Submitted: 10 April, 2024 Accepted: 07 June, 2024 Published: 15 August, 2024 DOI:10.22514/ejgo.2024.077

ORIGINAL RESEARCH

European Journal of Gynaecological Oncology

ZNF671 restrains endometrial cancer cell growth and stemness through the Wnt/β-catenin pathway

Bingjuan Fan[1], Wenjing Huang[1,*], Zhengwei Lai[1], Jun Han[1], Qun Xiao[1], Huabin Wang[1]

[1]Department of Gynaecology, The First People's Hospital of Xiaoshan District, 311200 Hangzhou, Zhejiang, China

***Correspondence**
huangwenjing40312@163.com
(Wenjing Huang)

Abstract

Endometrial cancer is a common malignant cancer in women, which poses serious health risks. Multifarious cancers have been shown to be suppressed by Zinc finger protein 671 (*ZNF671*). However, the precise regulatory impact of ZNF671 on endometrial cancer progression remains unclear. This study investigated *ZNF671*'s role in endometrial cancer. The Ualcan online database revealed that *ZNF671* exhibited low expression in uterine corpus endometrial carcinoma (UCEC) tissues. Endometrial cancer patients who exhibited low *ZNF671* expression suffered poor prognosis. *ZNF671* also retarded tumor growth in endometrial cancer. Cell invasion and migration were restrained by *ZNF671*. In addition, *ZNF671* inhibited endometrial cancer stemness. Eventually, ZNF671 suppressed the Wnt/β-catenin pathway. In conclusion, *ZNF671* restrained both cell growth and stemness in endometrial cancer through the Wnt/β-catenin pathway. *ZNF671* may serve as a bio-target for endometrial cancer treatment.

Keywords

ZNF671; Endometrial cancer; Stemness; The Wnt/β-catenin pathway

1. Introduction

Endometrial cancer is regarded as a common malignant cancer in women, especially in elderly women, and poses serious health risks [1]. Endometrial cancer is generally detected early, possess a good prognosis, and has a 5-year survival rate of 95% [2]. As endometrial cancer incidence rates increase rapidly, the number is expected to double by 2030 [3, 4]. Therefore, it is essential to fully elucidate its pathogenesis and identify more effective bio-targets for treatment.

Endometrial cancer is regulated by many proteins. For instance, Netrin-1 inhibition retards tumor growth and epithelial-mesenchymal transition (EMT) progress in endometrial cancer [5]. SPOP (a substrate-binding adaptor of the Cullin 3-RING E3 ubiquitin ligase complex (CRL3)) mutations modulate the interferon regulatory factor-1 (IRF1)/programmed cell death 1 (PD-L1) axis to aggravate immune escape in endometrial cancer [6]. A combination of SNORD60 and PIK3CA influences the phosphoinositide-3 kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway, facilitating endometrial cancer tumorigenesis and development [7]. Zinc finger protein 671 (*ZNF671*) belongs to the mammalian transcription suppressor Kruppel-associated box (KRAB)-Zinc-finger protein (ZFP) family, which includes Cys2His2 (C2H2) type zinc fingers and KRAB domains [8]. *ZNF671* has been demonstrated to suppresses tumor growth in multifarious cancers. By suppressing the Notch signaling pathway, *ZNF671* retards colorectal carcinoma [9]. In laryngeal carcinoma, methylated *ZNF671* facilitates mitogen-activated protein kinase 6 (MAPK6) transcription to restrain tumor progression [10]. Also, *ZNF671* is effective in preventing tumor growth and metastasis in non-small cell lung cancer [11]. Silencing *ZNF671* weakens cell cycle arrest to accelerate tumorigenicity in nasopharyngeal carcinoma [12]. However, *ZNF671*'s regulatory effects on endometrial cancer progression remain unclear.

This study revealed that *ZNF671* restrained endometrial cancer cell growth and stemness *via* the Wnt/β-catenin pathway. Possibly, this study will provide a bio-target to diagnose and treat endometrial cancer.

2. Materials and methods

2.1 Cell lines and culture

Endometrial cancer cell lines (HEC-1A and Ishikawa) were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). Cultivation was carried out with Roswell Park Memorial Institute (RPMI-1640) medium (31800022, Gibco, Grand Island, NY, USA) appended with 10% fetal bovine serum (10099-141, FBS, Gibco, Grand Island, NY, USA) in an incubator (5% CO_2, 37 °C).

2.2 Cell transfection

The pcDNA3.1 vectors targeting *ZNF671* (ZNF671) with negative control (Vector) purchased from Gene Parma (Shanghai, China) were transfected into HEC-1A and Ishikawa cells using Lipofectamine 2000 (11668019, Invitrogen, Carlsbad, CA,

USA).

2.3 Western blot

Proteins were extracted from HEC-1A and Ishikawa cells in radio immunoprecipitation assay (RIPA) buffer. 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate proteins. Next, moving proteins into polyvinylidene fluoride (PVDF) membranes (Beyotime, Shanghai, China). Post sealing, primary antibodies against ZNF671 (1:1000; ER1919-06; Huabio, Hangzhou, China), β-catenin (1:1000; ET1601-5), c-myc (1:500; 0912-2), Matrix metalloproteinase 7 (MMP7; 1:500; ER1913-08) and β-actin (1:10000; R1207-1) were appended for 12 h incubation. Incubation with the appropriate secondary antibodies (1:5000; HA1005) continued for another 2 h. Measurements were performed using the chemiluminescence detection kit (32106, Thermo Fisher Scientific, Inc., Waltham, MA, USA).

2.4 Cell counting kit-8 (CCK-8) assay

HEC-1A and Ishikawa cells (1000 cells/well) was plated in 96-well plate. 10 μL CCK-8 solution (CK04, Dojindo Laboratories, Kumamoto, Japan) in each well was incubated for 4 h. Lastly, the optical density (OD) value (at 450 nm) was determined with a spectrophotometer (ND-ONE-W, Thermo Fisher Scientific, Waltham, MA, USA).

2.5 Colony formation assay

HEC-1A and Ishikawa cells (1000 cells/well) was plated in 6-well plate. After 2 weeks, fixing (4% paraformaldehyde) and staining (0.1% crystal violet) were made for the colonies. Colonies were calculated.

2.6 Transwell assay

The upper Transwell chambers (Corning Life Sciences, Corning, NY, USA) covered with Matrigel (Becton Dickinson, USA) were appended with RPMI-1640 medium (200 μL) containing HEC-1A or Ishikawa cells (1×10^5). The lower chambers were appended with RPMI-1640 medium (600 μL) containing 20% FBS. After 48 h, the invaded cells were fixed (90% ethanol) and stained (0.1% crystal violet). The invaded cells were measured using a microscope (CX41, Olympus Corporation, Tokyo, Japan).

2.7 Wound healing assay

HEC-1A or Ishikawa cells (1×10^5) was plated in 6-well plate. A pipette tip was used to generate the wound. Light microscopy was used to monitor the wound at 0 and 24 h.

2.8 Sphere formation assay

In the 96-well plate (ultra-low attachment), HEC-1A or Ishikawa cells (1000/well) were cultured in serum-free RPMI-1640 medium (Gibco, Grand Island, NY, USA) containing epithelial growth factor (20 ng/mL), and fibroblast growth factor (20 ng/mL). After 14 days, a microscope (CX41, Olympus Corporation, Tokyo, Japan) was used to observe the spheroids.

2.9 Statistical analysis

Data were presented as mean $\pm$ standard deviation (SD). A statistical analysis was conducted using SPSS 22.0 statistical software (IBM Corp., Armonk, NY, USA). Three repetitions were conducted for each assay. For comparison, one-way analysis of variance (ANOVA) was used. $p < 0.05$ indicates a statistically significant difference.

3. Results

3.1 *ZNF671* was low expressed in endometrial cancer

Ualcan online database revealed a low expression of *ZNF671* in uterine corpus endometrial carcinoma (UCEC) tissues (Fig. 1a). The Kaplan-Meier Plotter database confirmed that patients with low *ZNF671* expression have a poor prognosis (Fig. 1b). In general, *ZNF671* was low expressed in endometrial cancer.

3.2 *ZNF671* retards tumor growth in endometrial cancer

ZNF671 protein expression was overexpressed after *ZNF671* amplification (Fig. 2a). Furthermore, *ZNF671* overexpression decreased cell viability (Fig. 2b). A decline in cell proliferation was confirmed by colony formation after *ZNF671* up-regulation (Fig. 2c). *ZNF671* retards tumor growth in endometrial cancer.

3.3 *ZNF671* restrains cell invasion and migration in endometrial cancer

Cell invasion capacity was reduced after *ZNF671* overexpression (Fig. 3a). Additionally, *ZNF671* augmentation also weakened cell migration rate (Fig. 3b). Overall, *ZNF671* restrains cell invasion and migration in endometrial cancer.

3.4 ZNF671 inhibits stemness in endometrial cancer

After *ZNF671* amplification, sphere formation assays revealed a decrease in stemness (Fig. 4a). Moreover, Oct4 and Sox2 protein expressions were both decreased after *ZNF671* overexpression (Fig. 4b). In endometrial cancer, *ZNF671* inhibited stemness.

3.5 ZNF671 suppresses the Wnt/β-catenin pathway

β-catenin and c-myc protein expressions both declined after *ZNF671* augmentation (Fig. 5a). Moreover, *ZNF671* overexpression also decreased MMP7 expression (Fig. 5b). In general, *ZNF671* suppresses the Wnt/β-catenin pathway.

4. Discussion

ZNF671 has been indicated to serve as a novel tumor repressor in multifarious cancers [9–12]. However, the *ZNF671* precise regulatory impact on endometrial cancer progression remains unclear. Using the Ualcan online database, first,

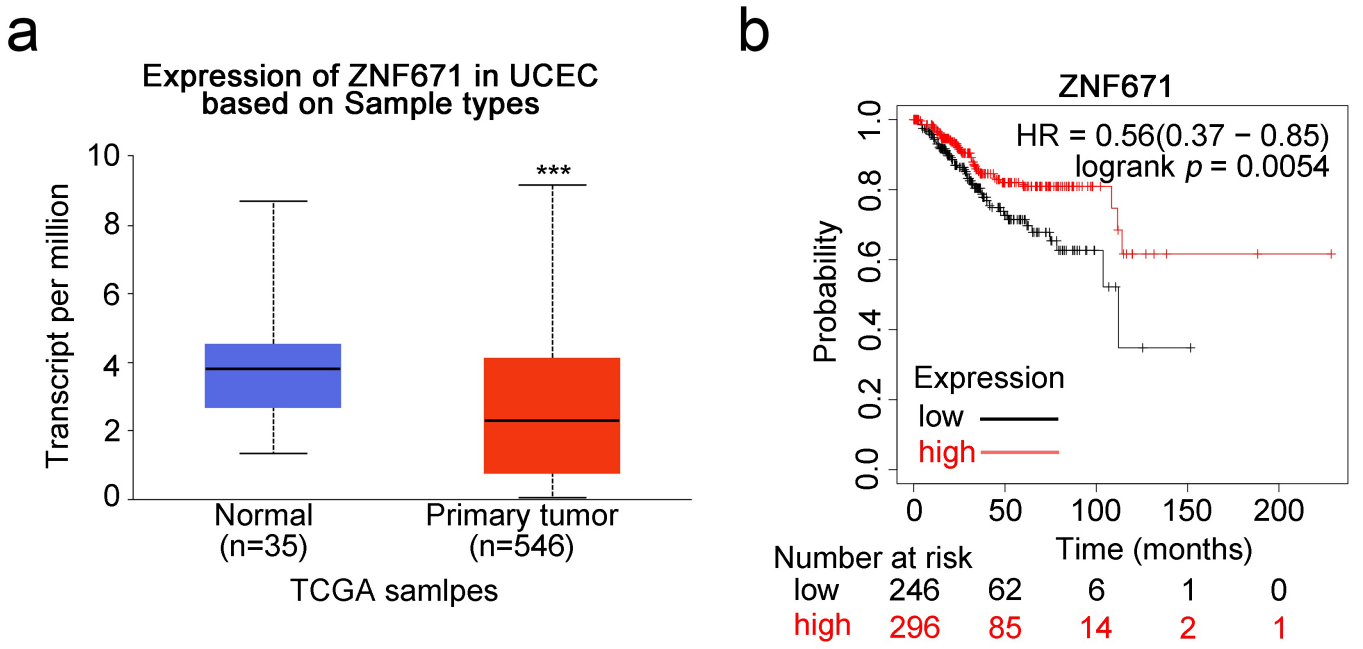

FIGURE 1. ***ZNF671* was low expressed in endometrial cancer.** (a) Ualcan online database confirmed *ZNF671* expression in uterine corpus endometrial carcinoma (UCEC) tissues and normal tissues. ***$p < 0.001$. (b) Prognosis of endometrial cancer patients with high or low *ZNF671* expression was confirmed through the Kaplan database. $p = 0.0054$. *ZNF671*: Zinc finger protein 671; TCGA: American Type Culture Collection; HR: Hazard ratio.

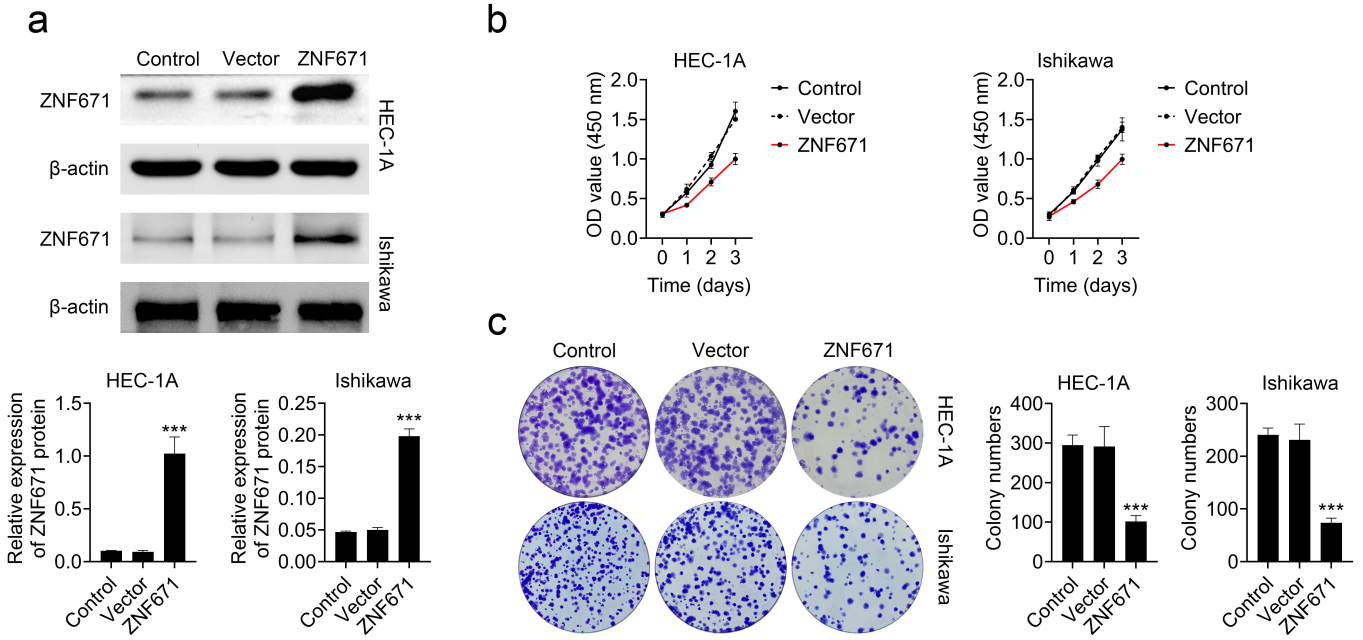

FIGURE 2. ***ZNF671* retards tumor growth in endometrial cancer.** Separated into three groups were Control, Vector and ZNF671. (a) ZNF671 protein expression was tested using western blot. (b) Cell viability was determined by CCK-8 assay. (c) Colony formation assay verified cell proliferation. ***$p < 0.001$. For comparisons, ANOVA was used. *ZNF671*: Zinc finger protein 671.

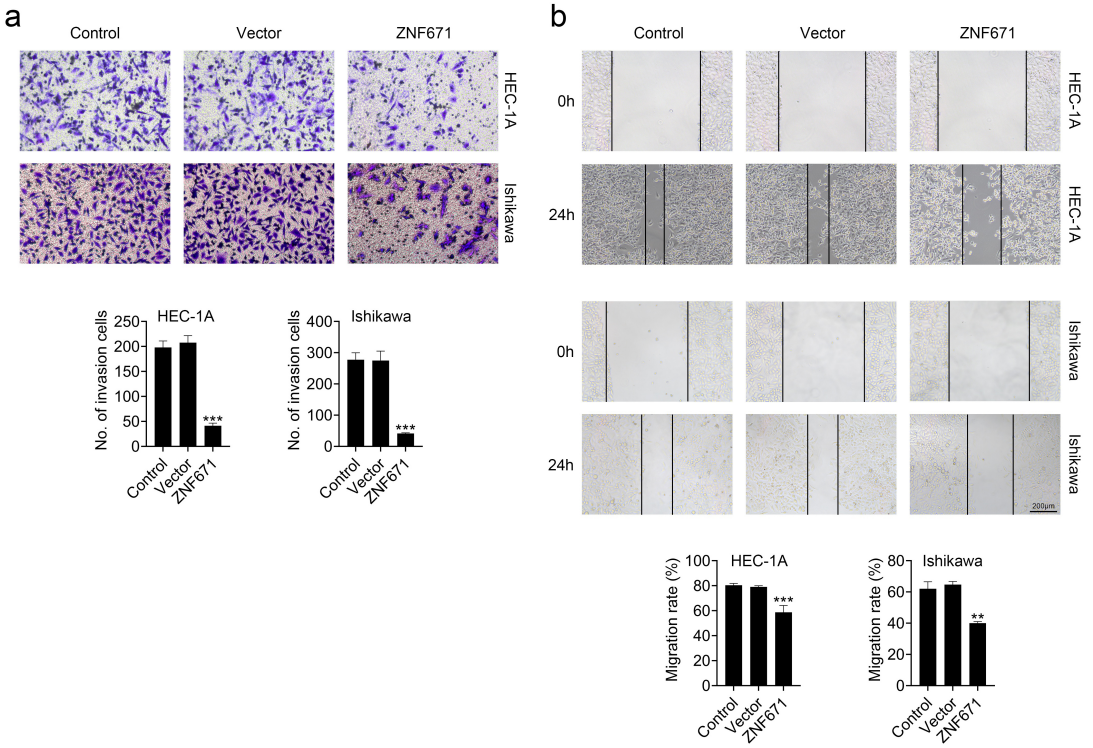

FIGURE 3. ***ZNF671*** **restrains cell invasion and migration in endometrial cancer.** Separated into three groups were Control, Vector and ZNF671. (a) Cell invasion was assessed through Transwell assay. (b) Cell migration examined with wound healing assay. $^{**}p < 0.01$, $^{***}p < 0.001$. For comparisons, ANOVA was used. *ZNF671*: Zinc finger protein 671.

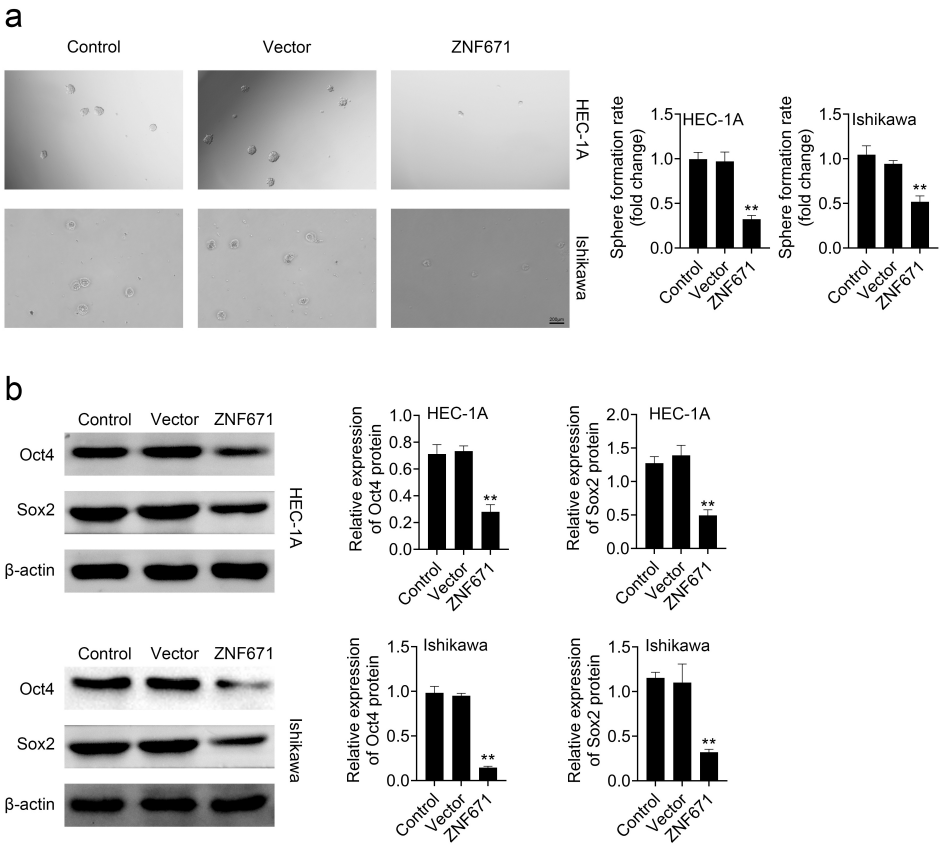

FIGURE 4. ZNF671 inhibits stemness in endometrial cancer. Separated into three groups were Control, Vector and ZNF671. (a) Stemness was evaluated through sphere formation assay. (b) Oct4 and Sox2 protein expressions were determined through western blot. $^{**}p < 0.01$. For comparisons, ANOVA was used. *ZNF671*: Zinc finger protein 671.

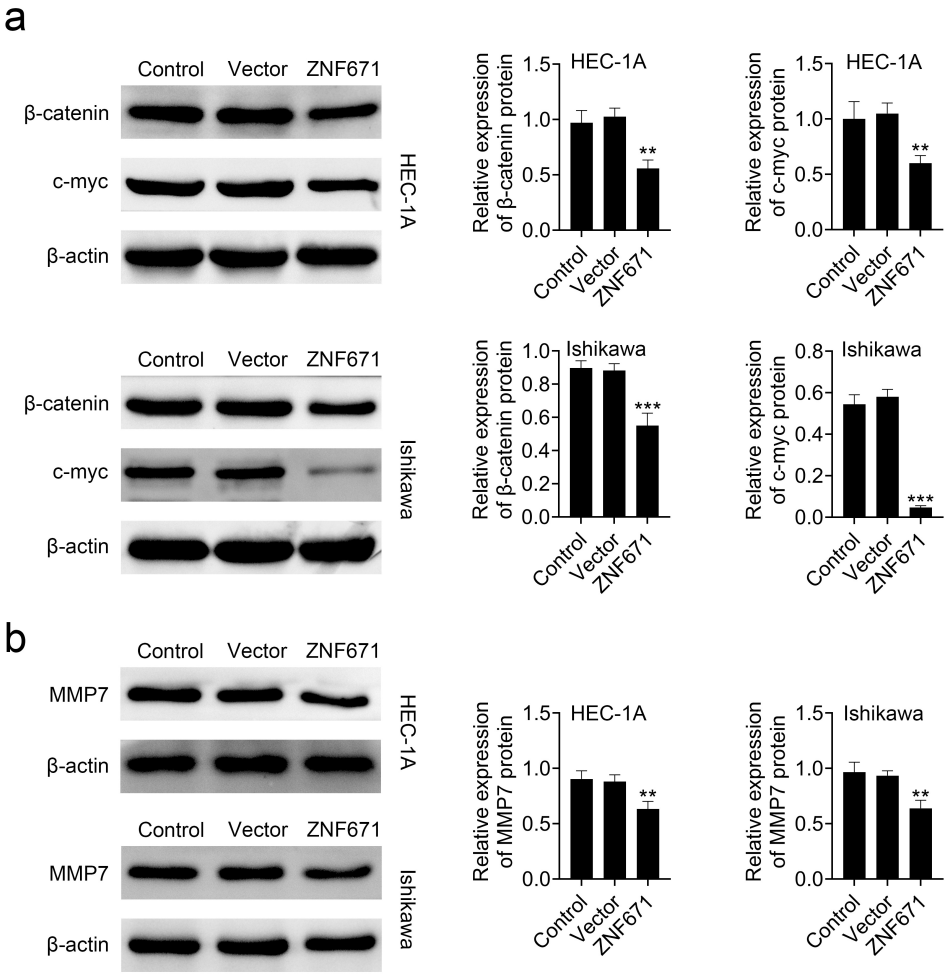

FIGURE 5. ZNF671 suppresses the Wnt/β-catenin pathway. Separated into three groups were Control, Vector and ZNF671. (a) β-catenin and c-myc protein expressions were detected through western blot. (b) MMP7 protein expression was determined through western blot. $^{**}p < 0.01$, $^{***}p < 0.001$. For comparisons, ANOVA was used. *ZNF671*: Zinc finger protein 671; MMP7: Matrix Metalloproteinase 7.

it was discovered that *ZNF671* is less expressed in uterine corpus endometrial carcinoma (UCEC) tissues. Low *ZNF671* expression is associated with a poor prognosis in endometrial cancer patients. Moreover, *ZNF671* retards tumor growth in endometrial cancer. *ZNF671* restrained endometrial cancer cell invasion and migration.

Due to the unlimited self-renewal ability of cancer stem cells, stemness is considered the major cause of metastasis and relapse after treatment [13, 14]. There has been extensive research on the role of stemness in endometrial cancer. For instance, vitexin modulates the PI3K/AKT pathway to restrain angiogenesis and stemness in endometrial cancer [15]. Additionally, RNA-binding region-containing protein 1 (RNPC1) stabilizes mammalian Ste20-like kinases 1/2 (MST1/2) to inhibit stemness in endometrial cancer [16]. It has been reported that Minichromosome maintenance protein 2 (MCM2) acts on the AKT/β-catenin pathway to increase stemness in endometrial cancer [17]. In endometrial cancer, Lysophosphatidylcholine acyltransferase 1 (LPCAT1) triggers the transforming growth factor (TGF)/β-Smad2/3 signaling pathway to exacerbate stemness and metastasis [18]. This study also found that *ZNF671* inhibited stemness in endometrial cancer as previously reported.

The Wnt/β-catenin pathway appears to be activated in manifold cancers, and has gained increasing attention [19]. When Wnt/β-catenin pathway is triggered, Wnt combines with coiled-coil receptors, next β-catenin is accumulated, accelerating target genes' transcription [20]. The Wnt/β-catenin pathway has also been explored in endometrial cancer. miR-15a-5p declines WNT3A expression to affect the Wnt/β-catenin signaling pathway, retarding endometrial cancer progression [21]. F-Box Protein 17 (FBXO17) blocks the Wnt/β-catenin pathway, mitigating the development of endometrial cancer [22]. Hypermethylation of the Wnt inhibitory factor-1 (WIF1) promoter affects the Wnt/β-catenin pathway, facilitating carcinogenesis in endometrial cancer [23]. Furthermore, high-mobility group A1 (HMGA1) stimulates the Wnt/β-catenin pathway to intensify tumor progression in endometrial cancer [24]. Interestingly, *ZNF671* has been demonstrated to suppress the Wnt/β-Catenin pathway in non-small cell lung cancer [11]. This study also showed *ZNF671* suppressed the Wnt/β-catenin pathway in endometrial cancer.

5. Conclusions

In conclusion, this study was the first to report that *ZNF671* restrained endometrial cancer cell growth and stemness *via* the Wnt/β-catenin pathway. There were, however, some limitations to this study. Further investigations (human samples, animal models and other malignant phenotypes) are ongoing.

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

BJF and WJH—designed the study and carried them out; prepared the manuscript for publication and reviewed the draft of the manuscript. BJF, WJH, ZWL, JH, QX and HBW—supervised the data collection, analyzed the data, interpreted the data. 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.

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How to cite this article: Bingjuan Fan, Wenjing Huang, Zhengwei Lai, Jun Han, Qun Xiao, Huabin Wang. *ZNF671* restrains endometrial cancer cell growth and stemness through the Wnt/β-catenin pathway. European Journal of Gynaecological Oncology. 2024; 45(4): 125-130. doi: 10.22514/ejgo.2024.077.