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ORIGINAL RESEARCH

AURKB Promotes the Metastasis of Gastric Cancer, Possibly by Inducing EMT

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Aim: To investigate the function of Aurora kinase B (AURKB) in gastric cancer (GC).

Methods: Immunohistochemistry was used to assay the expression of AURKB in 50 pairs of GC and adjacent tissues, and qRT-PCR was conducted to test *AURKB* expression in normal gastric epithelial and GC cell lines. Two segments of small interference RNAs (siRNAs) targeting *AURKB* were synthesized and inserted into GV248 lentivirus vector. After transfected with LV-AURKB-RNAis, CCK8, wound healing, transwell and flow cytometric assays were performed to determine the influence of silencing *AURKB* on cell proliferation, invasion, migration, cell cycles and apoptosis of GC cells, and the expression of EMT (epithelial–mesenchymal transition)-related markers was demonstrated by Western blots (WB).

Results: AURKB was highly expressed in GC and closely associated with lymph node metastasis and advanced stages of GC. Down-regulating *AURKB* suppressed the proliferation and promoted the apoptosis of GC cells, arrested the cell cycle in G2/M phase, and inhibited the invasion and migration of GC cells. The expression levels of AKT1, mTOR, Myc, MMP2, and VEGFA were decreased, while the expression levels of OCLN and JUP were increased after knocking down of *AURKB* in both AGC and MKN45 cells.

Conclusion: AURKB is overexpressed in GC and closely associated with clinicopathologic characteristics of GC. It is likely that by inhibiting VEGFA/Akt/mTOR and Wnt/β-catenin /Myc pathways, silenced *AURKB* could inhibit the invasive and migratory abilities of GC cells. However, because of the small sample size and the absence of in-vivo experiments, these results should be verified by further studies.

Keywords: AURKB, gastric cancer, metastasis, EMT

Introduction

Although the incidence is declining recently, gastric cancer (GC) remains a major public health concern, ranking as the fourth most common cancer and the second most frequent cause of cancer-related death worldwide.[1] The mortality rate of GC is highest in East Asia, including China. Approximately 60% of patients with GC are diagnosed in these regions.[2,3] The prognosis of GC is improved as a result of the progress in comprehensive treatments, including surgery, chemotherapy, and targeted therapy.[1] However, the five-year overall survival of GC remains very low.[3] Metastasis is one of the main reasons for the poor prognosis of GC as well.[1] Hence, it is urgent to explore the internal mechanisms of carcinogenesis and metastasis of GC.

Aurora kinase B (AURKB), which belongs to the mitotic protein kinase family, acts as a crucial regulator in mitosis. It serves as the enzymatic core of the chromosomal passenger complex (CPC),[4] which also contains other three non-enzymatic

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components: Survivin, inner centromere protein (INCENP) and borealin.[5] Several studies found that *AURKB* mainly functions on regulating chromatin modification and suppressing the procession of cytokinesis.[6] Overexpression or amplification of *AURKB* is usually detected in human cancers, including breast cancer,[7] ovarian cancer,[8] gastric/gastrointestinal cancer and other tumors,[9,10] and is proved to be related to poor prognosis.[11] Therefore, *AURKB* has become a promising therapeutic target in tumors. Some researchers found that high expression of AURKB was associated with the improved overall survival in GC,[12] however, others considered that inhibition of *AURKB* reduced GC cell viability and increased the sensitivity of resistant GC cell to cisplatin.[13,14] Hence, the exact effect of *AURKB* in GC remains unclear.

Studies found that epithelial–mesenchymal transition (EMT) played important roles in the development of carcinomas, including gastric cancer. In the process of EMT, the various biochemical changes of epithelial cells can cause the loss of polarity and migratory capacity of cells so that it contributes to the changes of cell shape and promotes immobile epithelial cells transform to motile mesenchymal cells, eventually strengthen cell metastasis.[15] It has been reported that EMT can lead to pathological migration and invasion in various cancer progressions, and EMT-induced carcinogenesis is the common cause for malignancies, including GC, neck squamous cell carcinoma, and ovarian cancer.[16–18] Recently, a study about breast cancer demonstrated that high expression of AURKB was associated with EMT activation and downregulated *AURKB* could inhibit EMT progression, which indicated that *AURKB* might induce EMT in breast cancer.[19] Therefore, *AURKB* is possibly associated with EMT, which takes part in the metastasis of various carcinomas.

In order to evaluate the effects of *AURKB* in gastric cancer and explore the possible mechanisms, in this study we regulated the expression of AURKB in gastric cancer cell lines, tested the change of biological behaviors, and then detected the expression of EMT-related proteins in vitro.

Methods

Ethics Approval

All procedures performed in this study involving participants were approved by the Ethical Review Committee of the First Affiliated Hospital of Guangxi Medical University (Nanning, Guangxi, China), and written informed consent was obtained from all patients.

Patients

Fifty patients (38 males and 12 females) who were pathologically diagnosed with gastric cancer between 2016 and 2017 were enrolled in this study. All the patients received radical gastrectomy or laparotomy. And the following clinical and pathological characteristics were collected: age, gender, tumor size, lymph node status, distant metastasis, pathological type, and TNM stage.

Cell Culture

Gastric cancer cell lines MKN45 (Signet-ring cell carcinoma) and AGS (Gastric adenocarcinoma) were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were incubated in RPMI-1640 (Gibco, USA) with 10% fetal bovine serum (FBS) (Gibco, USA), 50 mg/mL penicillin, and 100 mg/mL streptomycin (Solarbio, Beijing, China). Cells were grown at 37°C in humidified air with 5% CO2.

PCR

Total RNA was extracted using TRIzol reagent (Pufei Biotech Co., Shanghai, China), and 1 ug of total RNA was used to amplify complementary DNA, using the QuantiTect SYBR Green PCR Kit and real-time PCR (TAKARA Bio., Japan) with custom Primetime qPCR Primers (Genechem Co., Ltd, Shanghai, China): human *AURKB*, 50-CAGAAGAGCTGCACATTTGACG-30 (forward) and 50-CCTTGAGCCCTAAGAGCAGATTT-30 (reverse); and human GAPDH, 50-TGACTTCAACAGCGACACCCA-30 (forward) and 50-CACCCTGTTGCTGTAGCCAAA-30 (reverse). Polymerase chain reaction (PCR) amplification was performed in 50 μL reaction volumes containing 0.2 mmol/L each dNTP, 0.1 RM of each oligonucleotide primer, and 1.25 U Tag polymerase in PCR buffer by using a thermal cycler (model C1000, Bio-Rad). cDNA was amplified on a PCR thermal controller with an initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 20 s, 59°C for 30 sec, and 68°C for 30 s and a final extension step of 72°C for 10 min.

Western Blot

Cell lysates were harvested with radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, China) and centrifuged at 15,000×g for 10 min at 4°C. Then, proteins were separated by SDS-PAGE and transferred to the PVDF membrane (Millipore, USA). Subsequently, the PVDF membranes were blocked with a mixture of 5% non-fat

milk and Tris-buffered saline (TBS) for preventing non-specific binding. Specific primary antibodies against GAPDH (Santa Cruz, USA), AURKB (abcam, USA), MMP2 (CST, USA), AKT1 (CST, USA), mTOR (CST, USA), JUP (abcam, USA), Myc (abcam, USA), OCLN (abcam, USA), and VEGFA (abcam, USA) were utilized for incubating the membrane at 4°C overnight. Finally, the ECL Western Blotting Detection Kit (Thermo, USA) was applied to detect protein bands according to the manufacturer's instructions.

Lentivirus-Mediated RNA Interference (RNAi)

Two segments of small interference RNAs (siRNAs) targeting AURKB were designed. The sequences were presented as follows: 5′-CCAAACTGCTCAGGCATAA-3′ (KD1, 58,-972–1) and 5′-CCTTTGAGAGTGCATCACA-3′ (KD2, 58,-974–1). A negative sequence (5′-TTCTCCGAACGTGT CACGT-3′) served as a control. The siRNAs were synthesized and inserted into GV248 lentivirus vector (Shanghai Genechem Co., Ltd, China), and then verified by DNA sequencing (Sanger). The siRNAs packaged with lentivirus vector were transfected into cells at a multiplicity of infection of 10 PFU per cell (MOI = 10). Two GC cell lines (AGS and MKN45) were divided into four groups, respectively: GC cells in the control group without any treatment (CON), GC cells in the negative control group (NC) transfected with the negative control segment, GC cells in KD1 group transfected with 58,972–1, and GC cells in KD2 group transfected with 58,974–1.

Colony Formation, CCK8, Cell Cycle and Apoptosis Assays

Gastric cancer cells were transfected with the lentiviral vectors containing siRNA sequence targeting *AURKB* or negative control sequence. Two weeks later, the colonies were fixed in ice-cold methanol for 10 min and stained with 1% crystal violetin methanol for 15 min. Finally, the colonies were rinsed with water and then counted.

Cell counting Kit-8 (CCK-8, Sigma, US) was conducted to verify cell viability. 2×10^3 cells/well were seeded into 96-well plates and cultivated for 4 h. Briefly, adding 10 uL CCK-8, the

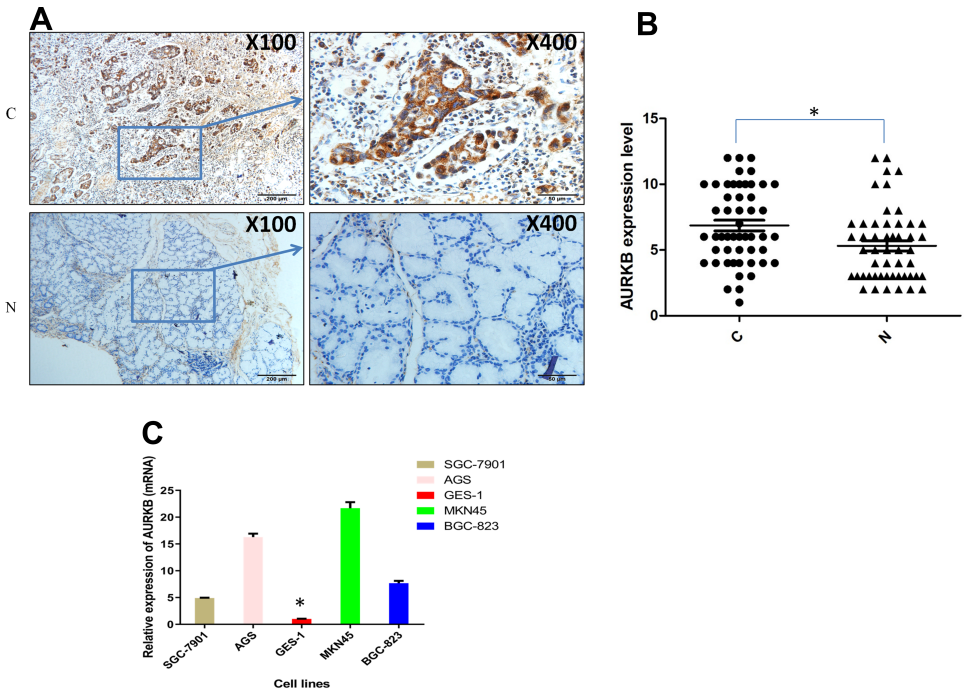

Figure 1 *AURKB* is highly expressed in gastric cancer tissues and cell lines. (**A**) The images of AURKB expression tested by IHC in gastric cancer and para-carcinoma tissues (X100 and X400, respectively). (**B**) The quantification of IHC in gastric cancer and para-carcinoma tissues of fifty-one patients (**C**) The mRNA level of *AURKB* in normal gastric epithelial cell lines and GC cell lines. (*$P < 0.05$).
Abbreviations: C, cancer tissues; N, normal tissues.

Table 1 The Association Between the Expression Level of AURKB and Clinical Pathological Characteristics of GC Patients

Clinical Pathological Characteristics	N	High AURKB Expression (%)	*P* value
Gender			
Male	38	31 (81.6%)	0.424
Female	12	8 (66.7%)	
Age			
≤58	23	17 (73.9%)	0.733
>58	27	22 (81.5%)	
Tumor size			
< 5cm	35	26 (74.3%)	0.468
≥ 5cm	15	13 (86.7%)	
Lymph node status			
Negative	39	34 (87.2%)	0.008
Positive	11	5 (45.5%)	
Tumor stage			
I,II	29	19 (65.5%)	0.016
III,IV	21	20 (95.2%)	

Note: Tumor stage was divided according to the 8th edition of American Joint Committee on Cancer.

absorbance of each well was evaluated at 450 nm. The growth curve was delineated with time as the horizontal axis and the absorbance as the vertical axis.

Gastric cancer cells were collected and trypsinized. After using ice-cold 70% ethanol to fix cells at 4°C overnight, those cells were incubated with RNase A (50 μg/mL) and stained by propidium iodide (50 μg/mL, Sigma, USA). Then, cell cycle distribution was analyzed by flow cytometry measurement of DNA content using a flow cytometer (Millipore, USA). The percentage of each population was analyzed using the InCyte software (Millipore, USA).

Apoptosis was performed using the Annexin V-FITC Apoptosis Detection kit (eBioscience, USA) based on the manufacturer's protocol. After being harvested and added to 200 μL 1X binding buffer, gastric cancer cells were incubated in 10 μL Annexin V -FITC for 10 min in dark. Apoptotic cells were quantified by a flow cytometer (Millipore, USA) using the InCyte software (Millipore, USA).

Matrigel Invasion and Wound-Healing Assays

Matrigel invasion assay was conducted using polycarbonate membrane transwell (Corning, United States) coated with the matrigel (BD Biosciences, San Jose, CA, United States). The transfected cells AGS (2.0×10^5 cells/mL) and MKN45 (1.5×10^5 cells/mL) were seeded into the upper chambers, respectively, and then cultured in a humidified atmosphere with 5% CO2 for 24 h at 37°C. The bottom chamber included medium with 5% FBS. The remaining cells on the upper surface were mechanically removed. Then, membranes were washed, fixed, and stained by Methyl Violet (Medion Diagnostics, Germany). Finally, the number of cells that had migrated to the lower side of the filter was counted under a bright field with a microscope (Olympus, Japan) to determine the invasive ability of cells.

Migration ability was tested by wound-healing assay. Stably transfected GC cells were cultured in 6-well plates and fused to approximately 90% (5×10^3 cells/well). The next day, a straight cell-free wound was created by a sterile pipette tip when ~100% of the surface was occupied. Washed twice by PBS, next, the detached cells were reincubated in RPMI 1640 medium with 1% FBS for 24 h at 37°C. The wound images were obtained using a fluorescence inverted microscope at 0 and 24 h, respectively. The wound distance was measured as follows:

$$\text{Migration distance} = \text{scratch distance at 0 h} - \text{scratch distance at 24 h}$$

Immunohistochemistry

Paraffin-embedded GC and para-carcinoma tissue blocks were subjected to immunohistochemistry. The tissues were deparaffinized, hydrated, and permeabilized with 0.5% Triton X-100/1 PBS for 10 min, then hybridized with the rabbit anti-human AURKB (abcam, USA) antibodies (1:200 dilution) for 2 h at room temperature. After that, an HRP-conjugated goat anti-rabbit antibody functioned as the secondary antibody. Then, the sections were immersed with the substrate-chromogenic solution and counter-stained with hematoxylin. Pictures were acquired by microscopy.

Statistical Analysis

Quantitative data were presented as mean ± standard deviation (SD). All experiments were repeated in triplicate. All analyses were calculated using Student's *t*-test or one-way ANOVA of GraphPad 6 (GraphPad Software, Inc). $P<0.05$ was considered statistically significant.

Results

AURKB Was Highly Expressed in Gastric Cancer and Associated with Clinicopathologic Characteristics of GC

In total, we tested the expression level of AURKB by using IHC in 50 pairs of tissues from GC and para-carcinoma, respectively. As a result, we found that AURKB was highly expressed in GC tissues compared with para-carcinoma (P=0.0068, Figure 1A and B).

Besides, the association between the expression level of AURKB and clinicopathologic characteristics of GC patients was evaluated. By using univariate analysis, we found that high AURKB expression was closely related to lymph node metastasis (P=0.008) and advanced stages (P=0.016) of GC (Table 1).

To confirm the association of *AURKB* and gastric cancer, we tested the *AURKB* mRNA level of the normal gastric epithelial cell line (GES-1) and GC cell lines. As a result, we found that the *AURKB* mRNA level of GC cell lines was much higher than that of GES-1 (P <0.05, Figure 1C).

Silencing of *AURKB* Inhibited the Proliferation and Promoted the Apoptosis of GC Cells

In order to evaluate the biological roles of *AURKB* in GC, we constructed two siRNAs targeted *AURKB* and packaged them into a lentiviral vector, which was then transfected into AGS and MKN45 cells, respectively. As shown in Figure 2A, the two siRNA segments were verified by DNA sequencing. After transfection, the transfection efficiency was observed under microscopy, and the expression level of *AURKB* was tested by qPCR. As shown in Figure 2B–D, *AURKB* was significantly down-regulated after RNA interference (P <0.05).

To evaluate the role of *AURKB* on the proliferation and apoptosis of GC, CCK8 assay, colony formation, and flow cytometry were used. As presented in Figures 3A–E and 4A and B, proliferation was significantly inhibited and apoptosis was significantly promoted by down-regulating *AURKB* in GC cell line AGS and MKN45 (P <0.05).

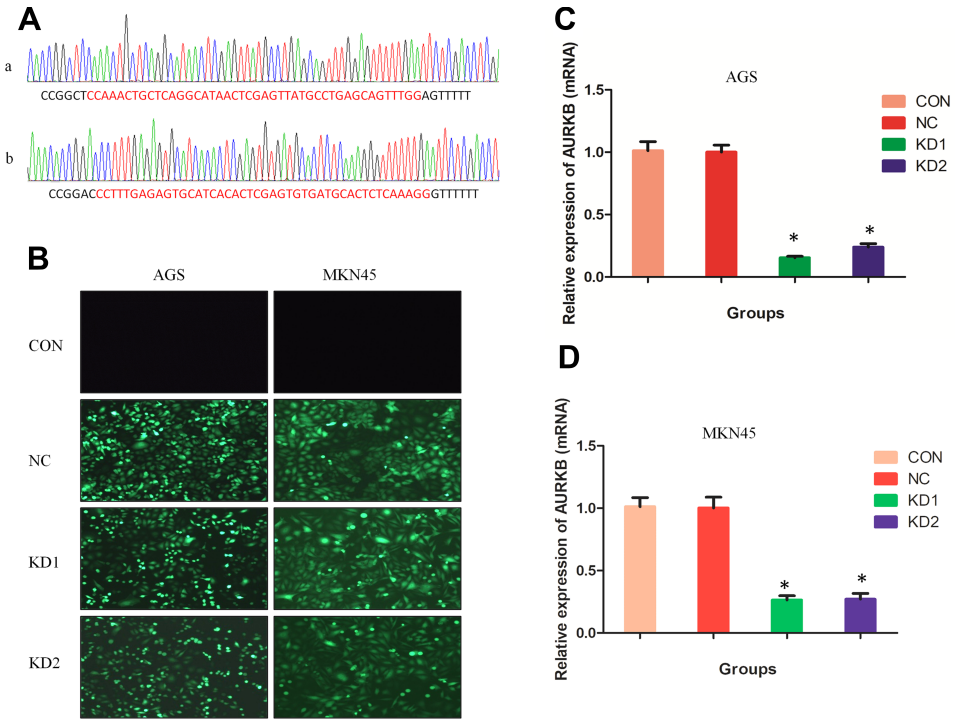

Figure 2 The construction and confirmation of lentivirus-mediated RNA interference plasmid targeting *AURKB*. (**A**) Two siRNAs targeted *AURKB* were constructed and confirmed by RNA-sequence, a: PSC58972-1, b: PSC58974-1. (**B**) Transfection efficiency of GC cell line AGS and MKN45 according to GFP signal. C, (**D**) The mRNA level of AURKB in CON, NC, and AURKB -KD group, (**C**) AGS, (**D**) MKN45. (*P < 0.05).

Abbreviations: CON, control group; NC, negative control group; KD, knockdown group.

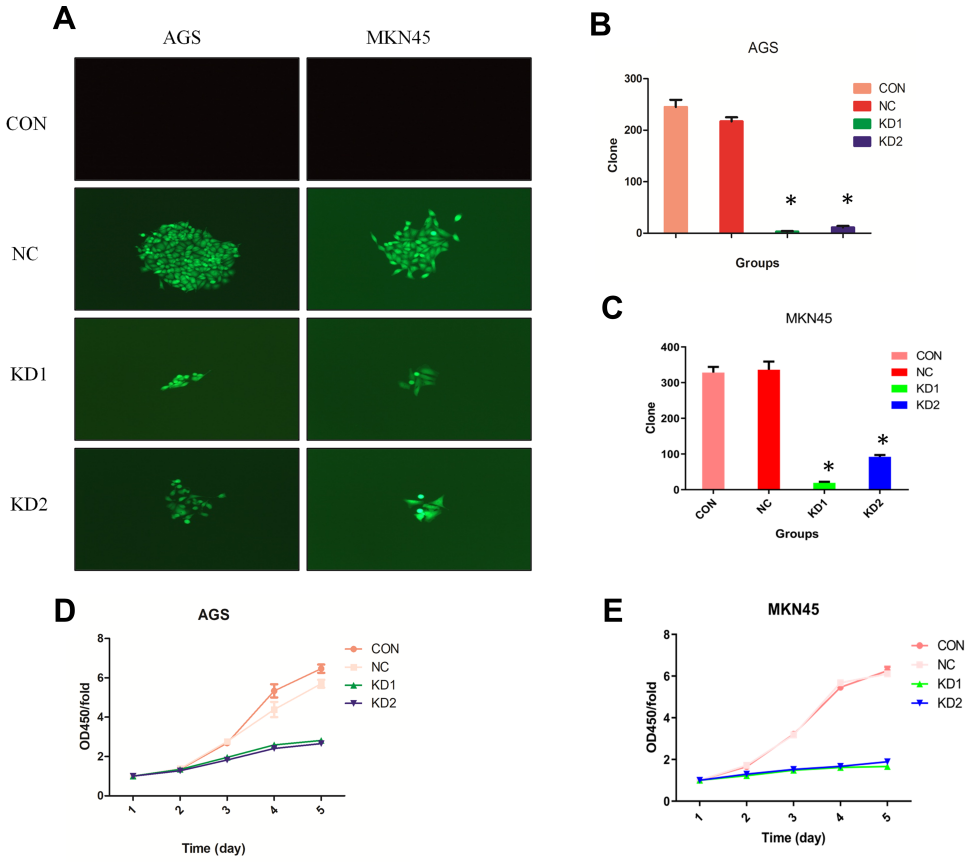

Figure 3 Proliferation (colony formation and CCK8 assay) of gastric cancer cells after silencing *AURKB*. (**A**–**C**) Colony formation assay showed that the ability of cell proliferation decreased significantly after knockdown of *AURKB* in AGS cells and MKN45 cells. After transfection, GC cells were then allowed to form colonies for approximately 6 days (**A**). Corresponding graphs show the mean number of colonies in AGS cells (**B**) and MKN45 cells (**C**). (**D**–**E**) CCK8 assay showed that the proliferation rate significantly decreased at each time point after silencing *AURKB* in AGS cells (**D**) and MKN45 cells (**E**). (*$P < 0.05$).
Abbreviations: CON, control group; NC, negative control group; KD, knockdown group.

Silencing of *AURKB* Arrested the Cell Cycle of GC Cells in G2/M Phase

To detect the effect of down-regulated *AURKB* on the cell cycle of GC, flow cytometry assay was conducted. As shown in Figure 4C–E, the cell counts in G1 phase in KD1 and KD2 groups decreased, and those in G2/M phase increased, compared with those in CON and NC groups (P <0.05). These indicated that down-regulating *AURKB* could arrest GC cells in G2/M phase so that it could prevent the proliferation of GC cells.

Silencing of *AURKB* Inhibited the Invasion and Migration of GC Cells

As we know, epithelial–mesenchymal transition (EMT) was closely associated with the invasion and migration of cancers. Therefore, we evaluated the role of *AURKB* on the invasion and migration of GC cells by using matrigel invasion and wound-healing assays. As a result, we found that the invasion and migration abilities of GC cells in KD1 group were significantly impeded compared with those in CON and NC groups (Figures 5A–C and 6A–D, P <0.05), but not in KD2 group (P >0.05). These showed that the silencing of *AURKB* could inhibit the invasion and migration of GC cells.

The Effect of *AURKB* on Metastasis of GC is Possibly Through Inducing EMT

As is well known, the aberrant activation of an epithelial–mesenchymal transition (EMT) promotes tumor cell invasion and dissemination. EMT is characterized by loss of epithelial markers and higher expression of mesenchymal markers. Therefore, we investigated the potential mechanisms that down-regulation of *AURKB* inhibited invasion and migration of GC cells. After silencing *ARUKB* in GC

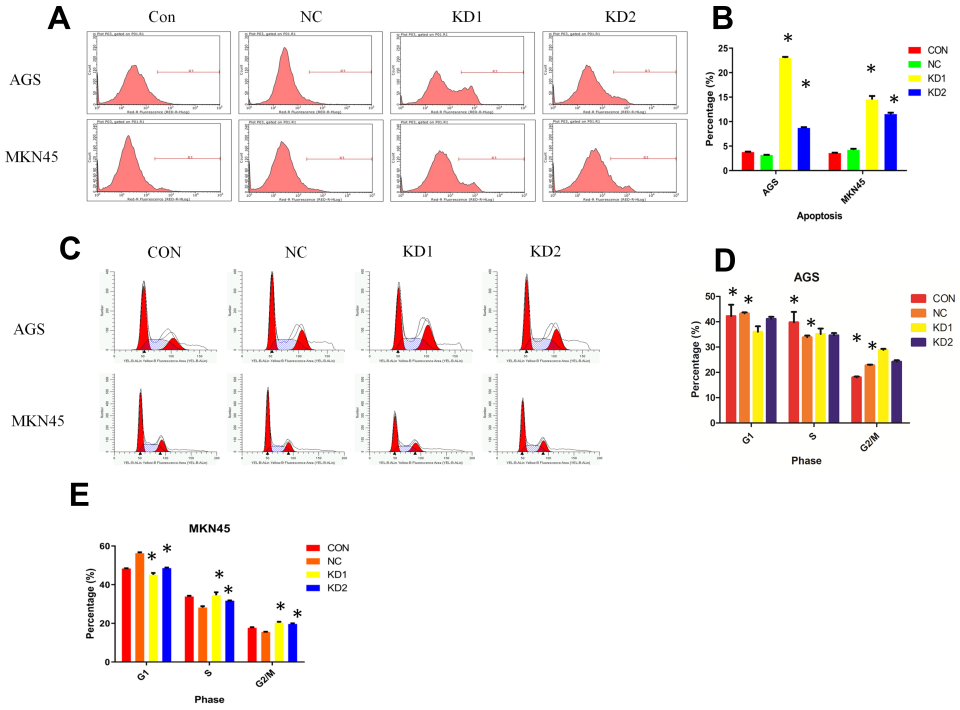

Figure 4 Cell cycle distribution and apoptosis (tested by flow cytometry) of gastric cancer cells after silencing *AURKB*. (**A, B**) Flow cytometry showed the apoptosis rate increased significantly after silencing of *AURKB* in AGS cells and MKN45 cells. (**C**–**E**) The proportion of cells in S phase and G2/M phase after silencing of *AURKB* increased significantly, but that of cells in G1 phase decreased markedly. (*$P < 0.05$).
Abbreviations: CON, control group; NC, negative control group; KD, knockdown group.

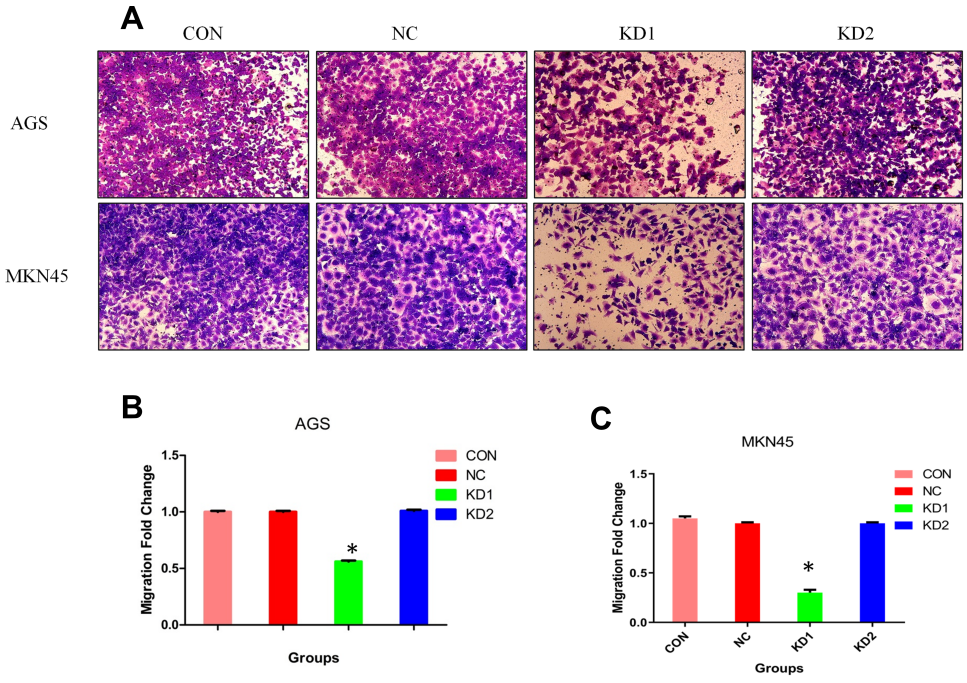

Figure 5 Invasion ability (matrigel invasion assay) of GC cell lines after silencing of *AURKB*. (**A**) The number of AGS cells and MKN45 cells crossing the basement membrane markedly decreased after silencing of *AURKB*. (**B, C**) Quantification of GC cells crossing the basement membrane after silencing of *AURKB*, (**B**) AGS, (**C**) MKN45. (*$P < 0.05$).
Abbreviations: CON, control group; NC, negative control group; KD, knockdown group.

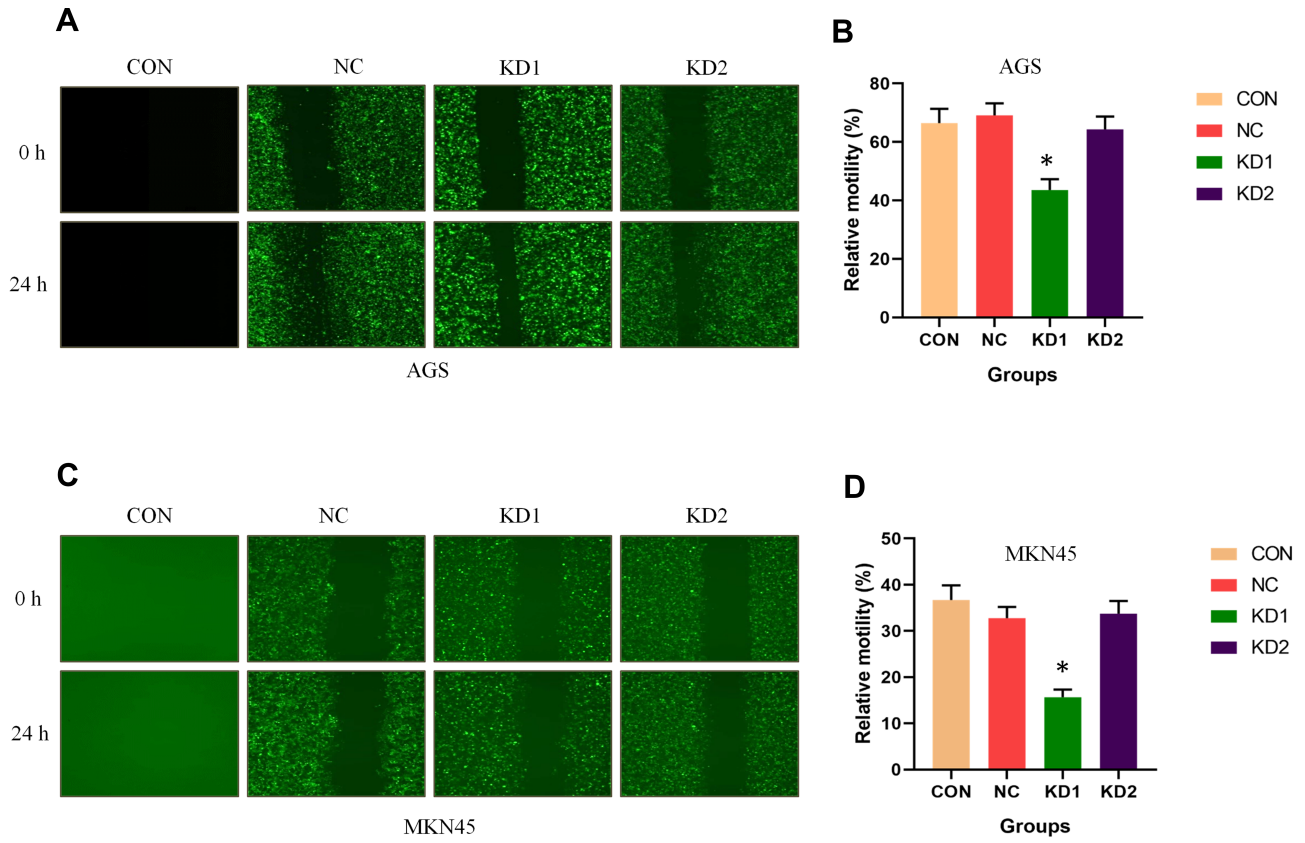

Figure 6 Migration ability (wound-healing assay) of GC cell lines after silencing of *AURKB*. (**A, C**) The migration potential of stably transfected GC cells was checked by wound-healing assays. Images were captured at a time interval between 0 and 24 h, (**A**) AGS cells, (**C**) MKN45 cells. (**B, D**) Percentage of migration was statistically analyzed, (**B**) AGS cells, (**D**) MKN45 cells. (*P < 0.05).
Abbreviations: CON, control group; NC, negative control group; KD, knockdown group.

cells, we detected the expression levels of EMT markers by WB, including AKT1, mTOR, JUP, MMP2, OCLN, Myc, and VEGFA. As performed in Figure 7A–D, the expression levels of AKT1, mTOR, Myc, MMP2, and VEGFA were all decreased after silencing *AURKB* in both AGC and MKN45 cells, and the changes were all statistically significant (P <0.05) except that of MMP2 (P >0.05). While the expression levels of OCLN and JUP were increased after knocking down *AURKB* in both AGC and MKN45 cells, although the change of OCLN was not statistically significant in AGS cells (P >0.05). Therefore, we considered that inhibition of invasion and migration by silencing of *AURKB* was possibly induced by EMT in GC.

Discussion

As a member of aurora kinase family, *AURKB* has been reported to be highly expressed in several cancer types and mainly plays critical roles in cancer cell mitosis.[20] *AURKB* mainly exerts its functions on suppressing the completion of cytokinesis and regulating chromatin modification. Studies have indicated that *AURKB* can promote the growth and metastasis of malignant tumors such as breast cancer,[7] ovarian cancer,[8] non-small cell lung cancer,[10] and osteosarcoma.[21] In fact, the effect of *AURKB* on GC was already studied, but the results remained controversial. Enjoji et al found that overexpression of *AURKB* was related to a favorable prognosis of GC.[12] However, Xiao Han et al reported that upregulated *AURKB* led to metastasis of GC.[14] Therefore, we conducted this study to assess the exact function of *AURKB* in GC.

By analyzing the expression level of *AURKB* in 50 GC patients, we found that *AURKB* was significantly high expressed and closely related to lymph node metastasis (P=0.008) and advanced stages (P=0.016) of GC. Then, in-vitro experiments were performed after silencing

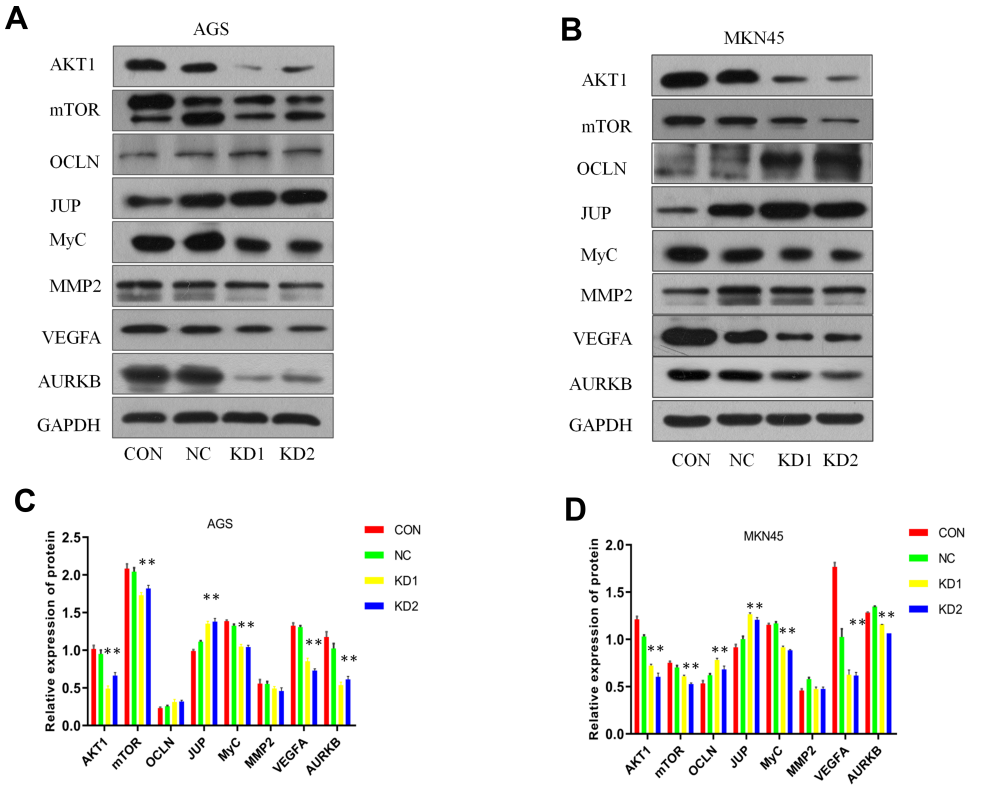

Figure 7 EMT relative protein expressions in GC cells after silencing of *AURKB*. (**A, B**) Western blot was used to test the expression of EMT relative protein in AGC cells (**A**) and MKN45 cells (**B**) after silencing of *AURKB*. (**C, D**) Quantification of EMT relative protein in AGC cells (**C**) and MKN45 cells (**D**) after silencing of *AURKB* according to Western blot. (*$P < 0.05$).
Abbreviations: CON, control group; NC, negative control group; KD, knockdown group.

AURKB. As a result, we found that the down-regulation of *AURKB* inhibited the proliferation and promoted the apoptosis of GC cancer cells, arrested the cell cycle of GC cells in G2/M phase, and inhibited the invasion and migration of GC cells. Our results were similar to previous studies, which found that *AURKB* was closely correlated with the prognosis, invasiveness, chemoresistance of various tumors.[7–11] Based on these results, we considered that *AURKB* could function as a novel oncogene and become a potential therapeutic target in GC.

Epithelial–mesenchymal transition (EMT), which is a process of cell remodeling critical during embryonic development and organogenesis, has been implicated in carcinogenesis and confers metastatic properties upon cancer cells by enhancing invasion and mobility. Studies found that several molecular mechanisms are known to promote the process of EMT, including regulation of the levels of specific cell-surface proteins, cell-extracellular matrix-degrading enzymes, and altering the expression of certain transcription factors.[22] In order to explore the possible mechanisms of *AURKB* in regulating the invasion and migration of GC, we tested the expression level of several biological markers, including AKT1, mTOR, JUP, MMP2, OCLN, Myc, and VEGFA, which were all reported to be closely associated with EMT.[22–24] As a result, we found that the expression levels of AKT1, mTOR, Myc, MMP2, and VEGFA were all decreased after silencing *AURKB* in both AGC and MKN45 cells, and the changes were all statistically significant except that of MMP2. While the expression levels of OCLN and JUP were increased after knocking down *AURKB* in both AGC and MKN45 cells, although the change of OCLN was not statistically significant in AGS cells ($P > 0.05$). Based on these results, we considered that *AURKB* possibly promoted the invasion and migration of GC by inducing EMT.

AKT, a Serine/Threonine kinase, is a key component in numerous processes. Recent studies found that AKT activation could phosphorylate and activate the EMT transcription factors, and AKT/mTOR pathway was one of the key signals regulating EMT in different cancer cells, including oral cancer, thyroid cancer and melanoma.[25–27]

Vascular endothelial growth factor A (VEGFA), exerting an important role in endothelial cell proliferation and vascular remodeling by activating its tyrosine kinase receptor, has been reported to be dysregulated and promote tumor progression in various carcinomas.[28,29] Recently, studies found that VEGFA could induce EMT and promote metastasis of various tumors by playing roles in the response to angiogenesis during tumorigenesis,[30] and this process was through activating PI3K/Akt/mTOR signaling pathway.[31] Matrix metalloproteinases (MMPs) are well known to degrade E-cadherin, which is an important epithelial marker.[32] It has been shown that EMT extension was associated with the level of MMP-2 activity and MMP-9 secretion,[33] and PI3K/AKT pathway has been shown to positively regulate MMPs in various cancers, including gastric cancer.[34] In our study, AKT, mTOR, VEGFA, and MMP2 (although statistical significance was not achieved) were all downregulated after silencing of *AURKB*, suggesting that *AURKB* might promote invasion and migration of GC cells by activating the VEGFA/AKT/mTOR pathway. And this was consistent with previous studies.

It is reported that Wnt/β-catenin signaling pathway has an important role in EMT during carcinoma progression.[35] During the process of EMT, the loss of E-cadherin may abnormally activate Wnt/β-catenin signaling pathway. Then, the degradation of β-catenin is inhibited, and β-catenin enters into the nucleus to promote the expression of downstream target genes such as Myc, which finally promotes the invasion and migration of tumor cells.[36] Both occludin (OCLN) and plakoglobin (JUP) are epithelial markers, which are often decreased during the process of EMT.[37,38] Studies found that decreased expression of OCLN and JUP was associated with malignant progression and poor prognosis of cancers.[39,40] These were verified in our studies, which found that the expression levels of OCLN and JUP were increased, while the expression level of Myc was decreased after silencing *AURKB*, suggesting that knockdown of *AURKB* could regain the expression of epithelial markers and inhibit Wnt/β-catenin pathway.

In conclusion, *AURKB* was highly expressed in GC, and closely associated with lymph node metastasis and advanced stages of GC. Down-regulating *AURKB* inhibited the proliferation and promoted the apoptosis of GC cells, arrested the cell cycle in G2/M phase, and inhibited the invasion and migration of GC cells. The process was possibly through inhibiting VEGFA/Akt/mTOR and Wnt/β-catenin/Myc pathways. However, owing to the small sample size and the lack of in-vivo experiments, these results should be verified by more studies in the future.

Ethics and Consent

All procedures performed in this study involving participants were approved by the Ethical Review Committee of the First Affiliated Hospital of Guangxi Medical University, Guangxi, China, and written informed consent was obtained from all patients.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Disclosure

The authors have no conflict of interest to declare for this work.

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