Submitted: 23 January, 2025 Accepted: 06 March, 2025 Published: 15 June, 2025 DOI:10.22514/ejgo.2025.086

ORIGINAL RESEARCH

European Journal of Gynaecological Oncology

KRT15 promotes proliferation, migration and invasion of cervical cancer cells

Mo Li[1], Chenchen Tian[1], Limei Zhang[1,*]

[1]Department of Obstetrics and Gynecology, Affiliated Hospital of Beihua University, 132011 Jilin, Jilin, China

***Correspondence**
zhanglimei106@163.com
(Limei Zhang)

Abstract

Background: Keratin 15 (KRT15) regulates invasion, metastasis, and tumor growth in a variety of gynecological malignancies outside cervical cancer. KRT15's role and mechanism in cervical cancer cells have not been reported. This study aims to investigate the mechanism of KRT15 knockdown on cervical cancer's malignant behavior. **Methods**: KRT15 expression in cervical cancer was assessed in multiple cervical cancer cell lines (HeLa, SiHa, CaSki and C33A) and examined using the Gene Expression Profiling Interactive Analysis (GEPIA) database. KRT15 was knocked down using siRNA technology, with siRNA Control (si-NC) as a control. Cell proliferation was assessed using cell viability and colony formation assays. Transwell tests were used to study cell invasion and migration. A Western blot analysis was performed using KRT15 knockdown HeLa and SiHa to assess Wnt/β-catenin pathway expression. Group differences were assessed using Student's *t*-test. **Results**: In western blot and bioinformatics analyses, cervical carcinoma had significantly elevated KRT15 expression. KRT15 knockdown inhibited cell growth. KRT15 knockdown also prevented HeLa and SiHa cells from migrating and invading. KRT15 knockdown reduced Wnt/β-catenin signaling pathway expression in cervical cancer cells. **Conclusions**: KRT15 knockdown suppresses the Wnt/β-catenin signaling pathway, thereby reducing cervical cancer cell mobility and proliferation. This suggests that KRT15 may serve as a potential therapeutic target for cervical cancer treatment.

Keywords

KRT15; Cervical cancer; Proliferation; Migration; Invasion; Wnt/β-catenin

1. Introduction

Cervical cancer (CC) is one of the most prevalent gynecological cancers worldwide. There are approximately 265,700 deaths caused by CC every year, making it a public health concern [1]. Infection with the high-risk human papillomavirus is a significant risk factor for CC. Patients with advanced CC typically face a poor prognosis, exhibiting a 5-year survival rate below 40% [2, 3]. Despite the fact that there are numerous treatment options available, such as surgery, radiation, chemotherapy and more, cervical cancer still poses a serious threat to women's health [4]. There are numerous gene alterations involved in the intricate molecular mechanism of cervical cancer growth and metastasis, as demonstrated by pathological investigations [5].

The human stratified epidermis and basal layer both express Keratin 15 (KRT15), a type I keratin. It's interesting to note that KRT15 encourages malignant keratin growth [6]. A prior study, for instance, demonstrated that KRT15 promotes colorectal cancer metastasis [7]. KRT15 silencing reduces liver cancer mobility and survivability while increasing doxorubicin chemosensitivity [8]. Women with endometrial cancer with elevated KRT15 also had cervical stromal invasion, lymphovascular invasion, and a lower chance of survival [9]. According to study, positive for KRT15 It is possible that crypt cells, which are resistant to radiation, arose from intestinal carcinogenesis [10]. Therefore, KRT15 contributes to cancer development as a multipurpose carcinogenic protein. However, the underlying mechanism is not well understood and needs to be clarified.

It is yet unknown how KRT15 contributes to cervical cancer. KRT15 expression in cervical cancer was examined in this study using an online database. Multiple cervical cancer cell lines were examined. This study also examined how KRT15 affects cervical cancer cells. Additionally, the function of invasion, migration, and proliferation as well as KRT15's regulatory impact on the Wnt/β-catenin pathway were investigated.

2. Methods

2.1 Bioinformatics analysis

The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) projects' tumor and normal sample RNA sequencing expression data are available in the GEPIA database (gepia.cancer-pku.cn). Using GEPIA, the expression

of KRT15 in cervical cancer tissues was evaluated.

2.2 Cell culture

The human cervical cancer cells HeLa, SiHa, CaSki and C33A, as well as the immortalized keratinocyte cell line HaCaT, were obtained from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). Cells were cultivated at 37 °C in a humidified incubator with 5% CO_2 in high-glucose media Dulbecco's modified eagle medium (DMEM, 2458509, Gibco, Grand Island, NY, USA) with 10% Fetal Bovine Serum (FBS, FB12999102, Gibco, Grand Island, NY, USA) and 1% penicillin-Streptomycin (C0222, Beyotime, Shanghai, China).

2.3 Cell transfection

KRT15-specific siRNA (si-KRT15, 5′-AGGAGTACAAGATG CTGCTTGACAT-3′) and a negative control (si-NC, 5′-AGGATACGATACGGTTCGTAGACAT-3′) were used to knockdown KRT15. Si-KRT15 and si-NC were transfected into cervical cancer cells (HeLa and SiHa) using the Lipofectamine 3000 kit (L3000015, Invitrogen, Carlsbad, CA, USA). KRT15 expression was measured 48 h after knockdown to assess the impact.

2.4 Cell viability assay (CCK-8)

3×10^3 HeLa and SiHa cells were seeded and cultured for 24 h. Cell viability was calculated using CCK-8 (10 μL/well; C0037, Beyotime, Shanghai, China) by reading optical density using a microplate reader. Six replicate samples were included in each group.

2.5 Colony formation assay

In separate 6-well plates, transfected HeLa and SiHa cells (5×10^3) were seeded. A 4% paraformaldehyde fixation and 0.1% amethyst staining (548-62-9, Sigma-Aldrich, Shanghai, China) were performed after cell clump formation to determine their colonial number. In colony formation experiments, if cells form colonies, this usually indicates significant proliferative capacity and viability.

2.6 Transwell assay

Invasion experiment: the upper chamber of Matrigel-coated Transwell plates (Corning Company) was injected with transfected HeLa and SiHa cells (4×10^4) suspended in FBS-free medium. 10% FBS-containing medium was added to the lower chamber culture media. An inverted light microscope (Leica DMi1, Leica Microsystems, Wetzlar, HE, Germany) was used to count the invasive cells after they had been cultivated for 24 h and stained with crystal violet (Y268091, Beyotime, Shanghai, China) for 20 minutes at room temperature.

Migration experiment: the experimental method is similar to the invasion experiment, except that the Transwell plate is not coated with Matrigel [11].

2.7 Western blotting

Radioimmunoprecipitation assay (RIPA) buffer (P0013B, Beyotime, Shanghai, China) was used to lyse cervical cancer cells. A Bicinchoninic acid assay (BCA) protein assay kit (P0009, Beyotime, Shanghai, China) was used to measure the total protein concentration. Using 20% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), 20 micrograms of proteins and transferred to a polyvinylidene fluoride (PVDF) membrane. Primary antibodies were incubated overnight at 4 °C after the membrane was blocked for 90 mins at 37 °C using 5% Bovine Serum Albumin (BSA, GC305010, Savis Biotechnology Co., Ltd., Wuhan, China), including KRT15 (ab52816; Abcam, Cambridge, UK), β-catenin (ab32572; Abcam, Cambridge, UK), cyclin D1 (ab239794; Abcam, Cambridge, UK), Wnt3a (ab219412; Abcam, Cambridge, UK), matrix metalloproteinase-2 (MMP-2, ab92536; Abcam, Cambridge, UK), MMP-9 (ab76003; Abcam, Cambridge, UK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, ab8245; Abcam, Cambridge, UK). Incubation was conducted for 1.5 h at 37 °C with diluted horseradish peroxidase (HRP)-labeled secondary antibody (ab6721; Abcam, Cambridge, UK). The membrane was detected using an enhanced chemiluminescence (ECL) kit (G2161, Wuhan Saiwei Biotechnology Co., Ltd., Wuhan, China), and grayscale values were analyzed using Image J (1.0, NIH).

2.8 Statistical analysis

Data plotting and statistical analysis were performed using GraphPad Prism 8 (Harvey Motulsky, San Diego, CA, USA). Group comparisons were analyzed using Student's *t*-test. Data are presented as the mean $\pm$ Standard Deviation (SD) from three independent experiments. Statistical significance was defined as $p < 0.05$. Each experiment was conducted three times independently.

3. Results

3.1 KRT15 was highly expressed in cervical cancer

Using GEPIA website analysis, we found higher KRT15 expression in tumor tissues of cervical cancer patients than in healthy subjects (Fig. 1A). Compared with immortalized keratinocytes (HaCaT cells), HeLa, SiHa ($p < 0.001$), CaSki and C33A cells ($p < 0.01$) showed increased KRT15 expression (Fig. 1B). In summary, cervical carcinoma showed elevated expression of KRT15. Since HeLa and SiHa cells expressed KRT15 at the highest levels, they were chosen for additional functional investigations.

3.2 Knockdown of KRT15 inhibited the proliferation of cervical cancer cells

After transfecting cells with si-KRT15, KRT15 expression decreased, indicating protein knockdown was effective ($p < 0.01$) (Fig. 2A). After transfection, si-KRT15 decreased the ability of cells to form colonies and maintain cell viability ($p < 0.01$) (Fig. 2B,C). Consequently, KRT15 plays an active role in cervical cancer cell proliferation.

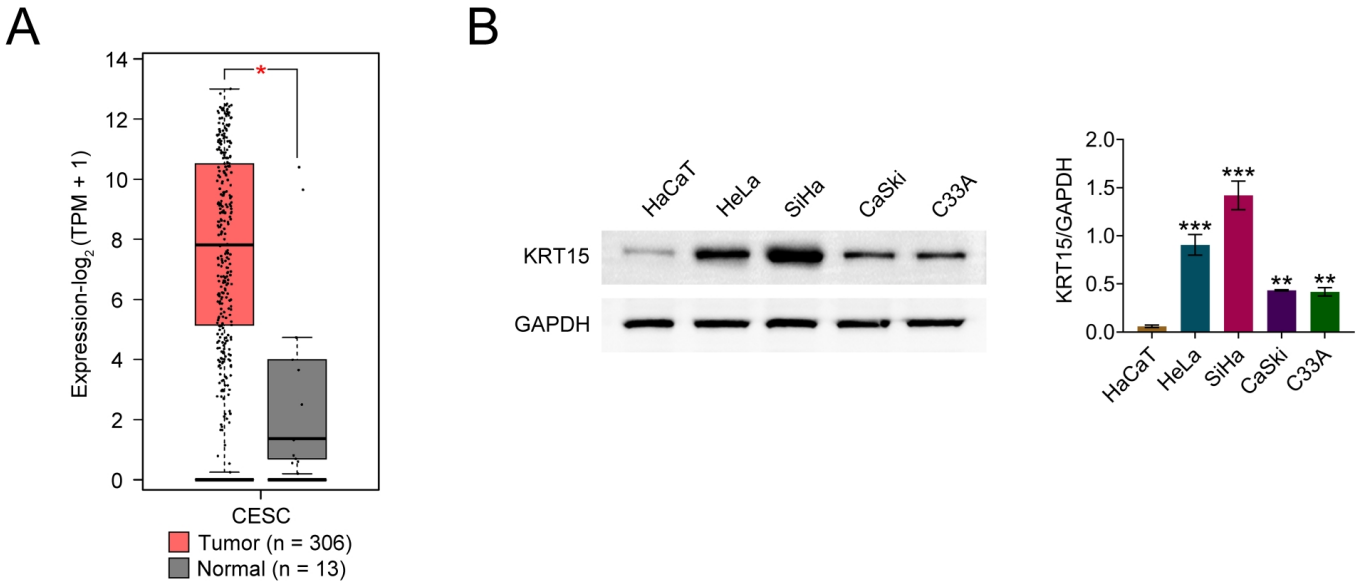

FIGURE 1. KRT15 expression in cervical cancer. (A) GEPIA database analysis of KRT15 expression in healthy subjects and cervical cancer patients. (B) Western blot analysis of KRT15 expression in the Human immortalized keratinocyte cell line HaCaT and human cervical cancer cell lines HeLa, SiHa, CaSki and C33A. Values are presented as mean ± SD. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. n = 3. TPM: Transcripts Per Million; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; KRT15: Keratin 15; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

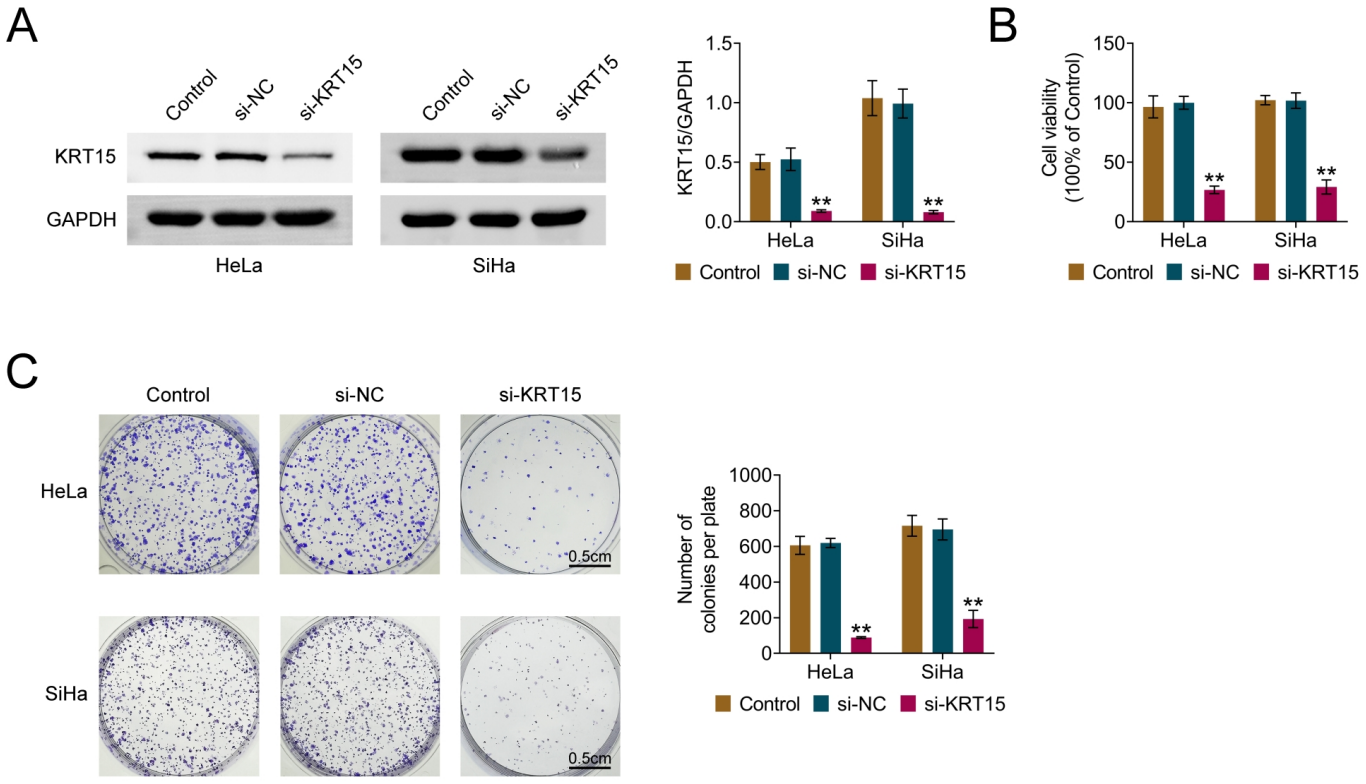

FIGURE 2. The role of KRT15 in cervical cancer cell proliferation. (A) KRT15 expression in transfected and untransfected cells. (B) CCK-8 kit detects cell viability. n = 6. (C) Crystal violet staining detects cell colony formation ability. Values are presented as mean ± SD. $**p < 0.01$. n = 3. KRT15: Keratin 15; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; si-NC: siRNA Control.

3.3 Knockdown of KRT15 inhibited cervical cancer cell migration and invasion

This study investigated KRT15's role in cell invasion and migration. Transwell experiments showed that HeLa and SiHa cells' capacity for invasion and migration was significantly reduced following KRT15 knockdown ($p < 0.01$) (Fig. 3A). KRT15 knockdown could decrease the expression of MMP-2 and MMP-9 proteins ($p < 0.01$) (Fig. 3B). Consequently, KRT15 actively contributed to cervical cancer cell invasion and migration. Enhanced migration and invasion capabilities may be indicators of tumor cells' metastatic potential.

3.4 Knockdown of KRT15 downregulated Wnt/β-catenin signaling pathway

KRT15 uses β-catenin to control cell invasion and migration in colorectal cancer [7]. Therefore, it is necessary to determine whether KRT15 regulates β-catenin. Western blotting was used to measure the amounts of β-catenin, Wnt3a, and Cyclin D1 proteins. KRT15 knockdown suppressed β-catenin, Wnt3a and Cyclin D1 expression in HeLa and SiHa ($p < 0.01$) (Fig. 4). In cervical cancer cells, KRT15 controlled the Wnt/β-catenin signaling pathway.

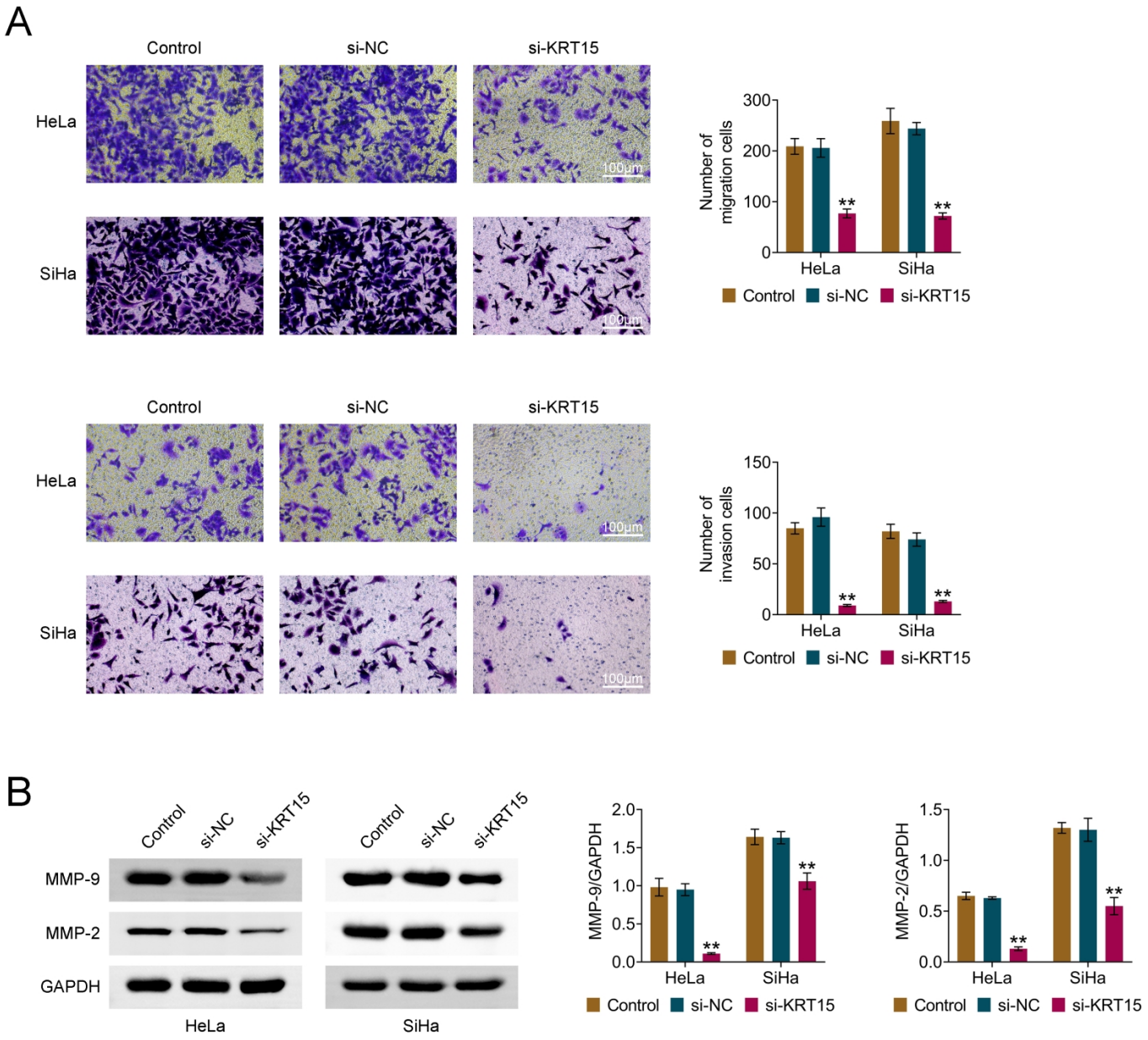

FIGURE 3. The role of KRT15 in the cervical cancer cell migration and invasion. (A) Cell migration and invasion numbers. (B) Western blot analysis of MMP-2 and MMP-9 expression in cells. Values are presented as mean ± SD. $^{**}p < 0.01$. n = 3. si-NC: siRNA Control; KRT15: Keratin 15; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; MMP: matrix metalloproteinase.

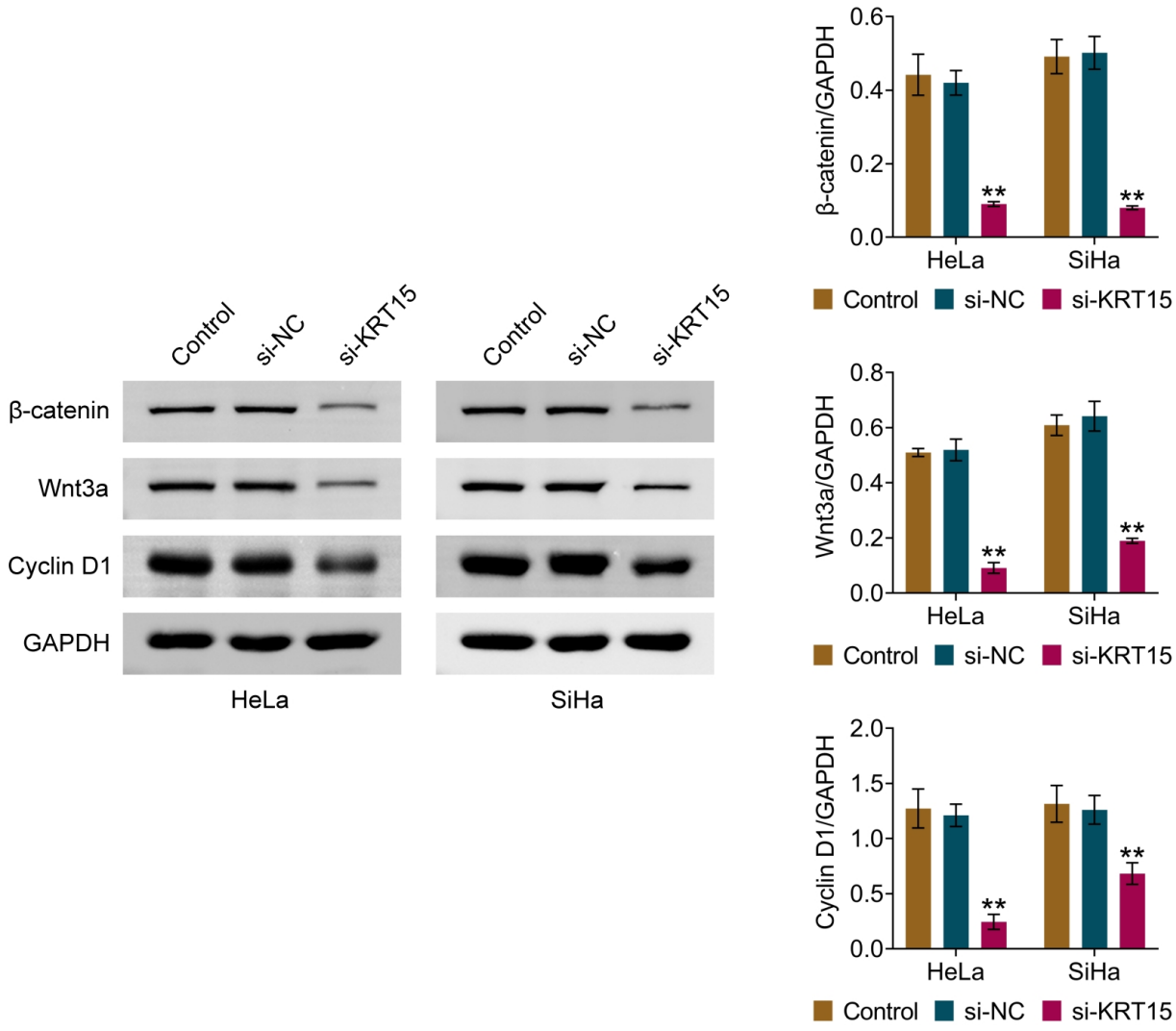

FIGURE 4. Effect of KRT15 on Wnt/β-catenin pathway in cervical cancer cells. Western blot analysis of β-catenin, Wnt3a, Cyclin D1 expression in cells. Values are presented as mean ± SD. $^{**}p < 0.01$. n = 3. si-NC: siRNA Control; KRT15: Keratin 15; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

4. Discussion

Cervical cancer is the most common cause of gynecological cancer-related death. Clinical treatment of cervical cancer requires an understanding of the carcinogenesis process [12]. KRT15 is crucial to cancer pathophysiology. In certain human malignant tumors, such as colorectal, breast and stomach cancers, KRT15 expression is elevated [7, 13, 14]. Bioinformatics analysis (GEPIA) showed cervical cancer was associated with increased KRT15 expression. The considerable rise in KRT15 expression levels in cervical cancer cells was validated by Western blotting of multiple cervical cancer cell lines. The high expression of KRT15 in cervical cancer is therefore reasonable.

KRT15 has also been linked to cervical stromal invasion, lymphatic invasion and poor prognosis in endometrial cancer patients. Furthermore, it may promote colorectal cancer cell migration and invasion. Oral squamous cancers also express high levels of KRT15. Based on the aforementioned research, it is crucial to control cancer's aggressive activity [7, 9, 15]. Cell migration ability may be enhanced by cell motility, extracellular signaling, and cell-cell interactions. In cancer research, enhanced migration capacity and cell invasion may indicate tumor cells' metastatic potential [16, 17]. Loss-of-function tests demonstrated that KRT15 knockdown decreased malignant characteristics of cervical cancer cell lines, in line with earlier findings. These results identify KRT15 as an oncogene and therapeutic target in cervical cancer pathophysiology.

Cervical cancer is oncogenic through the β-catenin pathway [18]. The classic β-catenin pathway entails Wnt binding to its membrane receptor, followed by β-catenin upregulation, further destruction of complex formation, and translocation to the nucleus. Th Transcriptional factors (like c-Myc and Cyclin D1) are activated, which results in carcinogenesis [19, 20]. Earlier research revealed that in colorectal cancer, KRT15 activates the β-catenin-mediated MMP7 pathway. Through the inhibition of the β-catenin pathway, KRT15 silencing reduces the mobility and survivability of liver cancer while increasing doxorubicin chemosensitivity [7, 8]. Similar to earlier research, KRT15 knockdown in this study rendered the signaling pathway of Wnt/β-catenin in cervical cancer cells inactive.

This study is restricted in several ways. KRT15 enrolment in cervical cancer was not confirmed in an animal model. The connection between KRT15 and β-catenin has not been fully clarified. Animal and clinical studies have not been conducted, and research into the mechanism has only been superficial. To fully investigate the function of β-catenin and establish experimental support for β-catenin as a therapeutic target for cervical cancer, more clinical and animal studies are required. Future research will be conducted in-depth.

5. Conclusions

In summary, this study confirmed for the first time that KRT15 promotes cervical cancer cell proliferation, migration, and invasion. In addition, KRT15 can promote Wnt/β-catenin pathway activity in cervical cancer. These findings will deepen our understanding of the mechanisms of cervical cancer tumorigenesis and progression and may provide new alternatives for current cervical cancer treatment.

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

ML—designed the study and carried them out. ML, LMZ, CCT—supervised the data collection; analyzed the data; interpreted the data. ML, LMZ—prepared 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

This article does not contain any studies with human participants or animals performed by any of the authors.

ACKNOWLEDGMENT

Not applicable.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Voelker RA. Cervical cancer screening. JAMA. 2023; 330: 2030.

[2] Sahasrabuddhe VV. Cervical cancer: precursors and prevention. Hematology/Oncology Clinics of North America. 2024; 38: 771–781.

[3] Yao S, Zhao L, Chen S, Wang H, Gao Y, Shao NY, *et al*. Cervical cancer immune infiltration microenvironment identification, construction of immune scores, assisting patient prognosis and immunotherapy. Frontiers in Immunology. 2023; 14: 1135657.

[4] Manrriquez EN, Zakhour M, Salani R. Precision medicine for cervical cancer. Current Opinion in Obstetrics & Gynecology. 2022; 34: 1–5.

[5] Lizano M, Carrillo-García A, De La Cruz-Hernández E, Castro-Muñoz LJ, Contreras-Paredes A. Promising predictive molecular biomarkers for cervical cancer (review). International Journal of Molecular Medicine. 2024; 53: 50.

[6] Chong LY, Cheok PY, Tan W, Thike AA, Allen G, Ang MK, *et al*. Keratin 15, transcobalamin I and homeobox gene Hox-B13 expression in breast phyllodes tumors: novel markers in biological classification. Breast Cancer Research and Treatment. 2012; 132: 143–151.

[7] Chen W, Miao C. KRT15 promotes colorectal cancer cell migration and invasion through β-catenin/MMP-7 signaling pathway. Medical Oncology. 2022; 39: 68.

[8] Wang J, Zhu G. Silencing of keratin 15 impairs viability and mobility while facilitating the doxorubicin chemosensitivity by inactivating the β-catenin pathway in liver cancer. Oncology Letters. 2023; 26: 447.

[9] Yang H, Li A, Li A, Zhao F, Zhang T. Upregulated keratin 15 links to the occurrence of lymphovascular invasion, stromal cervical invasion as well as unfavorable survival profile in endometrial cancer patients. Medicine. 2022; 101: e29686.

[10] Giroux V, Stephan J, Chatterji P, Rhoades B, Wileyto EP, Klein-Szanto AJ, *et al*. Mouse intestinal krt15+ crypt cells are radio-resistant and tumor initiating. Stem Cell Reports. 2018; 10: 1947–1958.

[11] Zhong G, Zhao Q, Chen Z, Yao T. TGF-β signaling promotes cervical cancer metastasis via CDR1as. Molecular Cancer. 2023; 22: 66.

[12] Yadav G, Srinivasan G, Jain A. Cervical cancer: novel treatment strategies offer renewed optimism. Pathology, Research and Practice. 2024; 254: 155136.

[13] Zhong P, Shu R, Wu H, Liu Z, Shen X, Hu Y. Low KRT15 expression is associated with poor prognosis in patients with breast invasive carcinoma. Experimental and Therapeutic Medicine. 2021; 21: 305.

[14] Zhang C, Liang Y, Ma M, Wu K, Dai D. KRT15, INHBA, MATN3, and AGT are aberrantly methylated and differentially expressed in gastric cancer and associated with prognosis. Pathology, Research and Practice. 2019; 215: 893–899.

[15] Khanom R, Sakamoto K, Pal SK, Shimada Y, Morita K, Omura K, *et al*. Expression of basal cell keratin 15 and keratin 19 in oral squamous neoplasms represents diverse pathophysiologies. Histology and Histopathology. 2012; 27: 949–959.

[16] Trepat X, Chen Z, Jacobson K. Cell migration. Comprehensive Physiology. 2012; 2: 2369–2392.

[17] Eccles SA, Box C, Court W. Cell migration/invasion assays and their application in cancer drug discovery. Biotechnology Annual Review. 2005; 11: 391–421.

[18] Zhang X, Dong N, Hu X. Wnt/β-catenin Signaling Inhibitors. Current Topics in Medicinal Chemistry. 2023; 23: 880–896.

[19] Liu J, Xiao Q, Xiao J, Niu C, Li Y, Zhang X, *et al*. Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities. Signal Transduction and Targeted Therapy. 2022; 7: 3.

[20] Yu F, Yu C, Li F, Zuo Y, Wang Y, Yao L, *et al*. Wnt/β-catenin signaling in cancers and targeted therapies. Signal Transduction and Targeted Therapy. 2021; 6: 307.

How to cite this article: Mo Li, Chenchen Tian, Limei Zhang. KRT15 promotes proliferation, migration and invasion of cervical cancer cells. European Journal of Gynaecological Oncology. 2025; 46(6): 121-126. doi: 10.22514/ejgo.2025.086.