Activation of β-adrenergic receptor promotes cellular proliferation in human glioblastoma

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Abstract. Glioblastoma multiforme is the most common and aggressive form of primary malignant brain tumor. Previous evidence demonstrates that β-adrenergic receptors (β-ARs) are closely associated with the occurrence and development of brain tumors. However, the functional role of β-ARs in human glioblastoma and the underlying mechanisms are not fully understood. In the present study, by using the MTT assay, western blotting, and the reverse transcription polymerase chain reaction, it was revealed that isoproterenol (ISO), an agonist of β-ARs, promoted the proliferation of U251 cells but not U87-MG cells, and that this effect was blocked by the β-ARs antagonist propranolol. It was also demonstrated that ISO transiently induced extracellular signal-related kinase 1/2 (ERK1/2) phosphorylation, and that blocking the mitogen-activated protein kinase pathway by U0126 inhibited ERK1/2 phosphorylation and suppressed U251 cell proliferation. In addition, β-ARs activation increased the expression of matrix metalloproteinase (MMP) family members *MMP-2* and *MMP-9* mRNA through ERK1/2 activation. In conclusion, these data suggest that β-ARs induce ERK1/2 phosphorylation, which may in turn increase MMPs expression to promote U251 cell proliferation. These results provide additional insight into the specific roles of β-ARs in glioblastoma.

Introduction

Glioblastoma multiforme (GBM), or grade IV astrocytoma as classified by the World Health Organization (1), is the most common and malignant primary brain tumor in humans, with an incidence rate of 3.19 cases per 100,000 person/year in the United States 2005-2009 (1). The important characteristics of GBM are the high cellular proliferation rate, genetic instability, diffuse infiltration and high angiogenesis, conferring high levels of aggression and drug resistance in GBM (2). In spite of previous significant improvements in treatment, including surgery, radiotherapy and chemotherapy, the overall median survival rate remains only 12-16 months at present (3). This poor prognosis suggests that therapeutic resistance is a significant problem of GBM. Thus, it is urgent to identify novel candidate factors involved in glioma proliferation and angiogenesis that may assist to develop effective targeted therapies for GBM.

A growing number of studies have demonstrated that exposure to stress may promote tumor progression in many types of cancers (4-6) and the adrenergic system has been widely recognized to serve a significant role in stress signaling (7). Exposure to stressful events induces the activation of the hypothalamic-pituitary-adrenal (HPA) axis, which in turn results in the release of glucocorticoids and catecholamines, including norepinephrine (NE) and epinephrine (E), from the adrenal gland and from the brain and sympathetic nerve terminals (8). Concurrently, the secretion of dopamine, which assists to control the reward and pleasure centers of the brain, is reduced. This alteration in homeostasis leads to a microenvironment that is beneficial to tumor growth and progression in experimental models of disease (5). The functions of NE and E are primarily mediated by the activation of adrenergic receptors (ARs) including α-ARs and β-ARs (9). There are 3 subtypes of β-ARs, specifically β_1-AR, β_2-AR and β_3-AR, which have been identified to date (10). β_1-ARs and β_2-ARs are widely expressed in the majority of mammalian cell types, while β_3-ARs are almost exclusively expressed in adipocytes that have seldom been studied (11).

β-ARs are members of the superfamily of G protein-coupled receptors (GPCR), which activate the Gαs protein to initiate multiple signaling cascades and therefore lead to numerous pathological conditions such as cardiac, psychiatric, immunological and endocrine disorders (12). In previous decades, numerous studies have demonstrated that β-ARs may also regulate different processes of cancer initiation and progression, through activating the classical cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway and

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mitogen-activated protein kinases (MAPK) pathway (13,14). For example, in pancreatic ductal adenocarcinomas cells, it has been suggested that β-AR agonists promote cell growth by activating signaling via adenylyl cyclase (AC) and its downstream effectors cAMP, PKA and nuclear transcription factor cAMP-responsive element-binding protein (15). In addition, the extracellular signal-related kinase 1/2 (ERK1/2) signaling pathway, activated by transactivating epidermal growth factor receptor (EGFR), is also associated with this process (16,17). Previously, the potential involvement of β-ARs in the modulation of astrocytoma cancer cell proliferation has been suggested (18). However, the detailed underlying mechanisms by which these events occur remain unknown, but are becoming characterized.

The present study was designed to investigate the function and mechanism of β-ARs in human glioblastoma U251 cells. The results demonstrated that the β_1-AR and β_2-AR subtypes were expressed in U251 cells but not U87-MG cells. In addition, the activation of the β-ARs by isoproterenol (ISO) significantly enhanced the rate of cell proliferation in U251 cells via activation of the ERK1/2 pathway and matrix metalloproteinase (MMP)-2 and MMP-9 mRNA expression. These results may provide additional insight into the specific roles of β-ARs in glioblastoma.

Materials and methods

Antibodies and reagents. Primary antibodies including phospho-ERK1/2 (Thr202/Tyr204) (cat no. 9101; 1:2,000), ERK1/2 (cat no. 9102; 1:2,000) and β-actin (cat no. 4970; 1:4,000), the secondary antibody horseradish peroxidase (HRP)-linked goat anti-rabbit IgG antibody (cat no. 7074; 1:20,000) and U0126 (a specific ERK1/2 inhibitor) were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). ISO and propranolol (PRO) were purchased from Tocris Bioscience (Bristol, UK). The culture medium and other solutions used for cell culture were purchased from Invitrogen, Thermo Fisher Scientific, Inc. (Waltham, MA, USA). MTT was purchased from Carl Roth GmbH & Co., KG (Karlsruhe, Germany).

Cell culture. Human glioblastoma cell lines U87-MG and U251 were grown in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/l glucose supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), 2 mM glutamine, 50 μg/ml streptomycin and 50 IU/ml penicillin. Cultures were maintained at 37˚C in a humidified atmosphere containing 5% CO_2.

Reverse transcription polymerase chain reaction (RT-PCR). The total cellular RNA from the U251 and U87-MG cells was extracted using TRIzol reagent according to the manufacturer's protocol (Thermo Fisher Scientific, Inc.). The RNA concentration was measured using NanoDrop 2000 (Thermo Fisher Scientific, Inc.). Then the RNA was reverse transcribed into cDNA using SuperScript III reverse transcriptase according to the manufacturer's protocol (Thermo Fisher Scientific, Inc.). PCR amplification was performed using 5 μl cDNA, 12.5 μl 2X Taq PCR Master Mix (Promega Corporation, Madison, WI, USA), 0.5 μl 10 μM forward primer, 0.5 μl 10 μM reverse primer and 6.5 μl ddH_2O. The specific primers used are summarized in Table I. The thermocycling protocol was as follows: Amplification step, 94˚C for 5 min; 30 cycles of denaturation for 1 min at 95˚C; 1 min of annealing at 55˚C; elongation at 72˚C for 1 min; and extension at 72˚C for 1 min. The PCR products were electrophoresed on a 1.5% agarose gel and the bands in the gels that were stained with ethidium bromide were visualized under ultraviolet light transilluminators (ChemiDoc XRS+ system; Bio-Rad Laboratories, Inc., Hercules, CA, USA). The gene β-actin was used as a control.

RT-quantitative PCR (qPCR). The total RNA and cDNA were obtained as aforementioned. The PCR primers used for the analysis are summarized in Table I. The 20 μl PCR system was composed of 2 μl cDNA, 10 μl SYBR® Premix Ex Taq II, 0.4 μl ROX Reference Dye II (Takara Bio, Inc., Otsu, Japan), 0.8 μl 10 μM forward primer, 0.8 μl 10 μM reverse primer and 6 μl double-distilled H_2O. The RT-qPCR amplification was performed using the ViiA7 system (Applied Biosystems; Thermo Fisher Scientific, Inc.) under the following thermocycler conditions: 95˚C for 30 sec; 40 cycles of denaturation for 5 sec at 95˚C; 34 sec of annealing at 60˚C; elongation at 95˚C for 15 sec; and extension at 60˚C for 1 min. The quantification method for qPCR data was the $2^{-\Delta\Delta Cq}$ method (19). At least three independent experiments were conducted and samples were assessed in triplicate for each experiment. The same PCR system as above was used without template cDNA was used as a negative control.

Western blot analysis. Cells were cultured in serum-free DMEM overnight at 37˚C prior to drug treatment. To determine the time course effect of ISO on ERK1/2 activation, cells were treated with ISO (10 μM) for 1, 2, 5, 10, 20 and 30 min at 37˚C. To determine the effects of propranolol (PRO) and U0126 on ISO-mediated ERK1/2 activation, cells were pretreated with PRO (10 μM) or U0126 (20 μM) for 30 min and then with ISO (10 μM) for 5 min at 37˚C. Following treatment, cells were washed with ice-cold PBS twice, and lysed in ice-cold lysis buffer. The lysate was sonicated and centrifuged at 12,000 x g at 4˚C for 5 min. The protein concentration of extracts was determined by using the Bradford reagent from Bio-Rad Laboratories, Inc.. Equal amounts of protein (20 μg/lane) were loaded and separated by 12% SDS-PAGE. Proteins were transferred to nitrocellulose membranes (EMD Millipore, Billerica, MA, USA), and blocked in 5% non-fat dry milk for 1 h at room temperature. Subsequently, the membrane was incubated with different primary antibodies including phosphor-ERK1/2 (Thr202/Tyr204), ERK1/2 and β-actin overnight at 4˚C and then incubated with HRP-conjugated goat anti-rabbit IgG secondary antibody (1:20,000) for 2 h at room temperature. Immunoreactive bands were visualized by enhanced chemiluminescence (Pierce; Thermo Fisher Scientific, Inc.) and semi-quantified using ImageJ software (version 1.47t; National Institutes of Health, Bethesda, MD, USA).

MTT assay. Cellular viability was measured using the MTT assay as described previously (20). Briefly, cells were seeded in flat-bottomed 24-well plates ($5x10^4$ cells/well) and grown for 24 h in DMEM supplemented with 10% FBS. Following

Table I. Primers used for reverse transcription PCR and quantitative PCR.

Gene	Forward	Reverse	Size, bp
β1-AR	5'-GGGAGAAGCATTAGGAGGG-3'	5'-CAAGGAAAGCAAGGTGGG-3'	270
β2-AR	5'-CAGCAAAGGGACGAGGTG-3'	5'-AAGTAATGGCAAAGTAGCG-3'	334
β-actin	5'-ATCGTGCGTGACATTAAGGAGAAG-3'	5'-AGGAAGGAAGGCTGGAAGAGT-3'	179
MMP-2	5'-CCGTCGCCCATCATCAAGTTC-3'	5'-GCAGCCATAGAAGGTGTTCAGG-3'	90
MMP-9	5'-TGGTCCTGGTGCTCCTGGTG-3'	5'-GCTGCCTGTCGGTGAGATTGG-3'	111

PCR, polymerase chain reaction; β-AR, β-adrenergic receptors; MMP, matrix metalloproteinase.

24 h, cells were starved overnight at 37˚C and incubated with different drugs. To determine the dose course effect of ISO on the proliferation of U251 and U87-MG cells, cells were treated with 0.1, 1, 5, 10, 30 or 50 μM ISO for 24 h at 37˚C. To determine the time course effect of ISO on the proliferation of U251 cells, cells were treated with ISO (10 μM) for 24, 48, 72 and 96 h at 37˚C. To determine the effects of PRO and U0126 on ISO-mediated proliferation, cells were pretreated with PRO (10 μM) or U0126 (20 μM) for 30 min and then with ISO (10 μM) for 48 h at 37˚C. Following drug treatment, 20 μl MTT (5 mg/ml) was added to each well, and the cells were allowed to grow in complete media at 37˚C for 3 h. The supernatant was removed, then 500 μl dimethyl sulfoxide was added to each well and mixed for 10 min to dissolve the crystal. Subsequently, the absorbance was determined using a microplate spectrophotometer assay reader at 570 nm.

Statistical analysis. Data are indicated as the mean ± standard error of the mean from at least three independent experiments. Statistical analysis was performed using the unpaired two-tailed Student's t-test (comparison of two groups) or one-way analysis of variance followed by the Tukey's test (comparison of >2 groups). $P<0.05$ was considered to indicate a statistically significant difference. All statistical analyses were conducted using Prism version 6.0 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Expression of β-ARs in human glioblastoma cell lines. To detect whether β-ARs were expressed in cultured glioblastoma cells, the two cell lines U251 and U87-MG were profiled for β_1-AR and β_2-AR mRNA expression. The RT-qPCR analysis revealed that β_1-AR and β_2-AR transcripts were expressed in U251 cells; however, they were undetectable in U87-MG cells (Fig. 1A). These results were confirmed by RT-qPCR analysis (Fig. 1B). The β_2-AR transcript levels were increased compared with β_1-AR in U251 cells (Fig. 1B; $P=0.0135$). These data suggest that β-ARs may be functional and serve a role in the development process of U251 cells.

Isoproterenol promotes the proliferation of U251 cells. Based on the expression profile of β-ARs in U251, but not U87-MG cells, whether β-ARs activation was involved in U251 cell proliferation was then investigated. Firstly, the dose-dependent effect of ISO, an agonist of β-ARs, on the proliferation of U251

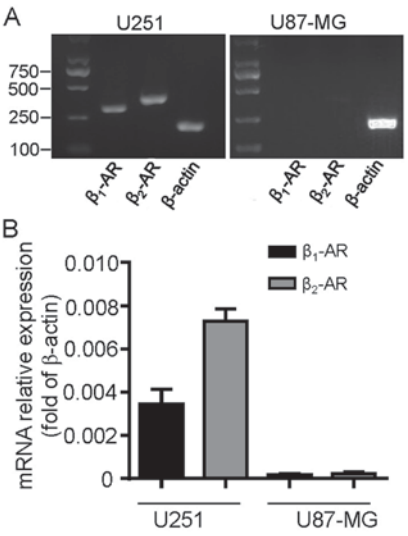

Figure 1. β-AR expression in human glioblastoma U251 and U87-MG cells. Expression of mRNA for β_1-AR, β_2-AR and β-actin by (A) reverse transcription PCR and (B) quantitative PCR in U251 and U87-MG cells. β-AR, β-adrenergic receptors; PCR, polymerase chain reaction.

cells was analyzed. The cells were treated with 0.1, 1, 5, 10, 30 or 50 μM ISO for 24 h and then proliferation was measured by MTT assay. These results revealed that ISO significantly increased the proliferation of U251 cells, which peaked at 10 μM ISO and decreased at higher concentrations of ISO (Fig. 2A). Notably, no effect of ISO-mediated cell proliferation was detected in U87-MG cells (Fig. 2B). This is in accordance with the results demonstrating no expression of β-ARs in U87-MG cells (Fig. 1A). Secondly, the effect of different time treatment of ISO on U251 cell proliferation was detected. The cells were incubated with 10 μM ISO for 24, 48, 72 or 96 h. As indicated in Fig. 2C, ISO significantly increased the proliferation of U251 cells, with the most marked effect at 48 h. Thus, 10 μM ISO was chosen as the reference concentration, and 48 h as the treatment time for the following studies unless specifically indicated.

To additionally confirm that ISO-induced proliferation was specifically mediated by β-ARs, the U251 cells were pretreated with β-AR antagonist PRO and then stimulated by ISO. As expected, PRO pre-treatment to block endogenous β-ARs activity significantly suppressed ISO-induced U251 cell proliferation (Fig. 2D). Taken together, these results indicate that specific activation of β-ARs is able to promote the cell proliferation in U251 cells.

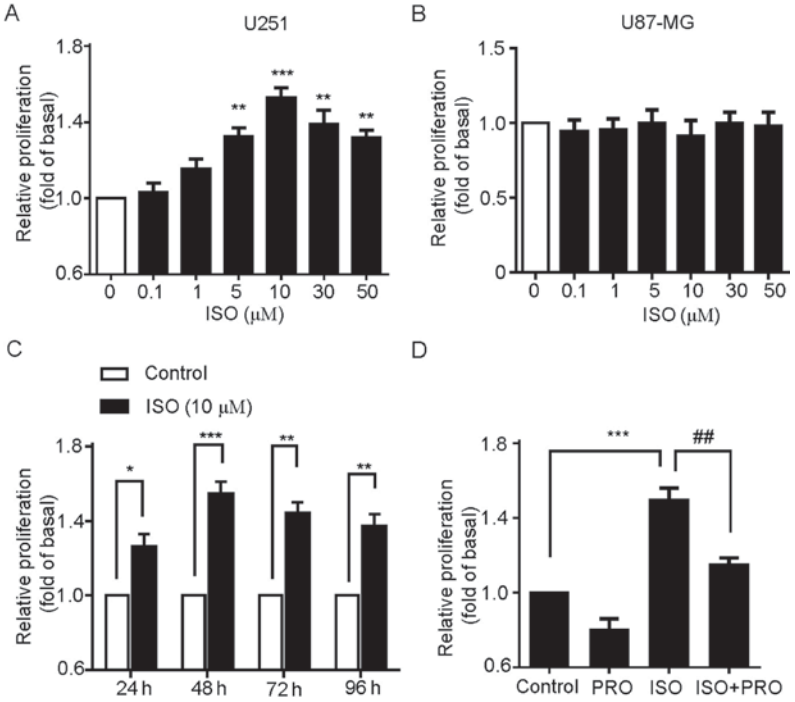

Figure 2. β-AR activation promotes U251 cell proliferation. (A) Effects of ISO on the cell proliferation of U251 cells. Cells were treated with various concentrations of ISO as indicated for 48 h, and cell proliferation was assessed by MTT assay. **P<0.01, ***P<0.001 vs. control group. (B) Effects of ISO on the cell proliferation of U87-MG cells. (C) Time-course stimulation of U251 cells with 10 μM ISO for 24, 48, 72 and 96 h. *P<0.05, **P<0.01, ***P<0.001 vs. control groups. (D) Effects of the β-AR antagonist PRO on ISO-induced cell proliferation. Cells were incubated with PRO for 30 min prior to and during treatment with ISO for 48 h. Values represent the mean ± standard error of the mean from at least three triplicate experiments. ***P<0.001 vs. control group, $^{\#\#}$P<0.01 vs. ISO-treated group. β-AR, β-adrenergic receptors; ISO, isoproterenol; PRO, propranolol.

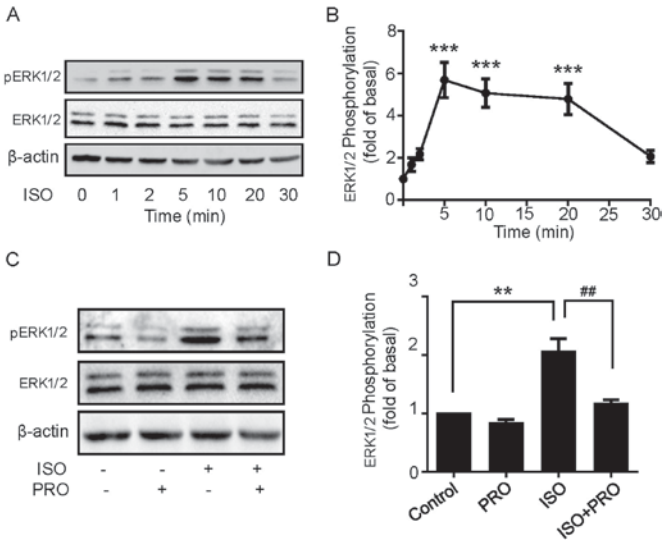

Figure 3. Activation of β-adrenergic receptors increases the ERK1/2 phosphorylation in U251 cells. (A) Effects of 10 μM ISO in the increase of transient ERK1/2 phosphorylation. Blots are representative of three independent experiments. (B) Quantitation of western blotting results from A. (C) Effects of PRO on ISO-induced ERK1/2 phosphorylation. (D) Quantitation of western blotting results from C. Values represent the mean ± standard error of the mean from at least three triplicate experiments. **P<0.01 vs. control group, $^{\#\#}$P<0.01 vs. ISO-treated group. ISO, isoproterenol; PRO, propranolol; ERK, extracellular signal-related kinase; p, phosphorylated.

β-ARs activation induces ERK$_{1/2}$ phosphorylation in U251 cells. The aforementioned results indicated that ISO treatment may significantly promote U251 cell proliferation. The underlying molecular mechanisms of ISO-induced cell proliferation in U251 cells were then explored. MAPK pathways have been widely recognized to regulate a variety of physiological processes including cell proliferation, differentiation and apoptosis in cancer cells (21). Thus, to define the role of MAPK cascades in the effect elicited by ISO, cells were treated with 10 μM ISO for 1, 2, 5, 10, 20 and 30 min, and then ERK1/2 phosphorylation levels were detected using western blotting. ISO induced ERK1/2 phosphorylation in a rapid and transient manner, which peaked at 5 min and decreased following 20 min, while ERK1/2 expression levels remained unchanged (Fig. 3A and B). Additionally, ERK1/2 phosphorylation was significantly blocked by PRO pretreatment (Fig. 3C and D), suggesting β-ARs activation is able to induce ERK1/2 phosphorylation in U251 cells.

ISO-induced proliferation is mediated by ERK$_{1/2}$ pathway. Whether ISO-mediated ERK1/2 activation was involved in cell proliferation of U251 cells was investigated. The effects of U0126, a specific MAPK/ERK (MEK)1/2 inhibitor, on ISO-induced ERK1/2 phosphorylation were first explored. As demonstrated in Fig. 4A and B, pretreating U251 cells with U0126 effectively abolished ISO-induced ERK1/2 phosphorylation. Next, the MTT assay showed that the proliferative effects of ISO were also significantly suppressed by U0126 (Fig. 4C). These results indicate that ERK1/2 pathway is essential for ISO-mediated proliferation in U251 cells.

β-ARs activation increases MMP-2 and MMP-9 mRNA expression through ERK1/2 activation. MMPs, particularly

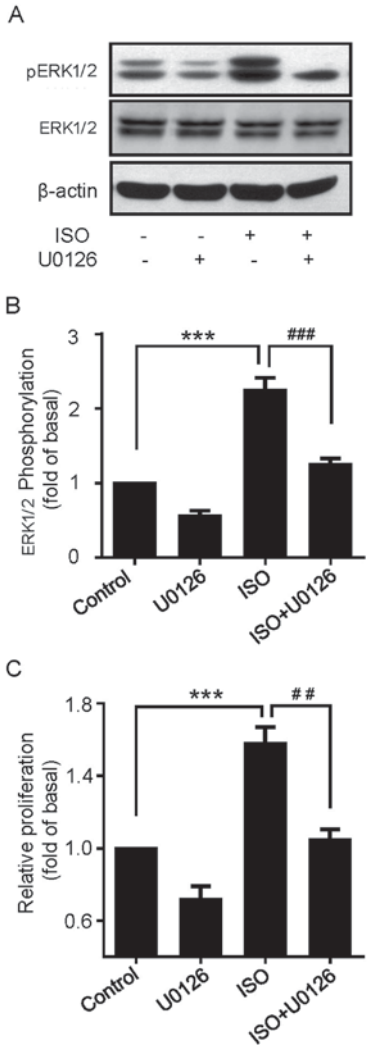

Figure 4. β-adrenergic receptor-dependent cell proliferation is mediated by ERK1/2 phosphorylation. (A) Effects of U0126 on ISO-induced ERK1/2 phosphorylation. Blots are representative of three separate experiments. (B) Quantitation of western blotting results in A, values represent the mean ± SEM from three separate sets of immunoblots. **P<0.01 vs. control group, $^{\#\#}$P<0.01 vs. ISO-treated group. (C) Effects of U0126 on ISO-induced cell proliferation. Values represent the mean ± SEM from at least three triplicate experiments. **P<0.01 vs. control group, $^{\#\#}$P<0.01 vs. ISO-treated group. ISO, isoproterenol; PRO, propranolol; ERK, extracellular signal-related kinase; p, phosphorylated; SEM, standard error of the mean.

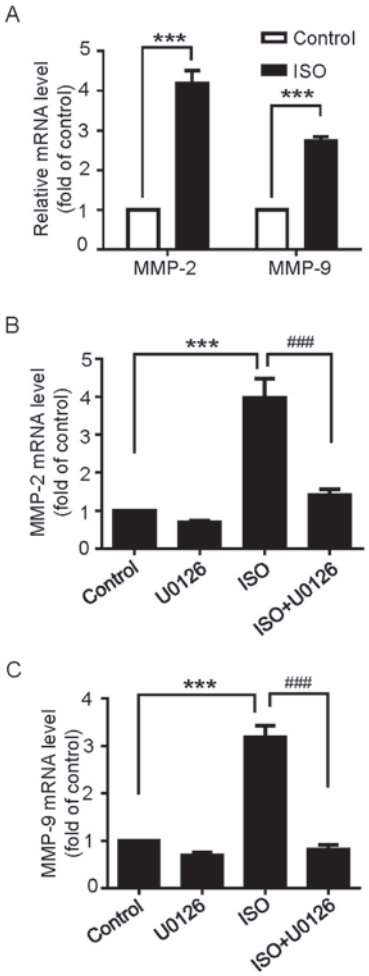

Figure 5. β-adrenergic receptors induce *MMP-2* and *MMP-9* mRNA expression, mediated by the extracellular signal-related kinase 1/2 pathway. (A) Effects of ISO on the expression of *MMP-2* and *MMP-9* mRNA. Cells were stimulated with 10 μM ISO for 48 h, then a quantitative reverse transcription polymerase chain reaction was performed to detect the mRNA level of *MMP-2* and *MMP-9*. ***P<0.001 vs. control groups. Effects of U0126 on ISO-induced (B) *MMP-2* and (C) *MMP-9* mRNA expression. ***P<0.001 vs. control groups, $^{\#\#\#}$P<0.001 vs. ISO-treated groups. Values represent the mean ± standard error of the mean from at least three triplicate experiments. ISO, isoproterenol; PRO, propranolol; MMP, matrix metalloproteinase.

the gelatinases MMP-2 and MMP-9, have been documented as key factors to the proliferation and invasion of cancer cells (22,23). Whether β-ARs activation regulates the expression of *MMP-2* and *MMP-9* mRNA in U251 cells was then examined. As demonstrated in Fig. 5A, the results indicated that *MMP-2* and *MMP-9* mRNA expression was significantly upregulated following ISO treatment. In addition, the pretreatment of U251 cells with U0126 inhibited ISO-induced *MMP-2* and *MMP-9* mRNA expression (Fig. 5B and C). These results suggest that β-ARs-mediated *MMP-2* and *MMP-9* mRNA expression occurs through the ERK1/2 pathway.

Discussion

The main results of the present study concern the mechanism of β-ARs-mediated cell proliferation of glioblastoma cells. It was demonstrated that: i) β_1-AR and β_2-AR are expressed in U251 cells but not in U87-MG cells; ii) activation of β-ARs by ISO promoted the proliferation of U251 cells through the ERK1/2 pathway; and iii) β-ARs activation increased MMP-2 and MMP-9 mRNA expression, which may be important for U251 cell proliferation.

Previous studies have demonstrated that β-ARs are expressed in human clinical glioblastoma specimens obtained from operated patients (24,25). Additionally, studies have indicated that β-ARs are also expressed in glioblastoma cell lines and primary cultures derived from human biopsies (26) and in the human-derived 1321N1 astrocytoma cell line (27). In addition, studies with the human-derived U118 glioma cell line indicate that there is a low but significant expression of β-ARs in these cells, but to a lesser extent compared with 1321N1 cells (18). However, the function of β-ARs in U87-MG cells remains debated. Previous studies demonstrate that *in vitro* U87-MG cells do not express functional

β-ARs (18,24). However, all three subtypes of β-ARs appear to be detectable in U87-MG tumors *in vivo*, indicating that β-ARs expression processes may take place and may be functional during tumor development (24). Additionally, (R,R')-4-methoxy-1-naphthylfenoterol, a selective β_2-AR agonist, was identified to inhibit cellular proliferation of U87-MG cells (28). All these data suggest that β-ARs may also be functional, by an unknown mechanism. Nevertheless, the exact function of β-ARs in U251 glioma cells and the underlying mechanism are not fully understood. In the present study, the expression of β-ARs in U251 and U87-MG cells was first measured by RT-PCR and RT-qPCR. The results demonstrated that β_1-ARs and β_2-ARs were expressed in U251 cells, but not in U87-MG cells, which was consistent with a previous study (24).

In previous years, evidence has suggested that β-AR agonists and antagonists affect cell growth and function, which may lead to the inhibition or induction of malignant diseases (29). The effect of β-AR activation is cell-specific as β_2-AR antagonists and agonists have been demonstrated to attenuate cell growth. For example, β-ARs activation may promote cell proliferation of certain cancer cells including lung cancer cells (20), ovarian carcinoma (5), human hepatocellular carcinoma cells (30), pancreatic (31), prostate (32), gastric (33) and colorectal cancer cells (34). By contrast, several studies have demonstrated that the β-AR agonist ISO suppresses the proliferation of MDA-MB-231 human breast cancer cells (35,36). In addition, in U118 glioma cells and 132N1 astrocytoma cells, treatment with β_2-AR agonists (R,R')-fenoterol or isoproterenol inhibited cell proliferation (18). However, the results of the present study indicated that the activation of β-ARs by ISO promoted U251 cell proliferation. How this difference occurs, and the underlying mechanism, requires additional investigation. As aforementioned, obtaining β-AR expression in U87-MG tumors *in vivo* would imply that β-AR may facilitate tumor formation by promoting cellular proliferation. Another potential probability is that this cell type-specific divergence on cellular proliferation may be due to cross-talk between β-ARs and other GPCR-linked signaling cascades including gamma-aminobutyric acid B receptors ($GABA_BR$) (37), α_2-AR (38), bradykinin B_2 receptor (39), oxytocin (40), and cannabinoid receptors (28). For example, the ISO-induced signaling cascade, cellular proliferation and cellular migration in pancreatic ductal adenocarcinoma cells may be potently inhibited following stimulation of $GABA_BR$ signaling (37).

The MMPs are a family of proteolytic enzymes that regulate various cell behaviors that are relevant in cancer biology through the degradation of the extracellular matrix surrounding tumors (41). Although MMPs, particularly MMP-2 and MMP-9, have been considered to be important factors in facilitating invasion and metastases through the degradation of type IV collagen, a major component of the basement membrane, additional evidence also demonstrates that MMPs may promote cancer cell proliferation (23). Previous, studies have suggested that NE may affect the progression of ovarian cancer by modulating the expression of MMPs and the angiogenic cytokine, vascular endothelial growth factor, in ovarian cancer cells (42). Additionally, β-ARs inhibition suppressed the expression of MMP-2 and MMP-9 in human brain microvascular endothelial cells (24) thus increasing the proliferative, invasive and metastatic potential of these cells. In the present study, it was revealed that MMP-2 and MMP-9 mRNA expression was significantly increased upon β-ARs activation. Consistently, the proliferation of U251 cells was also enhanced through the activation of β-ARs. In addition, pretreatment with U0126 to block ERK1/2 activity significantly reduced the upregulation of MMP-2 and MMP-9. Previous studies have demonstrated that the activation of the EGFR-MEK-ERK signaling pathway causes the overexpression of MMP-2 and MMP-9 in prostate cancer cells (43). However, whether EGFR transactivation pathway or/and other signaling cascades are involved in β-ARs-induced cell proliferation in glioblastoma cells requires additional study.

Acknowledgements

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References

1. Dolecek TA, Propp JM, Stroup NE and Kruchko C: CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2005-2009. Neuro Oncol 14 (Suppl 5): v1-v49, 2012.
2. Wen PY and Kesari S: Malignant gliomas in adults. N Engl J Med 359: 492-507, 2008.
3. Stupp R and Roila F; ESMO Guidelines Working Group: Malignant glioma: ESMO clinical recommendations for diagnosis, treatment and follow-up. Ann Oncol 20 (Suppl 4): S126-S128, 2009.
4. Nagaraja AS, Armaiz-Pena GN, Lutgendorf SK and Sood AK: Why stress is BAD for cancer patients. J Clin Invest 123: 558-560, 2013.
5. Thaker PH, Han LY, Kamat AA, Arevalo JM, Takahashi R, Lu C, Jennings NB, Armaiz-Pena G, Bankson JA, Ravoori M, *et al*: Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. Nat Med 12: 939-944, 2006.
6. Hassan S, Karpova Y, Baiz D, Yancey D, Pullikuth A, Flores A, Register T, Cline JM, D'Agostino R Jr, Danial N, *et al*: Behavioral stress accelerates prostate cancer development in mice. J Clin Invest 123: 874-886, 2013.
7. Timmermans W, Xiong H, Hoogenraad CC and Krugers HJ: Stress and excitatory synapses: From health to disease. Neuroscience 248: 626-636, 2013.
8. de Kloet ER, Joëls M and Holsboer F: Stress and the brain: From adaptation to disease. Nat Rev Neurosci 6: 463-475, 2005.
9. Fitzgerald PJ: Is norepinephrine an etiological factor in some types of cancer? Int J Cancer 124: 257-263, 2009.
10. Woo AY and Xiao RP: β-adrenergic receptor subtype signaling in heart: From bench to bedside. Acta Pharmacol Sin 33: 335-341, 2012.
11. Schuller HM and Al-Wadei HA: Neurotransmitter receptors as central regulators of pancreatic cancer. Future Oncol 6: 221-228, 2010.
12. Cohen S, Janicki-Deverts D and Miller GE: Psychological stress and disease. JAMA 298: 1685-1687, 2007.
13. Schuller HM: Beta-adrenergic signaling, a novel target for cancer therapy? Oncotarget 1: 466-469, 2010.
14. Braadland PR, Ramberg H, Grytli HH and Taskén KA: β-adrenergic receptor signaling in prostate cancer. Front Oncol 4: 375, 2015.
15. Al-Wadei HA, Al-Wadei MH and Schuller HM: Prevention of pancreatic cancer by the beta-blocker propranolol. Anticancer Drugs 20: 477-482, 2009.

16. Askari MD, Tsao MS and Schuller HM: The tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone stimulates proliferation of immortalized human pancreatic duct epithelia through beta-adrenergic transactivation of EGF receptors. J Cancer Res Clin Oncol 131: 639-648, 2005.
17. Weddle DL, Tithoff P, Williams M and Schuller HM: Beta-adrenergic growth regulation of human cancer cell lines derived from pancreatic ductal carcinomas. Carcinogenesis 22: 473-479, 2001.
18. Toll L, Jimenez L, Waleh N, Jozwiak K, Woo AY, Xiao RP, Bernier M and Wainer IW: {Beta}2-adrenergic receptor agonists inhibit the proliferation of 1321N1 astrocytoma cells. J Pharmacol Exp Ther 336: 524-532, 2011.
19. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
20. Hu P, He J, Liu S, Wang M, Pan B and Zhang W: β2-adrenergic receptor activation promotes the proliferation of A549 lung cancer cells via the ERK1/2/CREB pathway. Oncol Rep 36: 1757-1763, 2016.
21. Dhillon AS, Hagan S, Rath O and Kolch W: MAP kinase signalling pathways in cancer. Oncogene 26: 3279-3290, 2007.
22. Dong W, Li H, Zhang Y, Yang H, Guo M, Li L and Liu T: Matrix metalloproteinase 2 promotes cell growth and invasion in colorectal cancer. Acta Biochim Biophys Sin (Shanghai) 43: 840-848, 2011.
23. Egeblad M and Werb Z: New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2: 161-174, 2002.
24. Annabi B, Lachambre MP, Plouffe K, Moumdjian R and Béliveau R: Propranolol adrenergic blockade inhibits human brain endothelial cells tubulogenesis and matrix metalloproteinase-9 secretion. Pharmacol Res 60: 438-445, 2009.
25. Sardi I, Giunti L, Bresci C, Buccoliero AM, Degl'innocenti D, Cardellicchio S, Baroni G, Castiglione F, Ros MD, Fiorini P, *et al*: Expression of β-adrenergic receptors in pediatric malignant brain tumors. Oncol Lett 5: 221-225, 2013.
26. Prenner L, Sieben A, Zeller K, Weiser D and Häberlein H: Reduction of high-affinity beta2-adrenergic receptor binding by hyperforin and hyperoside on rat C6 glioblastoma cells measured by fluorescence correlation spectroscopy. Biochemistry 46: 5106-5113, 2007.
27. Wakshull E, Hertel C, O'Keefe EJ and Perkins JP: Cellular redistribution of beta-adrenergic receptors in a human astrocytoma cell line: A comparison with the epidermal growth factor receptor in murine fibroblasts. J Cell Biochem 29: 127-141, 1985.
28. Paul RK, Ramamoorthy A, Scheers J, Wersto RP, Toll L, Jimenez L, Bernier M and Wainer IW: Cannabinoid receptor activation correlates with the proapoptotic action of the β2-adrenergic agonist (R,R')-4-methoxy-1-naphthylfenoterol in HepG2 hepatocarcinoma cells. J Pharmacol Exp Ther 343: 157-166, 2012.
29. Evans BA, Sato M, Sarwar M, Hutchinson DS and Summers RJ: Ligand-directed signalling at beta-adrenoceptors. Br J Pharmacol 159: 1022-1038, 2010.
30. Yuan A, Li Z, Li X, Yi S, Wang S, Cai Y and Cao H: The mitogenic effectors of isoproterenol in human hepatocellular carcinoma cells. Oncol Rep 23: 151-157, 2010.
31. Pham H, Chen M, Takahashi H, King J, Reber HA, Hines OJ, Pandol S and Eibl G: Apigenin inhibits NNK-induced focal adhesion kinase activation in pancreatic cancer cells. Pancreas 41: 1306-1315, 2012.
32. Zhang P, He X, Tan J, Zhou X and Zou L: β-arrestin2 mediates β-2 adrenergic receptor signaling inducing prostate cancer cell progression. Oncol Rep 26: 1471-1477, 2011.
33. Shin VY, Wu WK, Chu KM, Koo MW, Wong HP, Lam EK, Tai EK and Cho CH: Functional role of beta-adrenergic receptors in the mitogenic action of nicotine on gastric cancer cells. Toxicol Sci 96: 21-29, 2007.
34. Coelho M, Moz M, Correia G, Teixeira A, Medeiros R and Ribeiro L: Antiproliferative effects of β-blockers on human colorectal cancer cells. Oncol Rep 33: 2513-2520, 2015.
35. Slotkin TA, Zhang J, Dancel R, Garcia SJ, Willis C and Seidler FJ: Beta-adrenoceptor signaling and its control of cell replication in MDA-MB-231 human breast cancer cells. Breast Cancer Res Treat 60: 153-166, 2000.
36. Carie AE and Sebti SM: A chemical biology approach identifies a beta-2 adrenergic receptor agonist that causes human tumor regression by blocking the Raf-1/Mek-1/Erk1/2 pathway. Oncogene 26: 3777-3788, 2007.
37. Schuller HM, Al-Wadei HA and Majidi M: GABA B receptor is a novel drug target for pancreatic cancer. Cancer 112: 767-778, 2008.
38. Cottingham C, Lu R, Jiao K and Wang Q: Cross-talk from β-adrenergic receptors modulates α2A-adrenergic receptor endocytosis in sympathetic neurons via protein kinase A and spinophilin. J Biol Chem 288: 29193-29205, 2013.
39. Hanke S, Nürnberg B, Groll DH and Liebmann C: Cross talk between beta-adrenergic and bradykinin B(2) receptors results in cooperative regulation of cyclic AMP accumulation and mitogen-activated protein kinase activity. Mol Cell Biol 21: 8452-8460, 2001.
40. Wrzal PK, Goupil E, Laporte SA, Hébert TE and Zingg HH: Functional interactions between the oxytocin receptor and the β2-adrenergic receptor: Implications for ERK1/2 activation in human myometrial cells. Cell Signal 24: 333-341, 2012.
41. Deryugina EI and Quigley JP: Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rev 25: 9-34, 2006.
42. Moreno-Smith M, Lutgendorf SK and Sood AK: Impact of stress on cancer metastasis. Future Oncol 6: 1863-1881, 2010.
43. Xiao LJ, Lin P, Lin F, Liu X, Qin W, Zou HF, Guo L, Liu W, Wang SJ and Yu XG: ADAM17 targets MMP-2 and MMP-9 via EGFR-MEK-ERK pathway activation to promote prostate cancer cell invasion. Int J Oncol 40: 1714-1724, 2012.