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Original Research

FKBP38 Suppresses Cervical Cancer Progression and Enhances Everolimus Sensitivity in Association With the mTOR Pathway

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Abstract

Background: The expression and functional role of FK506 binding protein 38 (FKBP38) in cervical squamous cell carcinoma (CESC) remain unclear. This study aimed to investigate FKBP38 expression in CESC cells and to analyze the mechanisms by which FKBP38 regulates CESC cell proliferation, migration, invasion, the mammalian target of rapamycin (mTOR) signalling pathway, and sensitivity to everolimus. **Methods**: We constructed FKBP38-overexpressing CESC cell lines to evaluate the protein's effects. Cell proliferation was assessed by colony formation assay and 5-ethynyl-2′-deoxyuridine (EdU) staining; cell invasion and migration were measured by Transwell assay; and protein levels of mTOR signaling pathway-related molecules were examined by Western blotting (WB). Additionally, the viability of everolimus-treated CESC cells and the half-maximal inhibitory concentration (IC_{50}) were determined using the Cell Counting Kit-8 (CCK-8) to assess the effect of FKBP38. **Results**: FKBP38 expression was low in CESC cells. Overexpression of FKBP38 significantly inhibited CESC cell proliferation, migration, and invasion (all $p < 0.05$), and markedly altered the expression of mTOR signaling pathway markers ($p < 0.05$). Furthermore, FKBP38 overexpression reduced the viability of everolimus-treated CESC cells. **Conclusions**: FKBP38 is underexpressed in CESC cells and exerts tumor-suppressive effects by inhibiting cell viability, proliferation, migration, and invasion, downregulating mTOR pathway activity, and enhancing sensitivity to everolimus.

Keywords: FKBP38; mTOR; cervical cancer; everolimus

1. Introduction

Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) is one of the four most common malignant gynecologic tumors, second only to breast cancer, and early detection and treatment are crucial [1]. For early-stage CESC, postoperative radiotherapy combined with chemotherapy results in a good prognosis; however, locally advanced and recurrent metastatic CESC often have a poor prognosis [2]. Conventional chemotherapy drugs have limited effectiveness in the treatment of locally advanced and recurrent metastatic CESC. Therefore, there is an urgent need to identify novel biomarkers to improve the diagnostic sensitivity of cervical lesions and CESC. In recent years, new targeted therapies and immunotherapeutic targets related to novel signaling pathways in CESC markers have become a research focus [3].

The FKBP38 protein has garnered growing attention in cancer research, particularly in breast and endometrial cancers [4,5]. FKBP38 regulates several signalling pathways, including Ras homolog enriched in brain (Rheb), phosphoinositide 3-kinase (PI3K), protein kinase B (Akt), mammalian target of rapamycin (mTOR), and sterol regulatory element-binding protein (SREBP), which play essential roles in cell proliferation, differentiation, and apoptosis [6,7]. Yan *et al.* [8] reported that FKBP38 inhibits proliferation and migration in endometrial carcinoma (EC) cells by suppressing the mTOR pathway. Zhou *et al.* [9] previously reported that FKBP38 deficiency-induced granulosa cell apoptosis in mice may be associated with ovarian dysfunction, potentially through activation of the mTOR signalling pathway. However, the mechanism of FKBP38 in CESC cells has not yet been reported.

Everolimus is an oral drug approved by the U.S. Food and Drug Administration (FDA). It is primarily used for the treatment of advanced renal cell carcinoma [10,11] and has demonstrated antitumor activity in multiple tumor models [12]. However, in CESC, everolimus is mainly used in combination with other drugs. Yilmaz *et al.* [13] reported that the combination of everolimus and paclitaxel downregulates B-cell lymphoma 2 (BCL2) and cyclin D1 (CCND1) mRNA levels in human CESC cells. Dong *et al.* [14] showed that paclitaxel inhibits apoptosis in CESC

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Table 1. Primers for mRNA quantification by RT-qPCR.

Genes	Primer sequence	Annealing temperature
FKBP38	F: TCCAGCGCCAAAGTGGACAT R: GAGCTCTGCGTGGATCGTCT	61 °C
GAPDH	F: AATGGGCAGCCGTTAGGAAA R: CGCCCAATACGACCAAATC	61 °C

F, forward; R, reverse; RT-qPCR, reverse transcription-quantitative real-time PCR.

cells via the PI3K/AKT/mTOR pathway. Nevertheless, the combined effect of FKBP38 and everolimus in CESC has not been investigated.

In this study, we examined FKBP38 protein expression in CESC cells and investigated its regulatory effects on cell viability, proliferation, migration, and invasion. We further explored how FKBP38 influences the sensitivity of CESC cells to everolimus. Our findings provide a theoretical foundation for future research to improve the clinical diagnosis and treatment of CESC.

2. Materials and Methods

2.1 Cell Culture and Reagents

HeLa cells (Procell, Wuhan, Hubei, China, Catalog number: CL-0101) and Ect/E6E7 cells (Shanghai Jingyuan Biotechnology, Shanghai, China, Catalog number: JY-J1126) were cultured in high-glucose DMEM medium (Weike Biotechnology, Shanghai, China, Catalog number: P04-03590) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Rockville, MD, USA, Catalog number: A5669701) and 1% penicillin-streptomycin (Thermo Fisher Scientific, Rockville, MD, USA, Catalog number: 15140122). Cells were maintained in a humidified incubator (Midi 40; Thermo Fisher Scientific, Waltham, MA, USA) at 37 °C with 5% CO_2. In addition, all cells underwent short tandem repeat (STR) authentication and tested negative for mycoplasma contamination.

2.2 Transfection

To investigate the role of FKBP38, HeLa cells seeded in 6-well plates were transfected with Lipofectamine 3000 reagent (Thermo Fisher Scientific, Rockville, MD, USA, Catalog number: L3000150) according to the manufacturer's instructions. Briefly, for each well, 2.5 µg of oe-FKBP38 plasmid or the corresponding empty vector control (both from Genomeditech, Shanghai) was diluted in Opti-MEM and combined with Lipofectamine 3000 reagent at a DNA (µg) to reagent (µL) ratio of 1:2. The complexes were then added to the cells. After transfection, the cells were maintained in a standard incubator at 37 °C with 5% CO_2.

2.3 Isolation of RNA and Reverse Transcription-Quantitative Real-Time PCR (RT-qPCR) Analysis

Total RNA was extracted using TRIzol (Thermo Fisher Scientific, Rockville, MD, USA, Catalog number: 15596018CN) and reverse transcribed into cDNA using a commercial kit (Invitrogen, Thermo Fisher Scientific, Rockville, MD, USA, Catalog number: 4368813). The mRNA levels of target genes and the internal control gene (*GAPDH*) were quantified by RT-qPCR with specific primers (sequences in Table 1) on a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA; Catalog number: 1861096).

Quantitative PCR amplification was performed using the following two-step cycling protocol: initial denaturation at 95 °C for 30 seconds, followed by 40 cycles of denaturation at 95 °C for 10 seconds and annealing/extension at 61 °C for 30 seconds, with fluorescence data acquisition. Relative gene expression was calculated using the comparative $2^{-\Delta\Delta Ct}$ method, with normalization of target gene Ct values to GAPDH and then to the control group. Each experiment included three technical replicates per sample and was independently repeated three times. The mean value of the technical replicates from each independent experiment was treated as a single data point (n = 3) for subsequent statistical analysis.

2.4 Colony Formation Assay

HeLa cells were seeded in 6-well culture dishes at a density of 6×10^2 cells per well and cultured for 7 days, with the medium replaced every 3 days. Subsequently, cells were fixed with 4% paraformaldehyde for 10 min and stained with 0.1% crystal violet for 10 min. Colonies were visualized and counted. A colony was defined as a cluster of $>$50 cells. The number of colonies in each well was determined manually or using ImageJ software (v1.53t; National Institutes of Health, Bethesda, Maryland, USA). The experiment was independently repeated three times, with each condition tested in duplicate wells per experiment. Colony counts from each well were averaged across the two technical replicates to yield one data point per experiment per condition (n = 3).

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2.5 5-Ethynyl-2′-Deoxyuridine (EdU) Assay

Cell proliferation was assessed with an EdU kit (RIBOBIO, Guangzhou, Guangdong, China, Catalog number: C00054) according to the manufacturer's instructions. Cells were examined using a fluorescence microscope (MIX60-FL; Mingmei, Guangzhou, Guangdong, China). The percentage of EdU-positive cells was calculated relative to the total number of Hoechst-stained nuclei. For each sample, five random microscopic fields (at least 200 cells per field) were analyzed. The experiment was independently repeated three times. The mean percentage of EdU-positive cells from the five fields in each independent experiment was used as a single data point (n = 3).

2.6 Cell Counting Kit-8 (CCK-8) Assay

Cell viability was assessed using CCK-8 assay. Cells were seeded in 96-well plates (5000 cells/well). After 24 hours, cells were transfected and cultured for an additional 48 hours. Subsequently, 10 µL of CCK-8 reagent (Sangon Biotech, Shanghai, China, Catalog number: E606335) was added to each well. Following a 2-hour incubation at 37 °C, the absorbance was measured at 450 nm with a microplate reader (HM-96A; Hengmei Zhizao, Suzhou, Jiangsu, China).

Each treatment condition was assessed in six replicate wells (technical replicates) within a single experiment. The experiment was independently repeated three times. The mean absorbance of the six technical replicates from each independent experiment was calculated and used as a single data point (n = 3) for cell viability.

2.7 Transwell Migration and Invasion Assays

Cell migration and invasion were evaluated using Transwell chambers (8 µm pore size; Catalog number: 3422, Corning, New York, USA). For the invasion assay, the membrane was pre-coated with 50 µL of Matrigel (diluted 1:8 in serum-free medium; Corning, Catalog number: CLS354234) and incubated at 37 °C for 2 h to allow solidification. HeLa cells (1×10^5) in 200 µL of serum-free medium were seeded in the upper chamber. The lower chamber contained 600 µL of medium supplemented with 20% FBS as a chemoattractant. Following a 24 h incubation, nonmigratory or noninvasive cells on the upper surface were removed. Cells that traversed the membrane were fixed, stained with 0.1% crystal violet, imaged, and counted in five random fields per well in three independent experiments. The counts from the five fields per well were averaged to represent the migratory or invasive capacity of that well. For each condition in each independent experiment, two replicate chambers (technical replicates) were used, and their averages were further averaged to yield one data point per experiment per condition (n = 3).

2.8 Western Blotting (WB)

Proteins were extracted from cells with radioimmunoprecipitation assay (RIPA) lysis buffer and quantified using a bicinchoninic acid assay (BCA) kit (Thermo Fisher Scientific, Rockville, MD, USA, Catalog number: 23235). Equal amounts of protein were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE, Meilunbio, Dalian Meilun Biotechnology Co., Ltd., Dalian, Liaoning, China, catalog no. MA0159) and then transferred onto a polyvinylidene difluoride (PVDF, Beyotime Biotechnology, Nantong, Jiangsu, China, catalog no. FFP70) membrane. The membrane was blocked with 5% skim milk in Tris-buffered saline with Tween 20 (TBST, Meilunbio, Dalian, Liaoning, China, catalog no. MA0091) for 1 hour at room temperature. After blocking, the membrane was incubated with the primary antibody overnight at 4 °C. Following three washes with TBST (10 min each), the membrane was incubated with an appropriate horseradish peroxidase (HRP)-conjugated secondary antibody for 1 hour at room temperature. After additional three washes with TBST, protein bands were visualized using enhanced chemiluminescence (ECL) reagent (Merck KGaA, Darmstadt, Germany, Catalog number: GERPN2134) and quantified with ImageJ software. Band intensity of the target protein was normalized to that of the loading control (GAPDH). Each WB experiment was independently repeated three times. The normalized protein expression level from each independent experiment was considered as one data point (n = 3).

The primary antibodies used were as follows: rabbit anti-FKBP38 (Abcam, Cambridge, UK, cat. no. ab129113, 1:1000 cat. #ab129113, 1:1000); rabbit anti-phospho-S6 (Thermo Fisher Scientific, Rockville, MD, USA, cat. #740057M, 1:500); rabbit anti-S6 (Thermo Fisher Scientific, Rockville, MD, USA, cat. #701374, 1:500); rabbit anti-phospho-Akt (Ser473) (Thermo Fisher Scientific, Rockville, MD, USA, cat. #700392, 1:500); rabbit anti-Akt (Thermo Fisher Scientific, Rockville, MD, USA, cat. #44-609G, 1:1000); rabbit anti-phospho-mTOR (Abcam, cat. #ab109268, 1:1000); rabbit anti-mTOR (Abcam, cat. #ab32028, 1:1000); rabbit anti-phospho-4EBP-1 (Thermo Fisher Scientific, Rockville, MD, USA, cat. #700238, 1:500); rabbit anti-4EBP-1 (Thermo Fisher Scientific, Rockville, MD, USA, cat. #AHO1382, 1:500); and rabbit anti-GAPDH (Abcam, cat. #ab9485, 1:2500).

2.9 Statistical Analysis

All data are presented as the mean ± standard deviation (SD) from at least three independent experiments, as detailed in each method section above. Statistical comparisons between two groups were performed using the unpaired Student's *t*-test. For comparisons among more than two groups, one-way analysis of variance (ANOVA) was used, followed by Dunnett's post hoc test for multiple comparisons with a single control group or Tukey's post hoc test

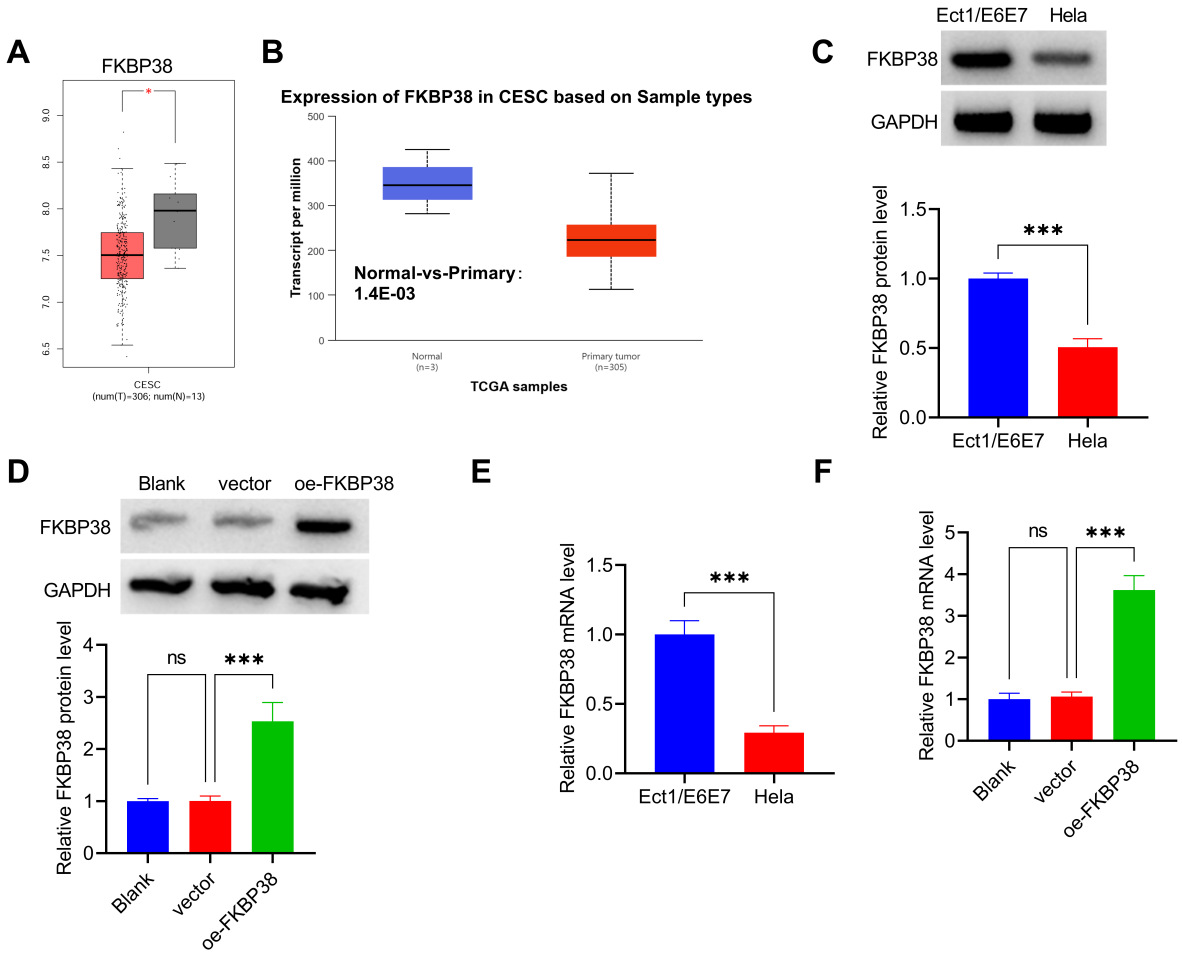

Fig. 1. Differential expression analysis of FKBP38 in cervical lesions. (A) GEPIA database. (B) FKBP38 gene expression in TCGA Samples (UALCAN database). (C,D) WB analysis of FKBP38protein levels. (E,F) RT-qPCR analysis of FKBP38 mRNA levels. The experiment was independently repeated three times. Data were analyzed using a *t*-test. ns > 0.05, *$p < 0.05$, ***$p < 0.001$. CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; GEPIA, Gene Expression Profiling Interactive Analysis; TCGA, The Cancer Genome Atlas; WB, Western blotting; RT-qPCR, reverse transcription-quantitative real-time PCR; UALCAN, University of ALabama at Birmingham CANcer.

for multiple comparisons among all groups. All statistical analyses were conducted using GraphPad Prism software (version 9.0; GraphPad, San Diego, California, USA). A *p*-value of less than 0.05 was considered statistically significant.

3. Results

3.1 Differential Expression of FKBP38 Protein in Cervical Lesions

Analysis of the Gene Expression Profiling Interactive Analysis (GEPIA) database (http://gepia.cancer-pku.cn/detail.php?gene=FKBP38) and UALCAN (University of ALabama at Birmingham CANcer) database (https://ualcan.path.uab.edu/cgi-bin/TCGAExResultNew2.pl?genenam=FKBP8&ctype=CESC) showed that FKBP38 is significantly underexpressed in CESC (Fig. 1A,B; both $p < 0.05$).

Following total protein extraction and RNA isolation from cultured cells, WB analysis (with BCA protein quantification and densitometric band analysis) and RT-qPCR assays, conducted in at least three independent biological experiments, showed that endogenous FKBP38 expression was significantly lower in HeLa cells compared with the normal immortalized cervical epithelial cell line Ect/E6E7, at both the protein and mRNA levels (Fig. 1C; both $p < 0.001$). The overexpression of FKBP38 (Blank/vector/oe-FKBP38) was used to further investigate its function (Fig. 1D).

As shown in Fig. 1E, FKBP38 mRNA expression was higher in HeLa cells than in Ect1/E6E7 cells ($p < 0.001$). Furthermore, compared with the untransfected (Blank) and empty vector (Vector) control groups, the cells transfected with the FKBP38 overexpression plasmid (oe-FKBP38) showed a marked and significant upregulation of FKBP38,

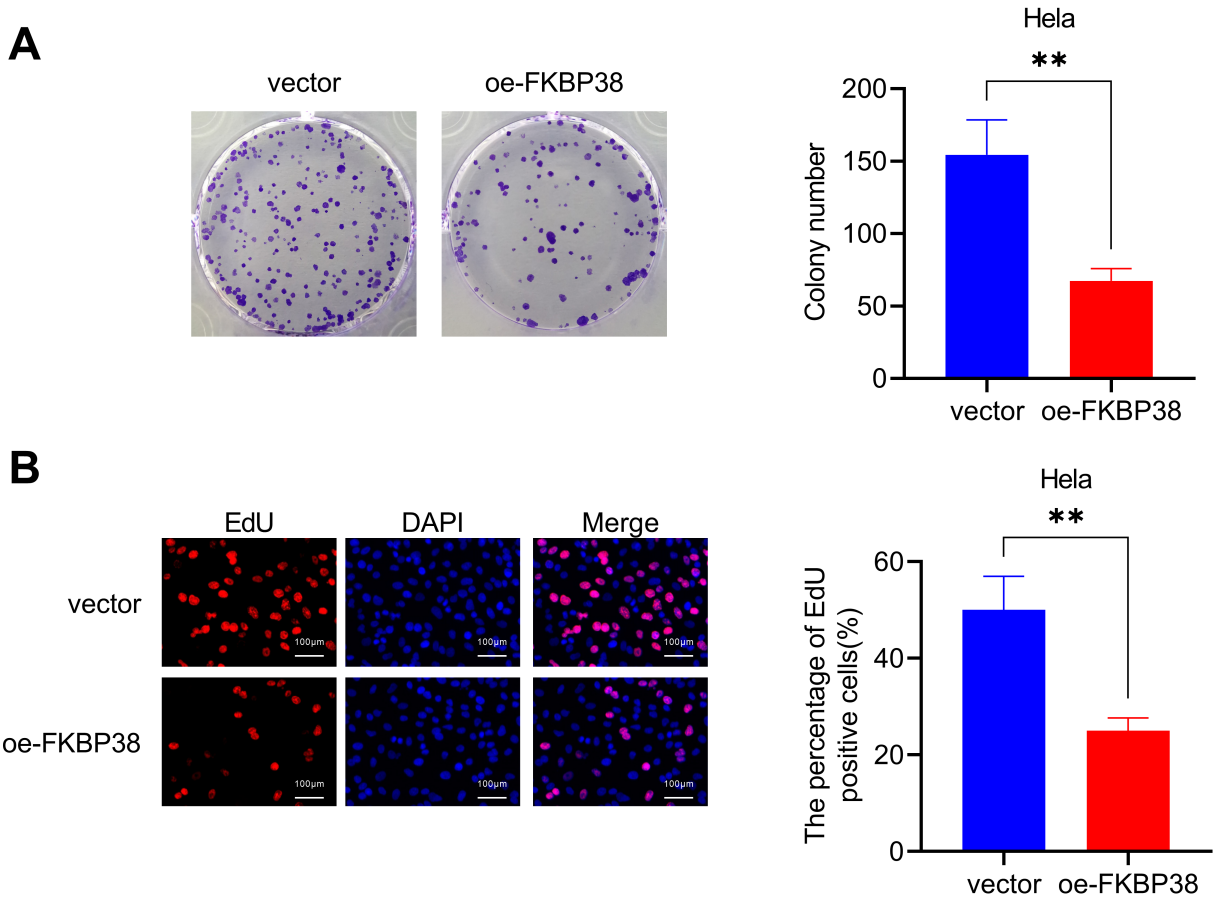

Fig. 2. Effect of FKBP38 on the proliferation of CESC cells. (A) Colony formation assay of cervical cancer cell reproductive capacity. (B) EdU assay of CESC cell proliferation. Scale bar: 100 μm. The experiment was independently repeated three times. Data were analyzed using a *t*-test. $^{**}p < 0.01$. EdU, 5-ethynyl-2′-deoxyuridine.

confirming the successful model establishment in HeLa cells (Fig. 1F; $p < 0.001$).

3.2 Effect of FKBP38 Protein on the Proliferation of CESC Cells

Colony formation assays showed that CESC cells with increased levels of FKBP38 displayed a prominent reduction in colony formation compared with the control group ($p < 0.01$) (Fig. 2A). Furthermore, EdU analysis indicated that FKBP38 overexpression resulted in a significant reduction in the fluorescence intensity associated with CESC cell proliferation, as demonstrated by both the number of EdU-positive cells and the relative cell proliferation rates ($p < 0.01$) (Fig. 2B) compared with the control group. These findings suggest that elevated FKBP38 expression effectively inhibits the growth of CESC cells.

3.3 Impact of FKBP38 on the Migration and Invasion of CESC Cells

Transwell assay were used to evaluate the impact of FKBP38 on CESC cell migrating and invasion. In comparison with the control group, FKBP38 overexpression significantly reduced the number of migrating cells ($p < 0.001$) (Fig. 3A). Additionally, the number of invading CESC cells was also significantly decreased in the FKBP38 overexpression group compared with the control group ($p < 0.01$) (Fig. 3B). These findings indicate that upregulation of the FKBP38 protein effectively supresses CESC cell migration and invasion.

3.4 Effect of FKBP38 on the Sensitivity of HeLa Cells to Everolimus

HeLa cells were transfected with vector and oe-FKBP38, followed by treatment with 100 μmol/L everolimus. As shown in Fig. 4A, FKBP38 overexpression of drastically decreased the viability of everolimus-treated HeLa cells compared with the vector group ($p < 0.01$).

HeLa cells transfected with vector and oe-FKBP38 were treated with 100 μmol/L everolimus. As shown in Fig. 4B, FKBP38 upregulation significantly decreased the half-maximal inhibitory concentration (IC_{50}) in everolimus-treated of HeLa cells compared with the vector group ($p < 0.01$). These findings suggest that overexpression of FKBP38 enhances the sensitivity of HeLa cells to everolimus.

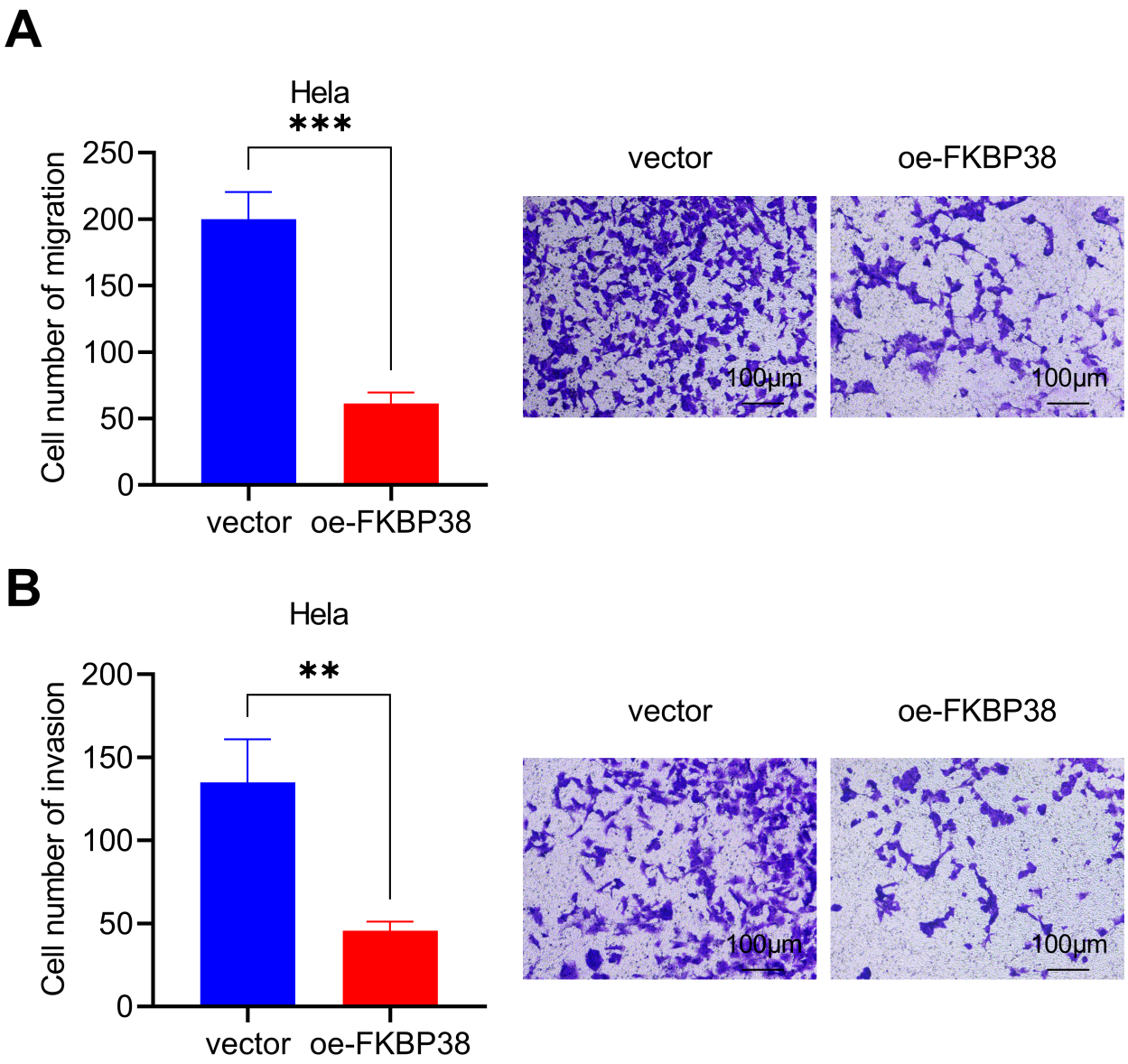

Fig. 3. Effect of FKBP38 protein on CESC migration and invasion. (A) Transwell detection of CESC cell migration. Scale bar: 100 μm. (B) Transwell assay of CESC cells invasion. Scale bar: 100 μm. The experiment was independently repeated three times. The data was subjected to analysis using a *t*-test. $^{**}p < 0.01$, $^{***}p < 0.001$.

3.5 FKBP38 Limits mTOR Pathway Activation in CESC Cells

WB analysis demonstrated that FKBP38 overexpression significantly suppressed the mTOR signaling pathway in CESC cells (Fig. 5A,B). This effect was evidenced by markedly reduced phosphorylation levels of key downstream effectors, including S6 ($p < 0.001$), Akt ($p < 0.05$), 4EBP-1 ($p < 0.05$), and mTOR itself ($p < 0.001$).

4. Discussion

This study is the first to demonstrate that FKBP38 is downregulated in CESC cells, and that its overexpression significantly suppresses CESC cell proliferation, migration, and invasion while enhancing sensitivity to everolimus. Notably, this work is the first to focus on the role of FKBP38 in CESC, which distinguishes it from previous studies that primarily focused on FKBP38's functions in other tumor types or non-tumor cells [4–7,9]. These findings are particularly relevant given the poor prognosis of locally advanced or recurrent metastatic CESC, for which effective targeted therapies remain limited [15–17]. By identifying FKBP38 as a tumor suppressor in CESC, these results address a critical gap in the understanding the molecular regulation of CESC progression and suggest a potential target for therapeutic intervention.

Our functional assays demonstrated that FKBP38 exerts tumor-suppressive effects in CESC by inhibiting the mTOR signaling pathway [8]. This mechanistic link is not

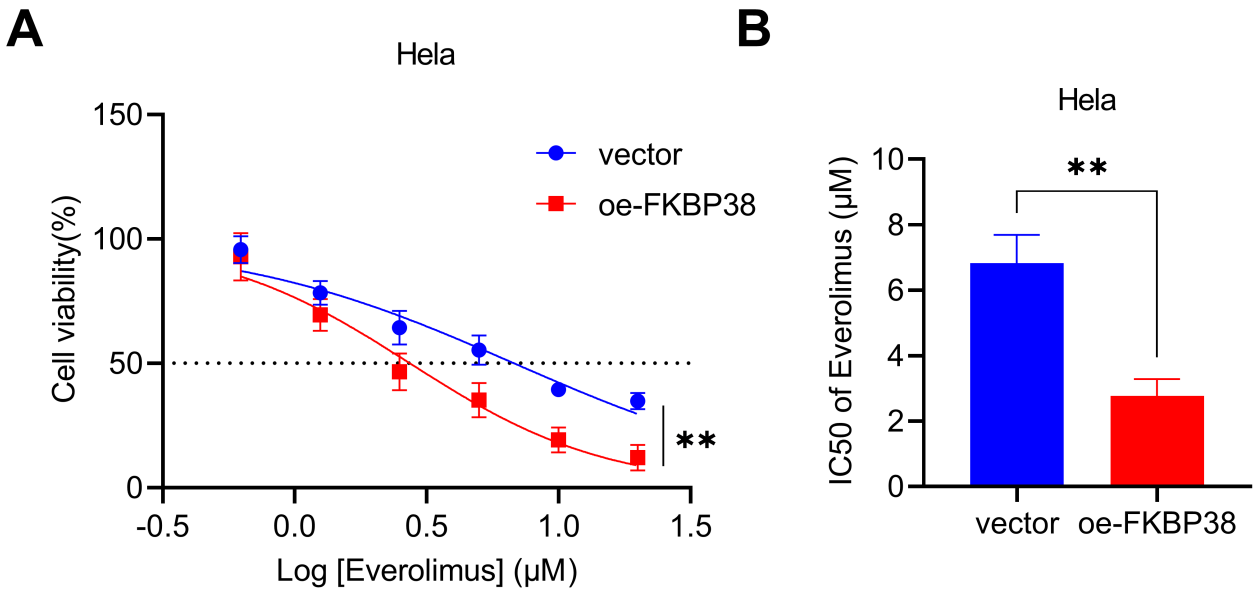

Fig. 4. FKBP38 enhances HeLa cell sensitivity to everolimus. (A) Effect of FKBP38 on the viability of HeLa cells treated with everolimus. (B) Half-maximal inhibitory concentration (IC_{50}) of everolimus in HeLa cells following FKBP38 overexpression. The experiment was independently repeated three times. Data were analyzed using a *t*-test. $^{**}p < 0.01$.

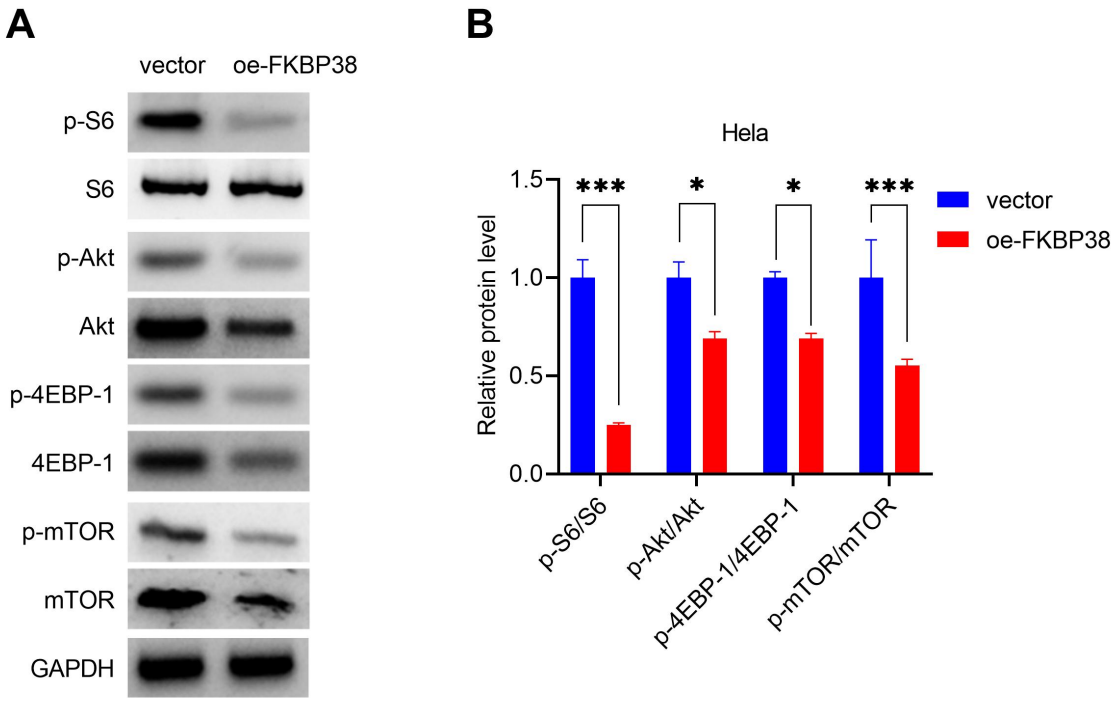

Fig. 5. FKBP38 constrains mTOR pathway activation in CESC cells. (A,B) WB analysis of S6, Akt, 4EBP-1, and mTOR phosphorylation after overexpression of FKBP38. The experiment was independently repeated three times. Data were analyzed using a *t*-test. $^{*}p < 0.05$, $^{***}p < 0.001$.

only specific to CESC but also defines a novel regulatory axis in this malignancy. Previous studies on the FKBP38-mTOR pathway have focused on non-tumor physiological processes or other cancer types [7,9,18], with no direct evidence for its role in CESC progression. This mechanistic link has clinical relevance because the mTOR pathway is frequently dysregulated in CESC and represents a well-established therapeutic target in multiple malignancies

[10,19]. In contrast to previous studies focusing on the general functions of FKBP38 in other cell types [5,20,21], our data specifically confirm that FKBP38 acts as an endogenous mTOR inhibitor in CESC. FKBP38 directly binds to mTOR and suppresses its activity [18,22], thereby blocking downstream signaling that promotes cell growth, migration, and invasion [23]. These findings suggests that restoring FKBP38 expression may provide a viable strategy to target the hyperactivated mTOR pathway in CESC, especially for tumors with mTOR overactivation.

A major clinical challenge in everolimus treatment is the lack of robust predictive biomarkers and effective strategies to overcome intrinsic or acquired resistance [24,25]. Our study directly addresses this by identifying FKBP38 as a key modulator of everolimus sensitivity in CESC. Mechanistically, this synergy arises from complementary modes of mTOR inhibition. Everolimus acts as an exogenous inhibitor through FKBP12 to acutely block overactive mTORC1 [26,27], whereas FKBP38 serves as an endogenous inhibitor that directly binds and suppresses basal mTOR activity [18,22]. In CESC with reduced FKBP38 expression, this endogenous inhibitory control is lost, potentially priming the mTOR pathway for hyperactivation and create conditions that favor everolimus resistance. Restoration FKBP38 expressionre-sensitizes the mTOR pathway, creating a cellular state in which everolimus can exert a more sustained and effective suppression. This finding has immediate translational implications: (1) FKBP38 expression may serve as a predictive biomarker to identify CESC patients who are most likely to benefit from everolimus-based therapy. (2) Therapeutic upregulation of FKBP38 (e.g., via gene therapy or pharmacological inducers) may represent a novel combination strategy to enhance everolimus efficacy and overcome resistance, offering a tangible approach to improve outcomes in a malignancy with limited targeted options [13–15].

Notably, FKBP38 and FKBP12 (another FKBP family member) differ in their modes of mTOR inhibition, and this distinction supports the synergistic effect observed between FKBP38 overexpression and everolimus, an interaction not previously reported in CESC. FKBP38 acts as an endogenous inhibitor that directly binds mTORC1 via its FKBP-C domain to maintain basal activity homeostasis [22,28], whereas FKBP12 exerts its effect by forming a complex with everolimus to achieve exogenous “precision blocking” of overactivated mTORC1 [26,27,29]. In CESC cells with downregulated FKBP38, endogenous inhibition of mTOR is impaired. Our data suggest that upregulation of FKBP38 can rescue this inhibitory function, thereby synergizing with everolimus to more effectively suppress mTOR activity [14,22]. This functional synergy, which reflects the molecular characteristics of CESC, provides a novel theoretical basis for combining FKBP38 upregulation with everolimus in clinical treatment strategies.

Clinically, these findings offer several practical implications. First, FKBP38 expression levels may serve as a prognostic biomarker in CESC, as reduced expression correlates with more aggressive phenotypes [8]. Second, targeting FKBP38 could represent a novel targeted therapy for CESC, either as monotherapy or in combination with everolimus [7,18]. Third, the FKBP38-mTOR axis may serve as a predictive marker of everolimus sensitivity, supporting personalized treatment selection for patients with CESC [24].

The dysregulation of Akt phosphorylation (p-Akt) observed after FKBP38 overexpression suggests the existence of a dynamic feedback circuit that may help explain the sustained low expression of FKBP38 in CESC. In multiple cancer models, inhibition of mTORC1 relieves negative feedback on upstream PI3K/Akt signaling, leading to compensatory Akt activation, a mechanism often linked to adaptive resistance [30]. Our data suggest that FKBP38-mediated mTOR suppression triggers a similar feedback response, resulting in elevated p-Akt. Notably, this reactivated Akt, or its downstream effectors, may in turn phosphorylate or transcriptionally repress FKBP38, thereby establishing a self-reinforcing loop that maintains low FKBP38 expression and sustains mTOR pathway activity. This proposed feedback axis not only provides a plausible mechanistic explanation for the endogenous downregulation of FKBP38 in CESC, but also highlights a potential resistance mechanism that may limit the long-term efficacy of mTOR-targeted therapies. Future studies validating this circuit and identifying the molecular links between feedback-activated signals and FKBP38 expression or function will be crucial for the development of strategies to disrupt this loop and achieve durable therapeutic responses.

In summary, our study makes three distinct and novel contributions. First, it is the first to identify FKBP38 as a tumor suppressor in CESC and confirm its downregulation in CESC cells. Second, it defines the FKBP38-mTOR regulatory axis in CESC, clarifying the specific molecular mechanism by which FKBP38 inhibits disease progression [8]. Third, it shows that FKBP38 overexpression enhances CESC cell sensitivity to everolimus and provides a mechanistic basis for this effect [14]. These findings not only advance our understanding of the molecular mechanisms underlying CESC progression but also provide a novel foundation for the development of targeted therapeutic strategies and personalized treatment approaches to improve the diagnosis, treatment, and prognosis of CESC [2,3,15].

Limitations and Future Perspectives

While this study establishes a foundational role for FKBP38 in CESC, several limitations warrant further investigation.

(1) Model Systems: Our findings are primarily based on the HeLa cell line. Future studies should validate the tumor-suppressive function of FKBP38 across a panel of

genetically diverse CESC cell lines and, importantly, in patient-derived organoids and *in vivo* xenograft models. These approaches are vital to confirm the pathophysiological relevance of these findings within a more complex tumor microenvironment.

(2) Therapeutic Synergy: We demonstrate that FKBP38 overexpression enhances everolimus sensitivity and independently supressed malignant phenotypes. A direct next step is to experimentally test the combined effect of FKBP38 upregulation and everolimus treatment on proliferation, migration, and invasion *in vitro* and on tumor growth *in vivo*, to quantify the potential therapeutic advantage.

(3) Upstream Regulation: The mechanisms leading to FKBP38 downregulation in cervical cancer remain unclear. Future studies should systematically explore the upstream regulatory network, including promoter methylation, transcriptional repression (e.g., by miRNAs or oncogenic transcription factors), and posttranslational modifications that control FKBP38 expression and stability.

(4) Clinical Translation: Although bioinformatics analyses suggest FKBP38 downregulation, its prognostic and predictive value requires rigorous clinical validation. Prospective studies that correlate FKBP38 protein expression (assessed via immunohistochemistry on patient tissue microarrays) with clinicopathological features, patient survival, and response to mTOR inhibitor therapy are needed to support translation of these findings into clinical application.

5. Conclusions

FKBP38 is underexpressed in CESC and acts as a tumor suppressor by inhibiting malignant behaviors, downregulating mTOR signaling, and increasing sensitivity to everolimus. These findings suggest FKBP38 is a potential therapeutic target in cervical cancer.

Availability of Data and Materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Author Contributions

WW and LH were responsible for conception and design. FT was responsible for guiding part of the experiments. HM and YW were responsible for collection and assembly of data. JW and GS were responsible for data analysis and interpretation. All authors contributed to critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflicts of Interest

The authors declare no conflicts of interest.

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