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ORIGINAL RESEARCH

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Dezocine inhibits proliferation as well as migration of endometrial carcinoma *in vitro*

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Abstract

Endometrial cancer (EC) includes several epithelial malignancies existed in the endometrium tissues. To improve the prognosis of EC, it is greatly needed to find new drugs. Dezocine, a organic compound, is an κ agonist and a μ receptor antagonist, is used in clinical anesthesia after cancer surgery with few side effects, and its anti-tumor effects have also been demonstrated in several tumors. However, the effects of Dezocine on EC progression have not been elusidated. Herein, we investigated the role of Dezocine in EC cell functions. Through a series of cellular assays, such as cell counting kit-8 (CCK-8), flow cytometry (FCM), transwell assays, we found that Dezocine suppressed the EC cell proliferation, and stimulated EC cell apoptosis *in vitro*. Furthermore, results revealed Dezocine restrained the motility, including migration and invasion of EC cells. Mechanically, Dezocine suppressed the Akt (protein kinase B, PKB)/mammalian target of rapamycin (mTOR) pathway in EC cells, thereby suppressing EC cell proliferation as well as migration *in vitro*. In conclusion, Dezocine inhibits proliferation as well as migration of EC *in vitro*.

Keywords

Endometrial cancer (EC); Dezocine; Apoptosis; Proliferation; Akt/mTOR pathway

1. Introduction

Endometrial cancer (EC) includes several epithelial malignancies found in the endometrium and usually occurs in perimenopausal as well as postmenopausal women [1]. EC ranks 15th among global malignancies and is the most common type of gynecological malignancy [2]. Surgical excision and postoperative adjuvant therapy of EC have been standardized, including hysterectomy and bilateral salpingo-oophorectomy, followed by conventional paclitaxel/platinum-based chemotherapy [3, 4]. However, women with recurrent or advanced disease have a very low response rate to conventional treatment and poor clinical outcomes [5]. It is greatly needed to find new drugs for EC treatment.

Anesthesia methods as well as drugs have been shown to affect the immune function in cancer patients, and even certain anesthetics can inhibit tumor development [6, 7]. Sevoflurane has been shown to have antitumor effects in different cancers, such as ovarian cancer [8]. Dezocine has also been widely used in clinical anesthesia and analgesia after cancer surgery without side effects [9]. Desocine inhibited ovarian cancer cell viability [9]. Dezocine inhibits the motility of ovarian cancer cells and promotes cell apoptosis [9]. In addition, the Akt/mTOR pathway is also restrained by Dezocine [9]. Dizocine induces apoptosis of HeLa cells through affecting endoplasmic reticulum stress [10]. But the effect of dezocine on endometrial cancer has not been clear.

Akt pathway plays a vital role in cell growth by inhibiting apoptosis of various cancer cells [11, 12]. Akt activation also promotes tumor metastasis, angiogenesis, and phosphorylated mTOR protein kinases [13]. The mTOR pathway is mediated by several cellular signaling, including hormones, nutrients, and cellular stress [14, 15]. Phosphoinositide 3-kinase (PI3K)/Akt/mTOR pathway, which is closely related to the regulation of cell proliferation as well as survival [16].

In summary, we investigated the potential effects of digoxin on EC and found that Digoxin inhibited endometrial cancer cell growth and migration, which can be mechanically attributed to its suppression of the Akt/mTOR pathway.

2. Materials and methods

2.1 Cell culture

Human EC cell line human endometrial cancer-1A (HEC-1A) and Ishikawa were purchased from Chinese Academy of Sciences. HEC-1A and Ishikawa cells were cultured with the roswell park memorial institute (RPMI)-1640 complete medium. After 12 hours of culture, cells were treated with Dezocine (lot number: H20080329, bought from Yangzijiang Pharmaceutical Group Co., Ltd., Taizhou, China) for 24 h at the concentration of 0, 20 and 40 μM. Then, the effect of Dezocine was verified for subsequent experiments.

2.2 Western blotting

Radioimmunoprecipitation assay (RIPA) lysate was added to fully lysate cells to extract protein, which was quantitated by bicinchoninic acid (BCA) reagent, separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and further transferred onto the polyvinylidene fluoride (PVDF) membranes. The proteins were blocked with tris-buffered saline with 0.1% tween® 20 detergent (TBST) containing 5% milk for 1 h, and then the corresponding primary antibodies were added. Primary antibodies aganist Bax (Abcam, ab32503, Shanghai, China, 1:2000), cleaved-caspase 3 (Abcam, ab32042, Shanghai, China, 1:2000), mTOR (Abcam, ab134903, Shanghai, China, 1:1000), p-mTOR (phospho S2448, Abcam, ab109268, Shanghai, China, 1:500), AKT (Abcam, ab8805, Shanghai, China, 1:1000), p-AKT (phospho-T308, Abcam, ab38449, Shanghai, China, 1:1000), β-actin (Abcam, ab8226, Shanghai, China, 1:3000), and then secondary antibodies were incubated for 1 h and photographed after chemiluminescence. The reagents used were purchased from Wuhan Google Co., LTD.

2.3 CCK-8 assay

1000 cells/well HEC-1A as well as Ishikawa cells were plated into 96-well plates as well as maintained for 48 h. Cells were subsequently incubated with CCK-8 for 4 h. Then the optical density (OD) 450 value was measured by using a microplate reader (BD).

2.4 Colony formation assay

HEC-1A as well as Ishikawa cells were plated into the 6-well plates (1000 cell per well) as well as maintained in media (10% fetal bovine serum, FBS) for 14 days at 37 °C. Then cells were fixed with paraformaldehyde (PFA) for 20 min as well as stained with 0.1% crystal violet for 20 min.

2.5 Transwell assay

The HEC-1A as well as Ishikawa cells were allowed to migrate into the transwell for 24 h. The, invaded cells on the upper chamber were fixed, stained with 2% crystal violet, and images were captured. The effect on cell invasion was observed by counting stained cells.

2.6 Wound-healing assay

A 10-μL pipette tip was used to create a scratch, after which the HEC-1A as well as Ishikawa cells were washed twice. Images of the wound were captured at 0 and 24 h to determine the extent of wound closure.

2.7 Cell apoptosis assay

The HEC-1A as well as Ishikawa cells were washed with phosphate buffered saline (PBS). Subsequently cells were fixed using 70% ethanol at −20 °C for 2 h. Subsequently stained with propidium iodide (PI) as well as fluorescein isothiocyanate (FITC) Annexin V at 4 °C and the apoptosis levels were measured using FACSCalibur flow cytometer and CellQuest Pro 5.1 (BD Biosciences, Inc., Franklin Lakes, NJ, USA).

2.8 Statistics

GraphPad 5.0 software (GraphPad Software Inc., Boston, MA, USA) was used and performed. Data were represented as mean $\pm$ standard deviation (SD). $p < 0.05$ was thought as significant.

3. Results

3.1 Dezocine treatment restrained the proliferation of EC cells

To evaluate the effects of Dezocine on the EC cell proliferation, we first investigated its effects on the viability of HEC-1A as well as Ishikawa cells at the concentration of 0, 20, 40 μM *via* CCK-8 assays. We noticed Dezocine treatment decreased the OD value in the HEC-1A as well as Ishikawa cells (Fig. 1a). We further performed the colony formation assays, and the data showed Dezocine treatment decreased the colony numbers at the high concentration of Dezocine (Fig. 1b). Therefore, Dezocine treatment restrained EC cell proliferation.

3.2 The treatment of Dezocine stimulated the apoptosis in EC cells

Interestingly, we further performed FCM assays to detect the effects of Dezocine on the apoptosis of HEC-1A as well as Ishikawa cells. We noticed that Dezocine treatment stimulated the apoptosis of HEC-1A as well as Ishikawa cells (Fig. 2a). Immunoblot assays also confirmed the increased Bax and cleaved caspase 3 expression upon Dezocine treatment (Fig. 2b). Therefore, Dezocine stimulated the apoptosis of EC cells.

3.3 Dezocine suppressed the motility of EC cells

Then we investigated the effects of Dezocine on the motility of EC cell line HEC-1A and Ishikawa. Through transwell assays, HEC-1A and Ishikawa cells were treated with Dezocine for 24 h at the concentration of 0, 20 and 40 μM. We found Dezocine treatment suppressed the invasion of HEC-1A as well as Ishikawa cells (Fig. 3a). Similarly, wound closure assay also confirmed Dezocine treatment restrained the migration of HEC-1A as well as Ishikawa cells, with the decrease of wound healing (Fig. 3b). Therefore, Dezocine suppressed the motility of EC cells.

3.4 Dezocine suppressed the Akt/mTOR pathway in EC cells

We detected the effects of Dezocine on PI3K/Akt pathway in HEC-1A as well as Ishikawa cells. Through Immunoblot assays, we found Dezocine treatment decreased the phosphorylation levels of Akt as well as mTOR in HEC-1A as well as Ishikawa cells (Fig. 4). Therefore, Dezocine suppressed the Akt/mTOR pathway in EC cells.

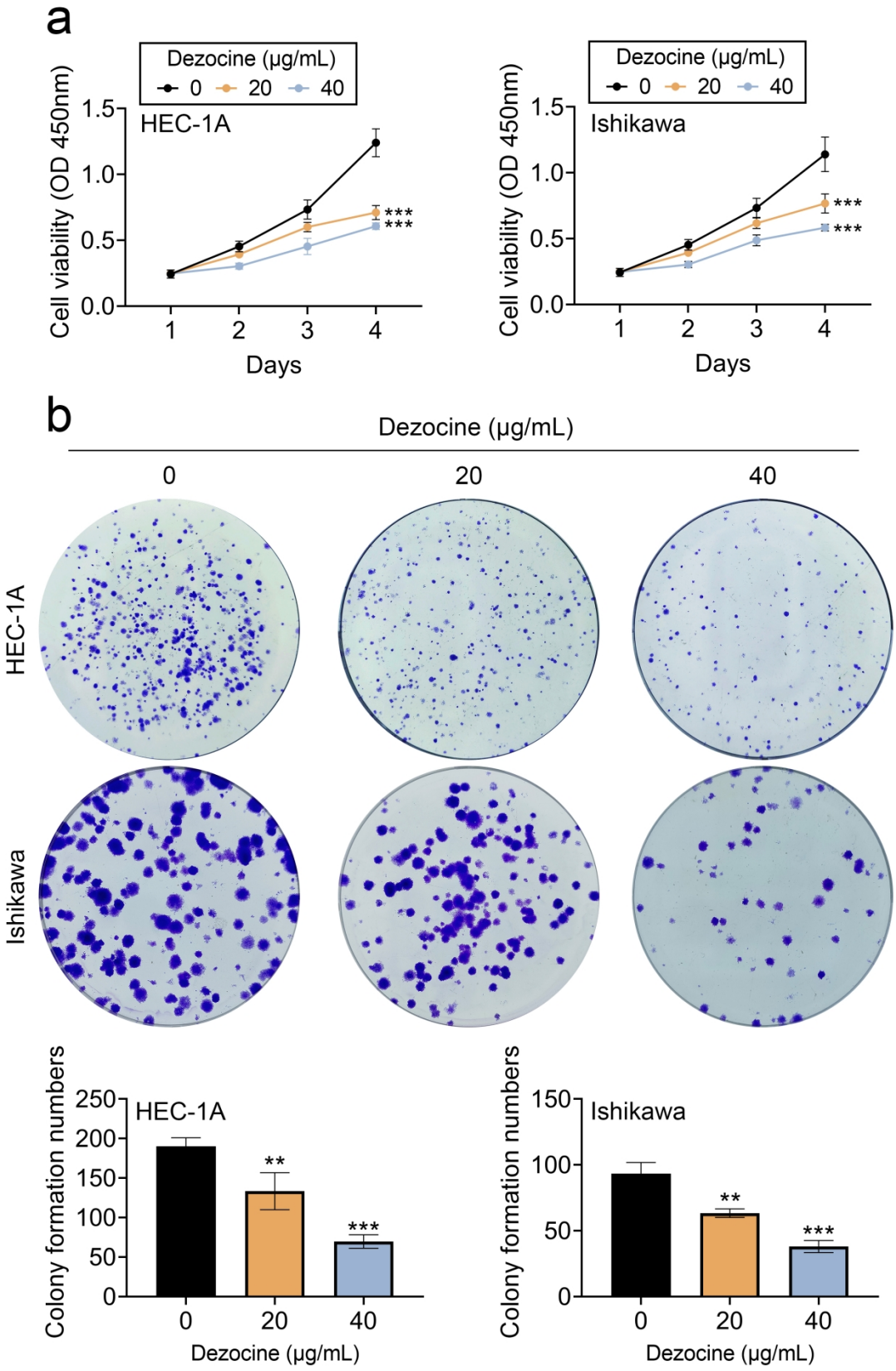

FIGURE 1. Dezocine treatment restrained the proliferation of EC cells. (a) CCK-8 assays showed the effects of Dezocine on the OD value at 450 nm wavelength at the concentration of 0, 20, 40 μM for 24 h in HEC-1A and Ishikawa cells. (b) Colony formation assays showed the effects of Dezocine on the viability of HEC-1A and Ishikawa cells at the concentration of 0, 20, 40 μM for 24 h. Data were represented as mean ± SD. $^{**}p < 0.01$, $^{***}p < 0.001$. OD: optical density; HEC-1A: human endometrial cancer-1A.

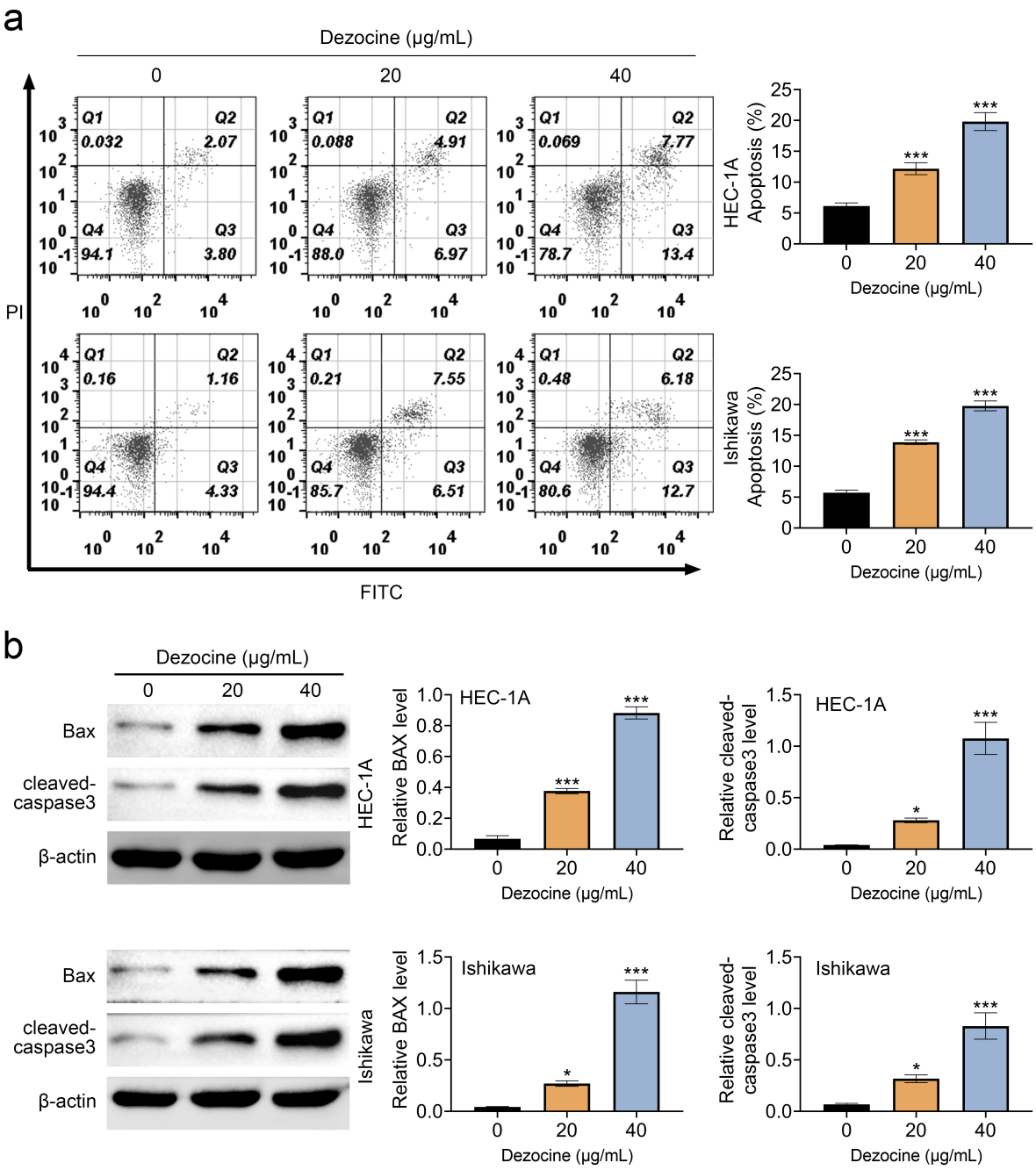

FIGURE 2. The treatment of Dezocine stimulated the apoptosis of EC cells. (a) FCM assays showed the effects of Dezocine on the apoptosis of EC cells at the concentration of 0, 20, 40 μM for 24 h in HEC-1A and Ishikawa cells. The percentage of apoptosis cells were calculated. (b) Immunoblot assays showed the effects of Dezocine on the expression of indicated proteins at the concentration of 0, 20, 40 μM for 24 h in HEC-1A and Ishikawa cells. Data were represented as mean ± SD. *$p < 0.05$, ***$p < 0.001$. PI: propidium iodide; FITC: fluorescein isothiocyanate; HEC-1A: human endometrial cancer-1A.

4. Discussion

Endometrial cancer is an epithelial malignancy existed in the endometrium tissues, most commonly occurring in perimenopausal as well as postmenopausal women [17]. Endometrial cancer is a tumor in the female reproductive system, and the third most common gynecological malignancy leading to death [5]. The treatment of endometrial cancer

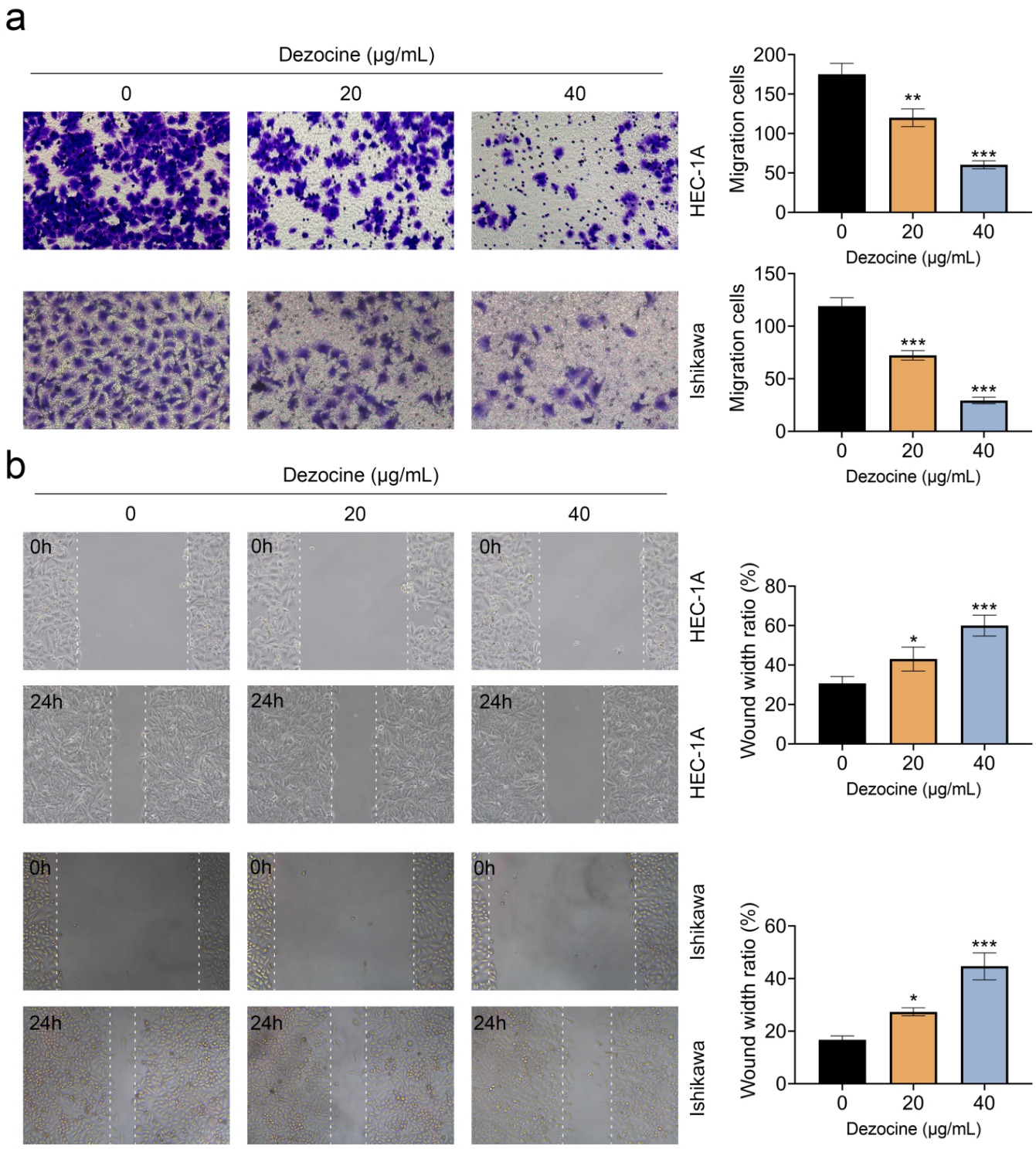

FIGURE 3. Dezocine suppressed the motility of EC cells. (a) Transwell assays showed the effects of Dezocine on the invasion of HEC-1A and Ishikawa cells at the concentration of 0, 20, 40 μM for 24 h in HEC-1A (up) as well as Ishikawa cells (down). (b) Wound healing assays showed the effects of Dezocine on the migration of cells at the concentration of 0, 20, 40 μM for 24 h in HEC-1A (up) as well as Ishikawa cells (down) cells. Data were represented as mean $\pm$ SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. HEC-1A: human endometrial cancer-1A.

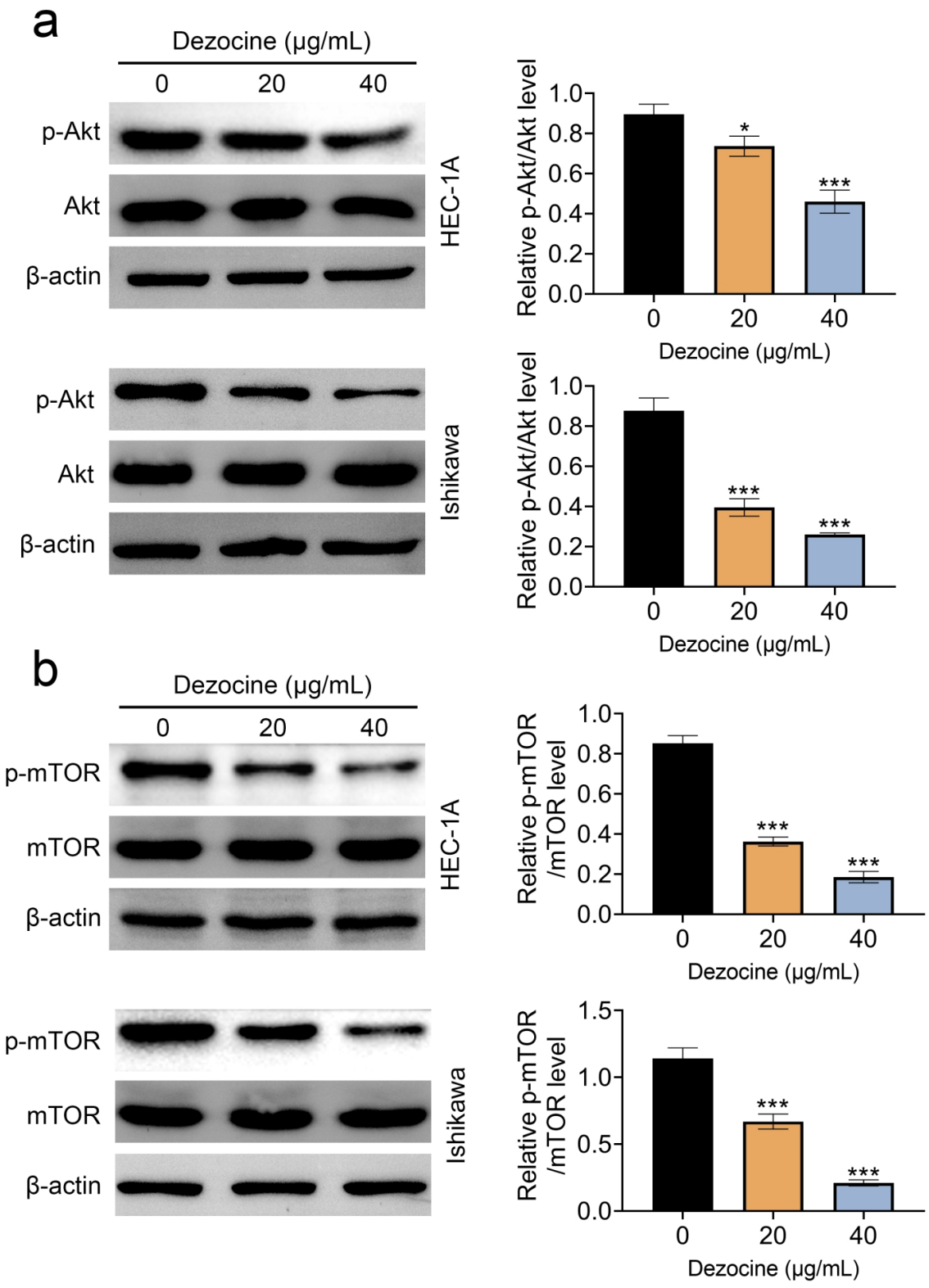

FIGURE 4. Dezocine suppressed the Akt/mTOR pathway in EC cells. (a) Immunoblot assays showed the effects of Dezocine on the phosphorylation of AKT in HEC-1A (up) as well as Ishikawa cells (down) cells at the concentration of 0, 20, 40 μM for 24 h. (b) Immunoblot assays showed the effects of Dezocine on the phosphorylation of mTOR in HEC-1A (up) as well as Ishikawa cells (down) cells at the concentration of 0, 20, 40 μM for 24 h. Data were represented as mean $\pm$ SD. *$p < 0.05$, ***$p < 0.001$. Akt: protein kinase B; HEC-1A: human endometrial cancer-1A; mTOR: mammalian target of rapamycin.

should be based on the patient's age and so on, and the appropriate treatment modalities should be selected. Since most endometrial cancers are adenocarcinoma, they are not very sensitive to radiotherapy, so surgery is the main treatment, and radiotherapy, chemotherapy and other comprehensive treatments are also used [3]. Most of the drugs used to treat endometrial cancer are endocrine drugs, such as medroxyprogesterone, mezolone and so on [2]. To combat this disease, new and more effective drugs are still needed. Interestingly, we found a clinical anesthesia, Dezocine, suppressed the progression of EC *in vitro* here.

Dezocine, an organic compound, is an κ agonist and a μ receptor antagonist. It is mainly used for postoperative analgesia and pain caused by visceral and cancer [18]. Dezocine is a powerful opioid analgesic [19]; It can relieve postoperative pain, and its analgesic intensity is comparable to morphine [20]. For all types of pain that require treatment with opioid analgesics. Importantly, the anti-tumor effects of Dizocine have already been demonstrated. Dezocine inhibited ovarian cancer cell viability [9]. Dezocine also inhibited the migration as well as the invasion of ovarian cancer cells [9]. Therefore, we thought it had potential to serve as a drug for EC treatment. Through CCK-8 as well as colony formation, we found Dizocine suppressed the proliferation of EC cells. Further through FCM assays, Dizocine stimulated the apoptosis of EC cells. We further revealed Dizocine suppressed the motility, thereby suppressing EC progression. However, the possible mechanism needs further study.

In recent years, the antitumor effects and mechanisms of anesthetics are being unraveled [18]. Research results have proved that anesthetics can affect the apoptosis, proliferation, metastasis and DNA demethylation of oncogenes in some tumor cells [6]. However, different local anesthetics have different antitumor effects on different malignant tumors. Anesthetics can not only inhibit growth signals, especially the mTOR signaling pathway, to inhibit the promotion of growth signals to the growth of cancer cells, but more importantly, they can inhibit the expression of anti-apoptotic proteins such as Bcl-2 and release substances inducing apoptosis of cancer cells such as cytochrome C by activating the Caspases signaling pathway [8]. It can also promote the expression of anti-tumor proteins such as Bax [6]. We here also revealed that Dizocine suppressed the EC progression *via* Akt/mTOR pathway.

The Akt pathway plays an important role in cell growth by inhibiting apoptosis of various types of human cancer cells [16]. Akt activation also promotes tumor metastasis, angiogenesis, and mTOR phosphorylation, thereby affecting tumor progression [11]. Next, we should further verify whether the EC inhibition of dezocine can be restored after treatment with mTOR inhibitors. It is worth to validate the data and go for animal model to establish it as a drug for EC.

5. Conclusions

In summary, we found Dezocine inhibited proliferation as well as migration of endometrial carcinoma, and promoted apoptosis of cells *via* Akt/mTOR pathway.

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

LL and BQ—designed the study and carried them out; LL, BQ, YL and XLZ—supervised the data collection, analyzed the data, interpreted the data, 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

This article does not contain any studies with human participants or animals performed by any of the authors. Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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