein levels in the tumor cells were greatly increased as compared with those in non-tumor cells. The specificity of the antibody was confirmed by blocking experiments with the antigenic peptides (data not shown). Together, these results indicated the expression of KATP channels in glioma cells and provide us initial evidence that KATP channels may play a role in glioma development.

**KATP channels promote glioma cell growth**

To investigate whether KATP channels are possibly involved in glioma cell proliferation, we examined the effects of KATP channels on U87 and
U251 cell growth. As shown in Figure 2a and b, tolbutamide (100 µM), an inhibitor of KATP channels (14,21,22), significantly decreased U87 and U251 cell numbers, as manifested by the right shift of the growth curve. However, diazoxide (100 µM), opener of KATP channels, promoted U87 and U251 cell proliferation (Figure 2a and b). These results suggest that KATP channels promote U87 cell growth.

To provide direct evidence that KATP channels are indeed involved in glioma cell proliferation, we specifically knocked down Kir6.2 subunits (α-subunit of KATP channel) using two siRNAs (Figure 2c).

Western blot analysis revealed that the Kir6.2 level in the cells transfected with the siRNA was 12.82–40.94% of that found in the cells transfected with non-silencing RNA (Figure 2d). Downregulation of KATP channel greatly reduced the cell number for U87 cell lines (75.48–81.44% of control group, Figure 2e). Further, we cotransfected Kir6.2 (α-subunit of KATP channel) and SUR1 (β-subunit of KATP channel) plasmids into U87 cells. Western blot analysis revealed...
that the Kir6.2 level in the cells transfected with the Kir6.2 and SUR1 plasmids was 1.5 times of that found in the cells transfected with green fluorescent protein (Figure 2f). Overexpression of K<sub>K<sub>A</sub>T</sub> channel significantly increased the cell number for U87 cell lines (118.33% of control group, Figure 2g). These results confirmed that K<sub>K<sub>A</sub>T</sub> channels are responsible for glioma cell growth in culture.

Blocking K<sub>K<sub>A</sub>T</sub> channels inhibit glioma cell growth by arresting U87 and U251 cell at G<sub>0</sub>/G<sub>1</sub> phase

To elucidate further the mechanism by which activity of K<sub>K<sub>A</sub>T</sub> channels influences proliferation of glioma cells, we examined the effect of tolbutamide and diazoxide on cell-cycle progression of U87 and U251 cells. As shown in Figure 3a and b, in 100 μM tolbutamide, the percentage of U87 cells in the G<sub>0</sub>/G<sub>1</sub> phase was significantly enhanced (61.92%, Figure 3a right), whereas that in the S phase was markedly reduced (1.96%, Figure 3a right). However, activating K<sub>K<sub>A</sub>T</sub> channels by diazoxide reduced U87 cells at the G<sub>0</sub>/G<sub>1</sub> phase (51.14%, Figure 3a middle) and increased cells at S phase (13.62%, Figure 3a middle). Similarly, inhibiting K<sub>K<sub>A</sub>T</sub> channels by tolbutamide arrested U251 cells at the G<sub>0</sub>/G<sub>1</sub> phase, whereas activating K<sub>K<sub>A</sub>T</sub> channels by diazoxide was characterized by a reduction of the fraction of the cells in the G<sub>0</sub>/G<sub>1</sub> phase and an increase in the percentage of cells in the S phase than those in control (Figure 3c and d). Together, these results suggest that K<sub>K<sub>A</sub>T</sub> channels are important for the proliferation of glioma cells.

K<sub>K<sub>A</sub>T</sub> channels influence the development of tumor in nude mice

To provide direct evidence that K<sub>K<sub>A</sub>T</sub> channels are responsible for tumor development, we injected subcutaneously 8 × 10<sup>6</sup> U87 or U251 cells suspension to the right flank of 5- to 7-week-old nude mice with K<sub>K<sub>A</sub>T</sub> channel inhibitors or activators. The control group was injected with U87 or U251 cells suspension with dimethyl sulfoxide. At day 18 after injection, all nude mice were killed and the tumors were taken out and blotted out the water. At the macroscopic observation, the difference at the size and volume of the tumors between three groups was marked (Figure 4a and c). In U87 cell-injected group, injection of tolbutamide slowed the development of tumor but diazoxide promoted the development of tumor compared with the controls in vivo. The tumor mass of the tolbutamide group was 0.30 ± 0.09 g and diazoxide group was 0.93 ± 0.21 g, whereas that

Fig. 5. K<sub>K<sub>A</sub>T</sub> channel regulated ERK activity. (a) K<sub>K<sub>A</sub>T</sub> channel agonist activated ERK; however, K<sub>K<sub>A</sub>T</sub> channel antagonist inhibited ERK. U87 cells were exposed to 100 μM diazoxide or 100 μM tolbutamide. Blots were evaluated for phosphorylated (active) and total ERK. Con for control, Dia for diazoxide treatment and Tol for tolbutamide treatment. (b) Downregulation of Kir6.2 inhibited ERK activity, NS means non-silencing RNA; 1 and 2 mean siRNA-1 and siRNA-2 against Kir6.2. (c) Overexpression of K<sub>K<sub>A</sub>T</sub> channel augmented ERK activity. Vector means transfected vector, K<sub>K<sub>A</sub>T</sub> means cotransfected Kir6.2 and SUR1 plasmids. Data are mean ± SD of three independent experiments in triplicates (n = 3, N = 3). *P < 0.05; **P < 0.01 versus control.
of control group was 0.59 ± 0.13 g (Figure 4b). Furthermore, injection of U251 cell also confirmed these results. The tumor mass measured in the tolbutamide-treated group was 0.32 ± 0.05 g and the diazoxide-treated group was 1.01 ± 0.22 g, whereas that measured in control group was 0.62 ± 0.16 g (Figure 4d). These results suggest that K<sub>ATP</sub> channels play an important role in the development of glioma in vivo.

**K<sub>ATP</sub> channel promote cell proliferation by activating ERK**

ERK is an important member of MAPK family and its activation is associated with cell survival and cell proliferation. To elucidate whether ERK is involved in the mechanism by which K<sub>ATP</sub> channels activity influence proliferation of glioma cells, we examined the effect of diazoxide and tolbutamide on ERK activity of U87 cells. To demonstrate that K<sub>ATP</sub> channels can modulate ERK activity, cells were stimulated with 100 μM diazoxide or 100 μM tolbutamide. We found that diazoxide increased ERK activity, whereas tolbutamide decreased ERK activity (Figure 5a). Similarly, downregulation of K<sub>ATP</sub> channel greatly attenuated ERK activity (Figure 5b) and overexpression of K<sub>ATP</sub> channel elicited ERK activity (Figure 5c). Western blot analysis of total ERK demonstrated equal loading of the proteins on the blots and no increase in total ERK. The effects of K<sub>ATP</sub> channels on ERK activity seems not to be mediated by transient regulation of cell membrane potential or intracellular calcium concentration because we did not detect significant difference in them between control, diazoxide and tolbutamide treatment (supplementary data and Figure S1 are available at Carcinogenesis Online). And diazoxide and tolbutamide did not influence autocrine or paracrine factor-mediated cell proliferation (supplementary data and Figure S2a are available at Carcinogenesis Online) and ERK activation (supplementary data and Figure S2b are available at Carcinogenesis Online).

**Inhibition of ERK activity blocks K<sub>ATP</sub>-channel-mediated proliferation in U87 cell**

To evaluate the contribution of ERK activity to U87 cell proliferation regulated by K<sub>ATP</sub> channel, we performed the following experiments. Pretreatment of cells for 30 min with 5 μM U-0126, a MAPK kinase inhibitor (23), before treatment with diazoxide or tolbutamide, inhibited ERK activation (supplementary data and Figure S3a are available at Carcinogenesis Online) and abolished the effect of K<sub>ATP</sub> channel on ERK activity (data not shown) and cell proliferation (Figure 6a) without changing cell membrane potential (supplementary data and Figure S3b are available at Carcinogenesis Online). Also, U-0126 could reverse U87 cell proliferation induced by K<sub>ATP</sub> channel overexpression (Figure 6b left). Downregulation of K<sub>ATP</sub> channels by siRNA could not further inhibit cell proliferation when cells were treated with U-0126 (Figure 6b right), which means ERK is downstream signal of K<sub>ATP</sub> channels in cell proliferation. Further, we transfected constitutively activated MEK plasmids (MEK CA) to U87 cell, and 24 h later, we added tolbutamide to the culture medium for 72 h. The results showed that continuous activation of ERK reversed the inhibitory effects of tolbutamide on proliferation in U87 cell (110.99% of control group, Figure 6c). Together, these results suggested that activation of K<sub>ATP</sub> channels augmented glioma cell proliferation via activating ERK activity, whereas inhibition of K<sub>ATP</sub> channels attenuated glioma cell proliferation via suppressing ERK activity.

**Discussion**

In the present investigation, we have shown that K<sub>ATP</sub> channels play an important role in the development of glioma and proliferation of U87 and U251 cells. Because of their roles in cell cycle and their increased expression in glioma tissue, K<sub>ATP</sub> channels may thus be a potential target for glioma therapy.

K<sup>+</sup> channels are known to regulate proliferation in many cell types (24), but the type of K<sup>+</sup> channel involved in this varies, as indicated by the identification of small conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels in fibroblasts (25), intermediate conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels in human endometrial cancer (18), voltage-activated and calcium-gated K<sup>+</sup> channels in lymphocytes (26) and K<sub>ATP</sub> channels in bladder cells (27). It has been shown that the expression of K<sub>ATP</sub> channels is upregulated in human uterine leiomyoma cells for estrogen-mediated cell proliferation (28). Consistent with these studies, the current work provides new evidence that expression of K<sub>ATP</sub> channel was also increased in human glioma. It is believed that K<sup>+</sup> channels are important in regulating cell volume,
which may have secondary effects upon mitogenesis as well (29). Also, membrane depolarization associated with inhibition of K\(^+\) channels could modulate calcium channels with perturbation of signal transduction mechanisms (30–32). However, the effect of K\(_{\text{ATP}}\) channel activity upon hepatocyte proliferation was not simply due to indirect modulation of intracellular calcium (8). Similarly, we did not detect transient alteration of membrane potential and intracellular calcium concentration following diazoxide or tolbutamide treatment (supplementary data and Figure S1 are available at Carcinogenesis Online).

Furthermore, mitochondrial K\(_{\text{ATP}}\) channel openers activate the ERK kinase by an oxidant-dependent mechanism (21). Therefore, one plausible explanation for the fact that K\(_{\text{ATP}}\) channels were responsible for the proliferation of glioma cells is that K\(_{\text{ATP}}\) channels opening trigger ERK activation to affect the proliferation of glioma cells. Our findings that activating K\(_{\text{ATP}}\) channel elicited ERK activation and cell proliferation but inhibiting K\(_{\text{ATP}}\) channel depressed ERK activation and cell proliferation in U87 cells are consistent with this explanation.

The activation of ERK is a critical signal for cell survival, proliferation and differentiation. Receptors for peptide growth factors, such as platelet-derived growth factor (33), epidermal growth factor (34) and insulin (35), are protein tyrosine kinases (36) that undergo sequential phosphorylation and activation of the Ras–Raf–MEK–ERK (34) and insulin (35), are protein tyrosine kinases (36) that undergo such activation. This explanation.

ERK activation and cell proliferation in U87 cells are consistent with this explanation. The activation of ERK is a critical signal for cell survival, proliferation and differentiation. Receptors for peptide growth factors, such as platelet-derived growth factor (33), epidermal growth factor (34) and insulin (35), are protein tyrosine kinases (36) that undergo sequential phosphorylation and activation of the Ras–Raf–MEK–ERK pathway (37–41). However, how K\(_{\text{ATP}}\) channels regulate ERK pathway poorly understood in our experiment, the regulation of ERK activity by K\(_{\text{ATP}}\) channels seemed not to be dependent on transient alteration of intracellular calcium concentration or autocrine/paracrine factors (supplementary data and Figure S2 are available at Carcinogenesis Online).

Recent studies supported the notion that opening of mitochondrial K\(_{\text{ATP}}\) channels resulted in free radical generation that is critical in the protection pathways of ischemia. Free radicals could then activate protein kinase C and the MAPK cascade in parallel pathways. But the precise mechanisms of all these processes need further research (42).

In summary, we have shown that K\(_{\text{ATP}}\) channels are proliferative molecules in glioma cells. In any case, it is predictable that K\(_{\text{ATP}}\) channel will become a relevant anticancer target in the coming years.

Supplementary materials

Supplementary data and figures S1–S3 can be found at http://carcin.oxfordjournals.org/

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Conflict of Interest Statement: None declared.

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