Let-7i-5p inhibits the proliferation and metastasis of colon cancer cells by targeting kallikrein-related peptidase 6

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Abstract. Colorectal cancer is one of the most common types of malignancies worldwide, and as it is often diagnosed at an advanced stage, it is a serious threat to human health. MicroRNAs are important regulators of the growth and metastasis of colon cancer (CC). In the present study, the results demonstrated that kallikrein-related peptidase 6 (KLK6) plays a critical role in suppressing colon carcinoma progression. To further investigate whether microRNAs affect the impact of KLK6, a bioinformatics approach was employed, which indicated that let-7i-5p may directly target KLK6. Furthermore, the expression level of let-7i-5p was significantly negatively correlated with the expression of KLK6 at the mRNA and protein levels in CC. Functionally, overexpression of let-7i-5p inhibited the proliferation and invasion of CC cells, and suppressed the growth of CC *in vitro*. The luciferase reporter assays revealed that let-7i-5p targeted the KLK6 3'-untranslated region. Collectively, these results indicated that let-7i-5p inhibited the proliferation and metastasis of CC cells by targeting KLK6, thereby blocking the cell cycle and promoting apoptosis in colon cells. Therefore, the present study revealed that the let-7i-5p/KLK6 axis may be a potential target for new therapeutic strategies to treat colon tumors.

Introduction

Colon cancer (CC) is the third most common cause of cancer-related mortality, and is characterized by several stages of inflammation, including tumor initiation, progression and malignant invasion (1,2). The majority of clinical diagnoses of CC are made during the late stages of progression, and thus, it is associated with poor prognosis (3-5). As the current treatment for CC, gene targeted therapy is an effective strategy. Although a number of factors have been considered as potential diagnostic and prognostic biomarkers in the process of tumor proliferation and metastasis, the prevention of cancer progression remains a challenge. Kallikrein-related peptidase 6 (KLK6), the kallikrein gene located on chromosome 19q13, is a latent biomarker for colon and gastric cancer. Previous studies have reported that KLK6, as an active trypsin-like serine protease, is highly expressed in CC and is dysregulated in tumorigenesis (6). Although KLK6, as a tumor-associated protein, has been widely reported, the molecular mechanisms underlying the regulation of the expression of KLK6 are currently not well understood.

MicroRNAs (miRNAs/miRs), a class of small endogenous non-coding RNAs, can silence target genes by specifically binding to the 3'-untranslated regions (UTRs) and inhibiting post-transcriptional gene expression. miRNAs, as tumor suppressors, play a key role in cell processes, including cell development, proliferation and apoptosis (7,8). Among the different types of miRNAs, the let-7 family has been associated with tumorigenesis in several types of cancer including, liver, breast and CC. At present, the let-7 family, which consists of a series of miRNAs, has been widely used as a tumor inhibitor and its expression has been observed to be attenuated in several types of human cancers (9). However, there are few studies concerning the biological function and mechanisms of let-7i-5p in CC, and thus, further investigation is required.

In the present study, let-7i-5p was revealed to play a tumor-suppressor role in CC cells. The results confirmed that the KLK6 gene may be a direct target of let-7i-5p, and that KLK6 may promote the proliferation and migration of CC cells. In addition, the overexpression of let-7i-5p suppressed the growth of CC xenografts in nude mice. These data indicated that let-7i-5p may be a target for CC diagnostics and treatment.

Materials and methods

Clinical sample collection. The clinical samples were obtained with patient written informed consent, and the study protocol was approved by the Ethics Committee of the Affiliated

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Hospital of Medical College of Qingdao University (Shandong, China). A total of 31 colon tissue samples, including 27 tumor tissues and 4 normal colon tissues, were obtained via surgical resection at the Affiliated Hospital of Qingdao University between March 2015 to September 2016. The average age of the patients was 50.3 (range, 42-75 years) and 66.67% were male patients. All patients did not receive any antitumor treatment, such as chemotherapy or radiotherapy, before surgery. Samples were immediately frozen in liquid nitrogen following surgical resection and stored at -80˚C for subsequent study.

Cell line culture. The human CC cell lines SW480, HT-29, LoVo and HCT-116, and the normal colon cell line FHC were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). All cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin/streptavidin (Gibco; Thermo Fisher Scientific, Inc.), and maintained at 37˚C in a humidified atmosphere of 5% CO_2.

Small interfering (si)RNA transfection. For siRNA transfection, $2x10^5$ cells per well were plated in a 6-well plate. After adhering for 24 h, control miRNA (miR-con), let-7i-5p mimic and its corresponding let-7i-5p inhibitor (Guangzhou RiboBio Co., Ltd., Guangzhou, China) were added to the transfection medium for 6 h at 37˚C in a CO_2 incubator. Loss of KLK6 expression was achieved using KLK6 siRNA (sense, 5'-GUGCUGGGGAUGAGAAGUAdTdT-3' and antisense, 3'-dTdTCACGACCCCUACUCUUCAU-5'; Guangzhou RiboBio Co., Ltd.) (10), and cell transfection was performed with Lipofectamine 2000™ reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Cells were supplemented with normal culture medium and cultured at 37˚C with 5% CO_2 for up to 48 h before harvesting.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. RNA was isolated from tissues or cells using a mirVana miRNA Isolation kit (Ambion; Thermo Fisher Scientific, Inc.) following the manufacturer's instructions. First-strand cDNA was synthesized using a PrimeScript First Strand cDNA Synthesis kit (Takara Biotechnology Co., Ltd., Dalian, China). The extraction concentration was determined with a NanoDrop spectrophotometer. The products were kept at -80˚C until further experimentation. The cDNA was then amplified using the Power SYBR-Green PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) with the appropriate primers and an ABI 7500-fast thermocycler (Applied Biosystems; Thermo Fisher Scientific, Inc.). The primers used were as follows: KLK6 forward, 5'-GAAGCATAACCTTCGGCAAA-3' and reverse, 5'-GGGAAATCACCATCTGCTGT-3'; GAPDH forward, 5'-GAAGGTGAAGGTCGGAGTC-3' and reverse, 5'-GAAGATGGTGATGGGATTTC-3' (6). In addition, primers for let-7i-5p and U6 were purchased from Tiangen Biotech Co., Ltd. (Beijing, China). U6 and GAPDH served as the internal controls. Relative expression was determined using the $2^{-\Delta\Delta Cq}$ method (11).

Western blot analysis. Following transfection experiments, the SW480, HT-29, LoVo, HCT-116 and FHC cells were harvested and the protein supernatants were isolated using cell lysis buffer (cat. no. 9803; Cell Signaling Technology, Inc., Danvers, MA, USA) with added phenylmethylsulfonyl fluoride. The extracted proteins were determined using the BCA method. The total protein content (30 mg) from cell lysates were resolved by 10% SDS-PAGE, and transferred to a 0.45-mm nitrocellulose membrane (EMD Millipore, Billerica, MA, USA) for 1 h. The membranes were washed with TBS-T containing 5% (w/v) bovine serum albumin (BSA; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). The membranes were incubated overnight with specific primary antibodies [use 5% skim milk in TBST (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.15% Tween-20) for 1:1,000 dilution] for KLK6 (cat. no. sc-374564; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), GAPDH (cat. no. 2118; Cell Signaling Technology, Inc.), cleaved-caspase-3 (cat. no. 9664; Cell Signaling Technology, Inc.) and cleaved peroxisome proliferator-activated receptor (PARP; cat. no. 5625; Cell Signaling Technology, Inc.). Membranes were then exposed to secondary antibodies conjugated to horseradish peroxidase [anti-mouse IgG (H+L), cat. no. 14709; Cell Signaling Technology, Inc.; anti-rabbit IgG (H+L), cat. no. 14708; Cell Signaling Technology, Inc.; and use 5% skim milk in TBST (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.15% Tween-20) for 1:2,000 dilution] for 2 h at room temperature. The membranes were washed three times with TBS-T at room temperature. Chemiluminescent signals were generated using the SuperSignal West Pico Trial kit (Thermo Fisher Scientific, Inc.) and detected with the Vilber Lourmat imaging system (Vilber Lourmat, Marne-la-Vallée, France).

Cell viability assay. Cell proliferation was determined using an MTT assay. Cells were seeded in a 96-well plate with density of the optimized cell number (5,000 cells/well). Following 24 h of seeding, cells were treated with siRNA or diluted chemicals at indicated working concentration. Cells were incubated for indicated time-points and then 20 μl MTT (5 mg/ml) was added into the wells and 4 h later the mixed medium was replaced by 150 μl dimethyl sulfoxide (DMSO; Sigma-Aldrich; Merck KGaA). Subsequently, the 96-well plate was agitated for 15 min at room temperature. Then the OD value of each well was determined using a fluorescence microplate reader (Sunrise Remote; Tecan Austria GmbH, Grödig, Austria) at a wavelength of 490 nm.

Luciferase reporter assay. Using the online platform TargetScan (http://www.targetscan.org), let-7i-5p was used to search for candidate miRNAs that can bind to KLK6. The human colon tumor cells were grown to 70-80% confluence in 24-well plates, and then co-transfected with the recombinant plasmid containing the wild-type/mutant KLK6 3'-UTR and miRNA mimics (50 nM) using Lipofectamine 2000™ reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Luciferase activity was analyzed 48 h after co-transfection using Dual-Luciferase assays (Promega Corp.) and values were normalized against *Renilla* luciferase activity.

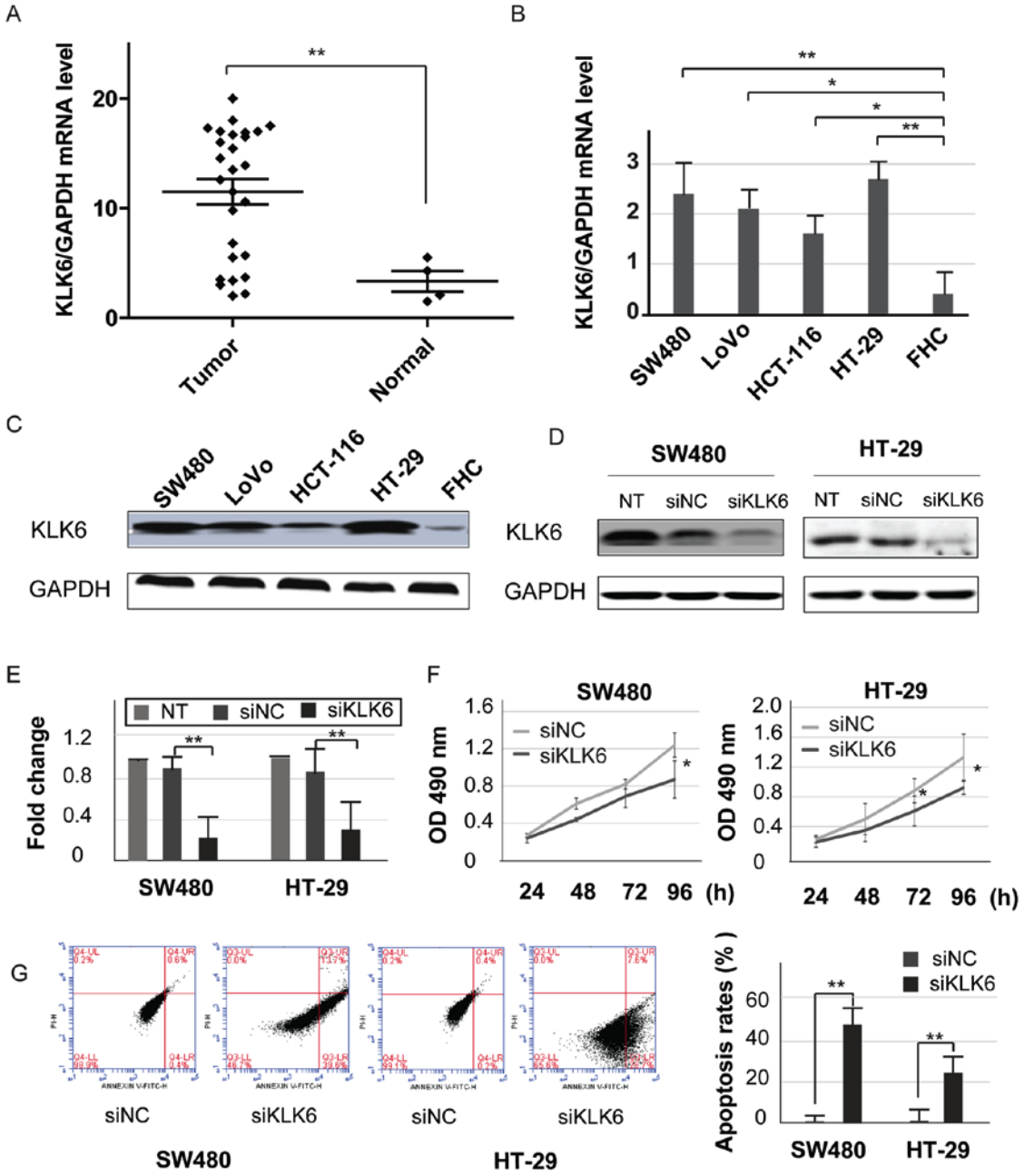

Figure 1. KLK6 is highly expressed in colon tumors and is essential for cell proliferation and apoptosis. (A) Reverse transcription-quantitative polymerase chain reaction was performed to examine the relative mRNA expression level of KLK6 in colon tumor tissues (n=27) compared with normal tissues (n=4). (B) Relative mRNA and (C) protein expression of KLK6 in four colon tumor cell lines (SW480, LoVo, HCT-116 and HT-29) and one colorectal mucosal cell line (FHC). (D and E) Transfection of KLK6 siRNA reduced the KLK6 protein expression level. (F) Cell proliferation was inhibited and (G) apoptotic rate was increased by siRNA-KLK6 in SW480 and HT-29 cells. All data are presented as the mean ± standard deviation. *P<0.05 and **P<0.01, as indicated. KLK6, kallikrein-related peptidase 6; siRNA/si-, small interfering RNA; NT, normal tissue; NC, negative control; OD, optical density.

Wound healing assay. Migration was analyzed by a wound healing assay. Cells were seeded into 6-well plates (5x10^5/well) and allowed to grow to 90-95% confluence. A linear wound was made by scraping the cells using a 10-μl micropipette tip and debris was washed away twice with PBS. At different time-points (0 and 24 h) images of the cells were captured with an inverted microscope (Olympus Corp., Tokyo, Japan), and the migration distances were determined by ImageJ software (ImageJ 1.47v; https://imagej.nih.gov/ij/).

Apoptosis detection. Apoptosis was determined by Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) double staining. Cells were cultured in 6-well plates at 37˚C overnight in a humidified atmosphere with 5% CO_2, and then underwent transfection for 48 h, as aforementioned. Cells were then trypsinized and stained with Annexin V-FITC/PI. The levels of apoptosis were measured using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA). Annexin V$^+$/PI$^-$ cells were considered to be apoptotic cells.

Xenograft experiments. All mice were obtained from the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences and maintained under specific pathogen-free

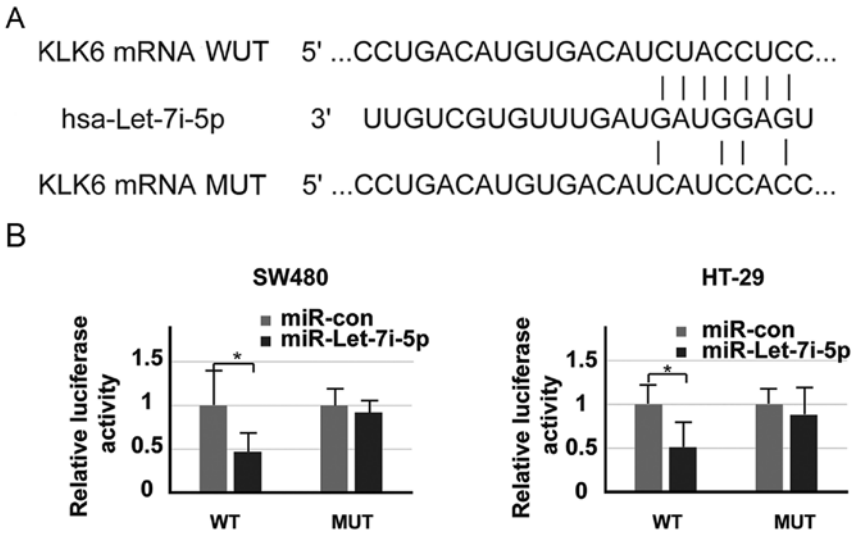

Figure 2. Let-7i-5p directly targets the KLK6 3'-UTR. (A) The binding sites of let-7i-5p in KLK6 mRNA 3'-UTR were predicted by TargetScan. The KLK6 WT and MUT types are presented. (B) SW480 and HT-29 cells were transfected with pMIR-3'-UTR or pMIR-3'-UTRMut combined with miR-con or let-7i-5p. After 48 h, relative luciferase activity was examined. All data are presented as the mean ± standard deviation. *P<0.05, as indicated. KLK6, kallikrein-related peptidase 6; UTR, untranslated region; WT, wild type; MUT, mutant; con, control; miR, microRNA.

conditions in accordance with the guidelines approved by the China Animal Welfare and approval was granted by the Ethics Committee of the Medical College of Qingdao University. The mice were divided into the following two groups: The miR-con group and the let-7i-5p group. A total of $3x10^6$ SW480 cells transfected either with let-7i-5p or miR-con were subcutaneously injected into the right armpits of 4-week-old BALB/c mice. During these experiments, the mice were weighed by table balance and the tumors were assessed with calipers each week. After 5 weeks, the mice were euthanized using a subcutaneous injection with sodium pentobarbital (150 mg/kg), and the volume of the tumor was assessed. The tumor volume (V) was calculated using the following formula: V (mm^3) = 0.5 x length x $(width)^2$.

Statistical analysis. All images were formatted for optimal presentation using Adobe Illustrator CS4 (Adobe Systems, Inc., San Jose, CA, USA). To determine statistical significance between two groups, a Student's t-test was performed to calculate the associated P-values. Statistical significance between multiple groups was evaluated by one way analysis of variance (ANOVA) followed by a Newman-Keuls post hoc test using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

KLK6 is significantly expressed in colon tumors. To examine the level of KLK6 in tissues and cell lines, the present study quantified KLK6 expression in 27 colon tumor tissues, 4 normal colon tissues, 4 CC cell lines and 1 normal colon cell line. The results of qPCR revealed that the mRNA expression level of KLK6 was significantly expressed in the colon tumor tissues compared with that noted in the normal colon tissues (Fig. 1A). Similarly, high mRNA and protein KLK6 expression levels were demonstrated in the colon tumor cell lines, including SW480, LoVo, HT-29 and HCT-116 cells (Fig. 1B and C). These results were consistent with those of a previous study (6). The expression levels of KLK6 were more significantly increased in SW480 and HT-29 cells than in the other two cell lines. To assess the biological role of KLK6, KLK6-specific siRNAs or its corresponding control siRNA were introduced into CC cells and the efficiency of KLK6 siRNAs was evaluated (Fig. 1D and E). Knockdown of KLK6 significantly decreased cell growth and enhanced apoptosis in the SW480 and HT-29 cells. (Fig. 1F and G). These results indicated that KLK6 may play a vital role in anti-apoptosis activity and cell proliferation in CC cells.

Let-7i-5p directly targets KLK6 3'-UTR in colon cancer cells. Using the online platform TargetScan (http://www.targetscan.org), let-7i-5p was revealed to be a potential KLK6 target gene. Let-7i-5p acts as an upstream factor that may be able to directly bind to the 3'-UTR of KLK6 mRNA (Fig. 2A). The present study cloned the 3'-UTR wild-type or 3'-UTR mutant-type of KLK6 into a pMIR-REPORT vector. As expected, the luciferase activity of the 3'-UTR wild-type in cells transfected with let-7i-5p was significantly lower than that of those transfected with miR-control, while the change in the luciferase activity of 3'-UTR mutant-type cells was minimal (Fig. 2B). Thus, these results indicated that KLK6 directly targets Let-7i-5p.

Let-7i-5p inhibits cell proliferation and invasion. To observe the effect of let-7i-5p on CC, the present study detected the expression of let-7i-5p in CC tissues and cell lines by qPCR. The expression of let-7i-5p in tumor samples was significantly lower when compared with that of the normal samples (Fig. 3A). Consistent with the results observed in cancer tissues, the let-7i-5p mRNA level was significantly lower in CC cells compared with the level noted in the normal colon cells (Fig. 3B). These data indicated that let-7i-5p may serve as an important regulator in CC.

Furthermore, colon tumor cell lines were transfected with let-7i-5p mimics, miR-con, anti-let-7i and anti-miR-con. qPCR

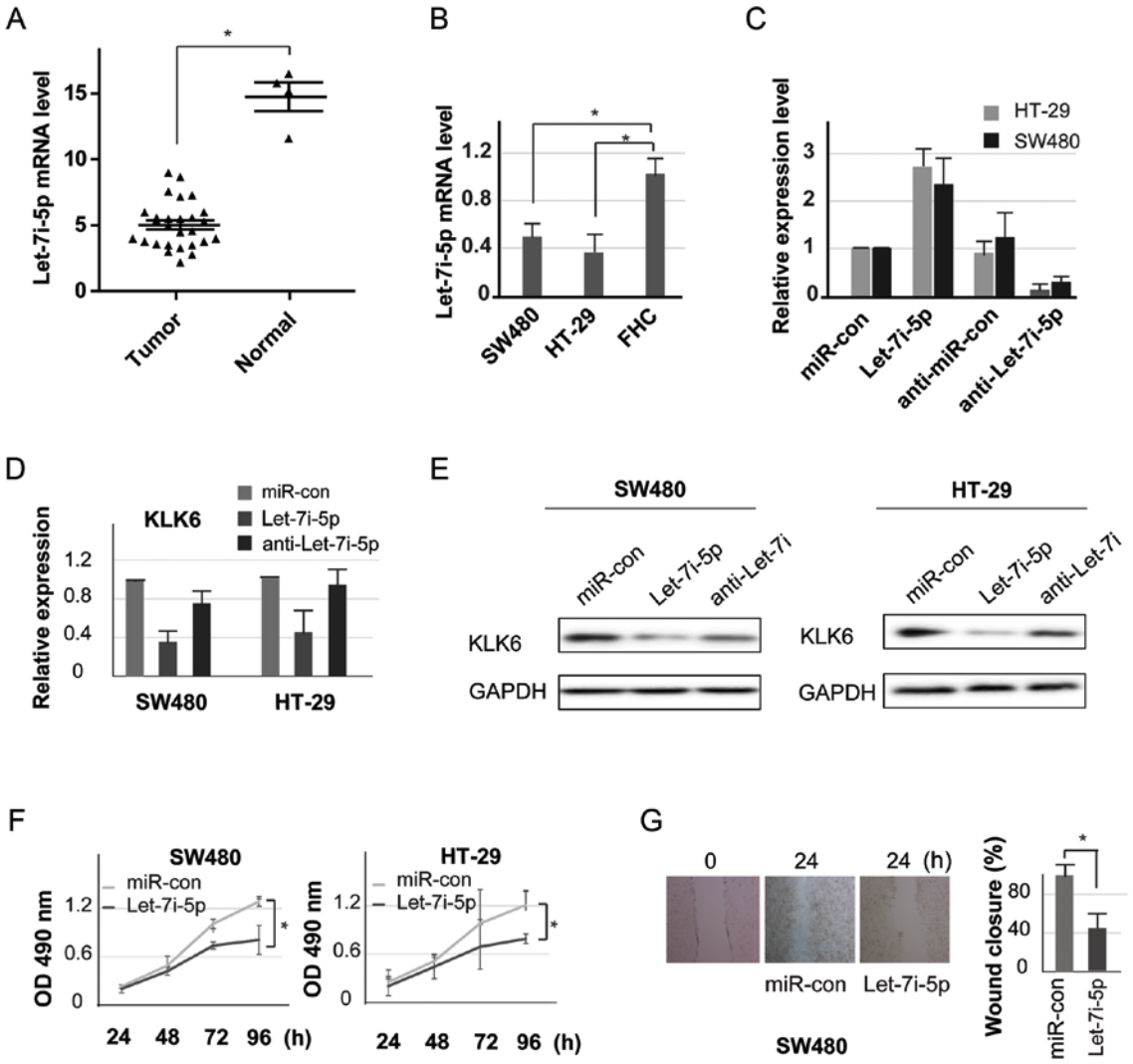

Figure 3. Let-7i-5p is downregulated in colon cancer (CC) and inhibits colon tumor cell proliferation and invasion. (A) Relative mRNA expression of let-7i-5p was investigated in colon tumor tissues compared with normal tissues by RT-qPCR. (B) The relative mRNA expression of let-7i-5p in colon tumor cell lines (SW480 and HT-29 cells) and normal colorectal mucosal cells (FHC). (C) After transfection with miR-con, let-7i-5p or anti-let-7i-5p, RT-qPCR was performed to determine the expression of let-7i-5p in SW480 and HT-29 cells. (D) The mRNA and (E) protein expression levels of KLK6 were evaluated following let-7i-5p transfection in SW480 and HT-29 cells. (F) MTT assay was performed to examine the effect of let-7i-5p on the cell proliferation of colon tumor cell lines. (G) Cell invasion was inhibited by let-7i-5p in SW480 cells. All data are presented as the mean ± standard deviation. *P<0.05, as indicated. KLK6, kallikrein-related peptidase 6; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; OD, optical density; con, control; miR, microRNA.

analysis was performed to determine the transfection efficiency in the SW480 and HT-29 cells (Fig. 3C). As displayed in Fig. 3D and E, there were markedly lower expression levels of KLK6 in cells transfected with the let-7i-5p mimics than in the other groups. MTT assay was used to evaluate cell proliferation. The results demonstrated that overexpression of let-7i-5p inhibited cell growth when compared with the negative control group in both SW480 and HT-29 cells (Fig. 3F). Cell migration is a crucial process in cancer metastasis (12), thus, a wound-healing assay was employed to assess the migration and invasion of colon tumor cells. High levels of let-7i-5p resulted in a significant decrease in cell migration compared with the miR-con group (Fig. 3G). Collectively these results indicated that let-7i-5p may directly target the KLK6 3'-UTR, inhibiting cell proliferation and invasion in CC cells.

Let-7i-5p promotes apoptosis and attenuates cell cycle progression in CC cell lines. Flow cytometry using Annexin V-FITC and PI double staining demonstrated that endogenous let-7i-5p was increased, and the rate of apoptosis was significantly increased in the SW480 and HT29 cells (Fig. 4A). It was revealed that the accumulation of let-7i-5p could increase the number of apoptotic cells. Several studies have indicated that KLK6 is associated with a caspase-dependent pathway in many types of tumor cells, including lung cancer, glioma and breast cancer. However, the underlying mechanism is not clear in CC (13). To determine whether let-7i-5p enhanced apoptosis via the caspase-dependent pathway, colon tumor cell lines were transfected with let-7i-5p mimics or miR-con for 24 h, and then further cultured for 48 and 72 h. Western blotting was carried out to analyze the levels of cleaved caspase-3 and PARP at 24, 48 and 72 h. The results revealed that let-7i-5p reduced the levels of cleaved-caspase-3 (Fig. 4B). These results demonstrated that caspase-dependent signaling may mediate the increased levels of apoptosis in colon tumor cells.

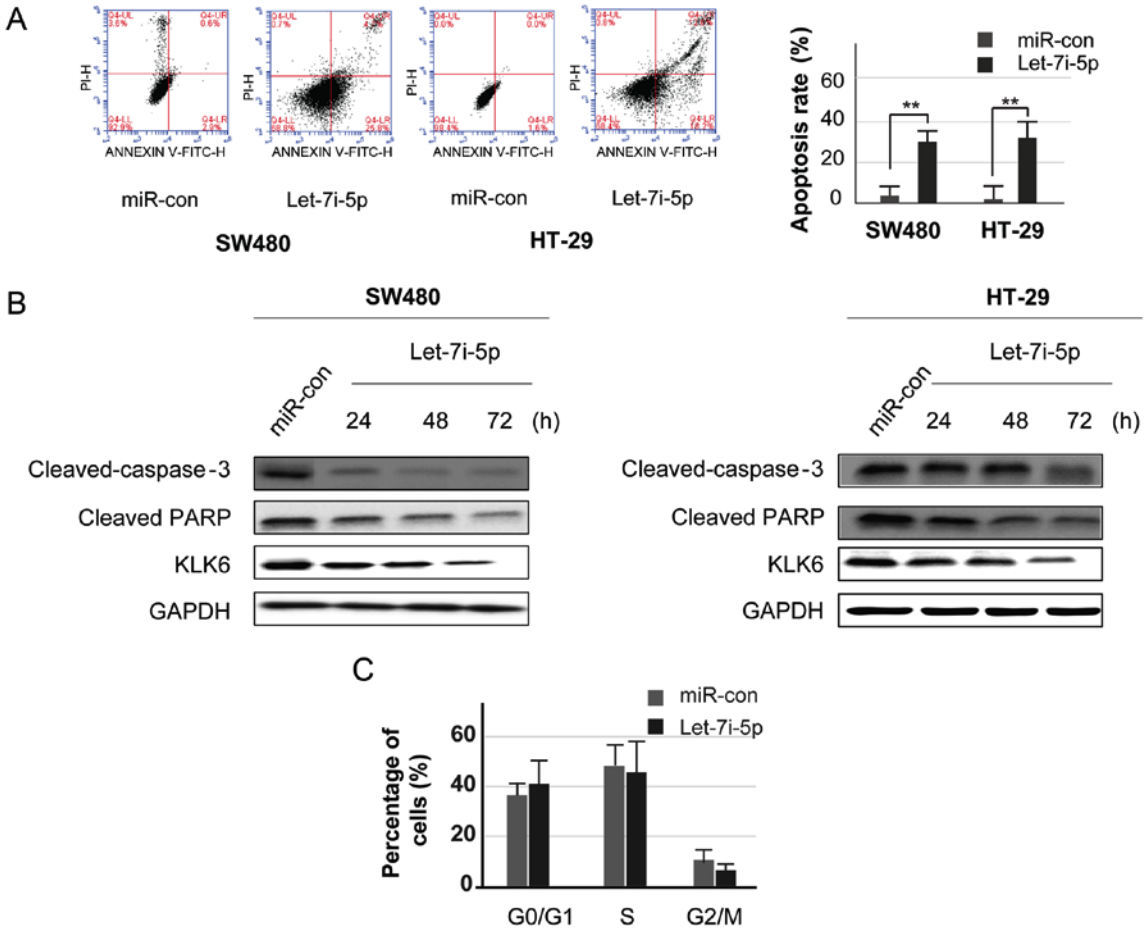

Figure 4. Effect of let-7i-5p on colon tumor cell growth and regulation of cell cycle progression. (A) The effect of let-7i-5p on cell apoptosis in colon tumor cell lines was detected by flow cytometry. (B) Western blotting was performed to detect the protein expression levels of cleaved caspase-3, cleaved PARP and KLK6. (C) SW480 cells transfected with let-7i-5p mimics were arrested in the G1 phase, compared with the miR-con group. All data are presented as the mean ± standard deviation. **P<0.01, as indicated. KLK6, kallikrein-related peptidase 6; PARP, peroxisome proliferator-activated receptor; con, control; miR, microRNA.

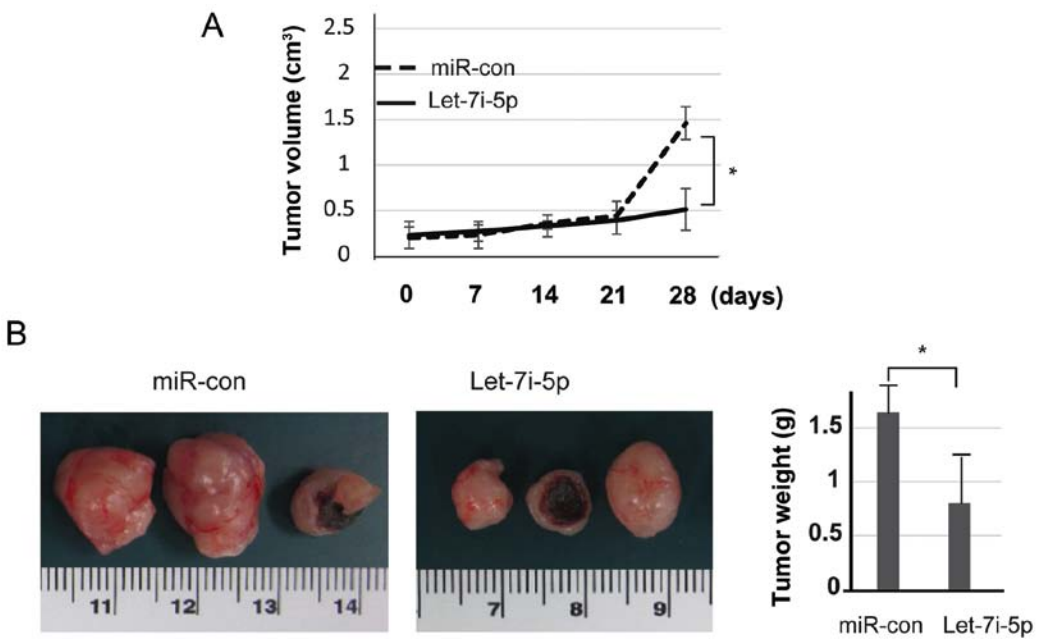

Figure 5. Let-7i-5p inhibits tumor growth *in vivo*. (A) Stably transfected SW480 cells were used to establish human tumor xenografts in a mouse model. The diameters of tumors were measured by calipers every 7 days. Tumor volumes were then calculated and are presented. (B) Images of tumor tissues and a graph of the tumor weights following the final measurements at the end of the experiments. All data are presented as the mean ± standard deviation. *P<0.05, as indicated. con, control; miR, microRNA.

Furthermore, flow cytometry was performed to evaluate cell cycle distribution, and thereby, verify whether the pro-apoptotic effect of let-7i-5p is associated with cell cycle arrest. Cells were transfected with let-7i-5p mimics and miR-con for 48 h. In the let-7i-5p group, there was a marked decrease in the proportion of cells in the S and G2 phase, as well as cell cycle arrest in the G1 phase. When compared with miR-con group, the number of cells was decreased in S and G2 phases (Fig. 4C).

Let-7i-5p suppresses the tumor growth of SW480 cells in vivo. The present study further evaluated the effects of let-7i-5p *in vivo* by measuring colon xenograft growth. SW480 cells were transfected either with let-7i-5p or miR-con. To establish the mouse xenograft models, stably transfected cells ($1x10^7$ cells per condition) were injected into nude mice subcutaneously; the tumors were then monitored every week. During the 4-week period, the tumor volumes were assessed. The results revealed that upregulation of let-7i-5p significantly inhibited tumor growth (Fig. 5A). Let-7i-5p markedly attenuated tumor growth compared with the miR-con group. After 4 weeks, the mice were sacrificed and the tumors were excised. As displayed in Fig. 5B, tumor weight was assessed using calipers. These data indicated that overexpression of let-7i-5p in SW480 cells markedly reduced their ability to form tumors.

Discussion

Colorectal cancer is a common malignant tumor of the digestive tract and is often clinically diagnosed at the late stages of the disease. It has been reported that cancer is a genetic disease induced by many complex factors (14,15). The development of colon cancer (CC) is associated with common genetic changes in Kirsten rat sarcoma viral proto-oncogene, B-Raf proto-oncogene serine/threonine kinase, tumor protein p53 and some genes related to the Wnt signaling pathway. Previous studies have demonstrated that the serine protease KLK6 was found to be highly expressed in several types of cancers, including pancreatic, colorectal, gastric and breast cancers, and it may serve as a biomarker for these cancers (16-19). It has also been reported that KLK6 may play a vital role in both cancer-inhibiting and cancer-promoting processes (20). The tissue type and the tumor microenvironment have different effects on the process of carcinogenesis due to the different roles of KLK6 (21). In the colon samples collected in the present study, the majority of the tumor tissues expressed high levels of KLK6 mRNA compared with the control tissue. In addition, both high KLK6 mRNA and protein expression was observed in the CC cell lines. Thus, the results indicated that high KLK6 expression in the colon may be associated with cell proliferation and malignant transformation. However, the underlying mechanism and therapeutic targets of CC have yet to be fully elucidated.

miRNAs regulate a variety of biological processes and they exert their functions under both physiological and pathological conditions, including cell proliferation, apoptosis, development and metabolism (22). To date, many studies have reported that miRNAs are associated with colon tumors. For example, miR-582-5p was observed to promote CC cell proliferation by inhibiting the expression of *Adenomatosis polyposis coli* (23). miR-552 is involved in the metastasis of CC cells by targeting a disintegrin and metallopeptidase domain 28 (24). Currently, there are few studies that have reported an association between let-7 and colon tumors, and few results have elucidated the mechanistic relation between them. In the present study, low expression of let-7i-5p was detected in colon tumor tissue samples compared with that noted in normal colon tissues. Similar results were observed in colon tumor cell lines. In addition, upregulation of let-7i-5p expression markedly inhibited colon tumor cell viability and invasion. Collectively, these results indicated that let-7i-5p expression may gradually decrease with increased colon tumor malignancy.

Previous research has revealed that miRNAs, as tumor inhibitors, play a vital role in the development and progression of cancer via complementary binding to the 3'-UTRs of target genes, causing the degradation or translational suppression of mRNA (25). The miRNA let-7 family, which is widely considered to have tumor-suppressor activity, was found to bind to the 3'-UTRs of target genes and serve an important role in the regulation of the cell cycle, cell differentiation and apoptosis in neuroblastoma, as well as liver and lung cancer (9,26). The present study identified KLK6 as a direct target gene of let-7i-5p. Enhanced expression of let-7i-5p was observed to lower the expression level of KLK6 in colon tumor cells and promoted malignant tumor progression. It was suggested that let-7i-5p attenuates the viability and invasion of colon tumor cells by targeting KLK6. The results of the present study also demonstrated that the accumulation of let-7i-5p affected the cell cycle, decreasing the proportion of cells in the S and G2 phases, and inducing cell cycle arrest in the G1 phase. Furthermore, upregulation of let-7i-5p was detected to alter several factors in the caspase signaling pathways. Additionally, the present study verified that mice injected with stably transduced let-7i-5p colon tumor cells had fewer metastases *in vivo*. This was in agreement with the data implicating let-7i-5p in the induction of cell apoptosis and cell cycle arrest in colon tumor cells by targeting KLK6, thereby inhibiting colon tumor cell growth. Therefore, these results support the notion that the let-7i-5p/KLK6 axis may be an ideal therapeutic candidate for improving the clinical outcomes of colon patients. However, further experiments are required to determine the use of let-7i-5p/KLK6 as an effective biomarker in the clinic.

In conclusion, the results of the present study revealed that let-7i-5p may be a potential target of KLK6, with regulatory roles in colon tumor cells. In addition, the results demonstrated that let-7i-5p may serve a vital role in the growth and metastasis of CC cells, affecting cell apoptosis and the cell cycle. Therefore, these findings may provide the basis for let-7i-5p as a diagnostic and prognostic marker for the treatment of colon tumor. In addition, the results of the present study may provide new methods for the diagnosis and treatment of CC patients. As KLK6 has been reported to be a valuable biomarker in other types of cancers, such as ovarian cancer (16-19), additional studies are required to assess the role of the let-7i-5p/KLK6 axis in other types of cancer. Furthermore, the present study highlights that integrated bioinformatic approaches on publicly available web tools, such as TargetScan, play critical roles in identifying candidate targets of miRNAs and potential mechanisms for cancers.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

NH and BW conceived and designed the study; JS, LW, NH and BW provided the study materials or patients; JS, QM, YY, ZY and NH collected and assembled the data; JS, BW and NH analyzed and interpreted the data. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The clinical samples were obtained with patient written informed consent, and the study protocol was approved by the Ethics Committee of the Affiliated Hospital of Medical College of Qingdao University (Shandong, China). Animal experiments were carried out in accordance with the guidelines approved by the China Animal Welfare and approval.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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