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PRKAA1 Promotes Proliferation and Inhibits Apoptosis of Gastric Cancer Cells Through Activating JNK1 and Akt Pathways

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PRKAA1 (protein kinase AMP-activated catalytic subunit α 1) is a catalytic subunit of AMP-activated protein kinase (AMPK), which plays a key role in regulating cellular energy metabolism through phosphorylation, and genetic variations in the PRKAA1 have been found to be associated with gastric cancer risk. However, the effect and underlying molecular mechanism of PRKAA1 on gastric cancer tumorigenesis, especially the proliferation and apoptosis, are not fully understood. Our data showed that PRKAA1 is highly expressed in BGC-823 and MKN45 cells and is expressed low in SGC-7901 and MGC-803 cells in comparison with the other gastric cancer cells. PRKAA1 downregulation by shRNA or treatment of AMPK inhibitor compound C significantly inhibited proliferation as well as promoted cell cycle arrest and apoptosis of BGC-823 and MKN45 cells. Moreover, the expression of PCNA and Bcl-2 and the activity of JNK1 and Akt signaling were also reduced in BGC-823 and MKN45 cells after PRKAA1 downregulation. In vivo experiments demonstrated that tumor growth in nude mice was significantly inhibited after PRKAA1 silencing. Importantly, inactivation of JNK1 or Akt signaling pathway significantly inhibited PRKAA1 overexpression-induced increased cell proliferation and decreased cell apoptosis in MGC-803 cells. In conclusion, our findings suggest that PRKAA1 increases proliferation and restrains apoptosis of gastric cancer cells through activating JNK1 and Akt pathways.

Key words: Gastric cancer; PRKAA1; Apoptosis; JNK1; Akt

INTRODUCTION

Malignant tumors are the second major cause of human death, and about 60–70% of the malignant tumors occur in the digestive system. Gastric cancer remains a major cause of death among malignant diseases[1], and the incidence of gastric cancer in China is ranked top among all kinds of tumors[2]. The number of deaths in China is up to 300,000, accounting for about 25–30% of all malignant tumor deaths[3]. The early onset of gastric cancer is occult and usually without special clinical symptoms or only with some nonspecific symptoms of the digestive tract, which makes it difficult for early diagnosis. Therefore, it is characterized by high mortality and incidence, as few gastric cancer cases are diagnosed in the early stage and most are in the advanced stage[4]. In addition, although gastric cancer has declined in global incidence, it is increased in young people, especially those under 30 years of age[5]. Therefore, finding genes and signaling pathways that affect the development of gastric cancer is of great significance for early diagnosis and molecular targeting therapy.

The PRKAA1 (protein kinase AMP-activated catalytic subunit α 1) protein is a catalytic subunit of the mammalian 5′-AMP-activated protein kinase (AMPK), which belongs to the serine/threonine protein kinase family and is a cell energy sensor in eukaryotic cells that regulates the level of intracellular nutrition and energy through glucose and lipid metabolic pathways[6]. AMPK has been found to participate in the regulation of tumor development through a variety of signaling pathways. The tumor suppressor gene LKB1 is the upstream gene of AMPK[7], which acts on the

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Thrl72 site of the α subunit N-terminal kinase domain and activates AMPK through energy stress feedback, and is further involved in the regulation of cell apoptosis, differentiation, and proliferation through its downstream pathway[8]. AMPK also regulates downstream tumor-related signaling pathways such as p53 and mTOR, by restarting cell metabolic process and altering the critical point of metabolism in cell cycle, to participate in the tumorigenesis of cancer[9,10]. The mutation of PRKAA1 in prostate cancer cells reduces p53 expression at both mRNA and protein levels and promotes the growth of cancer cells[11]. PRKAA1 deletion was recently considered as a promising strategy for cancer treatment[12], and the genetic variant of PRKAA1 is a risk factor for gastric cancer[13]. However, the underlying molecular mechanism by which PRKAA1 is involved in the gastric cancer is not known.

Cell survival and apoptosis are of great importance in the occurrence as well as development of cancer. Akt serves as an important regulatory factor of survival, influencing cancer treatment through inhibiting the activity itself and its downstream signaling pathways[14]. Akt phosphorylation negatively regulates Bad, blocking its inhibition of apoptosis inhibitor Bcl-2, and thus inhibiting apoptosis[15]. Akt is a signaling kinase known to be inactivated by activated AMPK[16,17]. A previous study showed that AMPK activation in ovarian cancer cells induced cell apoptosis through inhibiting Akt activity, which was increased for survival[18]. c-Jun N terminal kinase (JNK) signaling pathway plays an important role in mitogen-activated protein kinase (MAPK) and is central for a number of biochemical signals involved in various cellular processes, including differentiation, proliferation, development, and transcription regulation[19]. It has been reported that the JNK pathway is crucial for the induction of cancer cell apoptosis via regulating the Bcl-2 family proteins[20]. JNK activation is also associated with cell apoptosis of gastric cancer through a mitochondrial apoptotic pathway[21]. In addition, AMPK activation induced apoptosis through stimulation of JNK in MIN6 cells[22]. Thus, AMPK–JNK or AMPK–Akt might serve as an important signaling pathway for regulating cancer cell apoptosis as well as proliferation. However, its role in gastric cancer remains an open question.

In the present study, we sought to determine if the cell proliferation, cell cycle, and apoptosis of gastric cancer was regulated by PRKAA1. Additionally, the JNK and Akt signaling pathways' response to PRKAA1 in gastric cancer cells was also investigated.

MATERIALS AND METHODS

Bioinformatics

Gene set enrichment analysis (GSEA) was used to identify the pathways that were significantly enriched between gastric cancer patients with high and low PRKAA1 expression.

Cell Culture

MKN28, AGS, MGC-803, SGC-7901, BGC-823, and MKN45 human gastric cancer cell lines and a normal gastric cell line GES-1 were obtained from Life Technologies (Gaithersburg, MD, USA) and were maintained in a humidified incubator at 37°C and 5% CO_2. MGC-803, MKN45, and AGS cells were cultivated in RPMI-1640 medium (Life Technologies), and BGC-823, MKN28, and SGC-7901 cells were cultivated in DMEM medium (Life Technologies) plus 10 mM glucose, containing 10% fetal bovine serum, 100 μg/ml streptomycin, and 100 U/ml penicillin (all from Gibco, Grand Island, NY, USA).

Cell Transfection

Oligonucleotides encoding shRNA targeting human PRKAA1 (point 619–637 shRNA-1, 5′-GGTAGATAT ATGGAGCAGT-3′; point 1,320–1,338 shRNA-2, 5′-ACCCATATTATTTGCGTGT-3′; point 1,376–1,394 shRNA-3, 5′-ATGAGTCTACAGTTATACC-3′) as well as scramble shRNA were cloned into the pLKO.1 lentiviral vector. The cDNA encoding PRKAA1 was obtained by reverse transcription PCR and cloned into the pLVX-Puro for constructing the pLVX-Puro-PRKAA1 expression vector. The pLKO.1-scramble shRNA (shNC) and blank pLVX-Puro (vector) were used as negative control. 293T cells, were seeded in six-well plates and transfected with constructs at 37°C for 5 h using Lipofectamine reagent (Invitrogen, Grand Island, NY, USA) in accordance with the instruction of the manufacturer. Forty-eight hours after transfection, recombined lentivirus was collected and used for BGC-823, MKN45, or MGC-803 cell infection.

CCK-8 Assay

The Cell Counting Kit (CCK)-8 (Beyotime, Shanghai, P.R. China) assay was performed to examine gastric cancer cell proliferation. Briefly, BGC-823, MKN45, or MGC-803 cells with a density of 3×10^3 cells/well were performed following standard procedure in 96-well plates and maintained in a 5% CO_2 incubator at 37°C overnight. BGC-823 and MKN45 cells were transduced with pLKO.1-PRKAA1-shRNA or treated with AMPK inhibitor compound C (20 μM; EMD Millipore, Billerica, MA, USA), and MGC-803 cells were transduced with pLVX-Puro-PRKAA1 or blank pLVX-Puro (vector) and treated with JNK inhibitor SP600125 (20 μM; Abcam, Cambridge, UK) or Akt inhibitor MK-2206 (10 μM; Selleck Chemicals, Houston, TX, USA). After 0, 24, 48, and 72 h, CCK-8 solution (10 μl per well) was added into the cells, which were then maintained in a CO_2 incubator

for 1 h at 37°C, after which the absorbance readings were obtained at 450 nm.

Flow Cytometry Assay

Briefly, BGC-823, MKN45, or MGC-803 cells with a density of 3×10^5 cells/well were seeded in six-well plates and maintained in a humidified incubator at 37°C for 24 h. BGC-823 and MKN45 cells were transduced with pLKO.1-PRKAA1-shRNA or treated with compound C (20 μM), and MGC-803 cells were transduced with pLVX-Puro-PRKAA1 or blank pLVX-Puro (vector) and treated with JNK inhibitor SP600125 (20 μM) or Akt inhibitor MK-2206 (10 μM). After 48 h, cells were centrifuged at 1,000 × *g* for 5 min, fixed with 700 μl of precooled absolute ethyl alcohol, incubated with 1 mg/ml of RNase A (100 μl; Sigma-Aldrich, St. Louis, MO, USA) in the dark for 30 min, and stained with 50 μg/ml of propidium iodide (PI; 400 μl; Invitrogen) for 10 min for cell cycle assay or otherwise incubated with 5 μl of Annexin-V–FITC (BD Pharmingen, San Diego, CA, USA) for 15 min and 5 μl of PI for 5 min at 4°C. Cell cycle progression and apoptosis were assayed on a flow cytometer (Becton-Dickinson FACS Calibur, San Jose, CA, USA).

In Vivo Tumorigenesis in Nude Mice

Animal maintenance and experimental procedures were approved by the Xuzhou Central Hospital, Xuzhou Medical University Institutional Ethical Committee, P.R. China. We confirm that all research animals were obtained and used in compliance with the relevant guidelines and regulations of Xuzhou Central Hospital, Xuzhou Medical University Institutional Ethical Committee. For in vivo tumorigenesis assay, a total of 5×10^6 BGC-823 cells transduced with pLKO.1-PRKAA1-shRNA or shNC were trypsinized, resuspended in PBS, and then subcutaneously injected into the right armpit of 4- to 5-week-old BALB/c male nude mice (six per group) obtained from SLAC Laboratory Animal Center, Shanghai, P.R. China. Tumor volume was calculated as $0.5 \times \text{length} \times \text{width}^2$. Mice were sacrificed at 33 days after injection, and the tumors were weighed.

Quantitative Real-Time PCR

Total RNA was collected from gastric cancer cell lines and xenograft from nude mice using the miRNeasy kit (QIAGEN, Hilden, Germany). cDNA was synthesized using a PrimeScript reagent kit (Takara, Otsu, Shiga, Japan) in accordance with protocols of the manufacturer. Quantitative real-time PCR using SYBR Green (Takara) was performed using the GeneAmp PCR Systems 2700 (Applied Biosystems, Foster City, CA, USA). The primers used in the present study were: 5′-TTGA AACCTGAAAATGTCCTGCT-3′ (PRAKK1-F) and 5′-GGTGAGCCACAACTTGTTCTT-3′ (PRAKK1-R); 5′-AACCAGGAGAAAGTTTCAG-3′ (PCNA-F) and 5′-GCACAGGAAATTACAACAG-3′ (PCNA-R); 5′-CTGAGCGAGTGTCTCAAG-3′ (Bax-F) and 5′-CAG CCCATGATGGTTCTG-3′ (Bax-R); 5′-TCCCTCGCT GCACAAATAC-3′ (Bcl-2-F) and 5′-TGGAAGGCCA CATCTGAAC-3′ (Bcl-2-R); 5′-AATCCCATCACCATC TTC-3′ (GAPDH-F) and 5′-AGGCTGTTGTCATAC TTC-3′ (GAPDH-R). The internal control for mRNA is given as ratio to GAPDH, respectively. The relative quantification was calculated using the $2^{-\Delta\Delta Ct}$ cycle threshold method.

Western Blotting

Total protein was collected from gastric cancer cell lines and xenograft from nude mice using RIPA lysis buffer for 30 min at 4°C containing protease inhibitors, and the homogenates were centrifuged at 12,000 × *g* for 20 min at 4°C. Protein concentration was estimated by a BCA Protein kit (Thermo Scientific, Waltham, MA, USA). Equal amounts of proteins (25 μg) were separated by 10–15% SDS-PAGE and transferred into nitrocellulose membrane (Millipore). After blocking with 5% fat-free milk overnight at 4°C, the blots were incubated with anti-PRAKK1 (Abcam), anti-PCNA (Cell Signaling Technology, Danvers, MA, USA), anti-Bcl-2 (Santa Cruz Biotechnology, Dallas, TX, USA), anti-p-ERK1 (Abcam), anti-ERK1 (Abcam), anti-p-STAT3 (Abcam), anti-STAT3 (Cell Signaling Technology), anti-p-JNK1 (Abcam), anti-JNK1 (Abcam), anti-p-Akt (Cell Signaling Technology), anti-Akt (Cell Signaling Technology), and anti-GAPDH (Cell Signaling Technology) antibody overnight at 4°C. The blots were then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:1,000; Beyotime) for 1 h at 37°C. The membranes were developed using an enhanced chemiluminescence (ECL) kit (Applygen Technologies, Beijing, P.R. China) following the manufacturer's instructions.

Statistical Analysis

Data are presented as mean ± SD, and each test was repeated at least three times. The Statistical Package for the Social Sciences (SPSS, version 14) was used for statistical analysis. Comparison among data from various groups used one-way or two-way ANOVA. Significance was defined as a two-tailed value of $p < 0.05$.

RESULTS

PRKAA1 Expression in Gastric Cancer Cell Lines

To determine the function of PRKAA1 in gastric cancer tumorigenesis, PRKAA1 expression in different gastric cancer cell lines, including MKN28, AGS, MGC-803, SGC-7901, BGC-823, and MKN45, was measured

first. As shown in Figure 1A–C, PRKAA1 was highly expressed in all of the gastric cancer cells detected compared with a normal gastric cell line GES-1, and BGC-823. MNK45 cells showed higher mRNA and protein expression of PRKAA1, and MGC-803 and SGC-7901 cells demonstrated a lower PRKAA1 expression, compared with the other gastric cancer cells.

PRKAA1 Silencing Inhibits Cell Proliferation and Induces Apoptosis of Gastric Cancer

In view of the GSEA data that PRKAA1 expression is correlated with cell cycle and apoptotic execution phase (Fig. 2A), and thus the biological role of PRAKK1 in regulating cell proliferation, cell cycle and cell apoptosis of gastric cancer were measured. PRKAA1 was knocked down in BGC-823 and MNK45 cells, which expressed high levels of PRKAA1. Our results showed that three pLKO.1-PRKAA1-shRNAs' transduction significantly decreased the mRNA expression of PRKAA1 in both BGC-823 and MNK45 cells, with the higher knockdown efficiency detected in BGC-823 and MNK45 cells with pLKO.1-PRKAA1-shRNA-1 and pLKO.1-PRKAA1-shRNA-3 transduction (Fig. 2B and C), and were therefore used in our following experiments.

Next, gastric cancer cell proliferation, cell cycle, as well as apoptosis were measured by CCK-8 and flow cytometry assay. Depletion of PRKAA1 by shRNA or compound C treatment resulted in a significant reduction in cell proliferation compared with the shNC group in both BGC-823 and MNK45 cells at 24, 48, and 72 h, respectively (Fig. 2D and E). Depletion of PRKAA1 by shRNA or compound C treatment in BGC-823 and MNK45 cells significantly increased the number of cells in G_0/G_1 phase and decreased the number of cells in S and G_2/M phases, compared with the shNC group (Fig. 3A and B). Moreover, cell apoptosis was significantly increased in BGC-823 and MNK45 cells after depletion of PRKAA1 by shRNA or compound C treatment compared with the shNC group (Fig. 3C and D).

PRKAA1 Silencing Inhibits PCNA and Bcl-2 Expression as Well as JNK1 and Akt Activation in Gastric Cancer Cells

Subsequently, the expression of cell proliferation- and apoptosis-related factors such as PCNA, Bax, and Bcl-2 was also detected in vitro. As shown in Figure 4A–D, depletion of PRKAA1 by shRNA or compound C treatment markedly inhibited the PCNA and Bcl-2 expression in both BGC-823 and MNK45 cells compared with the shNC group, but the Bax mRNA expression was not changed in both BGC-823 and MNK45 cells after PRKAA1 knockdown (data not shown). Moreover, the activity of JNK1 and Akt signaling pathways was also measured in BGC-823 and MNK45 cells. Our results showed that depletion of PRKAA1 by shRNA or compound C treatment significantly decreased the protein expression of p-JNK1 and p-Akt in both BGC-823 and MNK45 cells (Fig. 4E and F), but had no effect on the JNK1 and Akt expression as well as on the activity of ERK and STAT3 (data not shown), compared with the shNC group. These results indicate that PRKAA1 may regulate gastric cancer cell survival through JNK1 and Akt signaling pathways.

PRKAA1 Silencing Inhibits Tumor Growth of Gastric Cancer In Vivo

To determine whether depletion of PRKAA1 in gastric cancer cells could reduce tumor growth in vivo, BGC-823 cells stably transduced with pLKO.1-PRKAA1-shRNA

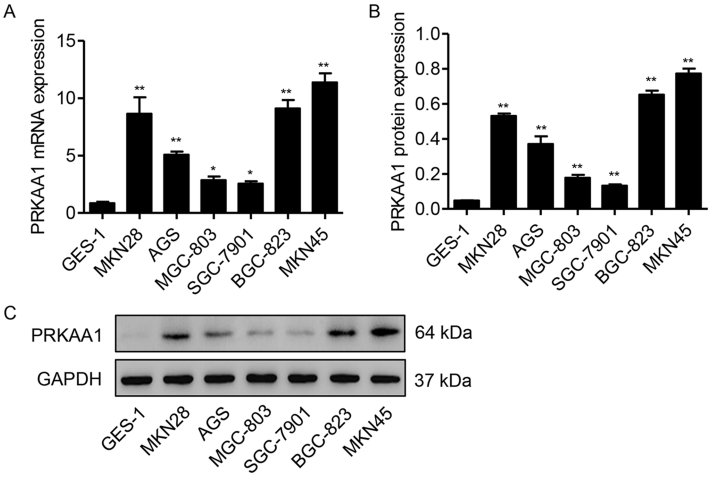

Figure 1. Protein kinase AMP-activated catalytic subunit α 1 (PRKAA1) expression in gastric cancer cell lines. PRKAA1 expression in different gastric cancer cell lines, including MKN28, AGS, MGC-803, SGC-7901, BGC-823, and MKN45, and a normal gastric cell line GES-1 were measured by quantitative real-time PCR (A) and Western blotting (B, C), respectively. $n = 3$, $^*p < 0.05$, $^{**}p < 0.01$ compared with GES-1.

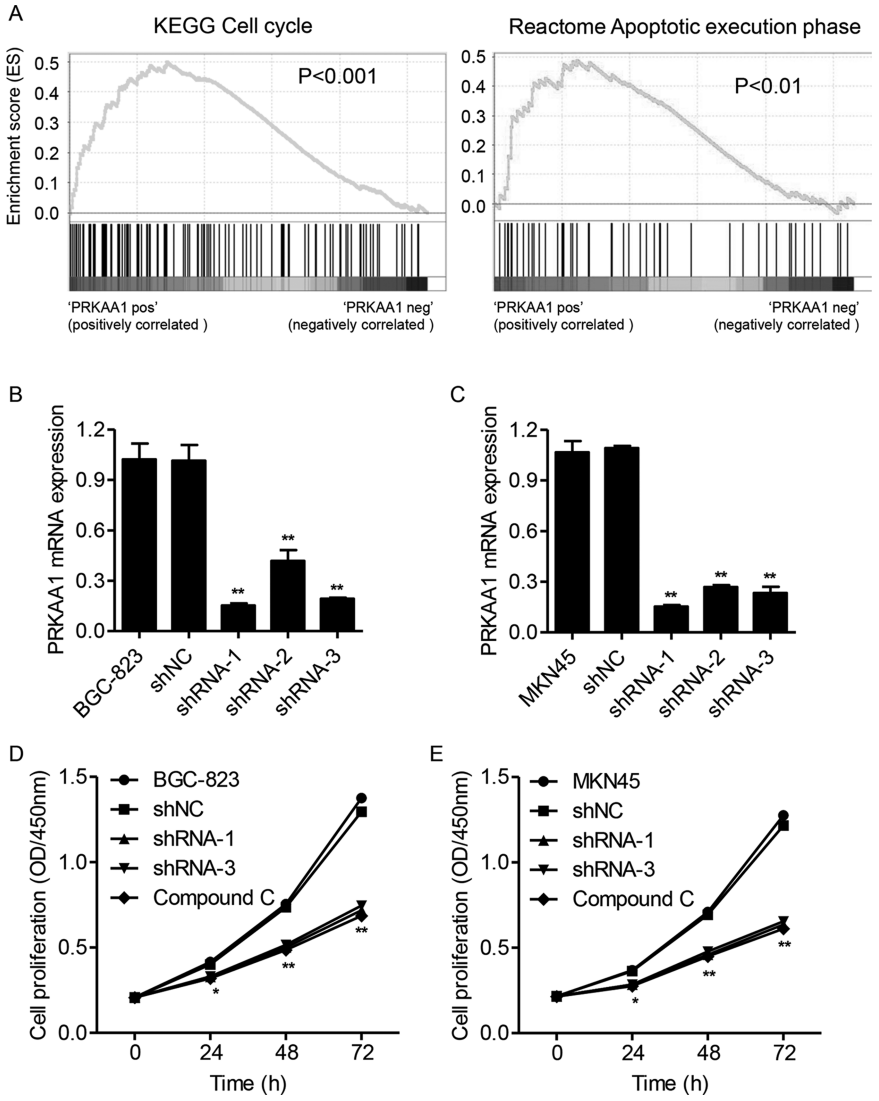

Figure 2. PRKAA1 silencing inhibits proliferation of gastric cancer cells. (A) Gene set enrichment analysis (GSEA) demonstrated that the genes of the cell cycle and apoptotic execution phase were correlated with gastric cancer patients with PRKAA1 low versus PRKAA1 high. After BGC-823 and MKN45 cells were transduced with pLKO.1-PRKAA1-shRNA-1, -2, -3, or shNC, PRKAA1 mRNA expression was measured by quantitative real-time PCR (B, C). After BGC-823 and MKN45 cells were transduced with pLKO.1-PRKAA1-shRNA, shNC or treated with 20 μM compound C, cell proliferation (D, E) was measured by cell counting kit (CCK)-8 assay, respectively. $n = 3$, $*p < 0.05$, $**p < 0.01$ compared with shNC.

or shNC were subcutaneously injected into nude mice. The mRNA and protein expression of PRKAA1 was significantly decreased in xenograft from the nude mice (Fig. 5A–C). pLKO.1-PRKAA1-shRNA-treated tumors grew much slower than the pLKO.1-NC-shRNA-treated tumors in nude mice (Fig. 5D). Mice were killed 33 days after injection, with average tumor weights of 1.44 ± 0.21 g and 0.35 ± 0.08 g in pLKO.1-NC-shRNA- and pLKO.1-PRKAA1-shRNA-treated mice, respectively (Fig. 5E and F). These data indicate an effect of PRKAA1 on the promotion of gastric cancer cell growth in vivo.

PRKAA1 Overexpression Promotes Cell Proliferation and Restrains Apoptosis of Gastric Cancer Through Activation of the JNK1 and Akt Pathways

To further investigate the involvement of JNK1 and Akt signaling pathways in PRKAA1-mediated gastric cancer, JNK1 and Akt signaling pathway inhibitors SP600125 and MK-2206 were introduced in the MGC-803 cells with PRKAA1 overexpression. PRKAA1 was overexpressed in MGC-803 cells, which expressed a lower level of PRKAA1. Our results showed that pLVX-Puro-PRKAA1 transduction significantly increased the

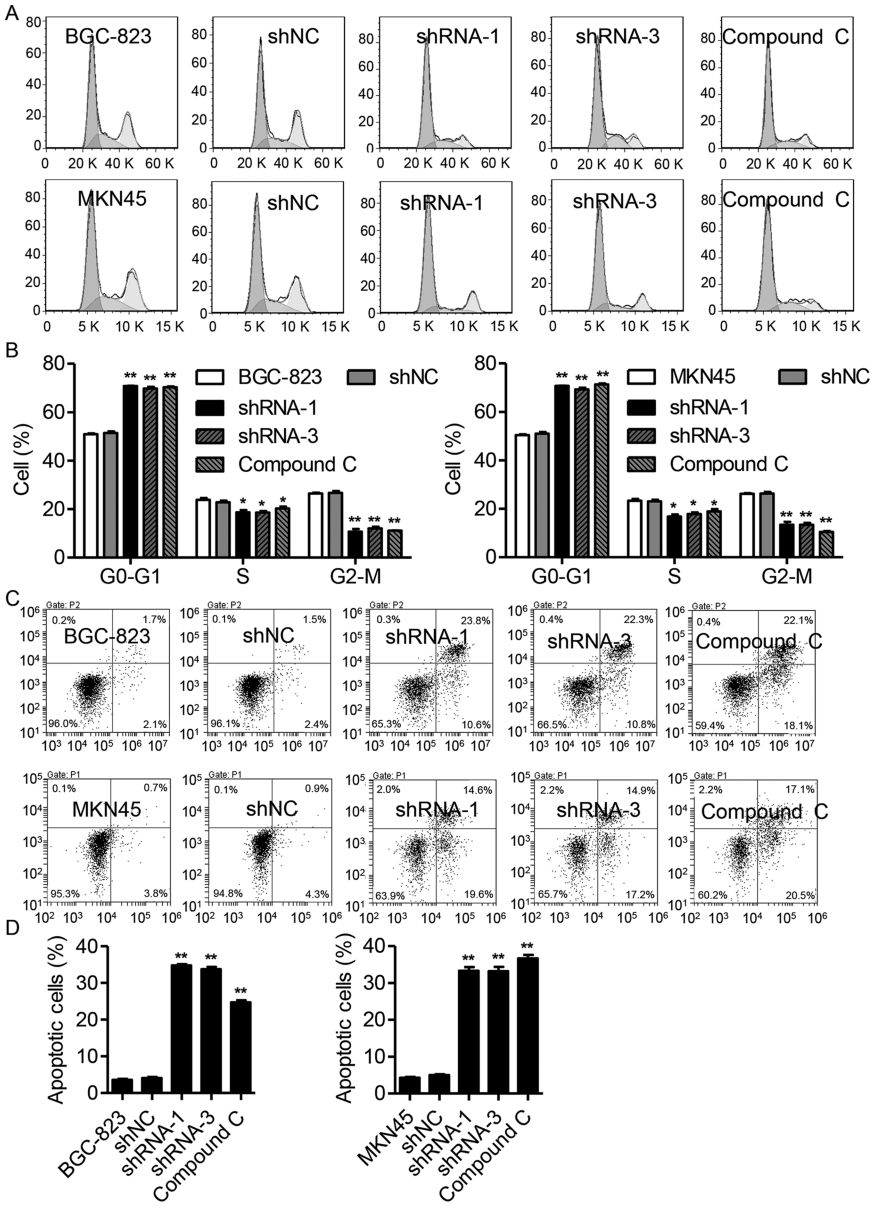

Figure 3. PRKAA1 silencing induced cell cycle arrest and apoptosis of gastric cancer cells. After BGC-823 and MKN45 cells were transduced with pLKO.1-PRKAA1-shRNA, shNC, or treated with 20 μM compound C, the cell cycle progression (A, B) and apoptosis (C, D) were measured by flow cytometry assay, respectively. $n = 3$, $^{*}p < 0.05$, $^{**}p < 0.01$ compared with shNC.

mRNA expression of PRKAA1 by 7.3-fold in MGC-803 cells compared with the blank pLVX–Puro (vector) group (Fig. 6A). CCK-8 assay demonstrated that overexpression of PRKAA1 resulted in a significant enhancement in cell proliferation compared with the vector group in MGC-803 cells at 24, 48, and 72 h, respectively (Fig. 6B). Overexpression of PRKAA1 significantly decreased the number of cells in the G_0/G_1 phase by 8.6% and increased the number of cells in the S phase by 18.6%, compared with the vector group (Fig. 6C and D). Moreover, cell apoptosis was significantly decreased by 66.7% in MGC-803 cells after PRKAA1 overexpression compared with the vector group (Fig. 6E and F). PRKAA1 overexpression also increased PCNA and Bcl-2 expression as well as JNK1 and Akt activation in MGC-803 cells. However, the effect of PRKAA1-induced cell behavior and PCNA and Bcl-2 expression were significantly reversed by treatment of SP600125 or MK-2206. Treatment of SP600125 or MK-2206 alone did not change the MGC-803 cell behavior and PCNA and Bcl-2 expression (data not

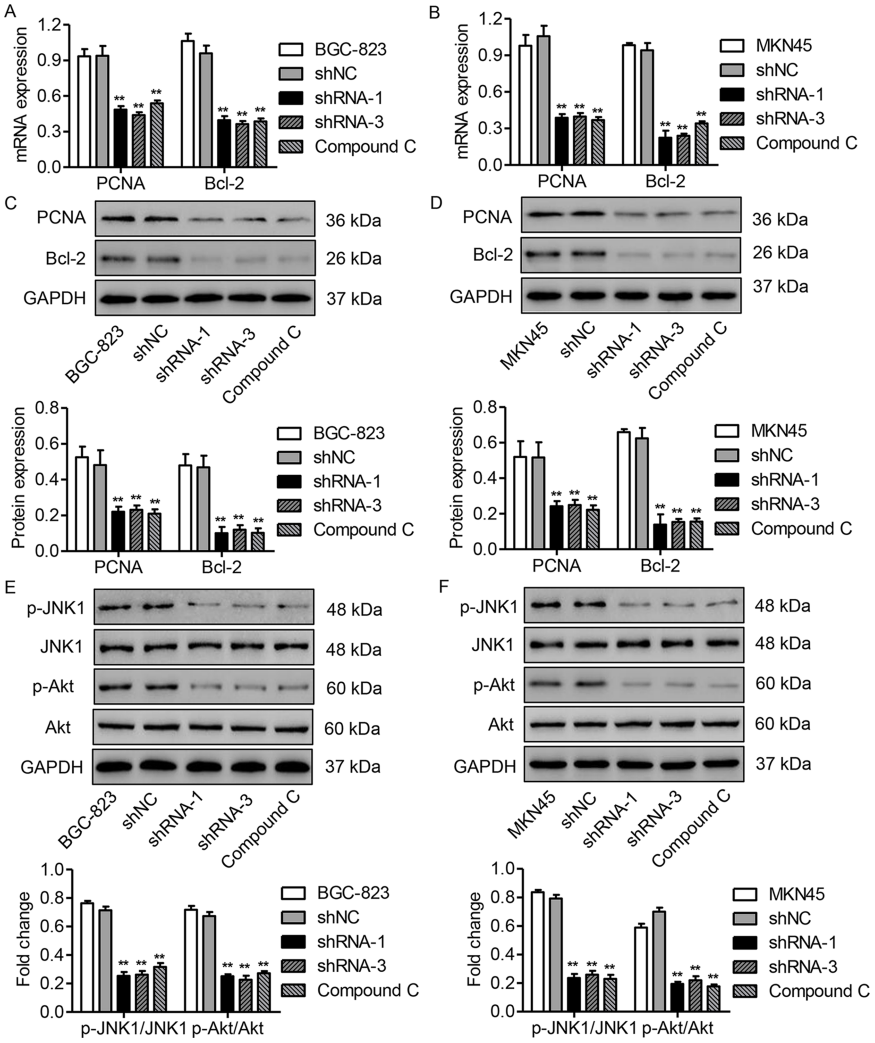

Figure 4. PRKAA1 silencing inhibits PCNA and Bcl-2 expression as well as JNK1 and Akt activation in gastric cancer cells. After BGC-823 and MKN45 cells were transduced with pLKO.1-PRKAA1-shRNA, shNC, or treated with 20 μM compound C, the mRNA and protein expression of PCNA and Bcl-2 was measured by quantitative real-time PCR (A, B) and Western blotting (C, D), respectively, and the protein expression of p-JNK1, JNK1, p-Akt, and Akt was measured by Western blotting (E, F). $n = 3$, $^{**}p < 0.01$ compared with shNC.

shown). These findings indicate an effect of PRKAA1 on the promotion of gastric cancer cell survival through activation of the JNK1 and Akt signaling pathways.

DISCUSSION

Tumor cells are characterized by uncontrolled proliferation and apoptosis evading. Our important findings, to our knowledge, are the first to determine the role of PRKAA1 in the proliferation and apoptosis of gastric cancer both in vitro and in vivo. PRKAA1 is critical to cancer progression as well as in clinical prognosis[23,24]. In previous studies, PRKAA1 was overexpressed in cervical cancer[25] and in prostate cancer[26], suggesting an oncogenic role of PRKAA1 in the tumorigenesis of cancer. However, the effect of PRKAA1 in gastric cancer tumorigenesis is not well characterized.

In this study, our results demonstrated that PRKAA1 was highly expressed in all of the gastric cancer cells detected compared with a normal gastric cell line GES-1, and PRKAA1 downregulation in BGC-823 and MKN45 gastric cancer cells showed significant decrease in cell proliferation and increase in cell apoptosis and cell cycle arrest. PRKAA1 overexpression in MGC-803 gastric cancer cells demonstrated an inverse effect, suggesting a proproliferation and antiapoptotic role of PRKAA1 in gastric cancer cells, which is similar to that in HCT116 colon cancer cells[27] and in LNCaP and CWR22Rv1 prostate cancer cells. However, contrary to our findings,

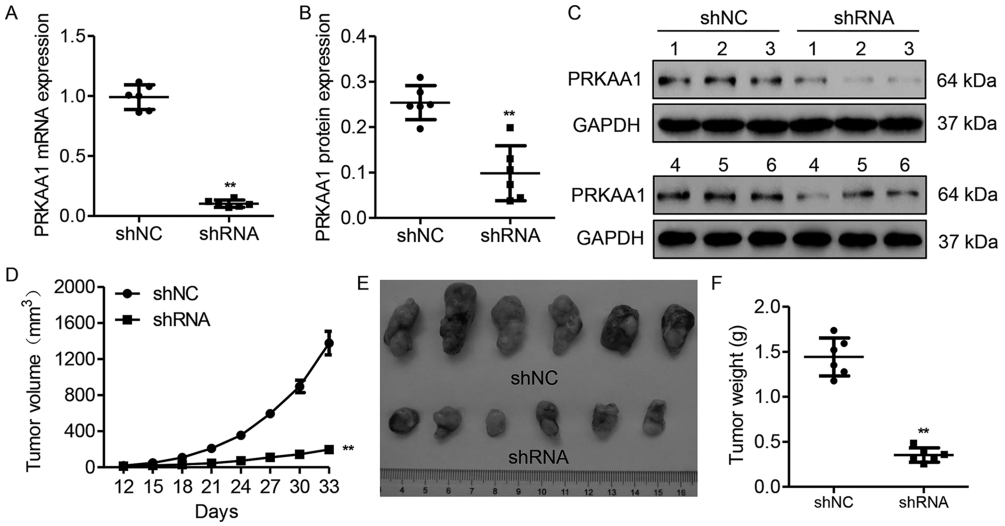

Figure 5. PRKAA1 silencing inhibits tumor growth of gastric cancer in vivo. BGC-823 cells transduced with pLKO.1-PRKAA1-shRNA ($n=6$) or shNC ($n=6$) were injected subcutaneously into the right armpit of nude mice. After 33 days, PRKAA1 expression in xenograft from the nude mice was measured by quantitative real-time PCR (A) and Western blotting (B, C), respectively. (D) Time course analysis of tumor growth after injection. (E, F) Mice were sacrificed, and the tumors were weighed after 33 days injection. $^{**}p<0.01$ compared with shNC.

knockdown of endogenous PRKAA1 expression showed a reduction in prostate cancer cell apoptosis[28], and activated PRKAA1 inhibited pancreatic cancer cell proliferation[29]. Our in vivo experiments were also consistent with our in vitro data that PRKAA1 downregulation in BGC-823 cells markedly inhibited tumor growth and PRKAA1 expression in xenograft from the nude mice.

In view of the important role of PI3K/Akt signaling pathway in regulating cell proliferation, apoptosis, transformation, angiogenesis, and metabolism, it is reasonable to believe that it is also involved in regulating the tumorigenesis of gastric cancer. Akt is at the center of the PI3K/Akt signaling pathway, and a variety of upstream signals are converged to Akt. Akt has been reported to be amplified in gastric cancer[30] and associated with angiogenesis, metastasis, drug resistance, and prognosis of gastric cancer[31,32]. Akt inhibited cell apoptosis via promoting p53 transportation to the nucleus and negatively regulating the function of p53 through phosphorylating MDM2, an E3 ubiquitin ligase[33]. In addition, Akt inhibited cell apoptosis and promoted cell survival by promotion of the expression of PCNA[34] and inhibiting Bax expression[35] and Bad-induced suppression of Bcl-2[15]. In line with the findings above, our results demonstrated that PRKAA1 silencing significantly inhibited the activity of Akt, as well as the expression of PCNA and Bcl-2, with no effect on Bax expression detected in gastric cancer (data not shown). PRKAA1 overexpression-induced increased cell proliferation and cell cycle progression and decreased cell apoptosis were reversed by Akt inhibitor MK2206, which suggest that PRKAA1 promotes gastric cancer cell survival through activating the Akt signaling pathway.

JNK is another important signal transduction pathway playing a dual role as proapoptotic and antiapoptotic in gastric cancer cells[21,36], which is dependent on the cell type, death stimuli, and other signaling pathways, suggesting that JNK signaling is complex and diverse in the cell stress response. Inhibition of JNK had been found to diminish proliferative response by a decrease in PCNA expression[37], but did not increase apoptosis after partial hepatectomy[38]. Additionally, JNK activation regulates the onset of cell apoptosis by regulating downstream apoptotic gene expressions and activity, including p53, Bcl-2, and Bax. JNK regulates Bax/Bcl-2 ratio through direct or p53-dependent indirect effect. Partially in line with our findings that PRKAA1 knockdown inhibited JNK1 activation and the expression of PCNA and Bcl-2, but no effect on Bax and p53 expression was detected in gastric cancer cells, and PRKAA1 overexpression induced increased cell proliferation and cell cycle progression and decreased cell apoptosis were reversed by JNK1 inhibitor SP600125, suggest that PRKAA1 promotes gastric cancer cell survival through activating the JNK1 signaling pathway.

PRKAA1 promotes p53 and Ulk1 activity through direct phosphorylation at serine 15 and 317, respectively, and this phosphorylation event is essential for mediating the effects of PRKAA1 on p53-dependent cell cycle arrest and on Ulk1-dependent autophagy[39,40]. Chen et al. reported that PRKAA1 induces JNK activation through

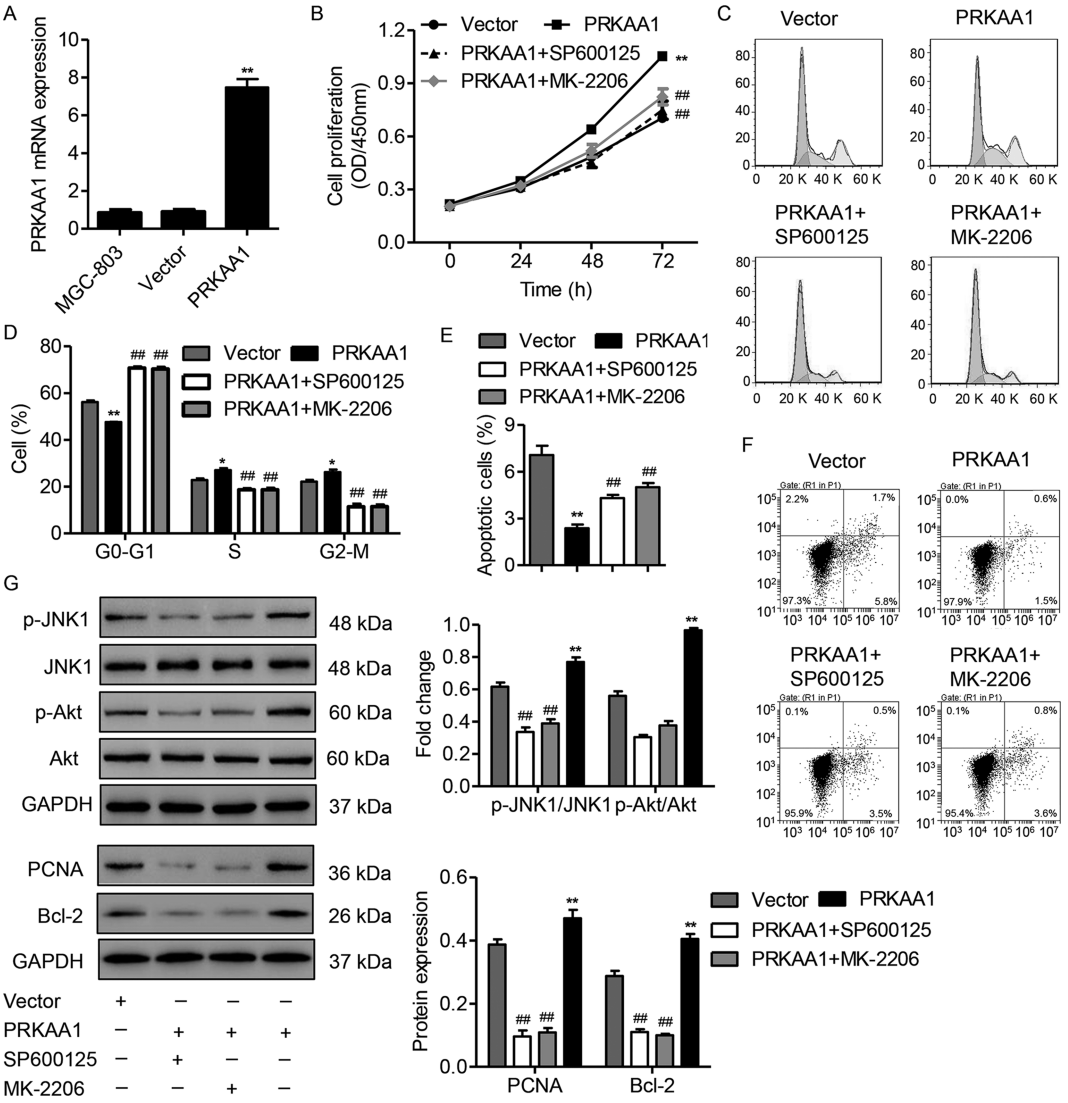

Figure 6. PRKAA1 overexpression facilitates proliferation and restrains apoptosis of gastric cancer cells through JNK1 and Akt pathways. After MGC-803 cells were transduced with pLVX-Puro-PRKAA1 or blank pLVX-Puro, the PRKAA1 mRNA expression was measured by quantitative real-time PCR (A), and cell proliferation (B), cell cycle progression (C, D), apoptosis (E, F), and the protein expression of p-JNK1, JNK1, p-Akt, and Akt (G) were measured by CCK-8, flow cytometry, and Western blot assay, respectively. $n=3$, $^{*}p<0.05$, $^{**}p<0.01$ compared with blank pLVX-Puro (vector). $^{\#\#}p<0.01$ compared with pLVX-Puro-PRKAA1.

inhibiting the expression of HSP70, which leads to the initiation of NF-κB signaling[41]. The inhibition of mTORC1 signaling by PRKAA1 may be responsible for the phosphorylation/activation of AKT observed in MCF-7, T47D, and BT474 ERα^{+} cells as well as in MDA-MB-468 triple negative cells[42]. The results of our study provide evidence that PRKAA1 may be an oncogene for gastric cancer, and JNK1 and Akt signaling might be the key mechanism for its action. However, further studies are needed to establish a cause–effect relationship between PRKAA1 and activation of JNK and Akt.

Moreover, the activity of ERK and STAT3 was also measured in gastric cancer cells with PRKAA1 knockdown. We demonstrated that PRKAA1 knockdown had no effect on the expression of ERK and STAT3 as well as their phosphorylation levels, ERK1 (phospho Tyr-204), and STAT3 (phospho Tyr-705) (data not shown). Inactivation of ERK by PD98059 promoted phosphorylation of STAT3 (phospho Tyr-705), and neither Akt nor JNK affected the STAT3 activity in gastric cancer cells after DIF-1 stimulus[43]. However, previous studies reported that knockdown of PRKAA1 expression reverses inhibition of ERK activity in pancreatic cancer cells[44], and

inhibition of AMPK by compound C significantly inhibits STAT3 activity in endometrial cancer cells[45]. Our data demonstrate that the effect of PRKAA1 on the activity of ERK and STAT3 is dependent on the different cell types and stimuli.

In conclusion, PRKAA1 is highly expressed in gastric cancer cells and promotes gastric cancer cell proliferation and cell cycle progression and inhibits apoptosis through activating JNK1 and Akt signaling pathways. Our study contributes to the evaluation of new therapeutic approaches for gastric cancer.

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