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ORIGINAL RESEARCH

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Knockdown of UBAP2L inhibits the growth and motility of endometrial cancer cells

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Abstract

Background: Endometrial cancer (EC) is a prevalent malignancy of the female reproductive system. To investigate the function of Ubiquitin-associated protein 2 (UBAP2L) has been linked to various cellular processes and cancer progression. However., its role in EC is still unclear. **Methods**: UBAP2L expression was analyzed in endometrial cancer tissues using data from the TCGA and Kaplan-Meier databases. siRNA was employed to knock down UBAP2L in EC cell lines. Cell growth and motility were assessed via CCK8 assays, wound healing, as well as Transwell, respectively. Immunoblot was used to explore the involvement of the phosphatidylinositol 3 kinase/protein kinase B (PI3K/AKT) axis. **Results**: UBAP2L expression was significantly upregulated in EC tissues. Knockdown of UBAP2L in human endometrial cancer-1A (HEC-1A) as well as Ishikawa cells suppressed cell growth, stimulated cell cycle arrest, and suppressed motility. Mechanistically, UBAP2L silencing suppressed the PI3K/AKT pathway. **Conclusions**: UBAP2L plays a critical role in promoting the growth and migration of endometrial cancer cells via the PI3K/AKT axis.

Keywords

UBAP2L; Endometrial cancer; PI3K/AKT pathway; Cell migration; Cell proliferation

1. Introduction

Endometrial cancer (EC) is a prevalent malignancy of the female reproductive system, primarily affecting post-menopausal women. However, recent years have seen increase in incidence among pre-menopausal women as well [1, 2]. EC originates from the lining of the uterus (the endometrium) [3]. While early-stage EC can often be managed with surgery and radiation, treatment options for advanced or recurrent disease remain inadequate, contributing to poor long-term survival rates [4]. Consequently, identifying new molecular targets for EC therapy is crucial for improving patient outcomes.

UBAP2L contains multiple arginine-glycine-glycine (RGG/RG) repeats [5]. UBAP2L has been linked to various cellular processes, including protein ubiquitination and degradation [6]. In recent years, its role in cancer biology has garnered attention, with studies demonstrating its involvement in promoting the growth, proliferation, and metastasis of cancers such as gastric, breast and liver cancers [7–9]. However, the role of UBAP2L in endometrial cancer remains largely unexplored.

The PI3K/AKT axis is a well-established oncogenic pathway that is frequently activated in various cancers, including EC [10]. The PI3K/AKT plays a pivotal role in the EC development and progression [11]. Abnormal activation of this pathway is frequently observed in many EC patients, promoting cell proliferation, survival, migration and invasion, thereby accelerating tumor progression [12]. PI3K is a serine/threonine kinase that is activated by phosphorylation of Phosphatidylinositol 4,5-bisphosphate (PIP2) to PIP3. After PIP3 is generated, 3-Phosphoinositide-dependent Protein Kinase 1 (PDK1) and AKT are recruited to the plasma membrane, where PDK1 phosphorylates AKT. Activated AKT, in turn, regulates downstream regulatory pathways that control cell survival and growth [13, 14]. Inhibitors targeting the PI3K/AKT pathway have been identified as potential therapeutic strategies for EC, with studies showing that blocking this pathway effectively suppresses tumor cell growth and metastasis, particularly in patients with hyperactivation of PI3K/AKT signaling [11, 15]. Therefore, key proteins in the PI3K/AKT pathway, such as PI3K, AKT and their upstream or downstream regulators, represent promising therapeutic targets of EC. Given its central role in promoting cell survival, proliferation and metastasis, the PI3K/AKT pathway remains a focus for targeted therapies. However, the effects of UBAP2L's and its specific mechanisms in EC progression require further elucidation.

This study aims to investigate the role of UBAP2L in endometrial cancer progression and explore its potential as a therapeutic target. Specifically, we evaluated the effects of UBAP2L knockdown on cell growth, migration and invasion, and investigate the underlying mechanisms, particularly its

influence on the PI3K/AKT axis.

2. Materials and methods

2.1 Cell lines and reagents

The human endometrial cancer cell lines HEC-1A and Ishikawa were obtained from American Type Culture Collection (ATCC). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, #D5796, Sigma, Saint Louis, MO, USA) supplemented with 10% Fetal Bovine Serum (FBS, #26140079, Gibco, Waltham, MA, USA). All cells were maintained in a 37 °C incubator with 5% CO_2.

2.2 siRNA transfection

siRNA targeting UBAP2L (si-UBAP2L) and the negative control (si-NC) were purchased from RiboBio. Cells were transfected using Lipofectamine 3000 (Thermo, #L3000015, Waltham, MA, USA). After 48 hours of transfection, cells were harvested for further analysis. The cells were divided into 3 groups, including control group (without transfection), si-NC group (with the transfection of negative control siRNAs) and si-UBAP2L (with the transfection of UBAP2L siRNAs).

2.3 Cell proliferation assay

The cell counting kit-8 (CCK-8) assay (Beyotime, #C0038, Beijing, China) was used to assess cell proliferation. Transfected HEC-1A and Ishikawa cells were seeded into 96-well plates at 3000 cells per well. Cell viability was measured at 0 h, 24 h, 48 h and 72 h by adding CCK-8 solution and measuring the absorbance at Optical density (OD) 450.

2.4 Colony formation assay

For colony formation, transfected cells were seeded at 500 cells per well in 6-well plates. Cells were allowed to grow for 10–14 days, fixed with 4% Paraformaldehyde (PFA, Beyotime, #P0099, Beijing, China) and stained with 0.1% crystal violet (Beyotime, #C0121, Beijing, China). Colonies were counted manually.

2.5 Wound healing assay

1×10^6 EC cells were seeded in the 24-well plate and transfected as described above. A wound was created using a 200 μL pipette tip. Images of the wound were taken at 0 h and 24 h using a microscope, and the wound width was measured using ImageJ software.

2.6 Transwell invasion assay

Cell invasion was analyzed using Transwell chambers (Corning, #3422, Corning, NY, USA) coated with Matrigel (BD Biosciences, #356234). 1×10^5 transfected cells were seeded in serum-free DMEM in upper chamber. After 24 hours, invaded cells were fixed with PFA and stained with crystal violet. The number of invasive cells was counted.

2.7 Immunoblotting

Proteins were separated by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE), transferred to Polyvinylidene Fluoride (PVDF) membranes (Millipore, #IPVH00010), and probed with the following primary antibodies: UBAP2L (Abcam, Cambridge, UK, #ab198452, 1:1000), PI3K (Abcam, Cambridge, UK, #ab32089, 1:1000), p-PI3K (Abcam, Cambridge, UK, #ab182651, 1:1000), AKT (Abcam, Cambridge, UK, #ab179463, 1:1000), p-AKT (Abcam, Cambridge, UK, #ab38449, 1:1000) and β-actin (Abcam, Cambridge, UK, #ab8226, 1:3000). After incubation with HRP-conjugated secondary antibodies (Abcam, Cambridge, UK, #ab6721, 1:5000), protein bands were visualized using Electrochemiluminescence (ECL) reagent (Beyotime, Beijing, China, #P0018S).

2.8 Flow cytometry

For cell cycle analysis, transfected cells were fixed in 70% ethanol and stained with propidium iodide (PI, Beyotime, Beijing, China, #C1052) containing RNase A (Beyotime, Beijing, China, #ST579). Cell cycle distribution was analyzed by flow cytometry (BD FACSCanto II, Franklin Lake, NJ, USA) and processed using FlowJo software (10.8, BD, Franklin Lake, NJ, USA).

2.9 Statistical analysis

Data are presented as mean ± Standard Deviation (SD). To check data normality, a Shapiro-Wilk test was conducted. Statistical significance was determined using Student's *t*-test or one-way Analysis of Variance (ANOVA) followed by Tukey's *post hoc* test. A p-value < 0.05 was considered statistically significant. Each experiment in this study was performed in triplicate.

3. Results

3.1 UBAP2L is highly expressed in endometrial cancer cells

To analyze the expression levels of UBAP2L in EC tissues compared to normal, bioinformation analysis was conducted. Using data from the Cancer Genome Atlas (TCGA) and Kaplan-Meier databases, we observed that UBAP2L expression was significantly upregulated in primary endometrial cancer tissues (Fig. 1A). Kaplan-Meier survival analysis revealed that patients with higher UBAP2L expression had poorer overall survival rates (Fig. 1B), suggesting UBAP2L is highly expressed in EC.

3.2 Knockdown of UBAP2L inhibits proliferation of endometrial cancer cells

To assess the effect of UBAP2L knockdown on the growth of EC cells, the *in vitro* assays were performed. UBAP2L expression was silenced in HEC-1A and Ishikawa cells using siRNAs. Immunoblot analysis confirmed a significant reduction in UBAP2L expression in the si-UBAP2L group (Fig. 2A). Cell growth, as measured by the CCK-8 assay, was remarkably reduced in cells with UBAP2L knockdown (Fig. 2B). Addi-

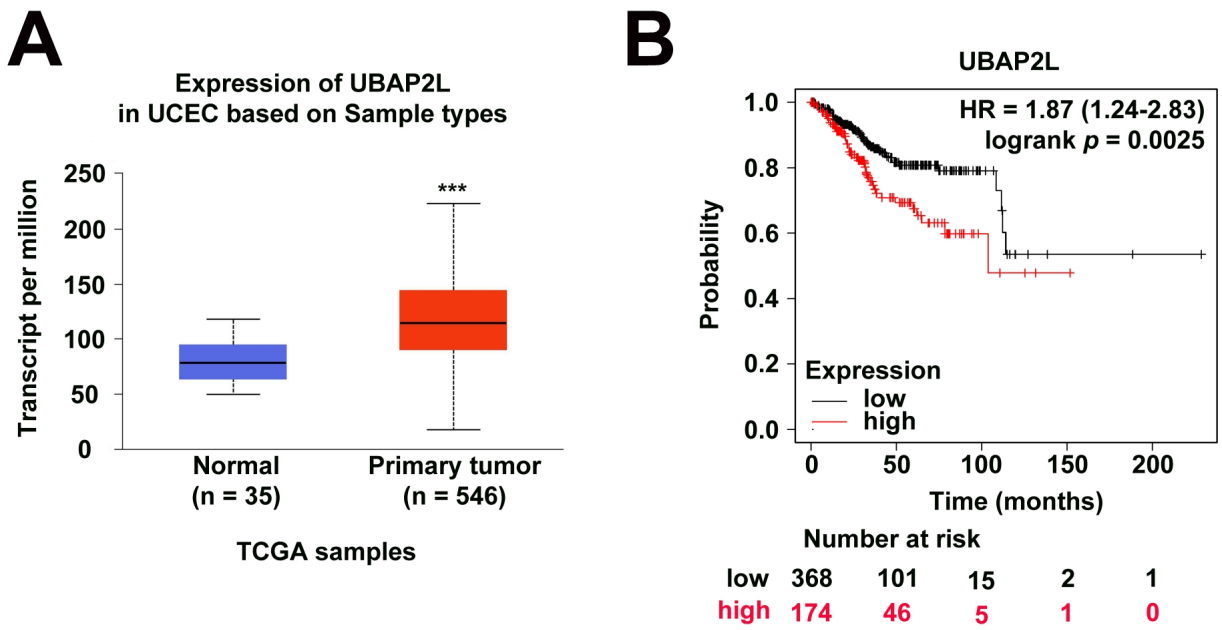

FIGURE 1. UBAP2L is highly expressed in endometrial cancer cells. (A) Expression levels of UBAP2L in normal endometrial tissues (n = 35) and primary endometrial cancer tissues (n = 546) based on TCGA samples, displayed as transcript per million (TPM). (B) Kaplan-Meier survival analysis showing overall survival of patients with endometrial cancer stratified by high (n = 174) and low (n = 368) UBAP2L expression levels. Hazard ratio (HR) and logrank *p* value are shown. TCGA: the Cancer Genome Atlas; UBAP2L: Ubiquitin-associated protein 2L; UCEC: Uterine Corpus Endometrial Carcinoma. ***: $p < 0.001$.

tionally, the colony formation assay showed a lower colony numbers following UBAP2L knockdown (Fig. 2C). Flow cytometry analysis revealed that UBAP2L knockdown caused generation 2/mitosis (G2/M) phase arrest in both cell lines (Fig. 2D,E). These results suggest UBAP2L knockdown inhibits cell growth in EC cells.

3.3 Knockdown of UBAP2L suppresses motility of EC cells

To determine whether UBAP2L knockdown affects cell motility, wound healing as well as Transwell invasion assays were performed. The migration of HEC-1A and Ishikawa cells was significantly impaired in the si-UBAP2L group compared to the control (Fig. 3A). Similarly, UBAP2L knockdown significantly reduced the number of invading cells in both cell lines (Fig. 3B). These findings indicate that UBAP2L is crucial in promoting the migratory and invasive capabilities of endometrial cancer cells.

3.4 UBAP2L knockdown inhibits the PI3K/AKT signaling axis in endometrial cancer cells

To investigate the molecular mechanism underlying UBAP2L's role in promoting endometrial cancer cell processes. Immunoblot analysis was performed to evaluate the effect of UBAP2L knockdown on the PI3K/AKT axis. In both HEC-1A as well as Ishikawa cells, knockdown of UBAP2L led to a significant decrease in phosphorylated PI3K and phosphorylated AKT levels (Fig. 4). These findings suggest that UBAP2L promotes endometrial cancer progression by activating the PI3K/AKT axis.

4. Discussion

EC exhibits complex mechanisms of tumorigenesis, with unchecked cell proliferation and enhanced migration being fundamental processes in tumor expansion and metastasis [12, 16]. These processes underpin the aggressive nature of EC and significantly impact patient survival. By focusing on interventions that halt proliferation and migration, it is possible to improve the clinical outlook for EC patients. Recent findings, including our own, demonstrate that UBAP2L knockdown impairs EC cell proliferation and migration, highlighting the importance of discovering new molecular targets in advancing EC treatment [17]. By disrupting essential signaling cascades, such as the PI3K/AKT pathway, which are integral to tumor development, there is a significant potential to transform therapeutic approaches for EC.

UBAP2L, part of the ubiquitin-associated protein family, is involved in several cellular functions, particularly protein degradation pathways. Its roles extend to cell proliferation, migration and regulation of the cell cycle, as demonstrated in various malignancies such as gastric, breast and hepatic cancers [18]. The involvement of UBAP2L in these processes suggests its potential contribution to EC progression, as evidenced by our findings. In our research, silencing UBAP2L in EC cells led to reduced cell proliferation and migration, indicating that it may modulate critical signaling mechanisms underlying tumor behavior. While its role in other cancers is well established, further investigation into its specific function

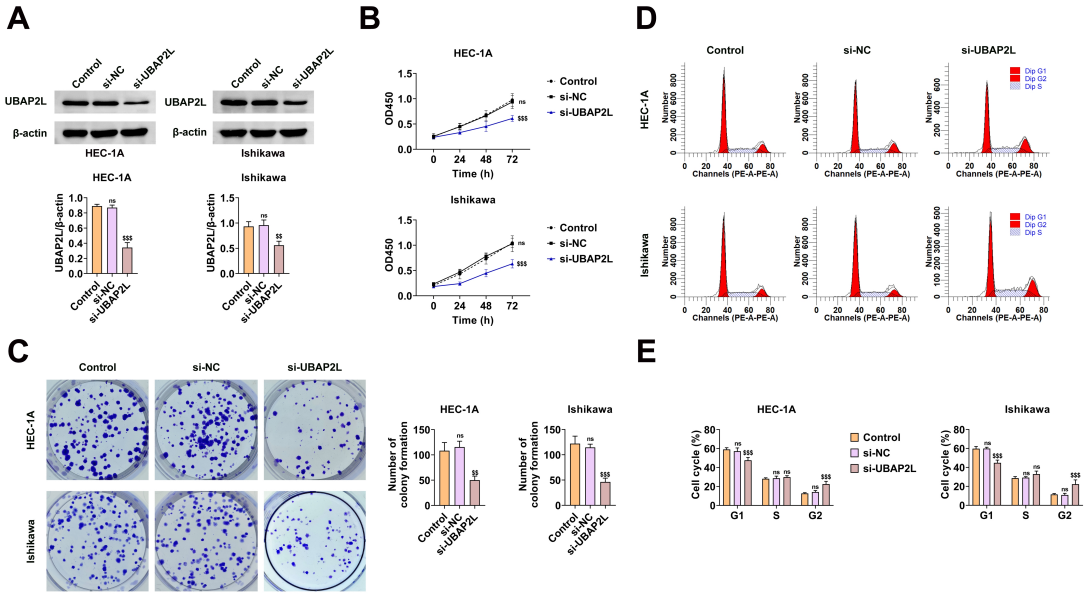

FIGURE 2. Knockdown of UBAP2L inhibits proliferation of endometrial cancer cells. (A) Immunoblot analysis showing UBAP2L expression in HEC-1A and Ishikawa cells after treatment with control, si-NC, or si-UBAP2L. β-actin is used as a loading control. Quantification of UBAP2L protein levels is shown below. (B) Cell viability was measured using CCK-8 assay at 0 h, 24 h, 48 h and 72 h in HEC-1A and Ishikawa cells transfected with control, si-NC, or si-UBAP2L. (C) Colony formation assay showing the number of colonies formed by HEC-1A and Ishikawa cells after transfection with control, si-NC or si-UBAP2L. Quantification of colony numbers is shown on the right. (D) Cell cycle analysis by flow cytometry in HEC-1A and Ishikawa cells after knockdown of UBAP2L, showing the distribution of cells in the G1, S and G2/M phases. (E) Quantification of cell cycle phase distribution in HEC-1A and Ishikawa cells transfected with control, si-NC or si-UBAP2L. ns: not-significant. NC: negative control. UBAP2L: Ubiquitin-associated protein 2L; HEC-1A: human endometrial cancer-1A; OD450: Optical density 450; G1: Generation 1; S: synthesis; G2: General 2; $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$.

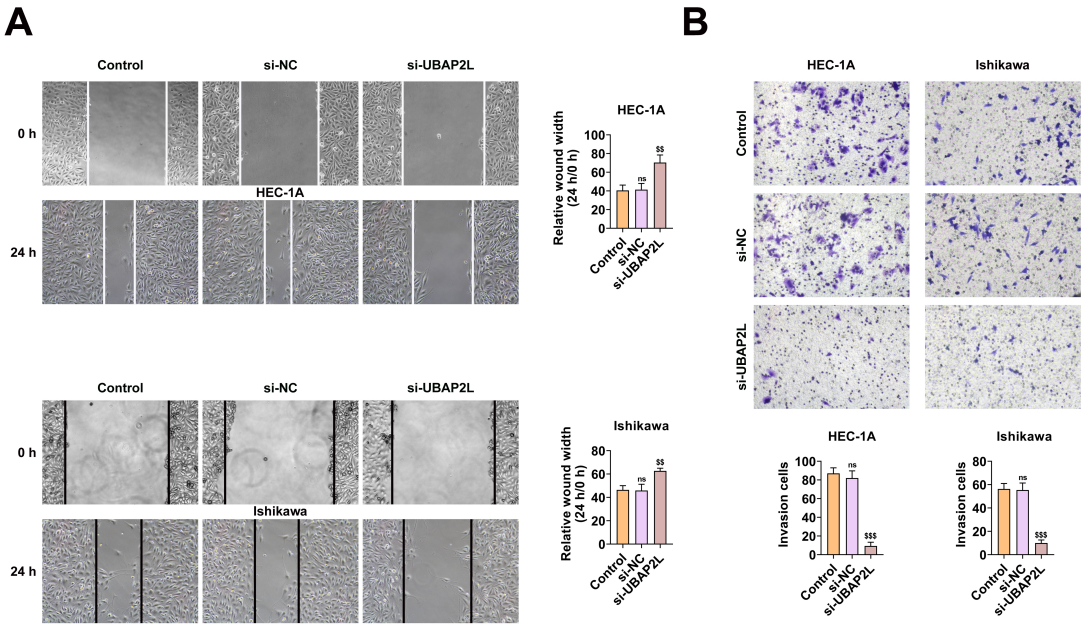

FIGURE 3. Knockdown of UBAP2L suppresses migration and invasion of endometrial cancer cells. (A) Wound healing assay showing the migration ability of HEC-1A and Ishikawa cells after treatment with control, si-NC or si-UBAP2L at 0 h and 24 h. Quantification of relative wound width is shown on the right. (B) Transwell invasion assay displaying the number of invasive cells in HEC-1A and Ishikawa cells treated with control, si-NC or si-UBAP2L. Quantification of invasive cells is shown below. ns: not significant. $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$. NC: negative control; UBAP2L: Ubiquitin-associated protein 2L; HEC-1A: human endometrial cancer-1A.

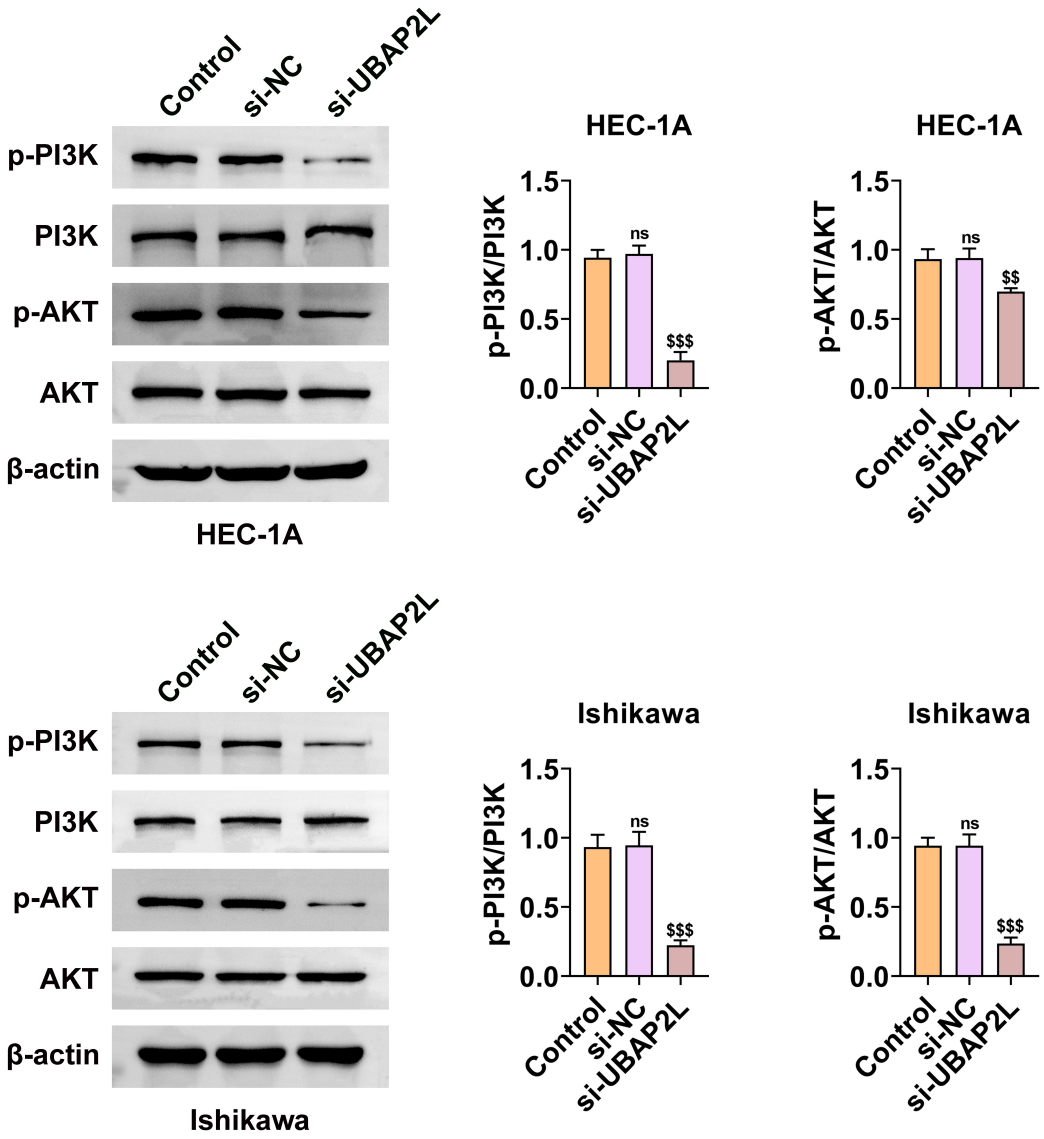

FIGURE 4. UBAP2L knockdown inhibits the PI3K/AKT signaling pathway in endometrial cancer cells. Immunoblot analysis of p-PI3K, PI3K, p-AKT and AKT protein levels in HEC-1A (up) and Ishikawa (down) cells after treatment with control, si-NC or si-UBAP2L. β-actin is used as a loading control. Quantification of the p-PI3K/PI3K and p-AKT/AKT ratios is shown on the right for both cell lines. ns: not significant; $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$. NC: negative control; PI3K: phosphatidylinositol 3 kinase; AKT: protein kinase B; UBAP2L: Ubiquitin-associated protein 2L; HEC-1A: human endometrial cancer-1A.

in EC is need exploration.

UBAP2L's impact on protein degradation and cell cycle regulation suggests that its modulation could directly influence cellular proliferation and migration. Our study demonstrates that silencing UBAP2L in EC cells inhibits both proliferation and migration, potentially linking these effects to broader molecular pathways involved in tumor progression. This connection highlights UBAP2L as a promising therapeutic target for EC, building on its previously reported roles in other cancer types and providing a compelling rationale for further exploration of its therapeutic potential.

The PI3K/AKT pathway is critical in regulating cellular proliferation and migration, contributing to cancer progression across many tumor types, including EC. Activation of this pathway leads to a series of downstream effects that promote survival and growth [19, 20]. Our study revealed that UBAP2L knockdown suppresses this pathway in EC cells, underscoring its pivotal role in tumor development. This finding suggests that targeting the PI3K/AKT pathway could represent an effective strategy in treating EC, particularly in cases where this pathway is aberrantly activated.

Regulation of the PI3K/AKT pathway in EC involves complex feedback mechanisms and interactions with other signaling networks. The pathway's central role in EC tumorigenesis, as demonstrated by our research, reinforces the need to target key components such as PI3K and AKT. Several PI3K/AKT inhibitors are already under investigation, and our results provide a strong foundation for pursuing such targeted therapies in EC patients, particularly when combined with UBAP2L inhibition.

While our study provides valuable insights, it is limited by its reliance on *in vitro* models. Our experiments focused on

the cellular level to verify the effect of UBAP2L on EC progression and draw relevant conclusions. However, subsequent validation at the animal and clinical level is necessary to further confirm our findings. Additionally, the precise mechanisms by which UBAP2L modulates the PI3K/AKT pathway require further investigation. Future research should also explore whether UBAP2L inhibition can act synergistically with current PI3K/AKT-targeting therapies.

5. Conclusions

In summary, our work identifies UBAP2L as a key regulator of EC progression, primarily through its impact on the PI3K/AKT pathway. Targeting UBAP2L offers a new therapeutic avenue for EC treatment, with potential to improve patient outcomes. Future studies should further elucidate the molecular mechanisms involved and assess the clinical applicability of targeting this pathway in 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

RZ, DL—designed the study and carried them out; prepared the manuscript for publication and reviewed the draft of the manuscript. RZ, FFZ, DL—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.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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