Rab6c is a new target of miR-218 that can promote the progression of bladder cancer

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Abstract. Bladder cancer has high morbidity and mortality rates among the male genitourinary system tumor types. MicroRNA-218 (miR-218) is associated with the development of a variety of cancer types, including bladder cancer. Rab6c is a member of the Rab family and is involved in drug resistance in MCF7 cells. The aim of the present study was to clarify the relationship between Rab6c and miR-218 in bladder cancer cell lines. In this study, the expression levels of miR-218 and Rab6c were evaluated via reverse transcription-quantitative PCR and western blotting, respectively. The association between Rab6c and miR-218 was recognized via TargetScan analysis and dual luciferase reporter gene detection. Cell proliferation was analyzed using Cell Counting Kit-8 and colony formation assays, and the invasive ability was measured via Transwell assays. Rab6c was highly expressed in bladder cancer, while miR-218 had abnormally low expression in bladder cancer. In addition, there was a mutual regulation between Rab6c and miR-218 in bladder cancer. It was found that overexpression of Rab6c significantly enhanced the proliferation, colony formation and invasion of T24 and EJ cells. Furthermore, miR-218 overexpression blocked the promoting effects of Rab6c on the malignant behavior of bladder cancer cells. Thus, Rab6c promotes the proliferation and invasion of bladder cancer cells, while miR-218 has the opposite effect, which may provide a novel insight for the treatment of bladder cancer.

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Introduction

Bladder cancer is diagnosed in more than 430,000 patients worldwide every year, making it the ninth most common malignancy (1). Ninety percent of bladder cancers are transitional cell carcinomas, and the other 10% are secondary deposits of squamous cell carcinoma, adenocarcinoma, sarcoma, small cell carcinoma and other cancers of the body (2). Bladder cancer has long been a threat to human health due to its high morbidity and mortality rates (3). In addition, genetic mutations and a variety of external risk factors such as exposure to carcinogens, smoking, chlorination of drinking water and cyclophosphamide can lead to bladder cancer (4). Unfortunately, the etiology and pathophysiology of bladder cancer are not fully understood.

The Rab protein family, a large number of small Rab GTPases, mediates secretion, endoplasmic reticulum membrane transport and the biogenesis of autophagosomes, and it is an essential component of the vesicle transport mechanism (5). Overexpression of Rab GTPases is related to cancer progression, and there are many mechanisms by which Rab protein dysfunction has been linked to cancer development (6). Elevated expression of oncogenic Rab1, along with Rab1a proteins, promotes the growth of tumors, often resulting in a poor prognosis (7). Overexpression of Rab23 has been linked to gastric cancer (8), and Rab23 overexpression has been shown to facilitate malignant cell growth and invasion in bladder cancer via the NF-κB pathway (9). Rab25 contributes to the invasiveness of cancer cells by regulating integrin trafficking (10). Upregulation of Rab27b promotes the malignant biological behavior, including F-actin recombination, G1/S phase cell cycle transformation, cell proliferation, and invasion enhancement of estrogen receptor-positive breast cancer cells (11). Rab2a GTPase promotes breast cancer stem cells and tumor progression via Erk signaling activation (12). Abnormal overexpression of Rab5a may stimulate the proliferation of ovarian cancer cells through the APPL1-related epidermal growth factor signal transduction pathway (13). Rab6c, a newly identified Rab6 subfamily member, has attracted recent attention because its aberrant expression might confer a selective advantage to drug-resistant breast cancer cells (14). However, the role of Rab6c in bladder cancer remains unknown.

miRNAs are small non-coding RNAs that regulate gene expression by binding to the 3' untranslated (3'UTR) of mRNA, resulting in mRNA degradation or protein translation inhibition (15). There are over 1,000 miRNAs in the human genome, each potentially regulating hundreds of mRNAs. Many miRNAs have been identified to exert important roles in various cellular biological processes (16). The miR-218 located on chromosome 4p15.31, is associated with tumor growth, invasion as well as metastasis (17). Accumulating evidence has shown that the expression of miR-218 is abnormally low in gastric cancer, cervical cancer, head and neck squamous cell carcinoma, and breast cancer (18,19). A recent bioinformatics analysis has suggested that miR-218 may be a candidate tumor suppressor gene for bladder cancer, potentially inhibiting the proliferation, migration, and invasion of bladder cancer cells (20). Additionally, several targets of miR-218 in bladder cancer have been reported including LASP1 (21), BMI1 (22), and Glut1 (23). Given that miRNA targeting transcripts is guided by complementary partial sequences, each miRNA may regulate hundreds of genes (16). miRNAs have been the focus of bladder cancer research in recent years (24). Therefore, the present study aimed to investigate the role of miR-218 in bladder cancer and its targets.

Materials and methods

Bioinformatics analysis of public datasets. The Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) databases provide an invaluable resource of publicly available gene expression data that can be integrated and analyzed to derive new hypothesis and knowledge. In this study, the difference in Rab6c expression between tumor and normal of bladder cancer samples were identified in the Gene Expression Omnibus (GEO) database (ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE3167) (25) and the database included 41 tumor tissue samples from bladder cancer patients and 9 normal tissue samples. The data was unpaired and had no gender distribution information. In addition, miR-218 expression in bladder cancer samples was analyzed from Cancer Genome Atlas (TCGA) database (portal.gdc.cancer.gov/) (26) and the database included 412 tumor tissue samples from bladder cancer patients and 19 normal tissue samples. The data was paired but there was no gender distribution information. In addition, TargetScan human 7.1 (targetscan.org/vert_72/) was used to analyze the target genes of miR-218 in bladder cancer.

Collection of clinical tissue specimens from patients. Tumor tissue and matched adjacent normal tissue samples were collected from 6 patients with bladder cancer (aged 55-67 years) undergoing surgery at the General Hospital of Shenyang Military from 2008/1/31 to 2014/3/31 (Shenyang, China). The inclusion criteria were male patients diagnosed with bladder cancer by histology or cytology. The exclusion criteria were those who had received systemic anti-cancer treatment for metastatic or persistent/recurrent disease, or had a disease involving the bladder during screening. Notably, <3 cm is adjacent normal tissue, 3-5 cm is near cancer tissue, and greater than 5 cm is distant cancer tissue (27). Permission for the collection and application of patient samples was granted by the Ethics Committee of the General Hospital of Shenyang Military. In addition, each patient signed a written informed consent before operation. All obtained clinical tissue specimens were immediately frozen in liquid nitrogen and stored at -80˚C.

Cell culture and transfection. Bladder cancer cell lines (SV-HUC-1, T24 and EJ) were purchased from the American Type Culture Collection and cultured for 24 h at 37˚C in a humidified atmosphere containing 5% CO_2 using DMEM (HyClone; Cytiva), containing 10% FBS (HyClone; Cytiva) and antibiotics (100 U/ml penicillin and 100 mg/ml streptomycin). All T24 and EJ cells were authenticated by short tandem repeat and confirmed negative for mycoplasma contamination prior to the experiments. The cells were transferred to the second generation for lentivirus transfection. Lentivirus vectors plasmid were constructed by GenePharma. Following the instructions of Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.), recombinant miR-218 lentivirus particles ($1x10^8$ TU/ml) constructed with 5 μg GV309, Rab6c lentivirus particles ($1x10^8$ TU/ml) constructed with 7.5 μg plent-EF1a-FH-CMV-GP were used to transfected cultured cells ($5x10^5$) using Lipofectamine 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) in DMEM with 10% FBS at a multiplicity of infection of 10 for 20 min. The lentiviral vector with green fluorescent protein (GFP) and resistance tag (Puromycin, 400 ng/ml) was used to select the cells. 72 h after transfection, the expression of GFP of the cells were observed under fluorescence microscope (x200 magnification; Olympus Corporation) and cultured for 24 h at 37˚C in a humidified atmosphere containing 5% CO_2 for subsequent experiments.

Reverse transcription-quantitative PCR (RT-qPCR). TRIzol reagent (Invitrogen, Germany) was used to obtain RNA from bladder cancer tissues and cells. According to the manufacturer's instructions, a TaqMan MicroRNA reverse transcription kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used for reverse transcription of total RNA to obtain cDNA to analyze miRNA expression. Subsequently, template cDNA was used for qPCR analysis with PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) and miR-218 or GAPDH primers, in which GAPDH was used as an internal reference. mRNA expression analysis of Rab6c was performed in the same manner. The thermocycling conditions were as follows: Initial denaturation 95˚C, 15 min; 40 of cycles of 55˚C for 15 min and final extension at 85˚C for 2 min. The primer sequences used in this study were as follows: Rab6c sense, 5'-AGGAGATCTGCC GCCGCGATCGC-3' and antisense, 5'-CGAGCGGCCGCG TACGCGTCCTC-3'; miR-218 sense, 5'-CGAGTGCATTTG TGCTTGATCTA-3' and antisense, 5'-TGGTGTCGTGGAGTC G-3'; U6 sense, 5'-CTCGCTTCGGCAGCACA-3' and antisense, 5'-AACGCTTCACGAATTTGCGT-3'; GAPDH sense, 5'-ACA ACTTTGGTATCGTGGAAGG-3' and antisense, 5'-GCC ATCACGCCACAGTTTC-3'. The relative mRNA expression of miR-218 and Rab6c was quantified with cycle threshold values and normalized using the $2^{-\Delta\Delta Cq}$ method (28). U6 was the internal control of miR-218 expression, and GAPDH was the internal control of Rab6c expression. The expression of miR-218 and Rab6c was relative to the fold change of the corresponding negative controls, which was defined as 1.0.

Western blotting. Total protein was extracted from cultured cells or tissues using RIPA lysis buffer (Beyotime). Subsequently, protein content was determined by the bicinchoninic acid method (BCA, Pierce; Thermo Fisher Scientific, Inc.) and 30 μg of protein from each group was separated by 10% SDS-PAGE and transferred to nitrocellulose membranes (Amersham Bioscience). After the membranes were blocked using TBST solution containing 5% skimmed milk and 0.5 ml/l Tween-20 at 4˚C for 1 h, incubated with primary antibody against Rab6c (1:500; Invitrogen; Thermo Fisher Scientific, Inc.; PA5-39409) and GAPDH (1:1,000; Santa Cruz Biotechnology, sc-47724) at room temperature for 2 h, they were incubated with horseradish peroxidase-labeled secondary antibody (Santa Cruz Biotechnology) with 1:5,000 dilution. The protein signal bands were visualized using an enhanced chemiluminescence detection reagent (ECL; Thermo Scientific, Inc.) and analyzed by ImageJ software 1.8.0.112 (National Institutes of Health).

CCK-8 assay. The T24 and EJ bladder cancer cell lines were seeded at $2x10^3$ cells/well and cultured in 6-well plates for 5 days. The manufacturer's instructions of the CCK-8 kit (Dojindo) were strictly followed to perform the cell counting experiment using a microplate reader (BioTek) to measure the optical density (OD) at 450 nm.

Colony formation detection. The colony formation assay was performed as previously described (29). Briefly, transfected T24 and EJ bladder cancer cells were cultured in 6-well plates at a density of 1,000 cells/well for 10 days. After the cell colonies were treated with methanol for 15 min, they were stained with 0.1% crystal violet for 10 min at room temperature. After the number of cells in a single clone was greater than 50, and the size between 0.3-1.0 mm, we started counting and taking pictures under an optical microscope (Olympus). The percentage of colony formation was calculated by setting the control group to 100%.

Cell Transwell invasion assay. Briefly, $2x10^4$ transfected T24 and EJ cells were seeded in the upper Transwell invasion chambers (24-well, 8-mm pore; Corning), which were coated with Matrigel (BD Biosciences) at 37˚C. The lower chamber was filled with medium containing 10% FBS. After 48 h, the unmigrated cells were removed, and the cells that migrated to the bottom were fixed with 70% ethanol and stained with 0.1% crystal violet for 20 min at room temperature. Next, the stained cells were photographed under fluorescent microscope (200x magnification, Olympus Corporation) and counted by ImageJ software 1.8.0.112 (National Institutes of Health).

Luciferase reporter assay. The sequences of h-Rab6c-3'UTR-wild-type (WT) or h-Rab6c-3'UTR-mutant (Mut) were synthesized, connected into the pSI-Check2 vector (Hanbio) and extracted plasmids. The 293T cells (Chinese Academy of Sciences, Shanghai, China) were cultured in 96-well plates for 24 h at 37˚C until the cell density reached $5x10^4$ cells/ml and co-transfected with the corresponding plasmids (0.16 μg) with Lipofectamine 2000 (0.3 μl, 0.8 mg/ml; Invitrogen;; Thermo Fisher Scientific, Inc.) at 37˚C. After 6 h of transfection, the cells were exchanged for fresh DMEM medium, and cultured for 48 h at 37˚C for subsequent *Renilla* luciferase detection. Follow the instructions of the Dual Luciferase Reporter Assay Kit (Promega Corporation), 100 μl Passive Lysis Buffer was added to the 96-well plate and centrifuged at 1,200 x g at 4˚C for 10 min, and then 100 μl Luciferase Assay Reagent II and 100 μl cell lysate were added in sequence and mixed by pipetting 2-3 times. 100 μl STOP & GLO® reagent (Luciferase Assay Reagent; Promega Corporation) was added and mixed 2-3 times to record the *Renilla* luciferase value, which was the reporter gene luminescence value.

Statistical analysis. The presented results were representative of experiments repeated at least three times and all data as the mean ± standard deviation (SD). Statistical analysis was conducted with SPSS 21.0 (IBM, Inc.) and GraphPad 8.0 (GraphPad Software). All tests were analyzed using paired t-test and one-way ANOVA followed by Bonferroni's post hoc test analysis. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Rab6c is upregulated and miR-218 is lowly expressed in bladder cancer. Previous studies have reported that miR-218 was associated with the development of a variety of cancers, including bladder cancer (23,30). In addition, Rab6c is a member of the Rab family and is involved in drug resistance in MCF7 cells (31). The relationship between Rab6c and miR-218 in bladder cancer cell lines was examined in the current study. First, the difference in Rab6c expression between tumor and normal of bladder cancer samples was identified in the GEO database. The results indicated that the expression level of Rab6c in tumor tissues was significantly higher compared with that in normal tissues (Fig. 1A). Conversely, TCGA database results demonstrated that miR-218 expression was significantly lower in bladder cancer tissues compared with that in normal tissues (Fig. 1B).

Subsequently, the expression levels of Rab6c and miR-218 were detected in clinical samples of bladder cancer. As expected, Rab6c mRNA expression was higher in tumor tissues compared with in normal tissues (Fig. 1C). At the same time, it was identified that miR-218 was expressed at low levels in bladder cancer (Fig. 1D). Consistently, Rab6c protein expression was abnormally elevated in tumor tissue compared with normal tissues (Fig. 1E). Due to the individual differences and pathological grade stages of these 6 patients with bladder cancer, the expression level of Rab6c varied among different patients. In fact, in patients with advanced bladder cancer (cases 2, 3, 6), Rab6c was expressed in normal tissues adjacent to the cancer, but it is still lower than that in tumor tissues. In addition, Rab6c expression in T24 and EJ cells was relatively higher compared with that in SV-HUC-1 cells (Fig. 1F). In general, Rab6c was upregulated in bladder cancer, while miR-218 was expressed at low levels.

Rab6c and miR-218 are mutually regulated in bladder cancer. Rab6c and miR-218 overexpressing cells were constructed to examine their effects in bladder cancer cells. As presented in Fig. 2A, miR-218 overexpression was established in T24 and EJ cells. It was found that Rab6c protein expression was decreased

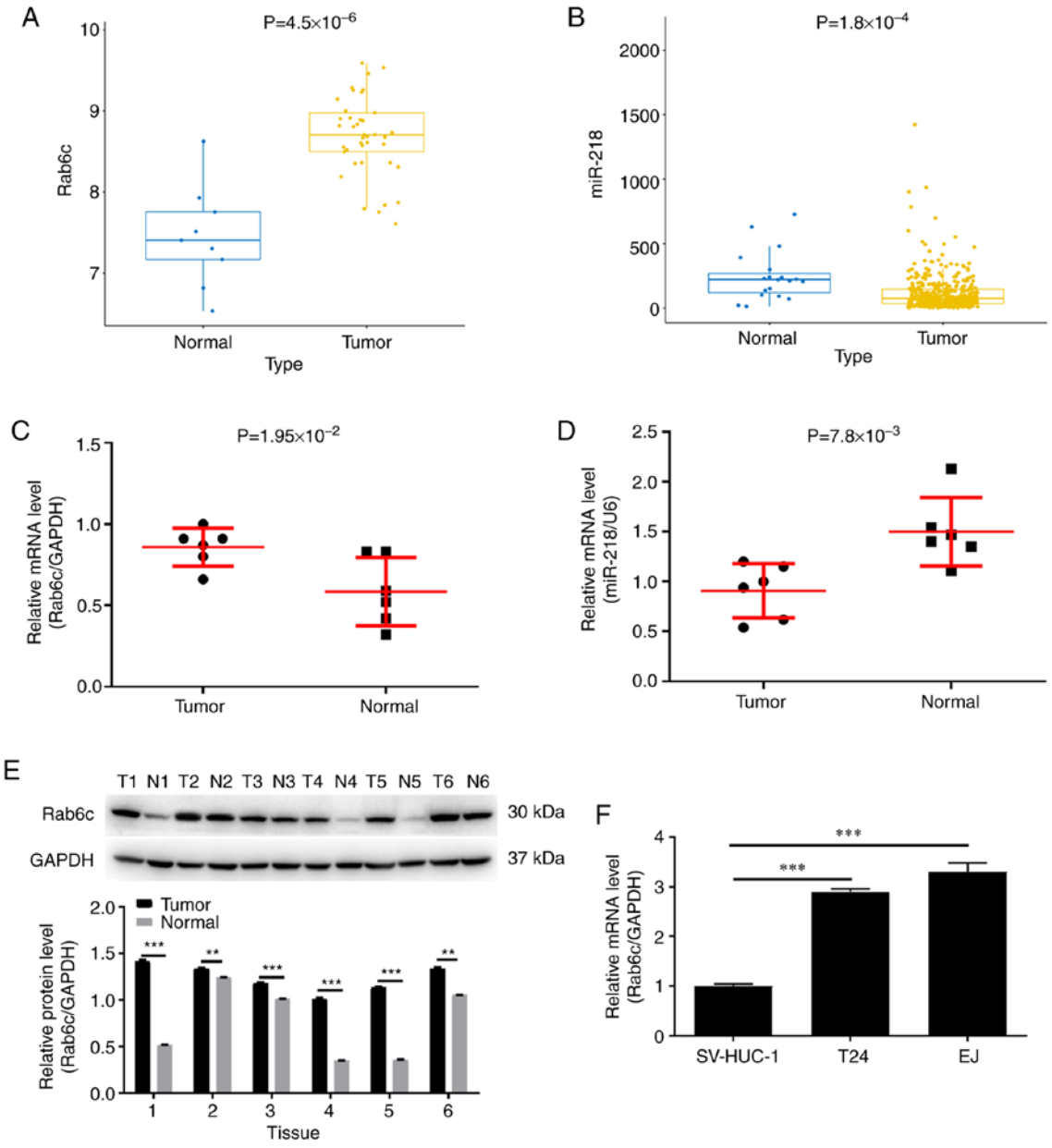

Figure 1. Rab6c expression is upregulated in bladder cancer tissue. (A) Difference in Rab6c expression between tumor and normal of bladder cancer samples was identified in the GEO database. (B) The Cancer Genome Atlas database showed the difference in miR-218 expression between tumor and normal of bladder cancer samples. mRNA expression levels of (C) Rab6c and (D) miR-218 were detected in clinical samples of bladder cancer. (E) Relative expression level of Rab6c was measured via western blotting in six pairs of bladder cancer tissues and adjacent normal tissues. GAPDH was used for normalization. The fold-changes are shown in the bar chart. (F) mRNA expression level of Rab6c in SV-HUC-1, T24 and EJ cells was detected. ***P<0.001. T, tumor; N, normal; miR, microRNA.

in miR-218-overexpressing T24 and EJ cells (Fig. 2B). Moreover, the expression level of miR-218 was downregulated in Rab6c-overexpressing T24 and EJ cells (Fig. 2C).

According to the conserved miR-218 binding site (-AAGCACAA-) in the 3'UTR of Rab6c mRNA, as indicated by TargetScan, a publicly available algorithm, Rab6c was preliminarily identified as a promising target for miR-218 (Fig. 3A). Compared with the NC group, hsa-miR-218-3p significantly downregulated the luciferase activity in h-Rab6c-3'UTR-wt group, indicating an interaction between RAB6C and miR-218. However, hsa-miR-218-3p failed to downregulate luciferase activity in h-Rab6c-3'UTR-mu group (Fig. 3B and C). These results suggested that there was a negative regulatory effect between Rab6c and miR-218 in bladder cancer.

Overexpression of miR-218 reverses the Rab6c-stimulated proliferation of bladder cancer cells. As the biological function of Rab6c in bladder cancer cells has not been revealed, to the best of our knowledge, proliferation was evaluated in T24 and EJ cells using CCK-8 and colony formation assays. The result of western blotting indicated that Rab6c expression was significantly upregulated following transfection with Rab6c lentivirus particles (Fig. 4A). The CCK-8 assay demonstrated that cell proliferation was notably promoted by Rab6c overexpression (Fig. 4B). Consistent with the results, Rab6c overexpression accelerated colony formation, as indicated by an increased number of colonies (Fig. 4C). To elucidate whether the effects of Rab6c overexpression were reversed by miR-218, restoration experiments were performed.

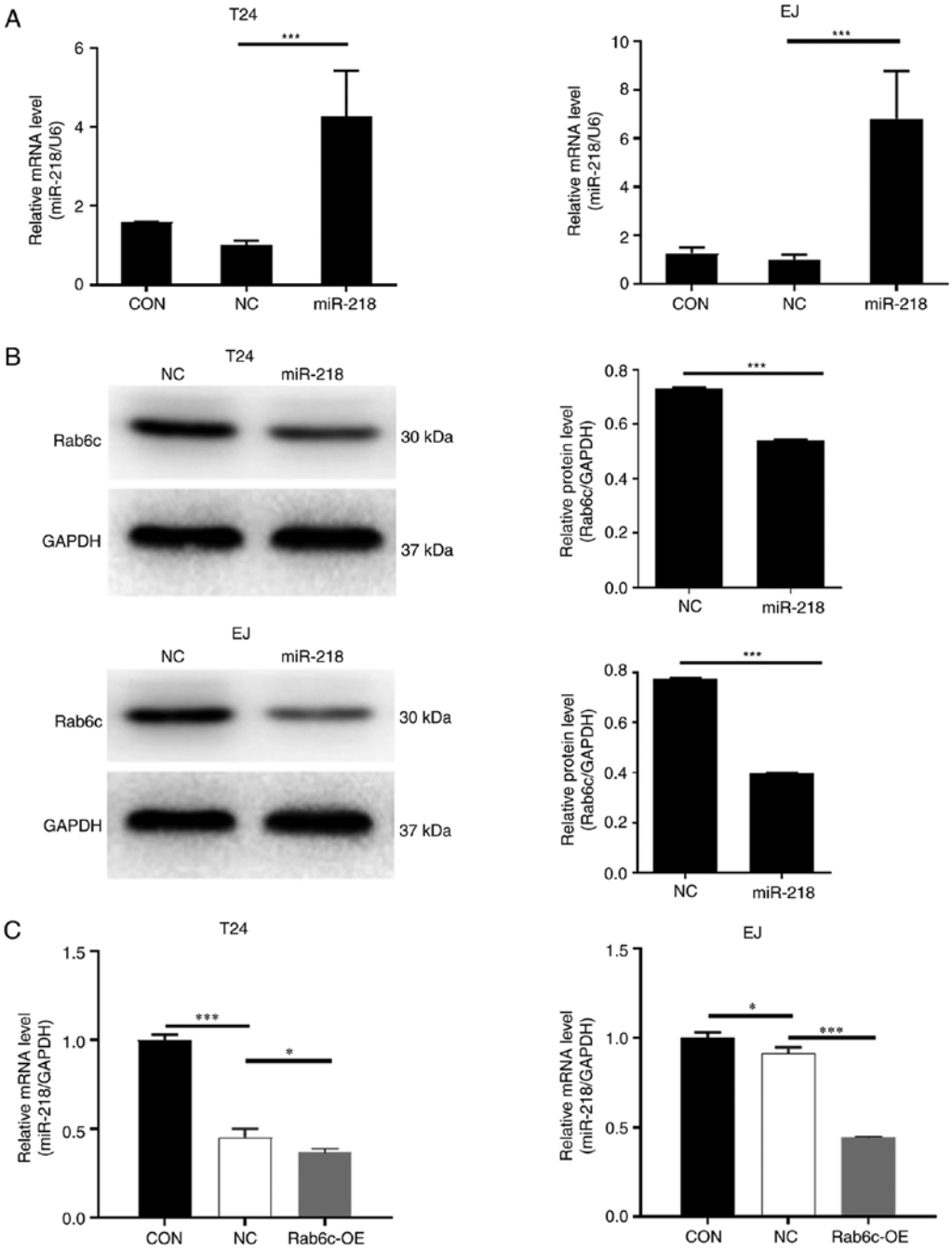

Figure 2. Establishment of miR-218 or Rab6c overexpression in T24 and EJ cells. (A) The relative mRNA expression level of miR-218 was determined via reverse transcription-quantitative PCR in the T24 and EJ cells transfected with recombinant miR-218 lentivirus particles or NC. U6 was used for normalization. (B) Rab6c protein expression in T24 and EJ cells was determined by western blotting following transfection with recombinant miR-218 lentivirus particles or NC. GAPDH was used for internal control. CON refers to cells without transfection; NC refers to cells transfected with the empty vector. (C) The relative mRNA expression level of miR-218 was determined via reverse transcription-quantitative PCR in the T24 and EJ cells transfected with recombinant Rab6c lentivirus particles or NC. *P<0.05, ***P<0.001. NC, negative control; miR, microRNA.

As shown in Fig. 4B and C, the cells overexpressing Rab6c and miR-218 exhibited a lower proliferation rate and fewer colonies compared with the cells overexpressing Rab6c alone.

Overexpression of miR-218 reduces Rab6c-promoted invasion of bladder cancer cells. Bladder cancer cell invasion was examined using a Transwell assays. Rab6c overexpression significantly promoted the invasion of bladder cancer cells (Fig. 5). However, co-transfection with miR-218 overexpression significantly reduced the invasion of T24 and EJ cells. Thus, it was concluded that Rab6c may serve a stimulative role in the proliferation and invasion of bladder cancer cells, which could be reversed by miR-218.

Discussion

Bladder cancer affects ~430,000 individuals and results in 165,000 deaths annually worldwide (32). Previous studies have reported several genes relevant to the progression of bladder cancer (33-35). For example, microarray data analysis

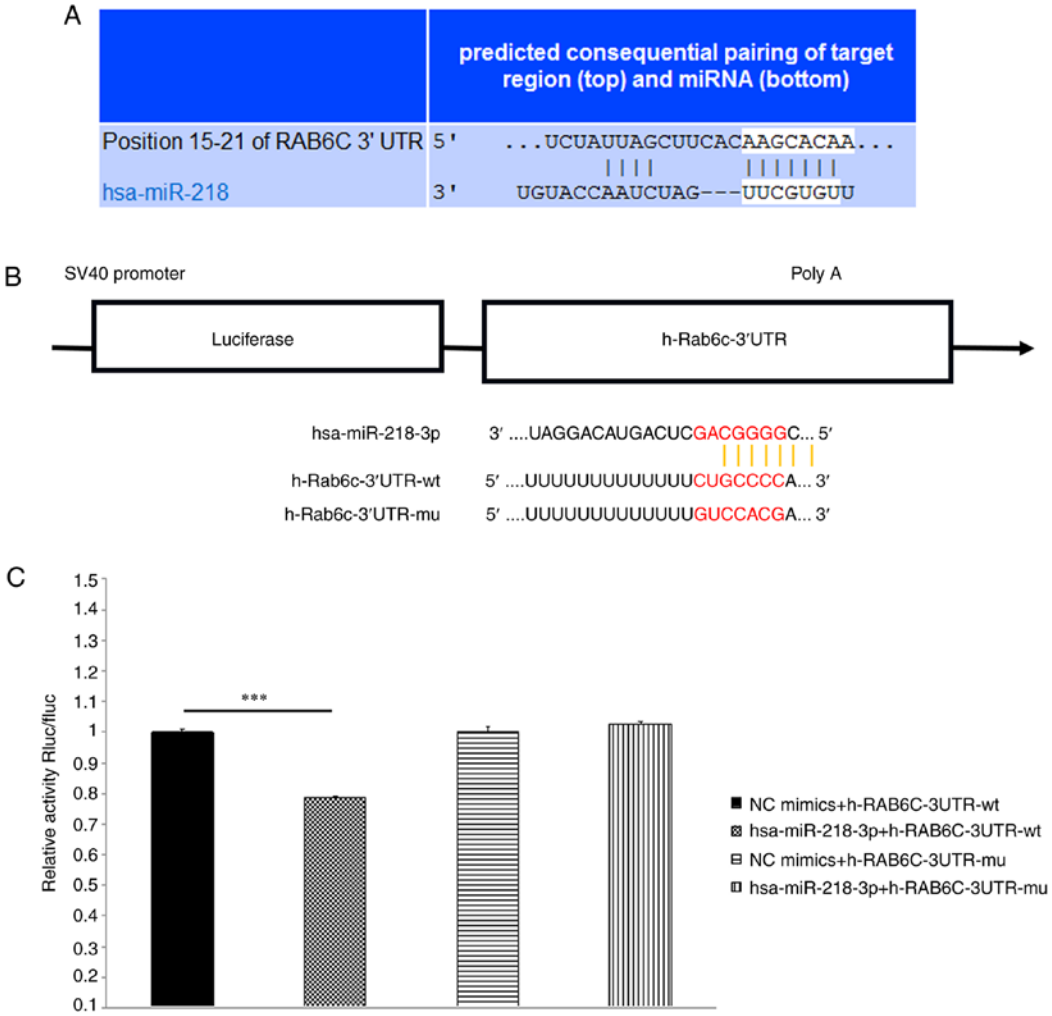

Figure 3. Rab6c and miR-218 are mutually regulated in bladder cancer. (A) The potential miR-218 binding site in the 3'UTR of Rab6c mRNA was computationally predicted using TargetScan. (B) The interaction between hsa-miR-218-3p and h-Rab6c-3'UTR was detected by (C) dual luciferase reporter assay. ***P<0.001. NC, negative control; miR, microRNA; mu, mutant; wt, wild-type; UTR, untranslated region.

has shown that PCMT1 is more highly expressed in bladder cancer than in normal urothelial tissue, and that it is positively correlated with myometrial invasion, lymph node metastasis, distant metastasis and clinical stage (33). Wnt7a activates canonical Wnt signaling and promotes bladder cancer cell invasion, and Wnt7a is associated with bladder cancer metastasis and predicts worse clinical outcome (34). Forkhead box M1 has been proposed to directly activate ATP binding cassette subfamily G member 2 (Junior blood group) to increase the drug efflux activation and drug resistance in bladder cancer cells (35). These studies have expanded the current knowledge on bladder cancer, providing a theoretical foundation for a new treatment of bladder cancer.

Accumulating evidence has revealed that miR-218 affects the progression of various cancer types by interacting with a variety of small molecules (36-40). In acute promyelocytic leukemia, overexpression of miR-218 significantly inhibits cancer cell proliferation, arrests the cell cycle in the G_0/G_1 phase and induces apoptosis by targeting BMI1 (22). High expression of RUNX family transcription factor 2 can restore the inhibitory effects of miR-218 on malignant behavior of ovarian cancer cells (17), while NEAT1 promotes cell invasion and proliferation by negatively regulating miR-218 in breast cancer (41). Moreover, in gastric cancer, miR-218 suppresses gastric cancer cell cycle progression via the CDK6/Cyclin D1/E2F1 axis in a feedback loop (42). Similarly, miR-218 functions as a tumor suppressor gene in cervical cancer (36). miR-218 suppresses the metastasis and epithelial-mesenchymal transition of hepatocellular carcinoma cells (43). Torres-Berrio *et al* (44) demonstrated that miR-218 is a molecular switch and potential biomarker of stress susceptibility. However, the relationship between miR-218 and bladder cancer remains unknown.

As an essential part of the vesicle transport mechanism, specific Rab proteins coordinate with homologous effectors to determine the destination of cargo proteins (45). Mutation of the Rab protein or posttranslational modification leads to the destruction of the regulatory network of vesicle transport, which impairs protein secretion, endocytosis, recycling and degradation, and is implicated in tumorigenesis (46,47). Accordingly, the mechanism of vesicle transport serves an important role in regulating the biological behavior of cancer cells. For example, overexpression of Rab1a activates the mTOR complex 1 signaling pathway, which stimulates the progression and invasion of colorectal cancer (48). Additionally, Rab2a mediates the activation of Erk signaling to drive the

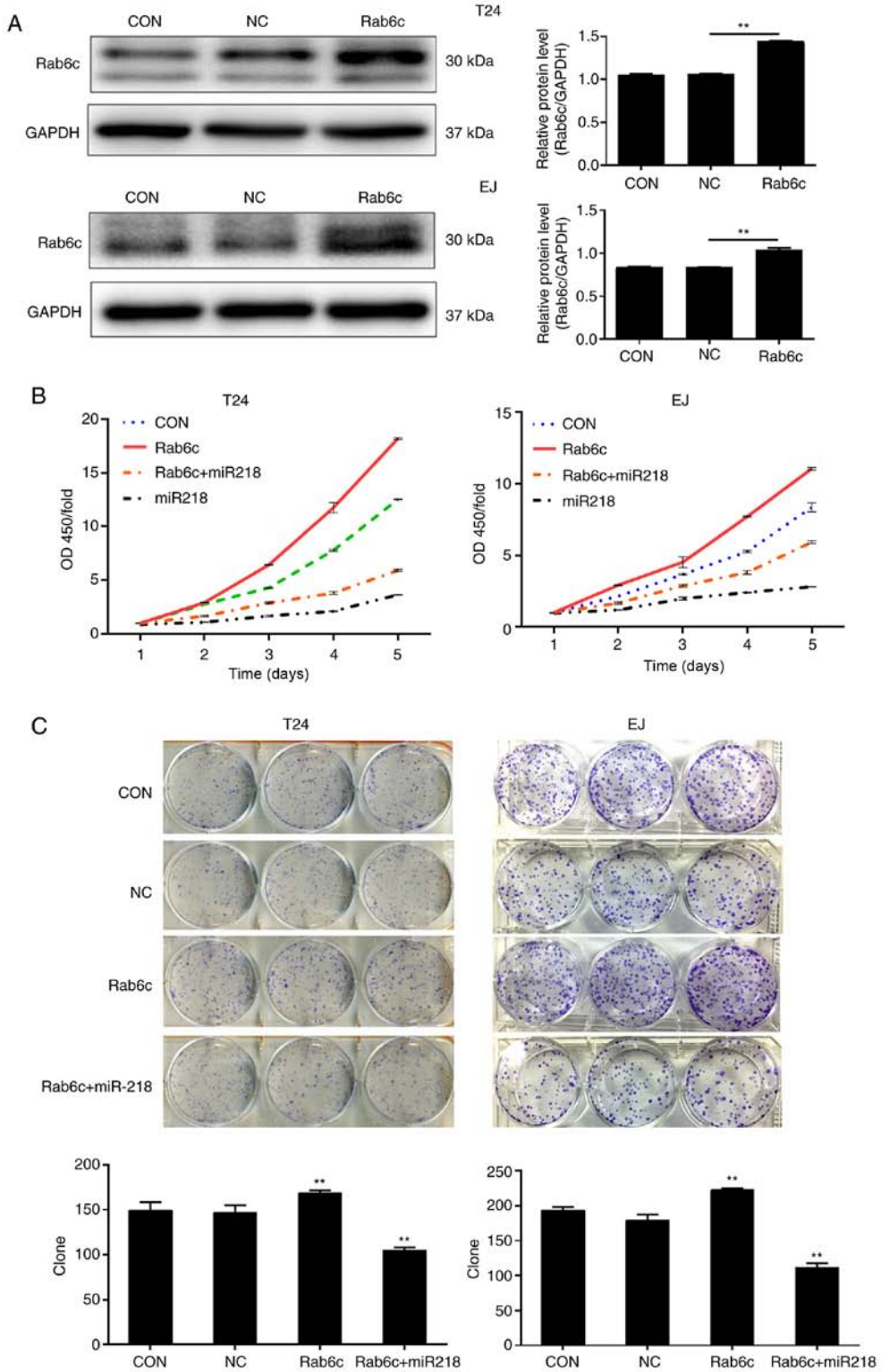

Figure 4. Overexpression of miR-218 reversed Rab6c-stimulated cell proliferation *in vitro*. (A) Rab6c expression was determined via western blotting in the T24 and EJ cells transfected with recombinant Rab6c lentivirus particles or NC. GAPDH was used for normalization. Cell proliferation was determined by (B) Cell Counting Kit-8 and (C) colony formation assays in the T24 and EJ cells with overexpressed Rab6c and/or miR-218 as indicated in the charts. CON refers to cells without transfection; NC refers to cells transfected with the empty vector. **P<0.01. NC, negative control; miR, microRNA; OD, optical density.

proliferation of breast cancer stem cells (12), and upregulation of Rab25 indicates a poor prognosis in breast and ovarian cancer (49). Furthermore, phosphorylation of Rab proteins is important for vesicle targeting and trafficking, and phosphorylation of Rab5a by protein kinase C (PKC) facilitates T-cell migration (50). Mechanistically, Rab5a phosphorylation leads to the activation of Rac1 to promote actin remodeling (51). Conventional PKC-mediated Rab11 and Rab6 phosphorylation contributes to impaired endosomal recycling and redistribution in the cytosolic fraction, respectively (52,53). The present study reported a novel role of Rab6c in bladder cancer progression and its regulation by miR-218.

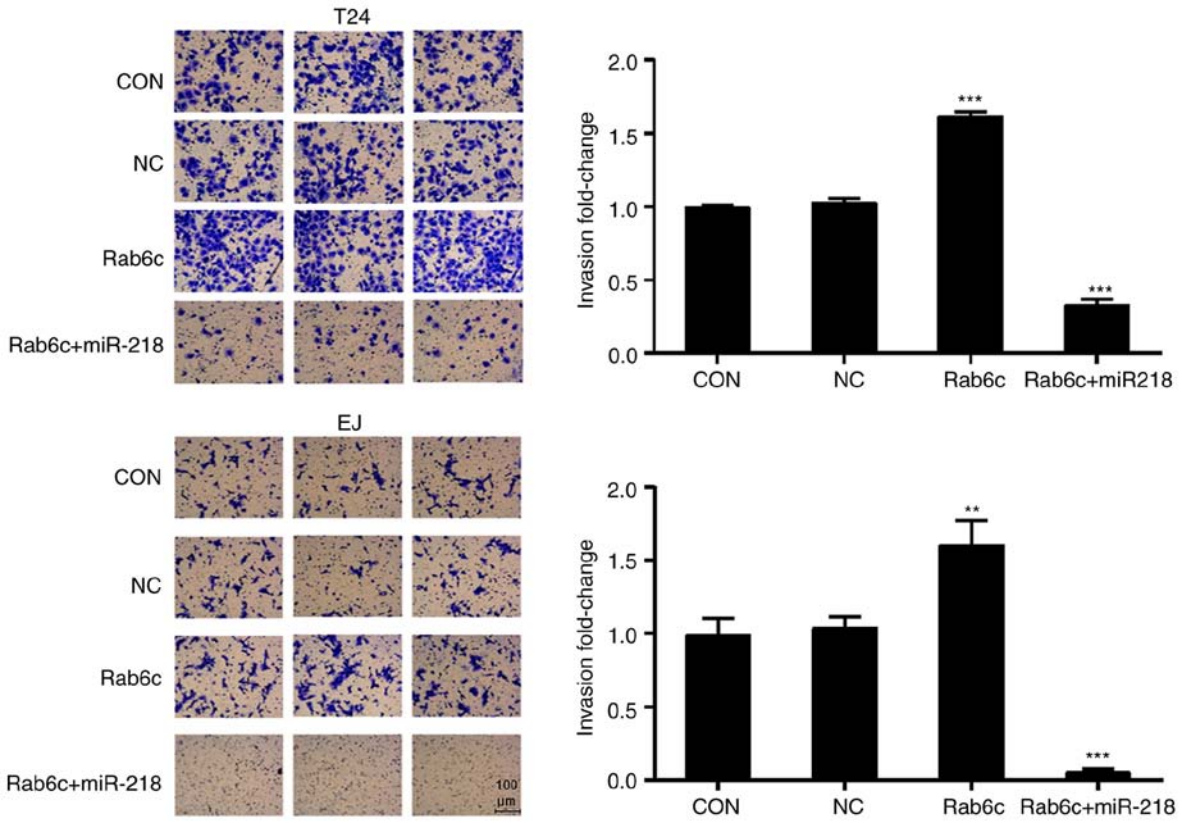

Figure 5. Overexpression of miR-218 compromises Rab6c promoted bladder cancer cell invasion. Cell invasion was determined using a Transwell assay in the T24 and EJ cells with overexpressed Rab6c and/or miR-218. CON refers to cells without transfection; NC refers to cells transfected with the empty vector. **P<0.01, ***P<0.001. NC, negative control; miR, microRNA.

Rab6c, a newly identified member of the Rab family, participates in the resistance of MCF7/AdrR cells (14). Moreover, Rab6c is a retrogene that encodes a centrosome protein involved in cell cycle progression (54). In addition, Rab6c is an independent prognostic factor for estrogen receptor positive/progesterone receptor negative breast cancer (55). The current study not only found that Rab6c was upregulated in bladder cancer tissues, but also identified that the upregulation of Rab6c enhanced the proliferation and invasion of bladder cancer cells *in vitro*. Thus, it was demonstrated that Rab6c exerted a tumor promoting role in bladder cancer. Moreover, western blot analysis revealed abnormally high expression of Rab6c protein in bladder cancer cells. Overexpression of miR-218 in cultured bladder cancer cell lines significantly inhibited Rab6c expression and reversed the malignancy induced by Rab6c. This evidence suggested that Rab6c was a target gene of miR-218 in bladder cancer. However, as the collected bladder cancer tissue and cell types in the present study were limited, additional detailed studies based on larger sample sizes are required to further confirm the role of miR-218 and Rab6c in the progression of bladder cancer.

In summary, the present study demonstrated that Rab6c served a stimulative role in bladder cancer progression, and that it was targeted and negatively regulated by miR-218. Therefore, miR-218 may serve as a promising innovative therapeutic target and Rab6c as a biomarker for bladder cancer treatment.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DH designed this project. LH, XP, YC, XW performed the cell experiments. LH, PC and DH performed the rest of the experiments. PC and CD conducted the data collection and analysis. LH produced the manuscript, which was checked and revised by DH. All authors read and approved the final manuscript. DH, LH and XP confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The collection and use of patient samples was approved by the Ethics Committee of the General Hospital of Shenyang Military (approval no. 201917), and written informed consent was obtained from each patient prior to surgery.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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