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Long non-coding RNA AK027294 promotes tumor growth by upregulating PCNA in gastric cancer

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Abstract. – OBJECTIVE: Recent researches have proved that long non-coding RNAs (lncRNAs) has an important role in many diseases. In this research, lncRNA AK027294 was explored to identify how it functions in the development of gastric cancer (GC).

PATIENTS AND METHODS: Real Time-quantitative-Polymerase Chain Reaction (RT-qPCR) was utilized to detect AK027294 expression in GC patients. Then, MTT assay, colony formation assay, and EdU incorporation assay were performed to identify its function in GC cells. Furthermore, the potential mechanism was also explored using mechanism assays.

RESULTS: AK027294 expression level was significantly higher in GC tissue samples and cell lines. Results of MTT assay, colony formation assay, and EdU incorporation assay showed that cell proliferation was inhibited through the silence of AK027294 in GC cells, while cell proliferation was promoted through overexpression of AK027294 in GC cells. Furthermore, the expression of PCNA was downregulated *via* silence of AK027294 in GC cells, while the expression of PCNA was upregulated *via* overexpression of AK027294 in GC cells. The correlation analysis showed that PCNA expression was positively correlated with AK027294 expression in GC tissues.

CONCLUSIONS: Our results suggest that AK027294 could enhance cell proliferation of GC cells by upregulating PCNA and might be applied as a novel target for therapy of GC.

Key Words:
Long non-coding RNA, AK027294, Gastric cancer, PCNA.

Introduction

Gastric cancer (GC) is the fourth most common cancer worldwide, especially in eastern Asia. Due to the high morbidity and mortality, GC represents the third leading cause of cancer-related death[1]. Approximately 28000 patients were diagnosed with gastric neoplasms in 2017[2]. Advances in surgical intervention and postoperative adjuvant therapy have raised the survival rate of GC patients in an early stage. However, the prognosis of patients at advanced stage remains poor, which brings a huge burden to both the patients and the society. Thus, a better understanding of the underlying molecular mechanism of GC is urgently needed.

It has been revealed that the majority (~98%) of the human genome have no protein-coding capacity, which are called non-coding RNAs (ncRNAs). As a class of ncRNAs, long non-coding RNAs (lncRNAs) have emerged as an important role in tumorigenesis. Moreover, lncRNAs have the potential to regulate gene expression which is frequently sequence homology-dependent and the particular mechanism of regulation can be associated with homology to different regions of the regulated gene. Recently, a number of regulatory promoters associated lncRNAs have been characterized. For example, lncRNA BACE1-AS inhibits the proliferation and invasion of ovarian cancer stem cell and functions as a novel target for treating ovarian cancer[3]. LncRNA AB073614 plays an important role in regulating cell proliferation and cell migration in colorectal cancer by modulating PI3K/AKT signaling pathway[4]. Through activating the Wnt/beta-catenin pathway, lncRNA EZR-AS1 functions as an oncogene in breast cancer cell proliferation, apoptosis, migration, and invasion[5]. Moreover, downregulation of lncRNA UCA1 modulated by CRISPR/Cas9 can depress the malignant phenotypes of bladder cancer, and UCA1 can be

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utilized as a novel non-invasive diagnostic biomarker for bladder cancer[6]. However, few studies have investigated the function of lncRNA AK027294 in GC and the underlying molecular mechanism until now.

We revealed that lncRNA AK027294 expression was significantly upregulated in GC samples. Moreover, experiments *in vitro* revealed that AK027294 regulated cell proliferation of GC cells. Some studies demonstrated that PCNA acts as an oncogene in tumor development. Furthermore, we discovered that lncRNA AK027294 played its function in GC cells by regulating PCNA.

Patients and Methods

Tissue Samples

58 patients were collected in this research. They underwent surgical resection at Gansu Provincial Hospital. Human GC tissues and adjacent non-tumor tissues were obtained from patients during the surgery. After surgical resection, all the tissue samples were snap-frozen in liquid nitrogen immediately. Before the surgery, no radiotherapy and chemotherapy treatment were obtained in any patient. This project received approval from the Review Board of Gansu Provincial Hospital. The written informed consents were gathered from all the patients enrolled.

Cell Culture

The GC cell lines (BGC-823, SGC-7901, HGC-27, and MKN-45) and human gastric epithelial cell (GES) were bought from American Type Culture Collection (ATCC; Manassas, VA, USA). Dulbecco's Modified Eagle Medium (DMEM; Invitrogen Life Technologies, Carlsbad, USA), supplemented with 10% fetal bovine serum (10% FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin, was used to culture the cells in a humidified incubator at 37°C with 5% CO_2.

Cell Transfection

According to the manufacturer's instructions, lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) was utilized to transiently transfect HGC-27 cells with negative control, cDNA oligonucleotides against AK027294 (sh-AK027294) or lentivirus against AK027294 (AK027294) respectively, which were provided by GenePharma (GenePharma, Shanghai, China). Cells were collected for following experiments after incubated for 24 h.

RNA Extraction and Real Time-Quantitative Polymerase Chain Reaction (RT-qPCR)

According to the manufacturer's instructions, TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was utilized to extract total RNA from tissue and cultured cells. By using a Reverse Transcription Kit (TaKaRa, Dalian, China), RNA was reverse transcribed to cDNA for RT-qPCR. By using SYBR Green (TaKaRa, Dalian China), the RT-qPCR was performed to detect the expression of AK027294 in tumor and non-malignant tissues with a normalizing control, glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Following are the primers using for RT-qPCR: AK027294 primers forward: 5′-ATGACACCTATTGGAGAA-3′, reverse: 5′-TAAGCACACCTGAGTAAT-3′; GAPDH primers forward: 5′-CACCCACTCCTCCACCTTTG-3′ and reverse: 5′-CCACCACCCTGTTGCTGTAG-3′. Thermal cycle was as follows: 30 sec at 95°C, 5 sec for 40 cycles at 95°C, 35 sec at 60°C.

Cell Proliferation Assay

Cell Proliferation Reagent Kit I (MTT; Roche, Basel, Switzerland) was used to detect cell proliferation. The transfected cells were seeded at a density of 2 $\times 10^3$ cells per well in 200 μL culture medium in 96-well plates. Following the manufacturer's protocol, cell proliferation was assessed every 24 h.

Colony Formation Assay

GC cells were placed in a 6-well plate for 10 days. The colonies were treated with 10% formaldehyde for 30 min. Then, 0.5% crystal violet was used for staining for 5 min. The analysis was conducted with Image-Pro Plus 6.0 (Silver Springs, MD, USA).

Ethynyl Deoxyuridine (EdU) Incorporation Assay

Cell proliferation of transfected cells was detected *via* an EdU Kit (Roche, Mannheim, Germany). Representative images were obtained through Zeiss Axiophot Photomicroscope (Carl Zeiss, Oberkochen, Germany).

Statistical Analysis

Statistical analysis was conducted by Statistical Product and Service Solutions (SPSS) 17.0 (SPSS, Chicago, IL, USA). Data analysis was conducted by the Student *t*-text method.

Data were presented as mean ± SD (Standard Deviation). $p<0.05$ was considered statistically significant.

Results

AK027294 Expression Level in GC Tissues and Cells

Firstly, AK027294 expression was monitored by RT-qPCR in 58 GC patients' tissues and 4 GC cell lines. AK027294 was remarkably higher-expressed in tumor tissue samples compared with adjacent tissues (Figure 1A). AK027294 expression level was higher in GC cells than that in GES (Figure 1B).

Cell Proliferation Was Repressed Via Silence of AK027294 in GC Cells

HGC-27 GC cell lines were chosen for the silence of AK027294. Then, AK027294 expression was detected by RT-qPCR (Figure 2A). MTT assay showed that cell growth ability of HGC-27 cells was inhibited *via* silence of AK027294 (Figure 2B). Moreover, colony formation assay also revealed that the number of colonies was significantly reduced *via* silence of AK027294 in HGC-27 cells (Figure 2C). Furthermore, EdU incorporation assay also showed that EdU positive cells were reduced after the silence of AK027294 in HGC-27 cells (Figure 3A).

Cell Proliferation Was Enhanced Via Overexpression of AK027294 in GC Cells

MKN-45 GC cell lines were chosen for the overexpression of AK027294. Then, AK027294 expression was detected by RT-qPCR (Figure 2D). MTT assay showed that cell growth ability of MKN-45 cells was promoted *via* overexpression of AK027294 (Figure 2E). Moreover, colony formation assay revealed that the number of colonies was significantly increased *via* overexpression of AK027294 in MKN-45 cells (Figure 2F). Furthermore, EdU incorporation assay showed that EdU positive cells were increased after overexpression of AK027294 in MKN-45 cells (Figure 3B)

Interaction Between PCNA and AK027294 in GC

We conducted RT-qPCR and Western blot assay to explore the association between PCNA and AK027294. The expression level of PCNA in HGC-27 cells in the sh-AK027294 group was remarkably lower than that in control group (Figure 4A). The expression level of PCNA in MKN-45 cells in AK027294 lentivirus group was remarkably higher than that in control group (Figure 4B). Besides, PCNA expression was remarkably higher in GC tissues compared with that in adjacent tissues (Figure 4C). Furthermore, PCNA expression was significantly higher in GC cells compared with GES cell (Figure 4D).

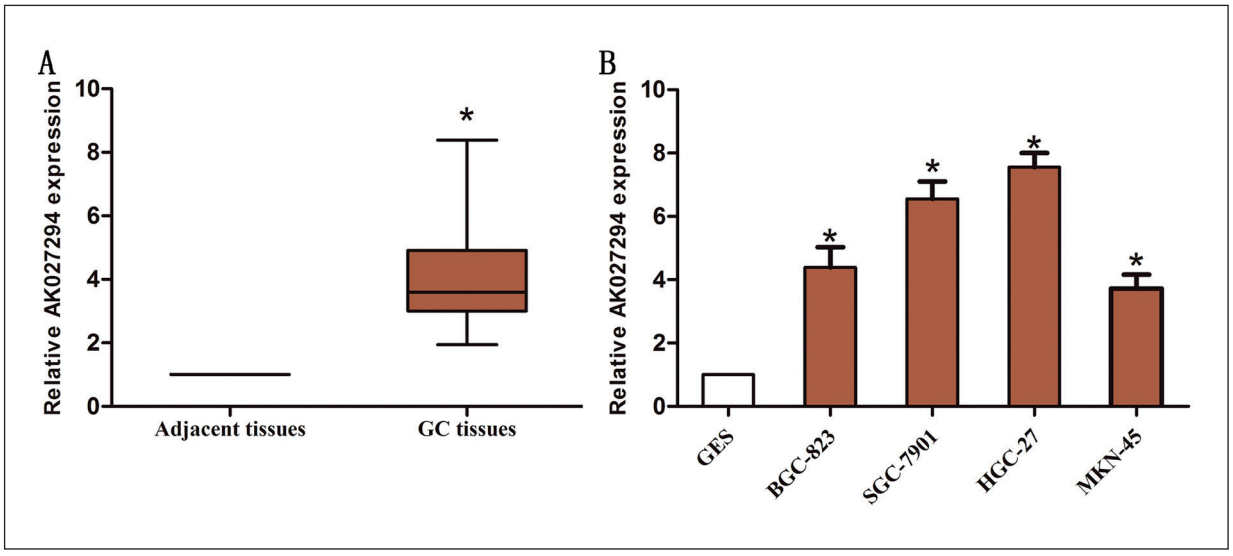

Figure 1. The expression levels of AK027294 in GC tissues and cell lines. ***A***, AK027294 expression was significantly increased in the GC tissues compared with adjacent tissues. ***B***, Expression levels of AK027294 were determined in the human GC cell lines (BGC-823, SGC-7901, HGC-27, and MKN-45) and normal human gastric epithelial cell (GES) by RT-qPCR. Data are presented as the mean ± standard error of the mean. *$p<0.05$.

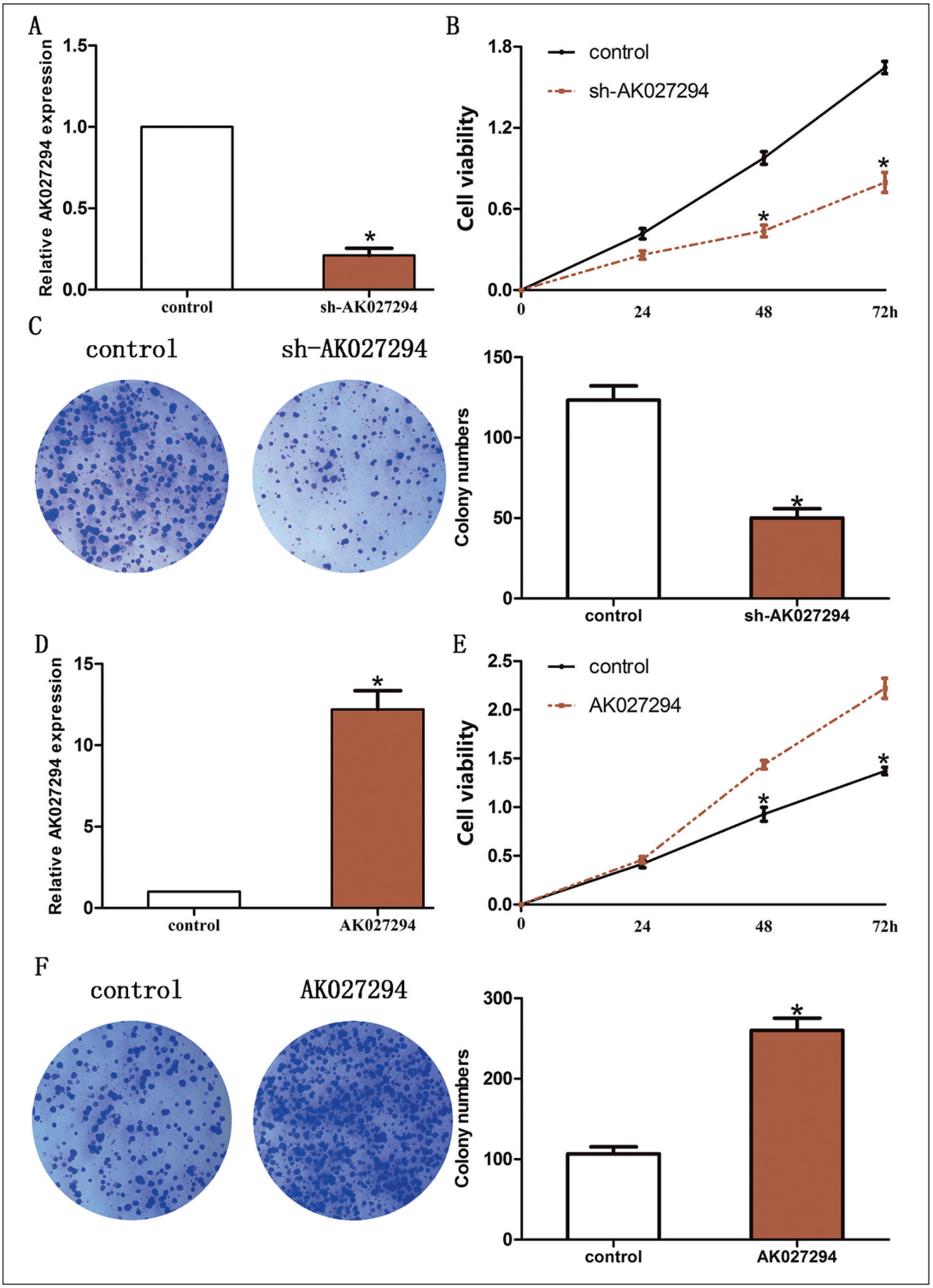

Figure 2. MTT assay and colony formation assay showed AK027294 promoted GC cell proliferation. ***A,*** AK027294 expression in GC cells transfected with sh-AK027294 and control was detected by RT-qPCR. GAPDH was used as an internal control. ***B,*** MTT assay showed that the silence of AK027294 significantly repressed cell proliferation in GC cells. ***C,*** Colony formation assay showed that the number of colonies was significantly decreased *via* silence of AK027294 in GC cells. ***D,*** AK027294 expression in GC cells transfected with AK027294 lentivirus (AK027294) and control was detected by RT-qPCR. GAPDH was used as an internal control. ***E,*** MTT assay showed that the overexpression of AK027294 significantly enhanced cell proliferation in GC cells. ***F,*** Colony formation assay showed that the number of colonies was significantly increased *via* overexpression of AK027294 in GC cells. *$p<0.05$, as compared with the control cells.

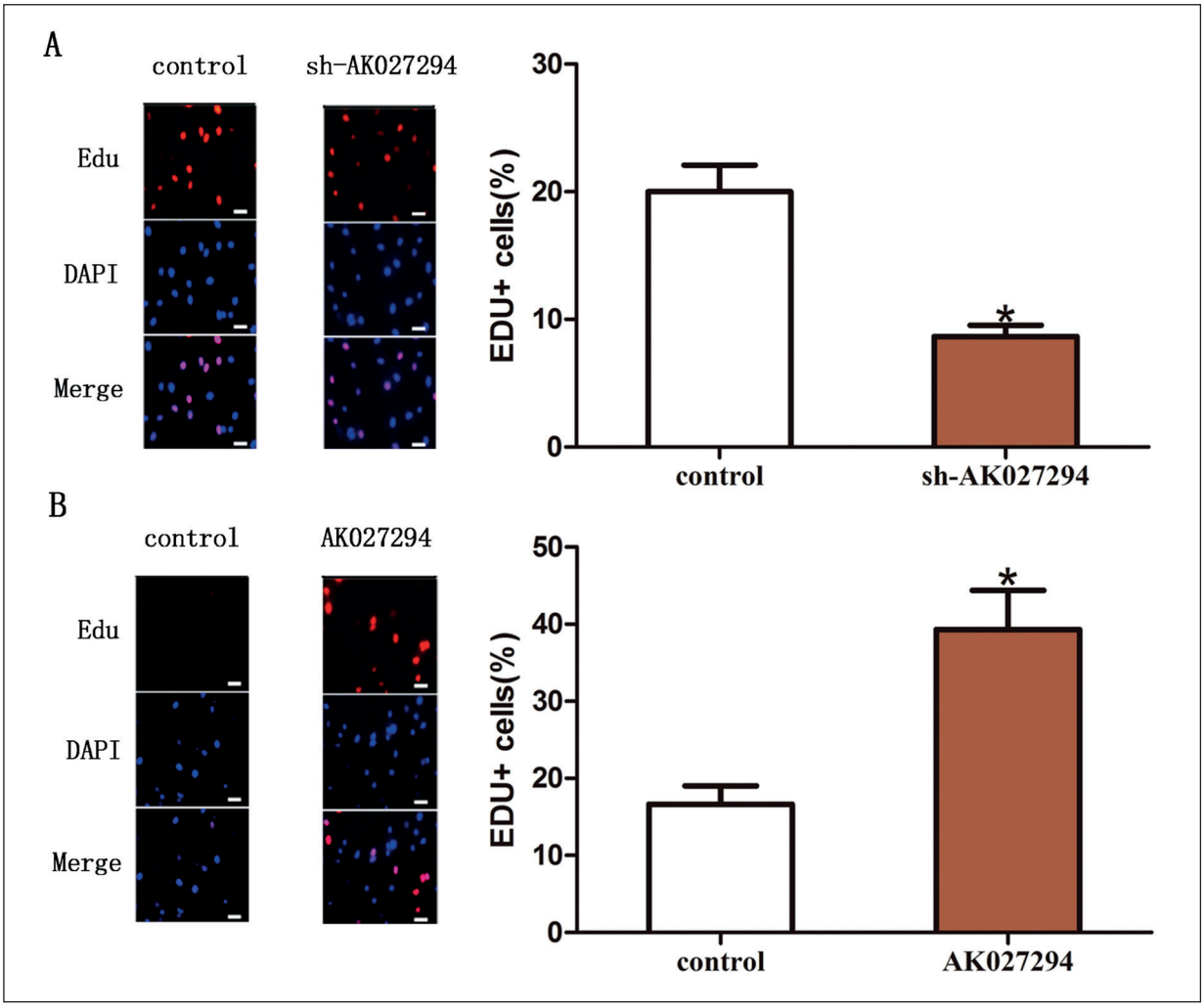

Figure 3. EdU incorporation assay showed that AK027294 promoted GC cell proliferation. ***A,*** EdU incorporation assay showed that the number of EdU positive cells was significantly reduced *via* silence of AK027294 in GC cells. ***B,*** EdU incorporation assay showed that the number of EdU positive cells was significantly increased *via* overexpression of AK027294 in GC cells. **p*<0.05, as compared with the control cells.

We further found the positive correlation between PCNA and AK027294 expression level in GC tissues (Figure 4E).

Discussion

Most of the human genome is transcribed, yielding a complex network of transcripts that includes tens of thousands of lncRNAs. LncRNAs, as a class of transcripts longer than 200 base pairs in length, have been implicated in regulating potential activity and splicing event *via* small RNA regulatory pathways. Conversely, transcription of some promoter lncRNAs induces an open chromatin formation that facilitates activator binding and transcription of the associated protein-coding gene.

Gastric carcinogenesis is a complicated biological process partly due to the dysregulation of many tumor-related genes. It is becoming clear that mammalian genomes encode thousands of lncRNAs. Although many advances have been made in the mechanisms of the development and progression of GC in the past few decades, the outcomes of the 5-year survival remain unoptimistic. A growing volume of studies has revealed that lncRNAs play an important role in the development of GC. For instance, by regulating vasculogenic angiogenesis, lncRNA

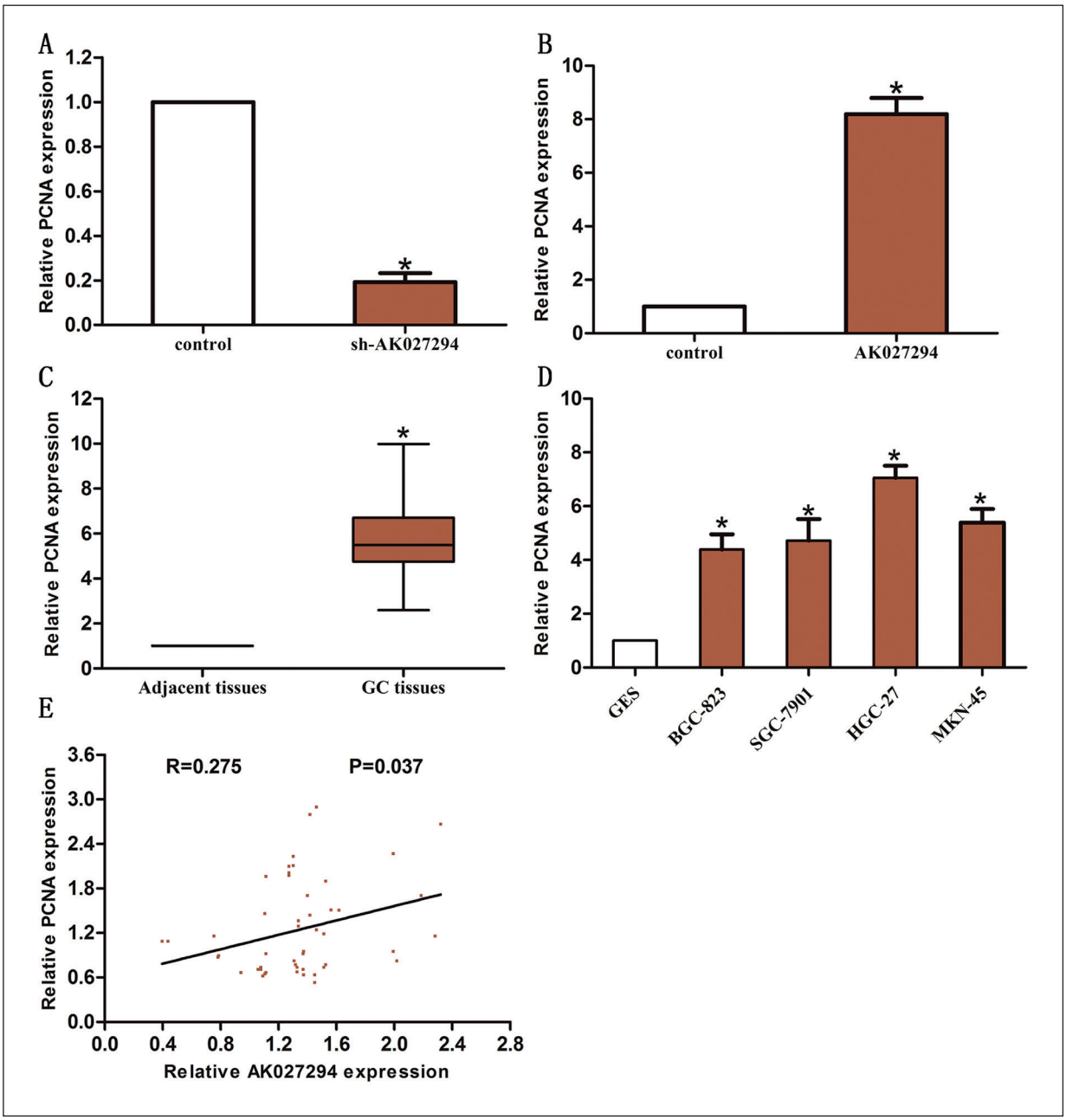

Figure 4. The interaction between PCNA andAK027294 in GC. ***A,*** RT-qPCR results showed that the PCNA expression was decreased in sh-AK027294 compared with the control group. ***B,*** RT-qPCR results showed that the PCNA expression was increased in AK027294 lentivirus (AK027294) compared with the control group. ***C,*** PCNA was significantly upregulated in GC tissues compared with adjacent tissues. ***D,*** Expression levels of PCNA were determined in the human GC cell lines (SGC-7901, BGC-823, HGC-27) and normal human gastric epithelial cell (GES) by RT-qPCR. ***E,*** The linear correlation between the expression level of PCNA and AK027294 in GC tissues. The results represent the average of three independent experiments. Data are presented as the mean ± standard error of the mean. *$p<0.05$.

MALAT1 is reported to promote tumorigenicity and cell metastasis in GC[7]. LncRNA LINC-PINT functions as a tumor suppressor in GC by crosstalk with miR-21 which is associated with a poor survival of GC patients[8]. Knockdown of lncRNA HOST2 inhibits cell proliferation and cell metastasis of GC, which may offer a potential therapeutic target and biomarker[9]. Through sponging miR-608, lncRNA NORAD facilitates cell proliferation and cell migration of GC[10]. In

this study, we explored the potential lncRNAs in GC development.

Long non-coding RNAs (lncRNAs) has been shown to have crucial roles in various biological regulatory processes. Most lncRNAs are transcribed by RNA polymerase II enzymes that lack open-reading frames and are expressed in specific tissues, demonstrating that the genes encoding these molecules are strictly regulated with respect to tissue development. LncRNAs have recently been found to be involved in development, differentiation, and proliferation, as well as cell cycle regulation and programmed cell death. They also have important roles in the progression and metastasis of various tumors. LncRNA AK027294 is a novel regulatory molecular in colorectal cancer. It enhances cell proliferation and cell migration in the progression by participating in the cycle process and DNA replication of colorectal cancer[11]. We showed that AK027294 was higher-expressed in GC tissues and cell lines. Besides, cell proliferation of GC cells was repressed by the silence of AK027294, while cell proliferation of GC cells was promoted by overexpression of AK027294. Above results suggested that AK027294 promoted the development and progression of GC *in vitro*.

Recently, PCNA (proliferating cell nuclear antigen) has been reported to be directly associated with tumor differentiation and progression in many tumors, especially in GC. Many researchers have identified that PCNA can be regulated by lncRNAs and functions as an oncogene in a variety of malignant tumors including GC. Therefore, we detected PCNA expression and AK027294 expression in GC tissues. Results showed that PCNA expression in GC tissues was positively related to AK027294 expression. For instance, PCNA is significantly upregulated in breast cancer, which indicates a poor prognosis for breast cancer patients[12]. The overexpression of PCNA reverse the repression of miR-363-3p on cell proliferation and colony formation in lung adenocarcinoma[13]. The upregulation of PCNA is closely related to a poor prognosis of patients with osteosarcoma which may be a potential biomarker[14]. In addition, PCNA is significantly overexpressed in colorectal cancer with liver metastasis, which may contribute to evaluate liver metastasis in patients with colorectal cancer[15]. We further found that PCNA expression could be suppressed by the silence of AK027294 in GC cells, and PCNA expression could be promoted by overexpression of AK027294 in GC cells. Then, we detected the expression of PCNA in GC tissues and cells. PCNA was higher-expressed in GC cell lines and tissues. All the results above suggested that AK027294 might promote the progression of GC through upregulating PCNA.

Conclusions

We identified that AK027294 could enhance GC cell proliferation through upregulating PCNA *in vitro*, indicating that AK027294 may act as a candidate target for therapy of GC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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