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ORIGINAL RESEARCH

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KRT17 promotes endometrial cancer cell migration as well as angiogenesis by regulating HIF-1α/VEGF pathway

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Abstract

Endometrial cancer (EC) is a common type malignant tumors in women. To combat this type of cancer, more effective treatments are still needed. EC cell growth and metastasis depend on angiogenesis. Keratin (KRT) is a family of proteins which are essential for hair formation. KRT17 affected a variety of cancers. Here, we reported that KRT17 expression was high in human EC. The depletion of KRT17 suppressed EC cell growth. We further showed the ablation of KRT17 led to the suppression of cell motility. Also, its depletion resulted in the inhibition of angiogenesis. We further demonstrated that KRT17 contributed to the progression of EC *via* regulating the HIF-1α/VEGF axis through a tumor growth assay in mice. In conclusion, KRT17 could serve as an EC target.

Keywords

Endometrial cancer (EC); Keratin 17 (KRT17); Angiogenesis; Motility; HIF-1α/VEGF axis

1. Introduction

Endometrial cancer (EC) is a common malignant tumor in women, and the incidence of endometrial cancer has been increasing rapidly in recent years [1, 2]. EC can be roughly divided into estrogen-dependent (type I) as well as type II [3, 4]. In China, 80% to 90% of new cases are type I [5]. In most type I cases, patients tend to be diagnosed at an early stage [6]. To combat this type of cancer, more effective treatments are still needed.

Angiogenesis is known as the formation of new blood vessels [7]. Recent studies have shown that HIF-1α/VEGF signaling pathway is involved in EC proliferation, differentiation, as well as permeability of blood vessels [8]. As with other solid tumors, EC cell growth as well as metastasis all depend on angiogenesis [9]. Targeting this process is a promising method to combat cancer.

Keratin (KRT) is a family of proteins essential for the formation of hair and abundant in human skin, thereby suppressing damage [10]. Keratin 17 (KRT17) is a type I keratin [10]. Abnormal expression of KRT17 is associated with the progression of skin diseases [10]. Recently, KRT17 was abnormally highly expressed in lung, cervical cancer, as well as oral squamous cell carcinoma (OSCC) [11, 12], which can promote function of lung adenocarcinoma cells [13]. In addition, KRT17 can induce epithelial mesenchymal transformation in esophageal squamous cell cancer [14], and promote bladder cancer progression and cisplatin resistance [15]. Knockdown of KRT17 inhibits HIF-1α expression, thereby inhibiting osteosarcoma cell proliferation and glycolysis [16]. Overexpression of KRT17 could contribute to the metastasis as well as angiogenesis in colon cancer [15]. However, its possible effects on EC are still unclear.

Studies have reported that high mRNA expression and positive immunohistochemistry of KRT17 are associated with reduced overall survival rate of EC, but the specific role and mechanism are not clear [11, 12, 15]. In this study, we revealed that KRT17 promoted EC cell proliferation, motility as well as angiogenesis *via* regulating HIF-1α/VEGF axis. We therefore believe that KRT17 could serve as a possible EC target.

2. Materials and methods

2.1 Bioinformatic analysis

We conducted bioinformational analysis *via* the GEPIA (http://gepia.cancer-pku.cn/) to analyze expression in Cancer Genome Atlas (TCGA) database.

2.2 Antibodies and siRNAs

Anti-KRT17 (1:500, ab51056, Abcam, Cambridge, UK), anti-HIF-1α (1:500, ab179483, Cambridge, UK), anti-VEGF (1:500, ab53465, Cambridge, UK), anti-β-actin (1:2000, ab8226, Cambridge, UK).

The siRNAs were bought from the Riobio plc (Guangzhou, China).

2.3 Cell culture and transfection

The EC cell line including Ishikawa, HEC-1B (Human endometrial cancer-1B), KLE, RL-952, human umbilical vein endothelial cell (HUVEC), and hEEC (Human endometrial cell) were all purchased from American type culture collec-

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tion (ATCC). These cells were all maintained in Dulbecco' s modified Eagle' s medium (DMEM), supplemented with fetal bovine serum (FBS, 10%) as well as incubated at 37 °C in a 5% CO_2 incubator.

Cells were seeded into 6-well plates as well as plasmids or siRNAs were used for transfection using 10 μL Lipofectamine RNAiMAX in each well. Subsequent assays were performed after 48 h.

2.4 Immunoblot assay

The Ishikawa and HEC-1B cell samples were lysed, and then separated by a 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) experiment, sequentially the total proteins were transferred onto nitrocellulose (NC) membranes (Millipore, USA). Then the membranes were blocked with 5% dry milk as well as antibodies. The membranes were treated with horse radish peroxidase (HRP)-labelled antibodies for 45 min. Each blot was then visualized using the Enhanced substrate chemiluminescence (#32106, GE, USA).

2.5 Cell viability assays

For 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assays, cells were seed into the 96-well plates (1000 cell per well) and maintained in media (10% FBS) at 37 °C. Ishikawa and HEC-1B cells were treated with MTT (C0009S, Beyotime, Beijing, China) at 37 °C for 4 h. Then the stained cells were dissolved by 150 µL dimethyl sulfoxide (DMSO). The relative cell viability was assessed at 490 nm wavelength (Bio-Rad, USA) for 3 d.

2.6 Transwell & wound healing assays

For transwell assays, 10^5 cells were placed in the upper chamber. Invaded cells underside were fixed with 4% paraformaldehyde, stained with the 0.2% crystal violet, and imaged. For wound healing assays, when the cell confluence reached 100%, scratches were made with 10 µL gun tip, washed twice with PBS, and then cultured with complete medium. The cells were observed under microscope at 0 h and 24 h.

2.7 Tube formation assay

HUVECs (10^5 per well) were plated onto 24-well plates precoated with Matrigel (1:1 diluted with serum-free medium). To examine tube formation, images were captured after 3 and 6 h using an Axio Observer light microscope (Axio Observer light microscope A1, Carl Zeiss AG, Oberkochen, Germany).

2.8 Tumor growth *in vivo* assay

Tumor growth assays Cells were stably transfected. Then, approximately 2×10^6 cells were subcutaneously implanted into athymic nude mice. The BALB/c nude mice (5 for each group) were used. The volume of tumors was measured and calculated every 7 days after 7 days until 28 days.

2.9 Statistics

GraphPad (6.0, GraphPad Software, San Diego, CA, USA) was used for statistical analysis. All data was expressed as mean $\pm$ SD. * indicates $p < 0.05$ and significant difference.

3. Results

3.1 KRT17 was highly expressed in human EC tissues as well as cells and promoted the viability of EC cells

To confirm whether the abnormal expression of KRT17 was existed in human EC tissues, we first searched it in the TCGA database. It revealed that KRT17 was highly expressed in uterine corpus endometrial carcinoma (UCEC) tissues (n = 546) compared to normal tissue (n = 35, Fig. 1A). Through the Tumor Immune Estimation Resource (TIMER) database, we noticed KRT17 was highly expressed in multiple cancer tissues, such as colon cancer and EC (Fig. 1B). Then, we further investigated the transcript per million (TPM) value of KRT17 in UCEC and normal tissues. The high KRT17 TRM value was also observed in human UCEC tissues (Fig. 1C). We then detected its expression levels in EC cell lines, and normal endometrial epithelial cell line, *i.e.*, hEEC. Through Immunoblot assays, we noticed KRT17 was highly expressed in EC cell lines compared to hEEC (Fig. 1D).

To further detect its effects on EC cell growth, we used two siRNAs to deplete its expression in Ishikawa and HEC-1B. We found transfection of KRT17 siRNA obviously decreased its expression in Ishikawa as well as HEC-1B cells (NC-siRNA, Fig. 1E). CCK-8 assays revealed the ablation of KRT17 suppressed the growth of Ishikawa as well as HEC-1B cells, with the decreased absolute value at 490 nm wavelength (Fig. 1F). Through colony formation assays, knockdown of KRT17 induced the decrease of the colony numbers in Ishikawa as well as HEC-1B cells (Fig. 1G). We therefore thought depletion of KRT17 restrained EC cell growth.

3.2 KRT17 stimulated the motility of EC cells *in vitro*

Since KRT17 could regulate the proliferation of EC cells, we further investigated its effects on the motility of EC cells though transwell assays. Performing transwell assays, we noticed KRT17 ablation dramatically restrained the invasion of Ishikawa and HEC-1B cells with decreased stained cell number, suggesting that KRT17 stimulated the migration of Ishikawa and HEC-1B cells (Fig. 2A). Subsequently we performed wound healing assays and the results revealed that knockdown of KRT17 suppressed Ishikawa and HEC-1B cell migration (Fig. 2B). We therefore thought KRT17 stimulated the motility of EC cells.

3.3 KRT17 depletion suppressed the angiogenesis of EC

We then detected the effects of KRT17 on the angiogenesis of ECs. The two siRNAs of KRT17 were first transfected into both Ishikawa and HEC-1B cells, to deplete its expression. Then the culture medium supernatant was collected, and added into the HUVECs. Subsequently, the tube formation assays were performed. Through tube formation *in vitro* assays, we found KRT17 depletion in both Ishikawa and HEC-1B cells

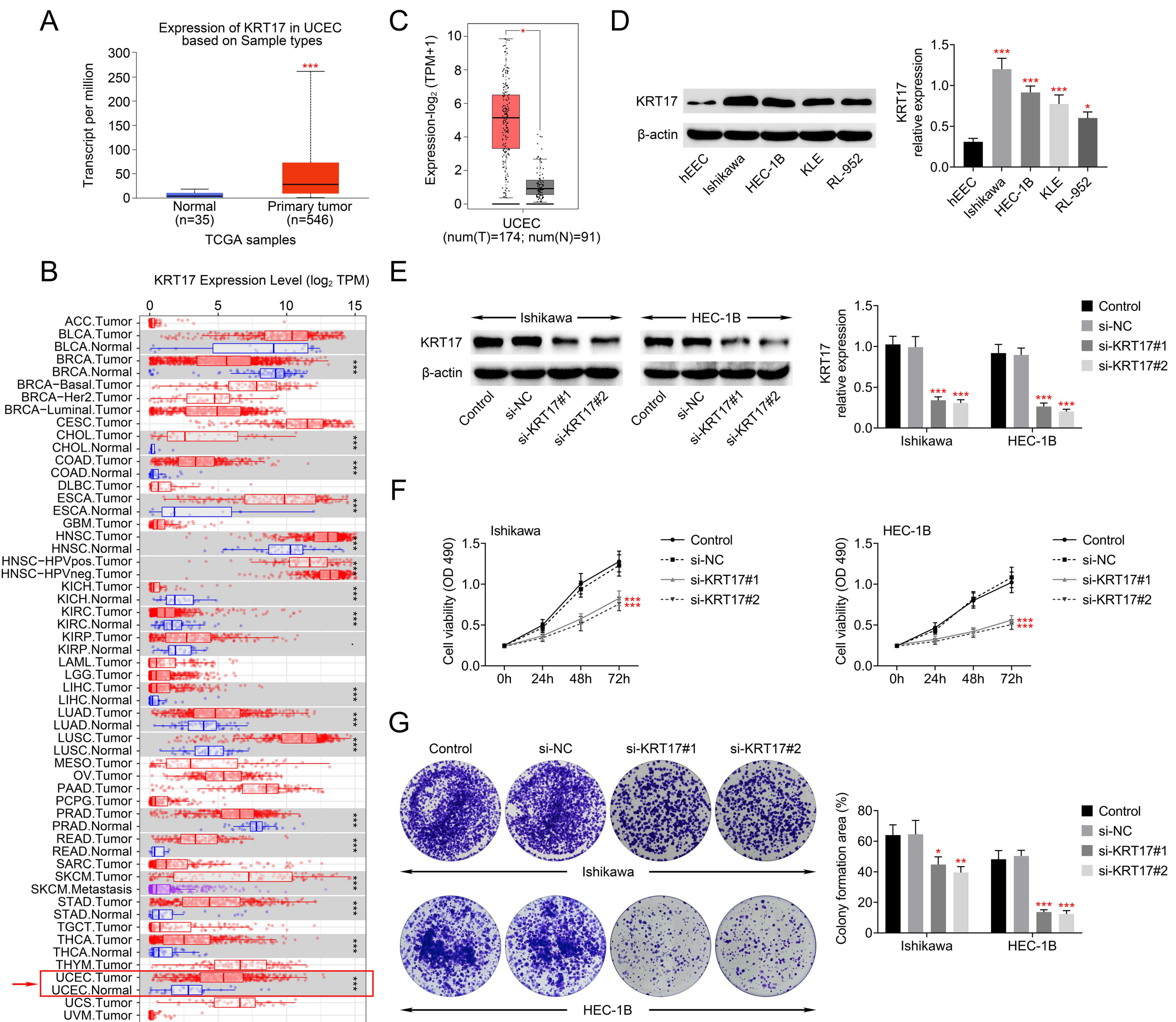

FIGURE 1. KRT17 was highly expressed in human endometrial cancer (EC) tissues as well as cells and promoted the viability of EC cells. (A) The expression of KRT17 in EC tissues. (B) The expression of KRT17 in Tumor Immune Estimation Resource (TIMER) database. (C) The expression of KRT17 in Ualcan database. (D) The expression of KRT17 in cell lines. (E) KRT17 in endometrial cancer cell line Ishikawa as well as HEC-1B. (F) The absorbance value at 490 nm wavelength in the indicated groups. (G) Colony numbers in the indicated groups. OD: absorbance; * $p < 0.05$, * $p < 0.01$, *** $p < 0.001$.

suppressed the tube formation of HUVECs, with the decrease numbers of branch points per field (Fig. 3). We therefore thought KRT17 depletion suppressed the angiogenesis of EC.

3.4 Downregulation of KRT17 inhibited the HIF-1α/VEGF pathway in EC

Previous study showed the effects of HIF-1α/VEGF pathway in EC progression. It was detected whether KRT17 promoted EC progression through this pathway. Through Immunoblot assays, KRT17 depletion decreased the expression levels of HIF-1α and VEGF in both Ishikawa and HEC-1B cells (Fig. 4). Therefore, downregulation of KRT17 inhibited the HIF-1α/VEGF pathway in EC.

3.5 Knockdown of KRT17 inhibited EC tumor growth *in vivo*

The tumor volume was measured every 7 days until to 28th days. Interestingly, KRT17 depletion obviously suppressed tumor growth of EC cells (Fig. 5A).

IHC assays, results revealed the expression levels of KRT17 were obviously decreased in KRT17 depleted tumor tissues (Fig. 5B). The expression levels of Ki67, VEGF and HIF-1α were decreased in KRT17 depletion tissues (Fig. 5B). Therefore, KRT17 suppresses EC tumor growth in mice.

4. Discussion

Endometrial cancer is one of the female reproductive system tumor [17]. In recent years, the incidence of EC has been increasing worldwide and can be transferred to many parts of the body [18]. However, the prognosis of metastatic EC is poor.

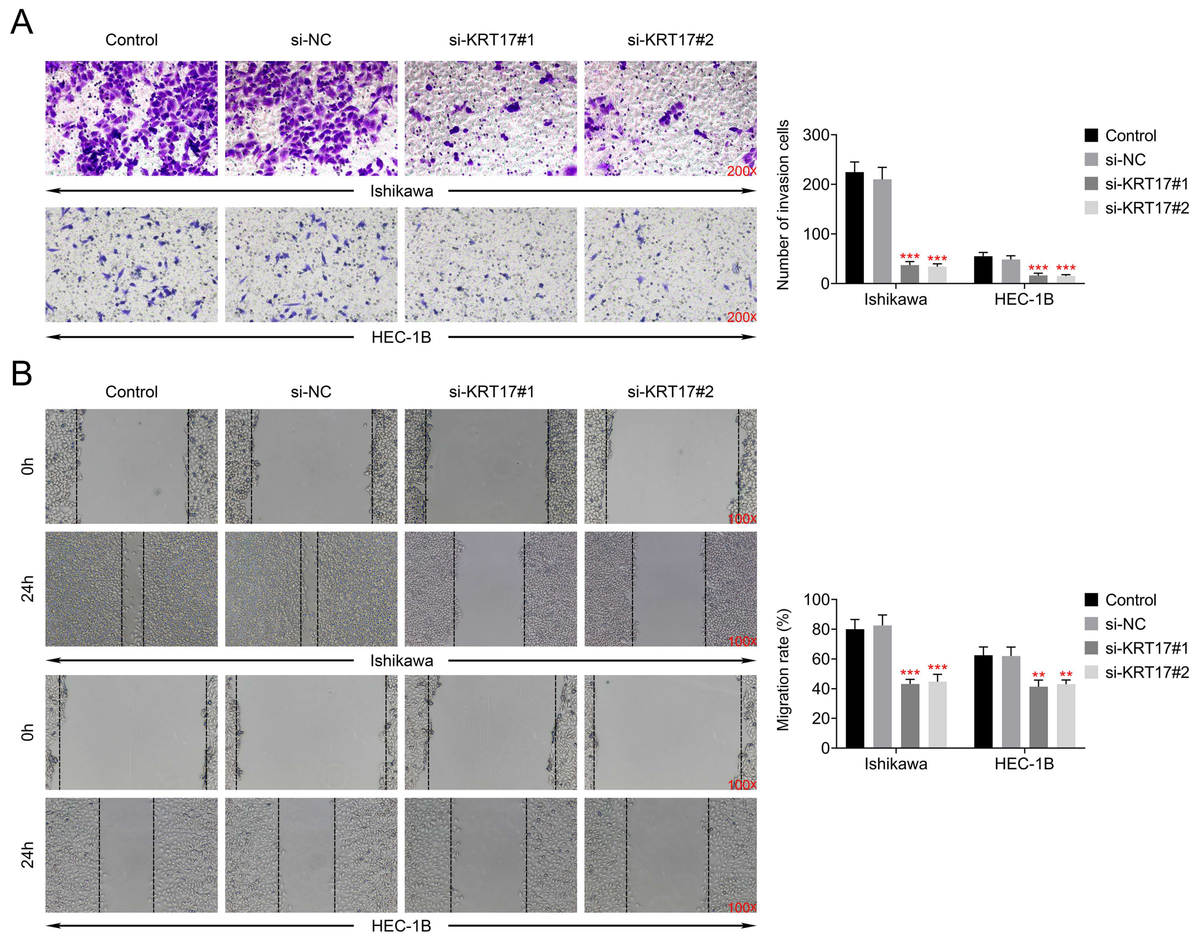

FIGURE 2. KRT17 stimulates the motility of endometrial cancer (EC) cells *in vitro*. (A) Transwell assays showed the invasion degree of Ishikawa as well as HEC-1B cells upon the transfection of siRNAs. (B) Wound healing assays showed the migration of Ishikawa as well as HEC-1B cells upon the transfection of siRNAs. ** $p < 0.01$, *** $p < 0.001$.

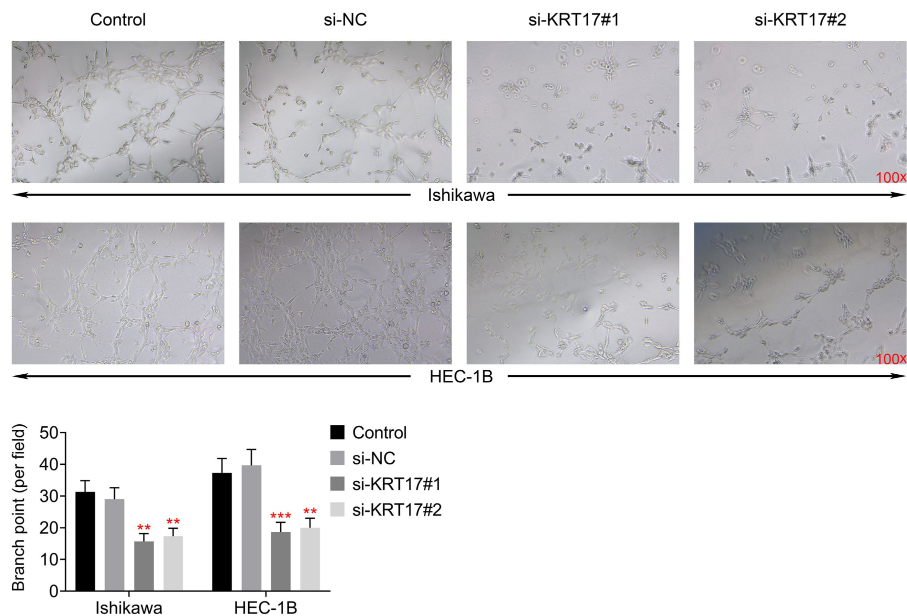

FIGURE 3. KRT17 depletion suppressed the angiogenesis of HUVECs. Tube formation assays showed the angiogenesis capacity of HUVECs upon the indicated treatment. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

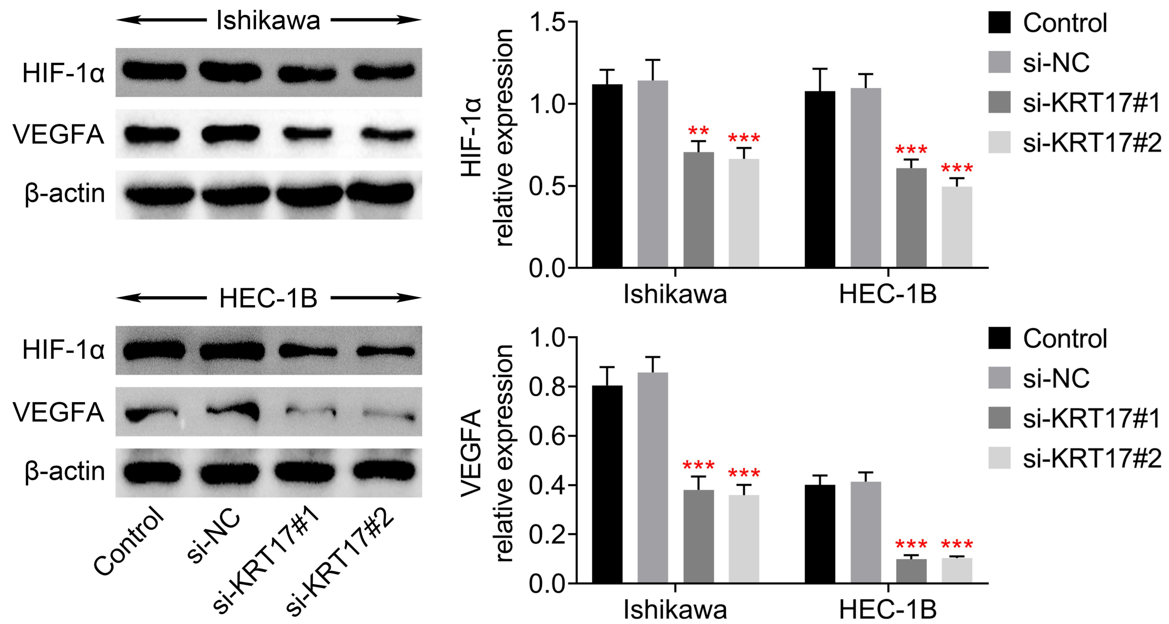

FIGURE 4. Downregulation of KRT17 inhibited the HIF-1α/VEGF pathway in endometrial cancer (EC). Immunoblot assays were performed to show hypoxia inducible factor-1 (HIF-1α) and vascular endothelial growth factor (VEGF) in Ishikawa as well as HEC-1B cells upon the transfection of siRNAs. ** $p < 0.01$, *** $p < 0.001$.

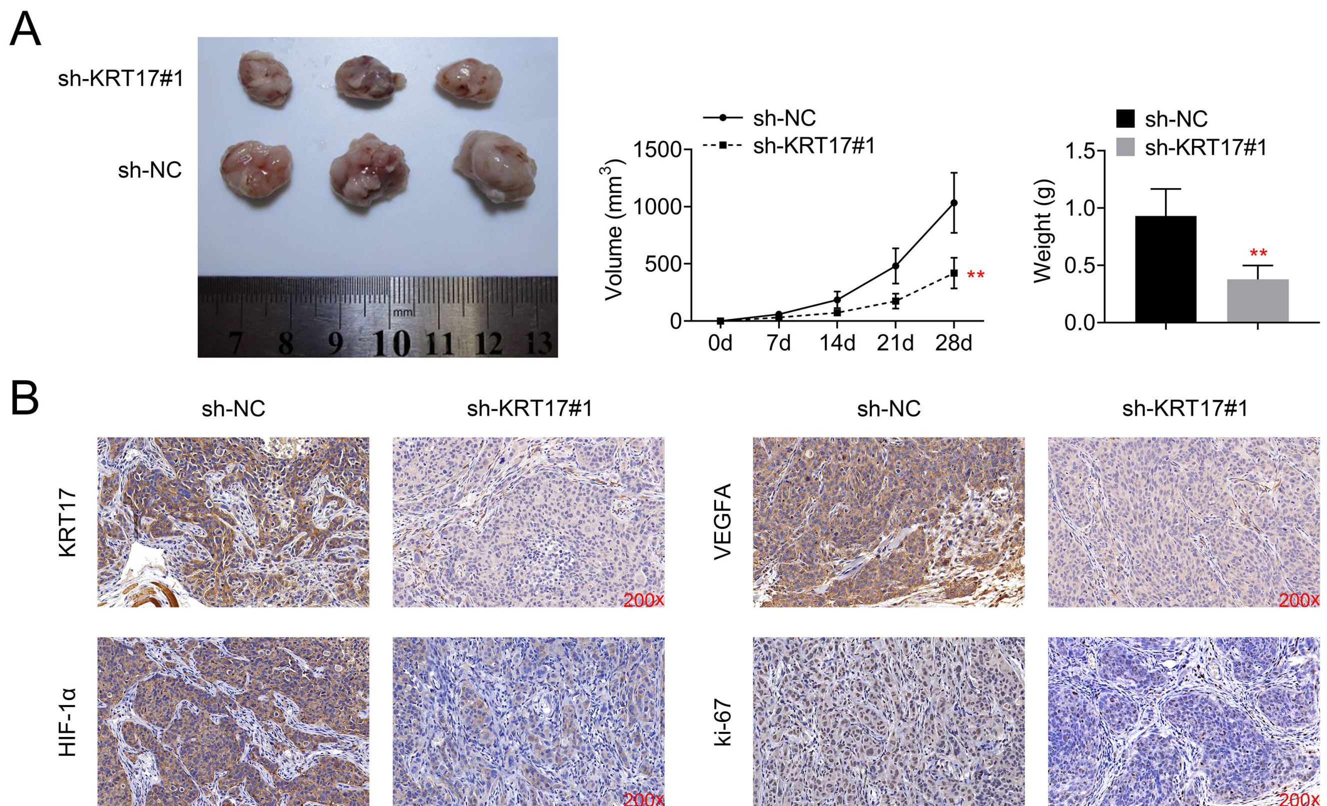

FIGURE 5. KRT17 knockdown inhibited tumor growth of endometrial cancer (EC) cells *in vivo*. (A) The representative images of tumors were shown. In addition, the tumor growth curves were conducted. The tumor weight was also shown (B) IHC assays showed the expression of the KRT17, Ki67, HIF-1α and VEGF in tumor tissues. ** $p < 0.01$.

To improve the prognosis of EC, we need to have a deeper understanding of tumor heterogeneity of EC and systematically unravel the molecular mechanisms of EC progression and metastasis, so as to find more effective intervention targets.

Through a series of *in vitro* assays, we confirmed that KRT17 affected the growth, motility, as well as angiogenesis of EC cells. Through CCK-8 as well as colony formation assays, the effects of KRT17 on EC cell growth was confirmed. Wound closure as well as transwell assays showed KRT17 affected the motility of EC cells. Further through tube formation assays, we found its effects on the angiogenesis of EC *in vitro*. KRT17 affected the progression of cancers [12]. For example, the upregulation of KRT17 could contribute to the cell metastasis as well as angiogenesis in colon cancer [15]. In addition, KRT17 could also promote the E-cadherin loss as well as the aggressiveness of gastric cancer [19]. KRT17 knockdown also restrained the malignancy as well as the cisplatin tolerance of bladder cancer cells [15]. KRT17 also affected DNA damage response and therefore affected the tumor initiation [20]. Knockdown of KRT17 inhibits HIF-1α expression, thereby inhibiting osteosarcoma cell proliferation and glycolysis [21]. KRT17 overexpression could contribute to the metastasis as well as angiogenesis in colon cancer [22]. Another study also indicated that KRT17 depletion decreased osteosarcoma cell growth as well as the Warburg effect [21].

Importantly, KRT17 could influence nuclear morphology as well as chromatin organization [23]. We guessed that it could therefore affected cancer progression. Nuclear KRT17 could affect nuclear morphology with an associated effect on mediating chromatin organization, gene expression, as well as the regulation of proliferation in epithelial cells [23]. We should next clarify whether KRT17 affected EC progression as well as angiogenesis *via* affecting nuclear morphology.

The effects of KRT17 on the angiogenesis and VEGF pathway of EC were investigated. Angiogenesis plays an important role in the growth, metastasis and prognosis of EC [24]. The culture medium supernatant from control or KRT17 depleted EC cell group was collected, and added into the HUVECs. Subsequently, the tube formation assays were performed. Through tube formation *in vitro* assays, we found KRT17 depletion in both Ishikawa and HEC-1B cells suppressed the tube formation of HUVECs. In addition, the precise mechanism needs to be furtherly studied.

5. Conclusions

In conclusion, we found KRT17 was highly expressed in human EC. KRT17 depletion suppressed the growth, motility as well as angiogenesis of EC *via* the HIF-1α/VEGF axis. We thought KRT17 could act as a target of EC.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

LX and FXX—designed the study and carried them out; LX, YBL, PX, LMZ, ML, YZ and LP—supervised the data collection, analyzed the data, interpreted the data, LX and FFX—prepare the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was obtained from the Beihua University Laboratory Animal Ethics Committee (Approval no. 2022070701). All patients signed informed consent forms.

ACKNOWLEDGMENT

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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