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Original Articles

IL-17 induces the proliferation and migration of glioma cells through the activation of PI3K/Akt1/NF-κB-p65

Bin Wang[a,1], Chen-Hui Zhao[b,1], Guan Sun[c,1], Zhi-Wei Zhang[d], Bao-Mei Qian[d], Yu-Feng Zhu[e], Meng-Yuan Cai[e], Samjhana Pandey[d], Dan Zhao[d], Ying-Wei Wang[d], Wen Qiu[d,*], Lei Shi[a,**]

[a] Department of Neurosurgery, The First People's Hospital of Kunshan affiliated with Jiangsu University, Suzhou, Jiangsu, 215300, PR China
[b] Department of Oncology, First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, 210029, PR China
[c] Department of Neurosurgery, The Fourth Affiliated Hospital of Nantong University, Yancheng City No.1 people's hospital, Yancheng, Jiangsu, 224000, PR China
[d] Department of Immunology, Nanjing Medical University, Nanjing, Jiangsu, 211166, PR China
[e] Clinical Medical Science of the First Clinical Medical College, Nanjing Medical University, Nanjing, Jiangsu, 211166, PR China

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ABSTRACT

Interleukin 17 (IL-17), as a pro-inflammatory cytokine, is up-regulated in the sera and tumor tissues of glioma patients; however the effects of IL-17 on glioma proliferation and migration remain unclear. In this study, the roles of IL-17 in the proliferation and migration of glioma cells and their potential mechanisms were determined. The results showed that IL-17 could not only enhance the proliferation and migration of cultured glioma cells (*in vitro*), but also promote the tumor formation of glioma cells in BALB/c nude mice (*in vivo*). Mechanical exploration revealed that IL-17 stimulation could increase the phosphorylation levels of Akt1 and NF-κB-p65 in glioma cells, and knockdown or inhibition of PI3K, Akt1 and NF-κB-p65 could also reduce the IL-17-induced proliferation and migration of the glioma cells. Moreover, PI3K/Akt1 was the upstream regulator of NF-κB-p65 activation in IL-17-incubated glioma cells. Furthermore, the inhibition of PI3K, Akt1 and NF-κB-p65 markedly suppressed the tumor formation of glioma cells induced by IL-17. Together, these data indicate that IL-17 can promote the proliferation and migration of glioma cells via PI3K/Akt1/NF-κB-p65 activation, and these findings might provide a new insight into glioma pathogenesis.

1. Introduction

Glioma accounts for the majority of primary malignant brain tumors around the world and is highly aggressive [1–3]. As the same as other tumors, glioma cells have a characteristic of proliferation and migration, and finally resulting in the death of patients [4–6]. However, the underlying mechanism of cell proliferation and migration in human Glioma has not been fully elucidated.

Recently, several documents have pointed out that the proliferation and migration of tumor cells can be induced by some pro-inflammatory cytokines or mediators in tumor microenvironment [7–9]. Interleukin 17 (IL-17), also known as IL-17A, is a newly identified pro-inflammatory cytokine [10,11]. Many groups have demonstrated that IL-17 in some tumors, such as lung cancer [12], liver cancer [13], and gastric cancer [14], is not only significantly increased, but also can lead to cell proliferation and migration. Besides, IL-17 production is also proven to be up-regulated in the sera and tumor tissues of gliomas patients [15–17]. However, the roles of IL-17 in glioma cell proliferation and migration and their mechanisms are still unclear.

Reportedly, IL-17-induced cell proliferation is often associated with the activation of certain signaling pathways, e.g. extracellular signal-regulated kinase 1/2 (ERK1/2), ERK5, and signal transducer and activator of transcription 3 (STAT3) [18–20]. It is well known that phosphoinositide-3-kinase (PI3K) is an important upstream regulator of Akt1, and PI3K/Akt1 signal pathway plays a pivotal role in gliomas growth [21–23]. Moreover, NF-κB signal pathway is also involved in the growth and metastasis of some cancers, including glioma [24–28]. For example, glioma-released fibulin-3 can cause tumor invasion through the activation of TNF-α/NF-κB [28], and NF-κB inhibition can induce anti-glioma activity [29]. Hence, whether IL-17 stimulation *in*

* Corresponding author. Department of Immunology, Nanjing Medical University, 101 Longmian Avenue, Nanjing, Jiangsu, 211166, PR China.
** Corresponding author. Department of Neurosurgery, The First People's Hospital of Kunshan affiliated with Jiangsu University, 91 Qianjin West Road, Suzhou, Jiangsu, 215300, PR China.
E-mail addresses: qiuwen@njmu.edu.cn (W. Qiu), shi.lei@ujs.edu.cn (L. Shi).
[1] These three authors contributed to this work equally.

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vitro can directly activate PI3K/Akt1 or NF-κB signal in glioma cells resulting in cell proliferation and migration, as well as the relationship between PI3K/Akt1 and NF-κB need to be explored.

In order to answer the above-mentioned questions, the roles of IL-17 stimulation in the proliferation and migration of glioma cells were detected in the current studies. Then, the possible signaling molecules involved in IL-17-induced glioma proliferation and migration were explored, and the increased phosphorylation of Akt1 and NF-κB-p65 in glioma cells was observed. Knockdown and inhibition experiments were performed to clarify the upstream and downstream relationship between PI3K/Akt1 and NF-κB-p65, and also determine the roles of Akt1 and NF-κB-p65 in glioma cell proliferation and migration exposed to IL-17. In addition, tumor formation experiments in nude mice were done to determine the effects of PI3K, Akt1 and NF-κB-p65 inhibition on tumor formation of glioma cells induced by IL-17.

2. Materials and methods

2.1. Reagents

Recombinant human IL-17 protein was from R&D Systems (Minneapolis, MN, USA). Monoclonal antibodies against human β-actin, total Akt1 (t-Akt1), Phospho-Akt1-Thr308 (p-Akt1-Thr308), Phospho-Akt1-Ser473 (p-Akt1-Ser473), total NF-κB-p65 (t-p65) and Phospho-NF-κB-p65-Ser536 (p-p65-Ser536) as well as HRP-conjugated anti-rabbit IgG and ECL detection system were purchased from Cell Signaling Technology (Danvers, MA, USA). PVDF membranes were from Millipore (Billerica, MA, USA). PE-labeled anti-human IL-17RA and PE-labeled isotype mouse IgG2a were supplied by BioLegend (San Diego, CA, USA). Lipofectamine 2000 was from Invitrogen (Carlsbad, CA, USA). Cell-Light™ EdU Apollo®567 In Vitro Imaging Kits were purchased from RiboBio Co., Ltd. (Guangzhou, China). PI3K siRNA (siPI3K), Akt1 siRNA (siAkt1), NF-κB-p65 siRNA (sip65) and control siRNA (siCTR) as well as PathScan® Intracellular Signaling Array Kits were from Cell Signaling Technology. LY294002 as a PI3K inhibitor, Perifosine as an Akt inhibitor and BAY11-7082 as an NF-κB inhibitor were supplied by Selleck (Houston, TX, USA). Crystal violet was from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco modified Eagle's medium (DMEM) was purchased from Gibco of Thermo Fisher Scientific (Waltham, MA, USA). Fetal bovine serum (FBS) was from ScienCell (Carlsbad, CA, USA).

2.2. Cell lines and animals

The human glioma cell lines of SHG-44 and U373 were obtained from Cell Bank of Chinese Academy of Sciences (Shanghai, China). Cells was cultured in DMEM supplemented with 10% (v/v) FBS and antibiotics (50 U/ml penicillin and 100 μg/ml streptomycin, Invitrogen) at 37 °C in 5% CO2.

Male BALB/c nude mice at the age of 6 weeks were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Mice were maintained under specific pathogen-free (SPF) conditions. All experiment protocols were approved by the Institutional Animal Care and Use Ethics Committee of The First People's Hospital of Kunshan affiliated with Jiangsu University.

2.3. Intracellular signaling array assay

SHG-44 cells were seeded in 35 mm cell culture dishes at 150000/per dish and cultured in DMEM with 10% FBS for 24 h. After serum withdraw for 24 h, cells were stimulated with 50 ng/ml of IL-17 for different time points under the condition of serum withdraw. Then, cells were lysed using PathScan® Sandwich ELISA Lysis Buffer at 4 °C for 30 min. Equal amounts (200 μg per sample) of protein were subjected to the PathScan® Intracellular Signaling Array Kits according to the instructions. Briefly, the array slide was blocked with array blocking buffer at RT for 15 min. Then, the diluted cellular lysate was added into each well of the array slide and incubated at 4 °C overnight. After washing with array wash buffer four times (5 min each time), the array slide was incubated with detection antibody cocktail at RT for 1 h. After washing four times, the array slide was incubated with HRP-linked streptavidin at RT for 30 min. Finally, image was captured by detecting chemiluminescent signals after covering the array slide with LumiGLO®/Peroxide reagent.

2.4. siRNA experiments and transfection

SHG-44 and U373 cells were transfected with siPI3K, siAkt1, sip65 or siCTR by using Lipofectamine 2000 according to the instructions.

2.5. Ethynyldeoxyuridine (EdU) detection

SHG-44 or U373 cells were seeded in 96-well plates at 3000/per well or 12000/per well and cultured in DMEM with 10% FBS. After 24 h, SHG-44 or U373 cells were cultured in DMEM without FBS or with 1% FBS for another 24 h. Next, SHG-44 or U373 cells were stimulated with different concentration of IL-17 for 36 h under the condition of serum withdraw (SHG-44), or for 48 h in DMEM with 1% FBS (U373). Cells were incubated with 10 μM EdU for last 6 h. Then, EdU staining and Hoechst 33342 staining were performed with Cell-Light™ EdU Apollo®567 In Vitro Imaging Kits according to the instructions. The ratios of EdU-positive cells/total cells (namely Hoechst 33342-positive cells) were calculated.

SHG-44 or U373 cells were seeded in 96-well plates at 3000/per well or 12000/per well and cultured in DMEM with 10% FBS. After 24 h of culture, corresponding siRNA was transfected into cells with Lipofectamine 2000. After 24 h of transfection, SHG-44 or U373 cells were cultured in DMEM without FBS or with 1% FBS for 12 h. Next, SHG-44 cells were stimulated with 50 ng/ml of IL-17, and then EdU and Hoechst 33342 staining was performed as above-mentioned.

2.6. Colony formation assay

SHG-44 or U373 cells were seeded in 6-well plates at 200/per or 500/per well and cultured in DMEM with 10% FBS for 24 h. Next, cells were treated with different inhibitors or DMSO control for 15 min, and then stimulated with 50 ng/ml of IL-17 for 15 min. After culture in DMEM with 2.5% FBS (SHG-44) or 5% FBS (U373) for 2 or 3 weeks, the cells were fixed with methanol at RT for 30 min and stained with 0.1% crystal violet in PBS at RT for 2 h. Visible colonies were calculated.

2.7. Cell scratch assay

SHG-44 or U373 cells were seeded in 6-well plates at 200000/per well or 300000/per well and cultured in DMEM with 10% FBS. After 24 h of culture, SHG-44 or U373 cells were cultured in DMEM without FBS or with 1% FBS for another 24 h. Then, SHG-44 or U373 cells were scratched with a 200 μl tip and stimulated with IL-17 for 48 h under the condition of serum withdraw (SHG-44), or for 72 h in DMEM with 1% FBS (U373).

SHG-44 or U373 cells were seeded in 6-well plates at 200000/per well or 300000/per well and cultured in DMEM with 10% FBS. After 24 h of culture, corresponding siRNA was transfected into cells with Lipofectamine 2000. After 24 h of transfection, SHG-44 or U373 cells were cultured in DMEM without FBS or with 1% FBS for 12 h. Then, SHG-44 or U373 cells were scratched and stimulated with IL-17 as above-mentioned.

2.8. Western blot analysis

Cells were lysed using RIPA lysis buffer. Equal amounts (30 μg/lane) of protein were subjected to 4–20% ExpressPlus™ PAGE Gel (Genscript,

Nanjing, China). Western blot analysis was performed as described before [30]. Primary antibodies against β-actin, t-Akt1, p-Akt1-Thr308, p-Akt1-Ser473, t-p65 and p-p65-Ser536 as well as anti-rabbit IgG were used to detect the expression of corresponding protein. The bands were captured by GE Amersham Imager 600 through ECL detection system. Finally, the density of radiographic band onto PVDF membranes was analyzed by using the software of Quantity One (Bio-Rad, Hercules, CA, USA).

2.9. Flow cytometry

SHG-44 or U373 cells were stained with PE-labeled anti-human IL-17RA or PE-labeled isotype mouse IgG2a at 4 °C for 30 min. After washing with PBS for two times, IL-17RA expression on cells was evaluated by flow cytometry on BD FACSCalibur (BD Biosciences, San Jose, CA, USA).

2.10. In vivo experiments

SHG-44 cells were cultured and incubated with or without 50 ng/ml of IL-17 for 15 min *in vitro*, and then injected into BALB/c nude mice (2000000 cells for each tumor) subcutaneously for tumor growth *in vivo* until 3 weeks according to published methods [14]. Tumor sizes were detected at different time points and tumor weights were obtained at last time points.

SHG-44 cells were incubated with LY294002, Perifosine, BAY11-7082 or DMSO control for 15 min, and then treated with or without 50 ng/ml of IL-17 for 15 min. Finally cells injected into BALB/c nude mice subcutaneously for tumor growth for 3 weeks. Tumor sizes were detected at different time points and tumor weights were obtained at last time points.

2.11. Statistical analysis

All statistical analysis was carried out by using SPSS 11.5 software. All data are given as mean ± SD. The statistical significance (defined as $P < 0.05$) of the groups was evaluated by T-Test or one-way ANOVA with simultaneous multiple comparisons between groups by the Bonferroni method.

3. Results

3.1. IL-17 induces the proliferation and migration of glioma cells in vitro

Firstly, the expression of IL-17 receptor (IL-17RA) was detected in two human glioma cell lines including SHG-44 and U373. Flow cytometry data showed that both SHG-44 and U373 cell lines significantly expressed IL-17RA (Figs. S1A and B). SHG-44 and U373 cells were then stimulated with human IL-17 protein at the doses from 0 to 500 ng/ml for 36 h or 48 h. EdU assay showed that IL-17 could markedly enhance the proliferation of both cell lines in a dose-dependent manner, with a maximum effect at the doses of 50 ng/ml and 500 ng/ml (Fig. 1A and B, Figs. S2A and B). Therefore, 50 ng/ml was chosen as the best stimulation dose of IL-17. Furthermore, the abilities of IL-17 to induce colony formation and migration (cell scratch assay) of glioma cells were measured, and the results showed that IL-17 could markedly increase the colony formation and migration of not only SHG-44 cells but also U373 cells (Fig. 2). We also checked the possible effects of IL-17 stimulation on IL-17RA expression on glioma cells. Flow cytometry analysis showed that IL-17 incubation could not change IL-17RA expression on both SHG-44 and U373 cells (Fig. S3).

3.2. IL-17 promotes the tumor formation of glioma cells in nude mice

Since we found that IL-17 could induce the proliferation, colony formation and migration of glioma cells *in vitro*, we continued to evaluate the ability of IL-17 to induce tumor formation of glioma cells in nude mice. Cultured SHG-44 cells were incubated with or without 50 ng/ml of IL-17 for 15 min *in vitro*, and then injected into BALB/c nude mice subcutaneously for tumor growth *in vivo* until 3 weeks according to published methods [14]. Tumor sizes were detected at different time points and tumor weights were obtained at last time point. The results showed that IL-17 incubation could obviously enhance the tumor formation of glioma cells in nude mice (Fig. 3).

3.3. The profile of signaling pathways in glioma cells in response to IL-17

In order to explore the potential mechanism of IL-17-induced proliferation and migration of glioma cells, the antibody chips of intracellular signaling array were used to screen some signaling molecules including p-ERK1/2 (Thr202/Tyr204), p-STAT1 (Tyr701), p-STAT3 (Tyr705), p-Akt1 (Thr308), p-Akt1 (Ser473), p-AMPKa (Thr172), p-S6 ribosomal protein (Ser235/236), p-mTOR (Ser2448), p-HSP27 (Ser78), p-Bad (Ser112), p-p70 S6 kinase (Thr389), p-PRAS40 (Thr246), p-p53 (Ser15), p-p38 (Thr180/Tyr182), p-JNK (Thr183/Tyr185), cleaved PARP (Asp214), cleaved Caspase-3 (Asp175) and p-GSK-3b (Ser9). SHG-44 cells were stimulated with IL-17 at the dose of 50 ng/ml for different time points (0, 5, 10, 20, 30, 60, 90, 120 min), and then the levels of above-mentioned molecules were determined. We observed that IL-17 could markedly increase the level of p-Akt1 (Ser473) at 10 min time point, but had no significant effect on other factors (Fig. 4A). Given that Akt1 phosphorylation at Thr308 is also essential for Akt1 activation, we further detected Akt1 phosphorylation at both sites of Thr308 and Ser473 by Western blot. We set the new time points at 0, 5, 10, 15, 20, 30 and 60 min to cover Akt1 activation time points better according to the antibody chip data. The results showed that IL-17 could markedly increase the level of p-Akt1 (Ser473) at 10 and 15 min time points, and obviously increase the level of p-Akt1 (Thr308) at 15 min time point in SHG-44 cells (Fig. 4B).

Given that other signaling molecules in intracellular signaling array were not activated, we continued to search some possible signaling molecules related to IL-17-induced proliferation and migration of glioma cells. Given that NF-κB-p65 activation is also related to glioma cell proliferation and migration [27,28], we further examined the activation of NF-κB-p65 in glioma cells exposed to IL-17. The results showed that IL-17 stimulation obviously increased the phosphorylation of NF-κB-p65 at Ser536 (as a marker of NF-κB-p65 activation) in SHG-44 cells in a time-dependent manner, with a maximum at 30 min (Fig. 4C). The similar up-regulation of Akt1 and NF-κB-p65 phosphorylation in U373 cells was also confirmed (Fig. 4D and E).

3.4. PI3K/Akt1/NF-κB-p65 axis contributes to the proliferation and migration of glioma cells exposed to IL-17

The roles of PI3K, Akt1 and NF-κB-p65 in the proliferation and migration of glioma cells caused by IL-17 were further checked. SHG-44 cells were treated with siRNA or inhibitors for PI3K, Akt1 or NF-κB-p65 respectively, and then incubated with IL-17 for different time points. PI3K knockdown or inhibition reduced Akt1 and NF-κB-p65 phosphorylation, and Akt1 knockdown or inhibition decreased NF-κB-p65 phosphorylation, but NF-κB-p65 knockdown or inhibition had no significant effect on Akt1 phosphorylation (Fig. 5), confirming the upstream and downstream relationship among PI3K, Akt1 and NF-κB-p65. Cellular proliferation, colony formation and migration experiments were further performed, and the results showed that knockdown or inhibition of PI3K, Akt1 and NF-κB-p65 (Fig. 5) could not only markedly reduce the proliferation (Fig. 6A and D) and colony formation (Fig. 6B and E), but also obviously inhibit the migration (Fig. 6C and F) of glioma cells induced by IL-17. The similar results were obtained in U373 cells in response to IL-17 (Fig. S4 and Fig. S5). Taken together, these data indicate that the activation of PI3K/Akt1/NF-κB-p65 axis contributes to the proliferation and migration of glioma cells exposed to

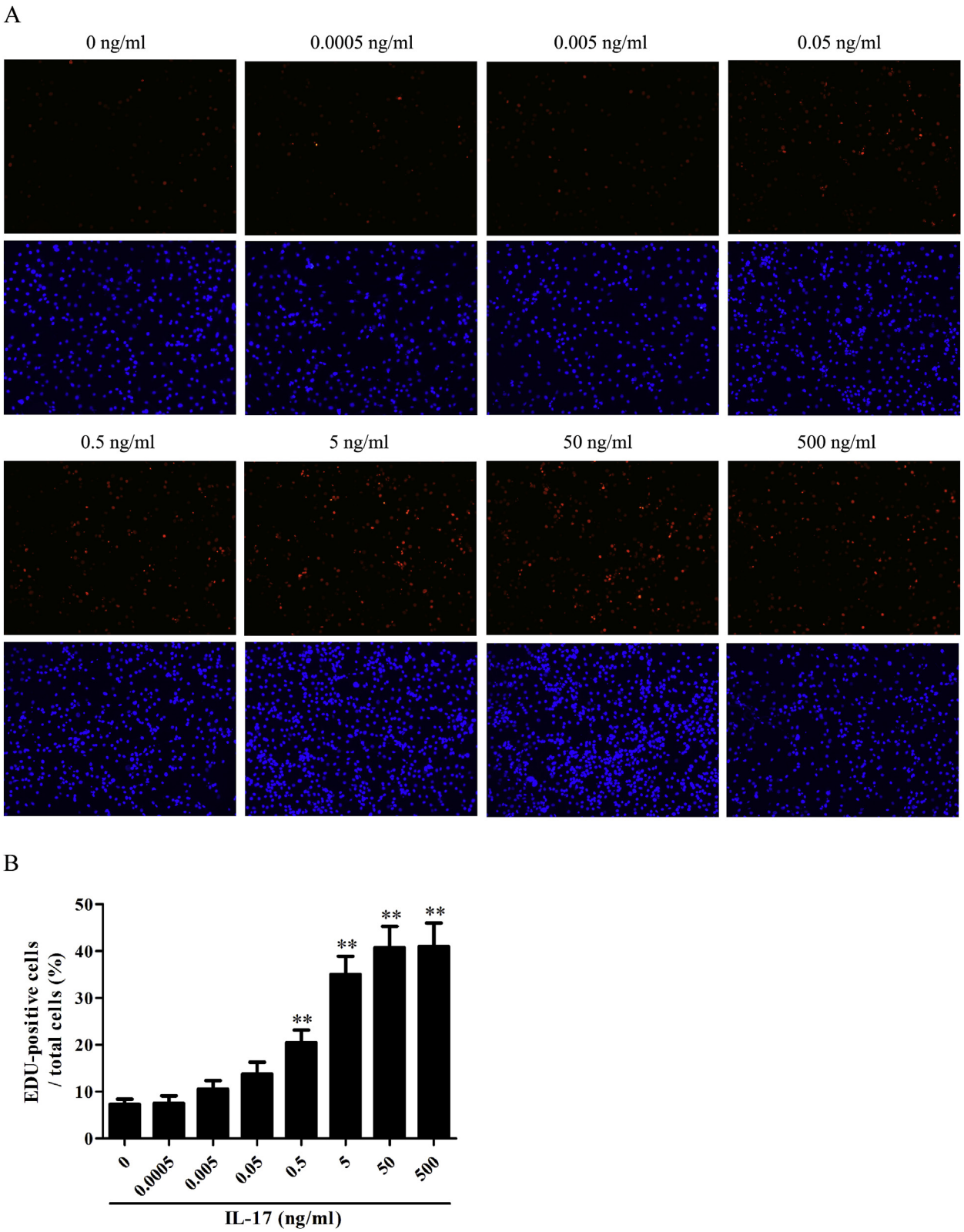

Fig. 1. IL-17 promotes the proliferation of SHG-44 cells. SHG-44 cells were stimulated with human IL-17 protein at different doses (0, 0.0005, 0.005, 0.05, 0.5, 5, 50, 500 ng/ml) for 36 h. The nuclei of proliferative cells were observed by EdU staining, and nuclei of total cells were observed by Hoechst 33342 staining under microscopy (A, magnification × 100). The ratios of EdU-positive cells/total cells were calculated (B). Data were represented as means ± SD (n = 4 in each dose). $^{**}P < 0.01$ vs. 0 ng/ml of dose.

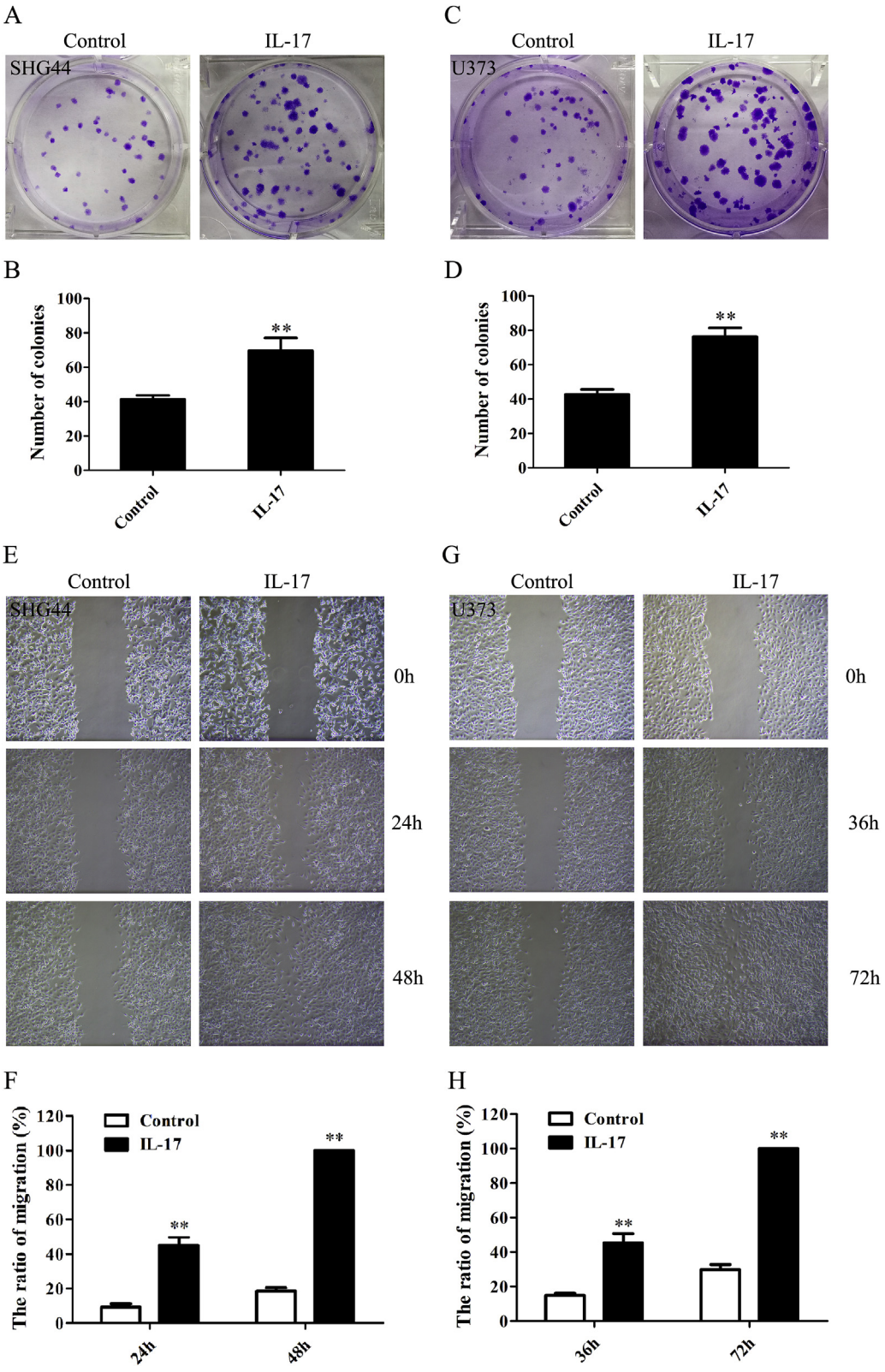

Fig. 2. IL-17 increases the colony formation and migration of SHG-44 and U373 cells. SHG-44 and U373 cells were stimulated with human IL-17 protein at the doses of 50 ng/ml (A–D) Colony formation experiments of SHG-44 (A and B) and U373 (C and D) cells were performed and stained by crystal violet. Data were represented as means ± SD (n = 3 in each group). $^{**}P < 0.01$ vs. control group. (E–H) Scratch assay was performed to evaluate the migration of SHG-44 cells (E and F, magnification × 100) and U373 cells (G and H, magnification × 100). Data were represented as means ± SD (n = 3 in each group). $^{**}P < 0.01$ vs. control group. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

IL-17.

3.5. Inhibition of PI3K, Akt1 and NF-κB-p65 reduces the tumor formation of glioma stimulated by IL-17

The roles of PI3K, Akt1 an NF-κB-p65 in IL-17-induced glioma tumor formation were further assayed *in vivo*. Cultured SHG-44 cells were incubated with PI3K, Akt1 and NF-κB-p65 inhibitors or DMSO control for 15 min, and then treated with or without 50 ng/ml of IL-17 for 15 min. After that, these treated cells were injected into BALB/c nude mice subcutaneously for tumor growth for 3 weeks. Tumor sizes were examined at different time points and tumor weights were detected at last time point. The results showed that inhibition of PI3K, Akt1 and NF-κB-p65 could result in the repression of tumor formation

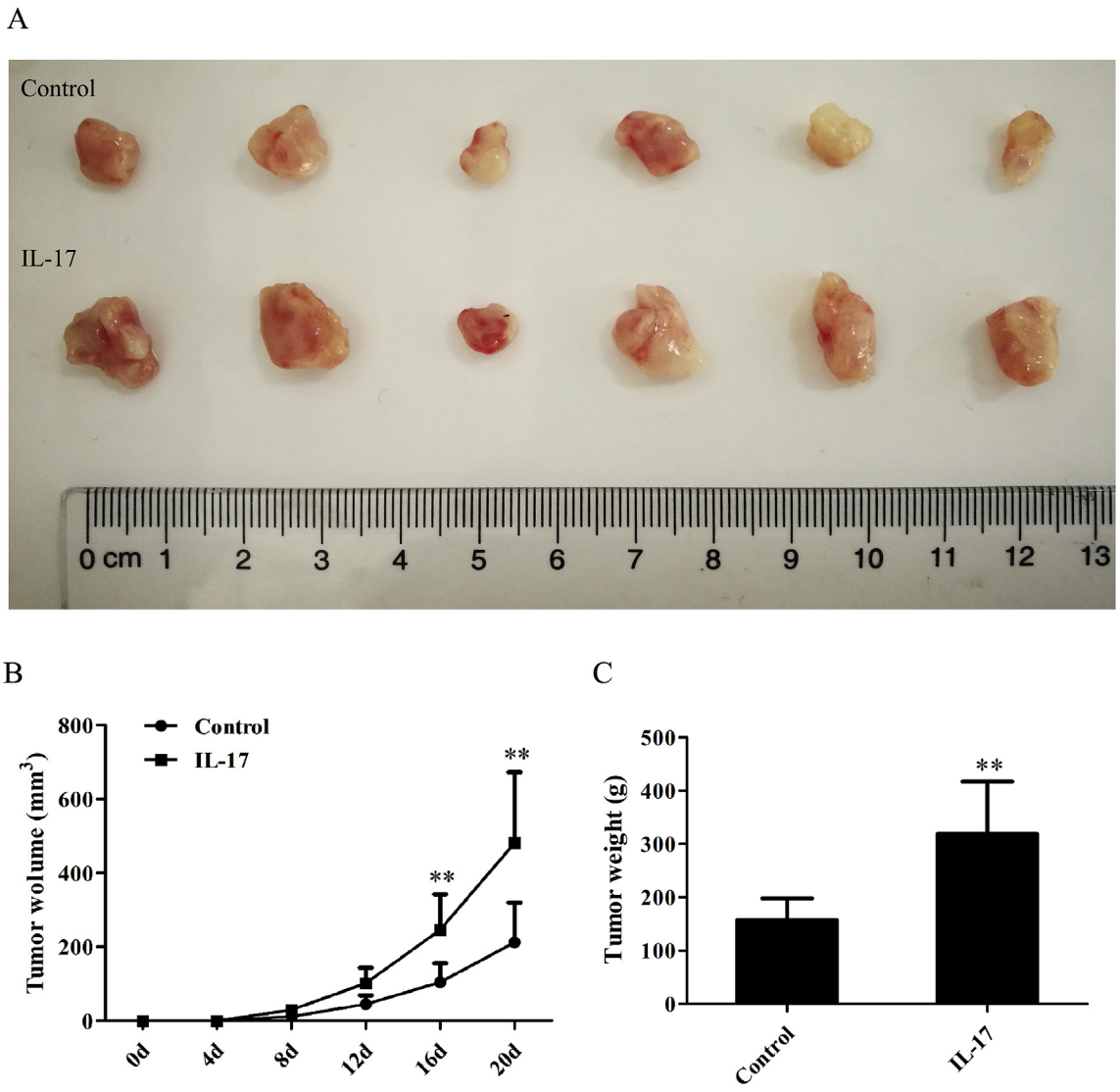

Fig. 3. IL-17 promotes the tumorigenesis of SHG-44 cells in nude mice. SHG-44 cells were incubated with or without 50 ng/ml of IL-17 for 15 min, and then injected into BALB/c nude mice subcutaneously for tumor growth until 3 weeks. Tumor sizes were detected at different time points and tumor weights were obtained at last time point. Data were represented as means ± SD (n = 6 in each group). $^{**}P < 0.01$ vs. control group.

of SHG-44 cells in response to IL-17 (Fig. 7). Taken together, these data indicate that IL-17 could induce the tumor formation of glioma cells through the activation of PI3K/Akt1/NF-κB-p65 axis.

4. Discussion

Glioma is the most aggressive and deadly form of adult brain cancer [31–33]. Reportedly, many pro-inflammatory cytokines such as IL-1β and IL-6 are closely involved in the proliferation and migration of glioma cells [7–9]. Recently, IL-17 elevation in some tumor microenvironment is confirmed and believed to contribute to tumor growth [13,14,18,34,35]. Although IL-17 secretion is also increased in the sera and tumor tissues of patients with glioma [15–17], the role and mechanism of IL-17 in the cell proliferation and migration of gliomas remain unknown. In the studies, we first evaluated IL-17 effects on the proliferation and migration of human glioma cell lines i.e. SHG-44 and U373, and the results showed that IL-17 simulation could not only increase the proliferation and migration of glioma cells, but also enhance the tumor formation and growth of glioma cells in nude mice, indicating that SHG-44 and U373 cells stimulated with IL-17 can promote the capability of cell proliferation and migration.

It has been known that tumor cell proliferation and migration induced by IL-17 is involved in the activation of ERK1/2, ERK5, STAT3 [18–20,36]. In current studies, to find the potential signaling pathways related to IL-17-induced proliferation and migration of glioma cells, some signal molecules were detected with antibody chip or Western blot. The data disclosed that IL-17-stimulated SHG-44 cells exhibited marked increase of p-Akt1 (Ser473) but not p-Akt1 (Thr308), which is also essential for Akt1 activation. In view of the sensitivity of antibody chip and distribution of time points, we further detected p-Akt1 (Thr308) by Western blot with new time points including 15 min, and the results exhibited that IL-17 could significantly elevate the level of p-Akt1 (Thr308) at 15 min. Here, the different time point distribution could explain why antibody chip did not show the increase of p-Akt1 (Thr308) in SHG-44 cells exposed to IL-17. Similar changes of Akt1 phosphorylation at these two sites were also observed in U373 cells after IL-17 incubation. Notably, although IL-17 treatment can increase the phosphorylation of other molecules such as ERK1/2 and STAT3 in other cancer cells [18–20], IL-17-treated glioma cells only exhibited marked phosphorylation of Akt1 in our experiments, reflecting the different signal responses of different tumor cells to IL-17 stimulation.

Reportedly, IL-17 can activate PI3K/Akt1 signal pathway in some

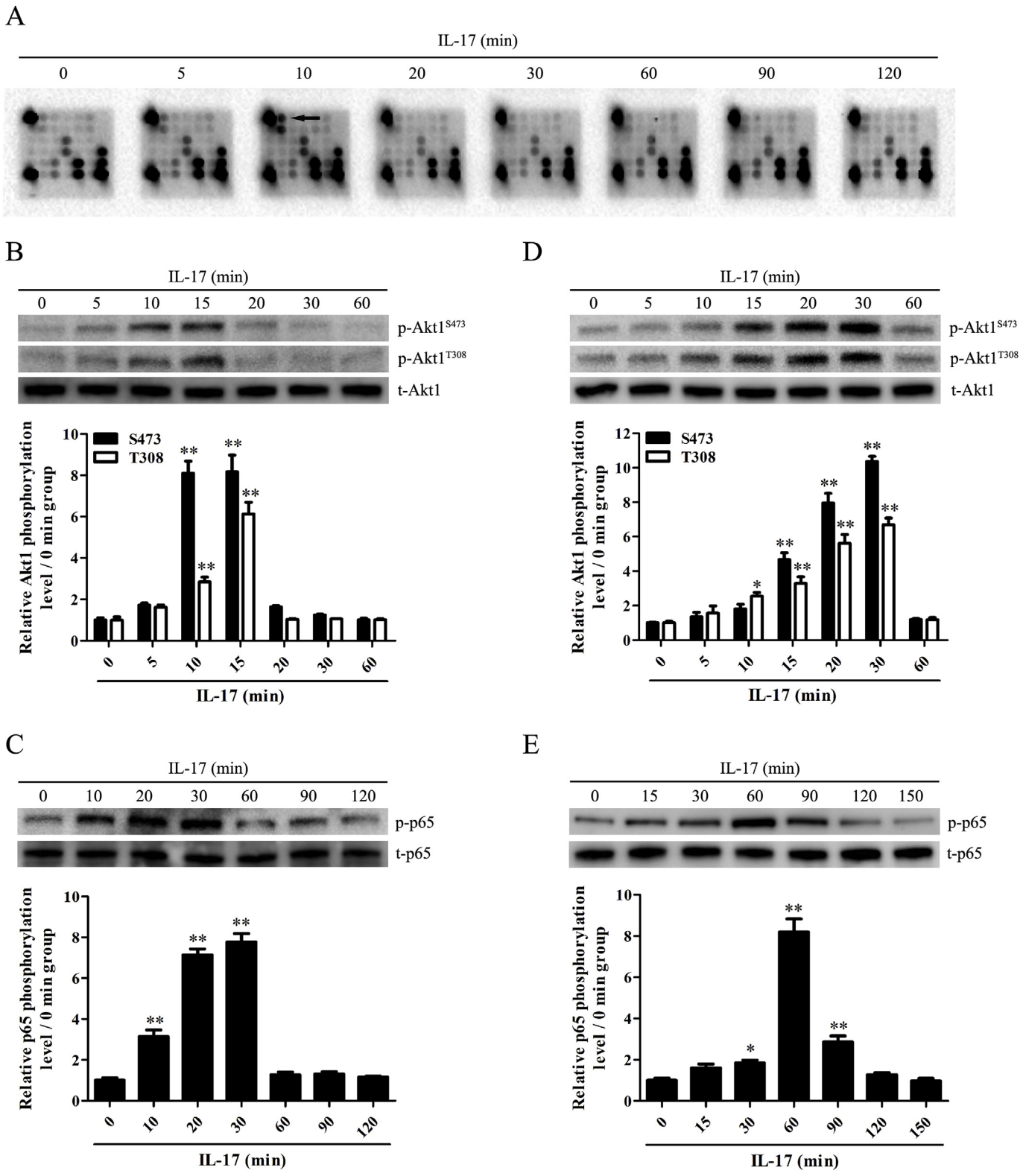

Fig. 4. The profile of signaling pathways in glioma cells induced by IL-17. (A) SHG-44 cells was stimulated with IL-17 at the dose of 50 ng/ml for different time points, the levels of different intracellular signaling molecules were determined with the antibody chips of intracellular signaling array. The site of p-Akt1 (Ser473) was pointed by an arrow. (B and C) SHG-44 cells were treated with 50 ng/ml of IL-17 for different time points, and the levels of p-Akt1 (Ser473), p-Akt1 (Thr308), t-Akt1, p-p65, t-p65 and β-actin were detected by Western blot. (D and E) U373 cells were incubated with 50 ng/ml of IL-17 for different time points, and the levels of p-Akt1 (Ser473), p-Akt1 (Thr308), t-Akt1, p-p65, t-p65 and β-actin were examined by Western blot. Data were represented as means ± SD (n = 3 in each time point). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. 0 min time point.

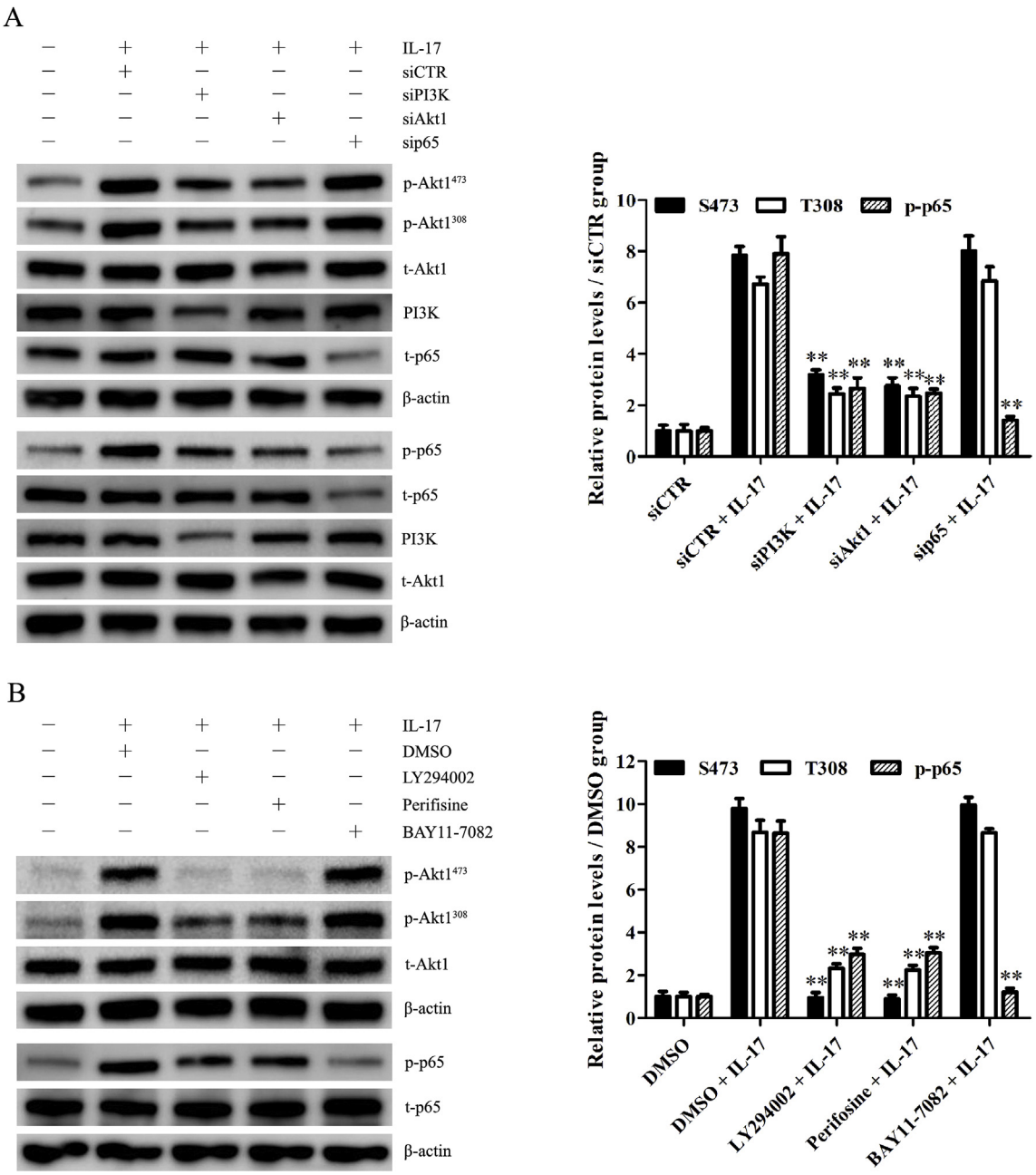

Fig. 5. The effects on siRNAs or inhibitors on PI3K, Akt1 and NF-κB-p65 expression and activation in SHG-44 cells in response to IL-17. SHG-44 cells were transfected with 100 nM of siPI3K, siAkt1, sip65 or siCTR (A), or treated with 10 μM of LY294002, Perifosine or BAY11-7082 (B), and then incubated with IL-17 for 15 and 30 min. The levels of p-Akt1 (Ser473), p-Akt1 (Thr308), t-Akt1 and β-actin at 15 min as well as p-p65, t-p65 and β-actin at 30 min were detected by Western blot. Data were represented as means ± SD (n = 3 in each group). $^{**}P < 0.01$ vs. siCTR + IL-17 group, or DMSO + IL-17 group.

types of cells, such as epidermal keratinocytes, bronchial epithelial cells and salivary gland cells [37–39]. Since PI3K/Akt1 signal pathway plays an important role in tumor growth including glioma [21,40], the effect of PI3K/Akt1 activation in the proliferation and migration of glioma cells induced by IL-17 need to be further explored. Our results proved that knockdown or inhibition of PI3K and Akt1 could markedly reduce the proliferation and migration of SHG-44 and U373 cells in response to IL-17 stimulation. Meanwhile, PI3K knockdown or inhibition obviously reduced Akt1 phosphorylation, suggesting that PI3K-dependent Akt1 activation contributes to the proliferation and migration of glioma cells exposed to IL-17 *in vitro*.

Because NF-κB-p65 is also confirmed to promote the proliferation and migration of glioma cells [27,28], we further determined the activation and role of NF-κB-p65 in the proliferation and migration of glioma cells exposed to IL-17. As expected, IL-17 stimulation greatly increased the phosphorylation of NF-κB-p65 at Ser536 (as a marker of NF-κB-p65 activation) both in SHG-44 and U373 cells in a time-dependent manner. Besides, PI3K or Akt1 knockdown or inhibition obviously reduced NF-κB-p65 phosphorylation, but NF-κB-p65 knockdown or inhibition had no significant effect on Akt1 phosphorylation, indicating that PI3K/Akt1 can affect NF-κB-p65 activation. Furthermore, the knockdown or inhibition of NF-κB-p65 *in vitro* could remarkably reduce the proliferation and migration of glioma cells induced by IL-17. Additionally, inhibition of PI3K, Akt1 and NF-κB-p65 activation *in vivo* could also significantly reduce the tumor formation and growth of glioma cells in response to IL-17 stimulation. Taken together, these data imply the activation of PI3K/Akt1/NF-κB-p65 pathway can really promote the proliferation and migration of glioma

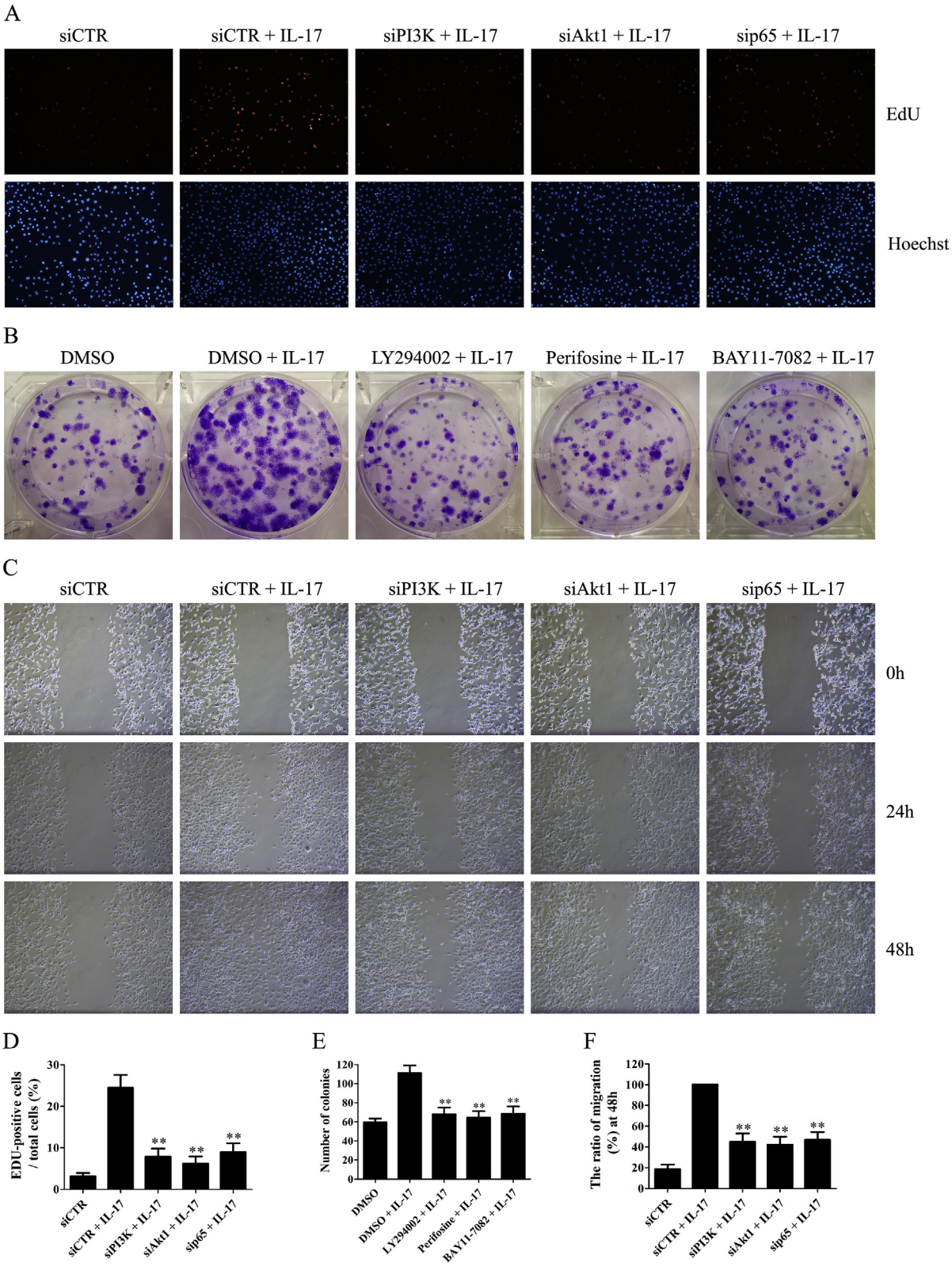

Fig. 6. The roles of PI3K, Akt1, NF-κB-p65 in the proliferation and migration of SHG-44 cells exposed to IL-17. SHG-44 cells were transfected with 100 nM of siPI3K, siAkt1, sip65 or siCTR (A, C, D, F), or treated with 10 μM of LY294002, Perifosine or BAY11-7082 (B and E), and then stimulated with IL-17 for different time points. EdU assay (A and D, magnification × 100), colony formation (B and E) and scratch experiment (C and F, magnification × 100) were done respectively. Data were represented as means ± SD (n = 3 in each group). $^{**}P < 0.01$ vs. siCTR + IL-17 group, or DMSO + IL-17 group.

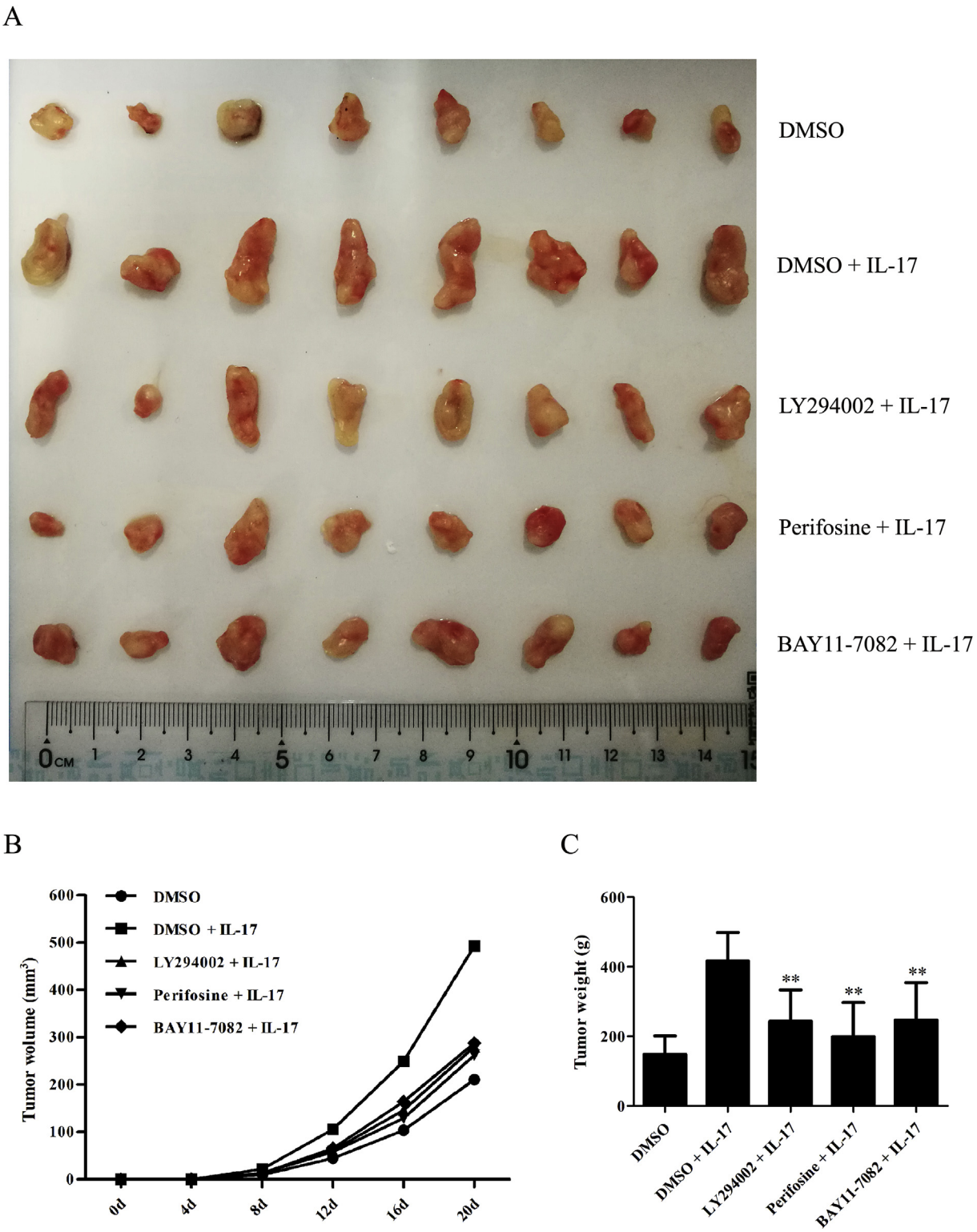

Fig. 7. Inhibition of PI3K, Akt1 and NF-κB-p65 reduces the tumor formation of SHG-44 cells stimulated by IL-17. SHG-44 cells were incubated with 10 μM of LY294002, Perifosine, BAY11-7082 or DMSO control for 15 min, and then treated with or without 50 ng/ml of IL-17 for 15 min. Finally cells injected into BALB/c nude mice subcutaneously for tumor growth for 3 weeks. Tumor sizes (B) were detected at different time points, tumors were taken photos (A), and tumor weights (C) were obtained at last time point. Data were represented as means ± SD (n = 8 in each group). $^{**}P < 0.01$ vs. DMSO + IL-17 group.

cells and elevate the ability of tumor formation and growth in glioma cells exposed to IL-17. As for the mechanisms about PI3K/Akt1 regulates NF-kB-p65 activation in IL-17-stimulated glioma cells, there are several possible mechanisms as follows: (1) PI3K/Akt1 activation leads to IκB kinase (IKK) activation and subsequent IκB degradation and NF-kB-p65 activation [41]. (2) PI3K/Akt1 activation results in an increase in IKK expression and sustained IκB degradation and NF-kB-p65 activation [42]. (3) PI3K/Akt1 might regulate NF-kB-p65 activation through other signaling molecules.

Here, it is worthy to mention that NF-κB activation is involved in the

induction of some pro-inflammatory cytokines or mediators, which also increase tumor cell proliferation and migration [43–45]. For example, the migration of glioma cells can be enhanced and facilitated via NF-κB-mediated IL-8 production [45]. Therefore, IL-17-induced production of inflammatory factors from glioma cells through NF-κB activation and their possible roles in glioma growth are worthy to be explored in our future studies.

In summary, our present studies first determined the roles and mechanisms of IL-17 in the proliferation and migration of glioma cells both *in vitro* and *in vivo*, and found that IL-17 could not only increase the proliferation and migration of cultured glioma cells (*in vitro*), but also promote the glioma formation and growth in nude mice (*in vivo*). During the process of proliferation and migration of glioma cells upon IL-17 exposure, the phosphorylation of Akt1 and NF-κB-p65 was elevated, and PI3K-dependent Akt1 phosphorylation was necessary for NF-κB-p65 activation. Knockdown or inhibition of PI3K, Akt1 and NF-κB-p65 could not only lessen the proliferation and migration of glioma cells in response to IL-17, but also alleviate *in vivo* tumor formation of glioma cells treated with IL-17. Collectively, these data implicate that IL-17 promotes the proliferation and migration of glioma cells via the activation of PI3K/Akt1/NF-κB-p65 axis and may provide new insight into the pathogenesis of glioma.

Conflicts of interest

All the authors declared no competing interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.canlet.2019.01.008.

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