

ORIGINAL RESEARCH

Sufentanil inhibits the invasion and epithelial-mesenchymal transition of endometrial cancer cells *in vitro*

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Abstract

This study investigates the impact of sufentanil on endometrial cancer (EC) cells. Human EC cell lines (HEC-1A and Ishikawa) were exposed to different concentrations (10, 20 and 40 nM) of sufentanil for 48 hours, following which cell-counting-kit 8 (CCK8), clone formation and transwell assays as well as western blot were conducted. The findings revealed that sufentanil reduced the cell viability, colony formation, migration and invasion of both HEC-1A and Ishikawa cells. Moreover, sufentanil treatment resulted in decreased levels of matrix metallo proteinase 9 (MMP-9), MMP-2, N-cadherin and alpha smooth muscle actin (α -SMA) proteins while increasing the expression of E-cadherin in treated cells. Furthermore, sufentanil treatment was associated with decreased phosphorylation of phosphatidylinositol 3-kinase (PI3K), Akt and mammalian target of rapamycin (mTOR), suggesting that its inhibitory effects on the proliferation, migration, invasion and epithelial-mesenchymal transition (EMT) of EC cells could be attributed to the inactivation of the PI3K/Akt/mTOR pathway.

Keywords

Endometrial cancer; Sufentanil; PI3K/Akt/mTOR pathway; Migration; Invasion; EMT

1. Introduction

Endometrial cancer (EC) is a form of epithelial cancer that arises in the endometrium [1]. Presently, its preferred treatment is surgery followed by adjuvant chemoradiotherapy [2, 3]. Additionally, targeted therapy and immunotherapy are considered for the treatment of advanced and recurrent EC patients [4, 5]. However, the effective management of EC remains challenging due to issues such as drug intolerance and medication-related side effects.

Epithelial-mesenchymal transition (EMT) plays a crucial role in various aspects of malignancy, including tumor invasion and metastasis [6]. It involves a gradual reduction in the expression of cell adhesion molecules by tumor cells, the acquisition of mesenchymal characteristics and the ability to migrate and invade, thereby promoting tumor progression and metastasis [7, 8]. Thus, research on strategies formulated to inhibit EMT represents a promising avenue for anti-cancer therapy.

Recent research has revealed the beneficial effects of anesthetic analgesic drugs on tumor cell growth and metastasis [9, 10]. For instance, sufentanil, an opioid narcotic analgesic, is widely used in the surgical treatment of various diseases, including cancer [11]. It has also been demonstrated that sufentanil can inhibit the growth and EMT of lung cancer, esophageal cancer and breast cancer by regulating the Wnt/beta-catenin, nuclear factor- κ -gene binding (NF- κ B), and

Snail signaling pathways [12–14]. However, the potential anti-tumor effects of sufentanil in EC remain unclear.

Herein, we designed this present study to investigate the effects of sufentanil on EC cell proliferation, migration, invasion and EMT *in vitro*.

2. Materials and methods

2.1 Cell culture and treatment

Human EC cell lines (HEC-1A and Ishikawa) and human endometrial stromal cells (hESC) were purchased from Procell (Wuhan, China) and cultured in 10% fetal bovine serum (FBS)-RPMI 1640 medium (Gibco, Rockville, MD, USA) containing 1% streptomycin/penicillin at a temperature of 37 °C in a 5% CO₂ environment. For sufentanil treatment, the cells were randomly allocated into four groups. One group served as a control and remained untreated, while the other three groups were exposed to 10, 20 and 40 nM sufentanil concentrations for 48 hours, respectively.

2.2 CCK8 assay

A volume of 100 μ L of cell suspensions containing 1000 cells was added to individual wells of 96-well plates. After adding 10 μ L of cell-counting kit-8 (CCK8) reagent (CK04, Dojindo, Beijing, China) to each well, the plates were incubated for 4 hours, following which absorbance at 450 nm (OD 450 nm)

was measured.

2.3 Clone formation assay

Cell suspensions, each containing 1000 cells, were introduced into 6-well culture plates and subjected to a 14-day incubation period. Following fixation with a 4% paraformaldehyde solution, the cells were subsequently stained utilizing a 0.1% crystal violet solution. The quantification of colony formation was then conducted by counting the number of stained colonies with the naked eye.

2.4 Transwell assay

Transwell assays were conducted using 24-well Transwell plates (Corning, NY, USA), with or without prior Matrigel coating (BD Pharmingen, NJ, USA). Briefly, a cell suspension devoid of serum, containing 2×10^4 cells, was loaded into the transwell inserts, while the lower chambers of the plates were filled with 600 μL of medium containing 10% FBS. Following incubation, the transwell inserts were fixed using 4% paraformaldehyde, and non-migratory cells were stained using 0.1% crystal violet. Subsequently, photographs were taken, and the migrated or invaded cells on the lower surface of the transwell inserts were counted using an Eclipse Ti2 inverted microscope (Nikon, Tokyo, Japan).

2.5 Western blot

RIPA lysis buffer (R0010, Solarbio, Beijing, China) was used for cell lysis in each treatment group. Following separation using 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), the cell lysates were transferred onto polyvinylidene fluoride (PVDF) membranes, which were subsequently incubated with primary antibodies targeting the proteins shown in Table 1. They were then incubated with secondary antibodies, following which protein bands were visualized, captured and quantified using ImageJ 1.8 software (National Institutes of Health, Bethesda, MD, USA).

2.6 Statistical analysis

The experiments were conducted in triplicate. GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA) was used for statistical analysis, and the data are presented as mean \pm standard deviation (SD). Multiple group comparisons were assessed using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* analysis. $p < 0.05$ was used to determine statistical significance.

3. Results

3.1 Sufentanil inhibits the proliferation of EC cells

Fig. 1A shows the structural formula for sufentanil. After HEC-1A and Ishikawa cells were exposed to 0, 10, 20 and 40 nM sufentanil for 48 hours, their cell viability and clone formation capacity were assessed. As shown in Fig. 1B, cell viability in the 20 and 40 nM sufentanil treatment groups was significantly reduced. However, different concentrations of sufentanil did not affect the cell viability of hESC cells. Results

from the clone formation assay indicated a significant decrease in the number of colonies formed in the sufentanil treatment groups compared to the control group (Fig. 1C). These findings suggest that sufentanil may effectively inhibit the proliferation of EC cells.

3.2 Sufentanil suppresses the migration and invasion of EC cells

Next, HEC-1A and Ishikawa cells' migratory and invasive abilities were assessed. As shown in Fig. 2A, the migration and invasion capacities of cells subjected to sufentanil treatment were significantly decreased compared to the control group. Furthermore, sufentanil treatment led to a significant reduction in the protein expression levels of MMP-9 and MMP-2 in the cells (Fig. 2B). These findings suggest that sufentanil may effectively impede the migration and invasion of EC cells.

3.3 Sufentanil inhibits EC cell EMT

Levels of E-cadherin, N-cadherin, and α -SMA, which mark EMT, were assessed using western blot, and the findings revealed an elevate in E-cadherin levels and a reduction in N-cadherin and α -SMA levels in both HEC-1A and Ishikawa cells upon sufentanil treatment compared to controls (Fig. 3), indicating the inhibitory effects of sufentanil on the EMT process in EC cells.

3.4 Sufentanil inhibits PI3K/Akt/mTOR protein expression

Western blot analysis revealed reduced levels of phosphorylated PI3K, Akt and mTOR in HEC-1A and Ishikawa cells treated with sufentanil compared to controls (Fig. 4). Moreover, the detection of PTEN mRNA and protein in HEC-1A cells significantly increased PTEN expression in sufentanil-treated cells than controls (Fig. 4B,C). Taken together, these data indicate that sufentanil treatment could suppress the PI3K/Akt/mTOR pathway in EC cells.

4. Discussion

EC is a prevalent gynecological malignancy [15], and approximately 70% of EC cases diagnosed in the uterine body, particularly those in the early clinical stages, have favorable prognostic outcomes [16]. However, patients with advanced disease and extrauterine metastasis have dismal prognoses [17]. Substantial evidence indicates that the development of EMT results in the weakening of cell-cell adhesion and the reinforced cell motility in tumor cells, facilitating their invasion of neighboring tissues and metastasis to distant organs [18, 19]. Findings from this study indicate that sufentanil effectively inhibits the proliferation, migration, invasion and EMT process in EC cells, suggesting its potential utility in reducing EC cell invasion and metastasis as part of EC treatment.

Matrix metalloproteinases (MMPs) play a pivotal role in influencing the invasion and dissemination of tumor cells by regulating the extracellular matrix (ECM) and basement membrane (BM) [20]. Specifically, among the MMPs, MMP-9 and MMP-2 are recognized as the primary matrix proteolytic

TABLE 1. Antibodies used for western blot.

Antigen	Code	Working dilution	Supplier
matrix metallo proteinase 9 (MMP-9)	ab137869	1:1000	Abcam
MMP-2	ab97779	1:1000	Abcam
E-cadherin	ab227639	1:100	Abcam
N-cadherin	ab207608	1:1000	Abcam
alpha smooth muscle actin (α -SMA)	ab88979	1:500	Abcam
phosphorylated-phosphatidylinositol 3-kinase (p-PI3K)	ab182651	1:500	Abcam
PI3K	ab140307	1:1000	Abcam
phosphorylated-Akt (p-Akt)	ab38449	1:1000	Abcam
Akt	ab8805	1:500	Abcam
phosphorylated-mammalian target of rapamycin (p-mTOR)	ab314037	1:1000	Abcam
mTOR	ab2732	1:1000	Abcam
protein tyrosine phosphatase (PTEN)	ab133532	1:10,000	Abcam
glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	ab59164	1:2000	Abcam

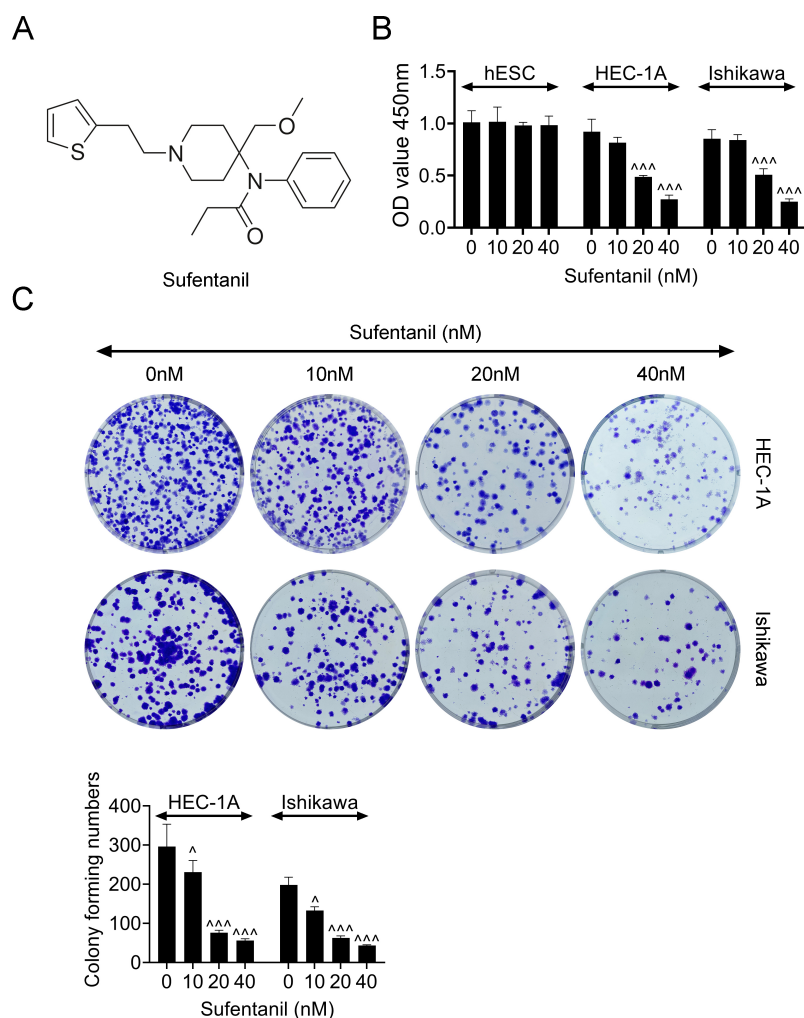


FIGURE 1. Sufentanil inhibits the proliferation of EC cells. (A) Structural formula of sufentanil. (B) Cell viability, assessed by CCK8 assay, following 48-hour sufentanil treatment ($n = 3$). (C) Clone formation ability evaluated after 48-hour sufentanil treatment *via* clone formation assay ($n = 3$). Statistical significance is indicated as $\hat{p} < 0.05$ and $\hat{\hat{p}} < 0.001$, *vs.* control (0 nM group). hESC: human endometrial stromal cells; HEC: Human endometrial cancer.

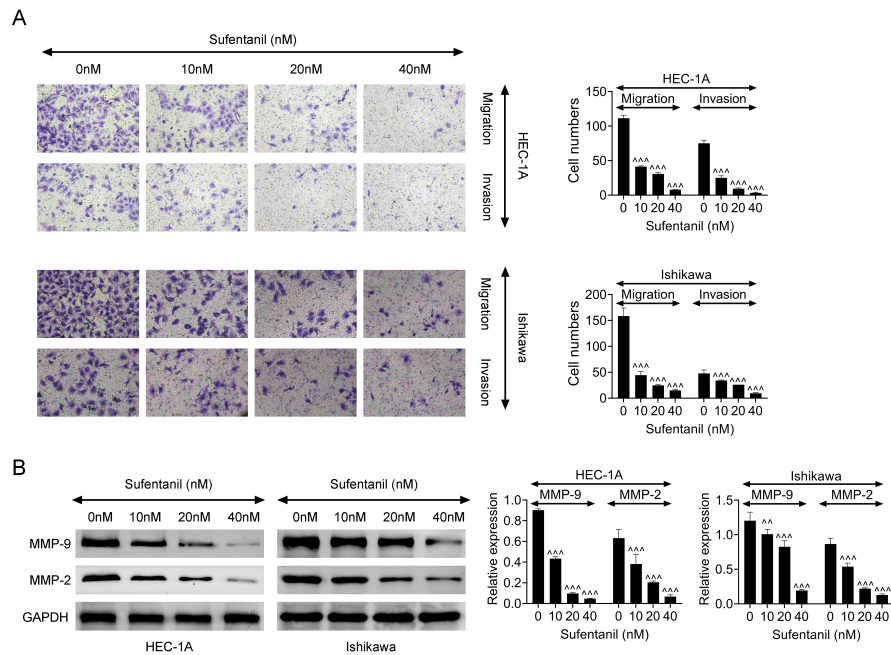


FIGURE 2. Sufentanil suppresses the migration and invasion of EC cells. (A) Transwell assay examining the migration and invasion of HEC-1A and Ishikawa cells treated with sufentanil for 48 hours ($n = 3$). (B) Western blot analysis of MMP-9 and MMP-2 protein expression ($n = 3$). Statistical significance is represented as $^{\wedge}p < 0.01$ and $^{\wedge\wedge}p < 0.001$, vs. control (0 nM group). HEC: Human endometrial cancer; MMP: matrix metalloproteinase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

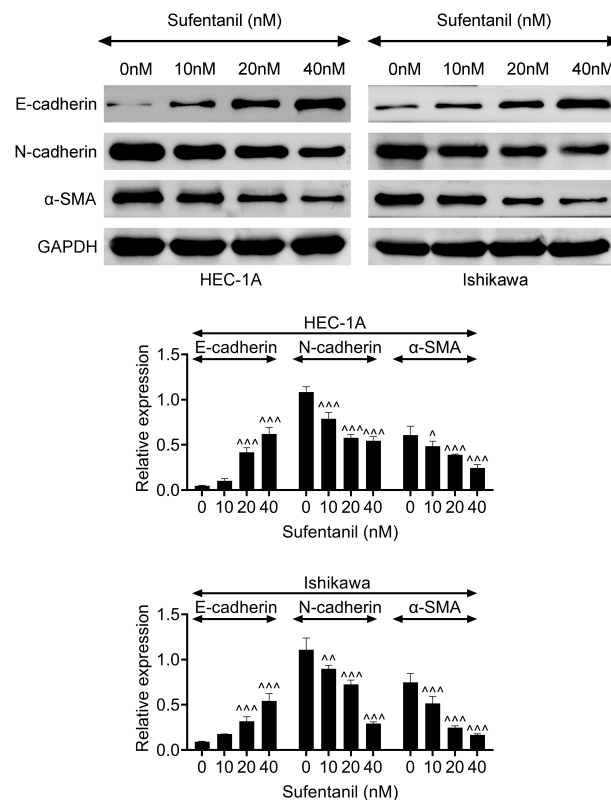


FIGURE 3. Sufentanil inhibits EC cell epithelial-mesenchymal transition (EMT). Western blot analysis of E-cadherin, N-cadherin, and α -SMA protein expression in different treatment groups ($n = 9$). Statistical significance is denoted as $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$ and $^{\wedge\wedge\wedge}p < 0.001$, vs. control (0 nM group). HEC: Human endometrial cancer; SMA: smooth muscle actin; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

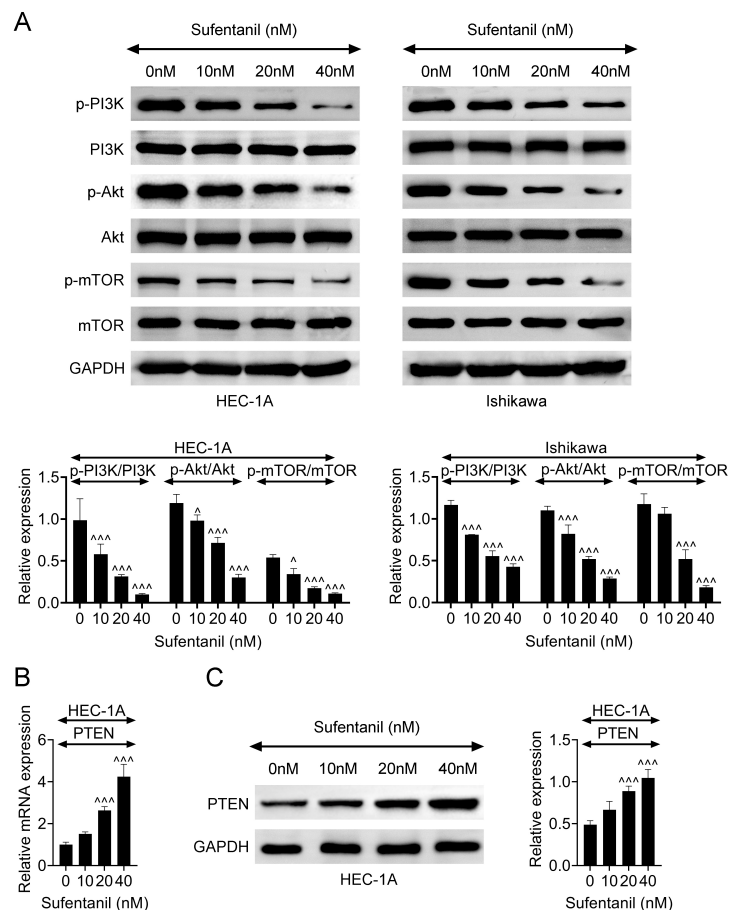


FIGURE 4. Sufentanil inhibits PI3K/ Akt/mTOR protein expression. (A) Western blot assessment of p-PI3K, PI3K, p-Akt, Akt, p-mTOR, and mTOR protein levels (n = 9). (B,C) Assessment of mRNA and protein expression of PTEN in HEC-1A cells *via* qRT-PCR and western blot. Statistical significance is indicated as $\hat{p} < 0.05$ and $\hat{\hat{p}} < 0.001$, vs. control (0 nM group). p-PI3K: phosphorylated-phosphatidylinositol 3-kinase; p-mTOR: phosphorylated-mammalian target of rapamycin.

enzymes capable of degrading type IV collagen, a critical structural component of the ECM [21]. Thus, MMP-9 and MMP-2 can disturb the equilibrium of matrix degradation and facilitate cancer cells to cross the tissue blood barrier, which is composed of the ECM and BM, thereby enabling the invasion of neighboring tissues and the metastasis to distant tissues [22, 23]. The results of our western blot analysis in this study demonstrated that sufentanil suppressed MMP-9 and MMP-2 proteins expression in EC cells. Furthermore, a sufentanil treatment resulted in increases in E-cadherin expression, while a decrease in N-cadherin and α -SMA was observed. These findings suggest that sufentanil can partially reverse the EMT process in EC cells, thereby validating its inhibition of cell migration and invasion.

The PI3K/Akt/mTOR pathway is frequently activated in human malignancies and contributes to the progression of tumor cells, making it a crucial target for anti-tumor therapies [24]. A previous study suggested that apoptosis in cervical cancer cells was inhibited by sufentanil by inactivating the PI3K/AKT/mTOR pathway [25]. PI3K/Akt/mTOR pathways are also frequently altered in EC [26]. Multiple evidence confirmed that as well as inhibiting the deterioration of EC cells, inhibition of the PI3K/Akt/mTOR pathway also has a positive effect on the treatment of advanced or recurrent EC [27–

29]. In line with these findings, our current research provides additional data indicating that sufentanil treatment can significantly reduce the phosphorylation levels of PI3K, Akt and mTOR and higher levels of PTEN expression. Collectively, these data suggest that the inactivation of the PI3K/Akt/mTOR pathway may be responsible for sufentanil inhibition of EC cell malignant behaviors.

5. Conclusions

In conclusion, this study highlights the inhibitory effects of sufentanil on the proliferation, migration, invasion and EMT of EC cells and reveals that the potential mechanism underlying these effects is mediated by the inactivation of the PI3K/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

HXC, GQH and QG—designed the study and carried them out; supervised the data collection, analyzed the data, interpreted the data, 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.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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