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ORIGINAL RESEARCH

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Oxycodone modulates VEGFA to inhibit cervical cancer cell migration and angiogenesis

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Abstract

Background: Cervical cancer (CC) is a leading cause of cancer-related deaths among women worldwide, necessitating the development of novel therapeutic strategies. Oxycodone, a semi-synthetic opioid, is primarily known for its analgesic properties but has shown potential anti-cancer effects in various malignancies. However, its impact on cervical cancer cells and the underlying mechanisms remain unclear. **Methods**: Human cervical cancer cell lines Hela and Siha were treated with varying concentrations of oxycodone (10, 20, 40 μg/mL). Cell proliferation was assessed using Cell Counting Kit-8 (CCK-8) and clonogenic assays. Apoptosis was measured by flow cytometry. Cell migration and angiogenesis were evaluated using wound healing and tube formation assays, respectively. VEGFA expression was analyzed by Western blotting. **Results**: Oxycodone inhibited the proliferation of cervical cancer cells. It induced apoptosis in cervical cancer cells. Oxycodone suppressed the migration and angiogenesis of cervical cancer cells. Mechanistically, oxycodone regulated vascular endothelial growth factor A (VEGFA) expression, leading to the inhibition of cell migration and angiogenesis. **Conclusions**: Oxycodone exhibits significant anti-cancer effects on cervical cancer cells by inhibiting proliferation, inducing apoptosis, and suppressing migration and angiogenesis through the regulation of VEGFA expression. These findings suggest that oxycodone has potential as a therapeutic agent for cervical cancer treatment.

Keywords

Oxycodone; Cervical cancer; Growth; Apoptosis; VEGFA

1. Introduction

Cervical cancer (CC) ranks fourth among cancer types in women worldwide and leads to tumor-related deaths [1]. Cervical cancer burden is particularly high in low- and middle-income countries [2]. CC is primarily caused by HPV infection, which leads to precancerous lesions and, eventually, invasive cancer [3]. Despite advances in CC treatment and screening methods, early diagnosis and treatment have significantly reduced mortality rates, survival rates remain low, particularly in advanced stages of the disease [4]. Therefore, studying drugs that affect the mechanisms and progression of cancer is of paramount importance for the diagnosis and treatment of CC.

The use of anesthetic drugs and techniques in cancer surgery may affect cancer cell invasion and migration capabilities, potentially affecting patients' long-term prognosis [4]. To improve life quality and treatment compliance, cancer patients are required to undergo pain management. Oxycodone, a semi-synthetic opioid derived from the alkaloid thebaine, exhibits an affinity for μ-opioid receptors that is one-fifth to one-fortieth that of morphine but can fully activate κ-opioid receptors [5–8].

In diverse tumor cell lines, oxycodone has been demonstrated to have anticancer effects. For instance, A549 lung cancer cells are effectively inhibited by oxycodone, causing them to undergo apoptosis by increasing p53 and Bax gene expression, and decreasing B-cell lymphoma-2 (Bcl-2) expression and inhibiting VEGF [9]. In liver cancer, oxycodone inhibits colony formation and migration of cancer cells [10]. In a hepatocellular carcinoma (HCC) xenograft mouse model, oxycodone significantly reduced HCC tumor growth [11]. As an analgesic after radical surgery for cervical cancer, oxycodone may play a role in influencing cancer outcomes beyond pain management.

Oxycodone's effects and mechanisms, however, are unclear. Understanding how oxycodone impacts cervical cancer cell growth, migration and angiogenesis could provide valuable insights into its potential therapeutic applications. We aim to investigate the role of oxycodone in mediating VEGFA expression and its impact on CC cells' migration and angiogenesis. In this study, CC cell growth and migration are significantly inhibited by oxycodone, and apoptosis is induced.

2. Materials and methods

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2.1 Cell culture and experimental design

Human CC cell lines Hela and Siha were used in this study. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, 11965092, Grand Island, NE, USA) supplemented with 10% fetal bovine serum (FBS, 10437-028, Gibco, Grand Island, NE, USA) at 37 °C in a humidified atmosphere containing 5% CO_2 (Carbon dioxide). We divided cells into four groups: Control; Oxycodone (10 μg/mL, Sigma-Aldrich, O1378, St. Louis, MO, USA); Oxycodone (20 μg/mL); Oxycodone (40 μg/mL). pcDNA3.1-vector and pcDNA3.1-VEGFA plasmids were transfected into HeLa cells using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). Human Umbilical Vein Endothelial Cells (HUVEC) were obtained from Lonza (Walkersville, MD, USA) and cultured in Endothelial Cell Growth Medium (EGM-2) supplemented with 10% FBS (Gibco). Oxycodone's effects on angiogenesis were assessed using HUVEC cells, which have been successfully used to study endothelial cell function.

2.2 Cell growth assay

A Cell Counting Kit-8 (CCK-8, Beyotime, C0038, Beijing, China) was used to measure cell growth. Absorbance was measured at 450 nm using a microplate reader (Bio-Rad, 1681135, Hercules, CA, USA).

2.3 Clonogenic assay

For the clonogenic assay, Hela and Siha cells were seeded in 6-well plates (500 cells/well) for 10 days. Colonies were fixed with methanol, stained with 0.1% crystal violet, and counted manually.

2.4 Apoptosis assay

Cell apoptosis was detected using an Annexin V-Fluorescein Isothiocyanate/Propidium Iodide (V-FITC/PI) Apoptosis Detection Kit (Beyotime, C1062M, Beijing, China). An analysis of the apoptosis rate was performed using a flow cytometry (BD Biosciences, FACSCanto II, San Jose, CA, USA).

2.5 Wound healing assay

With a sterile pipette tip, we scratched the monolayer of cells and treated them with Oxycodone. Images were captured using an inverted microscope (Olympus), and wound closure was quantified.

2.6 Tube formation assay

Angiogenesis was assessed by tube formation assay. HUVEC cells were seeded in Matrigel-coated 24-well plates and treated with media from Hela and Siha cells pre-treated with Oxycodone (10, 20, 40 μg/mL) for 24 h. Under an inverted microscope (Olympus), tube formation was observed and quantified.

2.7 Immunoblot analysis

Equal amounts of protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore). Membranes were blocked with 5% non-fat milk and incubated with primary antibodies against VEGFA (Abcam, ab1316, Cambridge, UK) and β-actin (Abcam, ab8226, Cambridge, UK) overnight at 4 °C. Membranes were incubated with horseradish peroxidase (HRP)-secondary antibodies (Abcam, ab6721, Cambridge, UK) and visualized using an enhanced chemiluminescence (ECL) detection kit (Beyotime, P0018, Cambridge, UK).

2.8 Statistical analysis

Data analysis was performed by SPSS 27 (SPSS Sump Software, Chicago, IL, USA). Data was presented as mean ± standard deviation (SD). Statistical significance was determined using a one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test. $p < 0.05$ indicates statistically significant differences.

3. Results

3.1 Oxycodone inhibits cervical cancer cell growth

To determine whether oxycodone effects CC cell growth, varying concentrations of oxycodone were applied to Hela and Siha cells, and cell viability was assessed. The CCK-8 assay showed significant inhibition of cell growth at 24, 48 and 72 h post-treatment with 10, 20 and 40 μg/mL oxycodone (Fig. 1A). Similarly, the clonogenic assay demonstrated that oxycodone significantly reduced the number of colonies formed by both Hela and Siha cells (Fig. 1B). Oxycodone effectively inhibits CC cell growth.

3.2 Oxycodone induces CC Cell apoptosis

To evaluate the pro-apoptotic effect of oxycodone on CC cells, flow cytometry was performed. Oxycodone induced apoptosis in both Hela and Siha cells. Percentage of apoptotic cells increased significantly with higher oxycodone concentrations (Fig. 2). Oxycodone promotes CC cell apoptosis, contributing to its anti-cancer effects.

3.3 Oxycodone suppresses CC cell migration and angiogenesis

The impact of oxycodone on CC cells' motility and angiogenesis was investigated. The wound healing assay demonstrated that oxycodone significantly suppressed Hela and Siha cells' motility, as indicated by the wider relative wound width in oxycodone-treated cells (Fig. 3A). Additionally, the tube formation assay showed that conditioned media from oxycodone-treated Hela and Siha cells reduced capillary-like structures formation by HUVECs, indicating inhibited angiogenesis (Fig. 3B). Immunoblot analysis further confirmed that oxycodone downregulated VEGFA expression in Hela and Siha cells (Fig. 3C). Oxycodone suppresses CC cell motility and angiogenesis, partially through VEGFA downregulation.

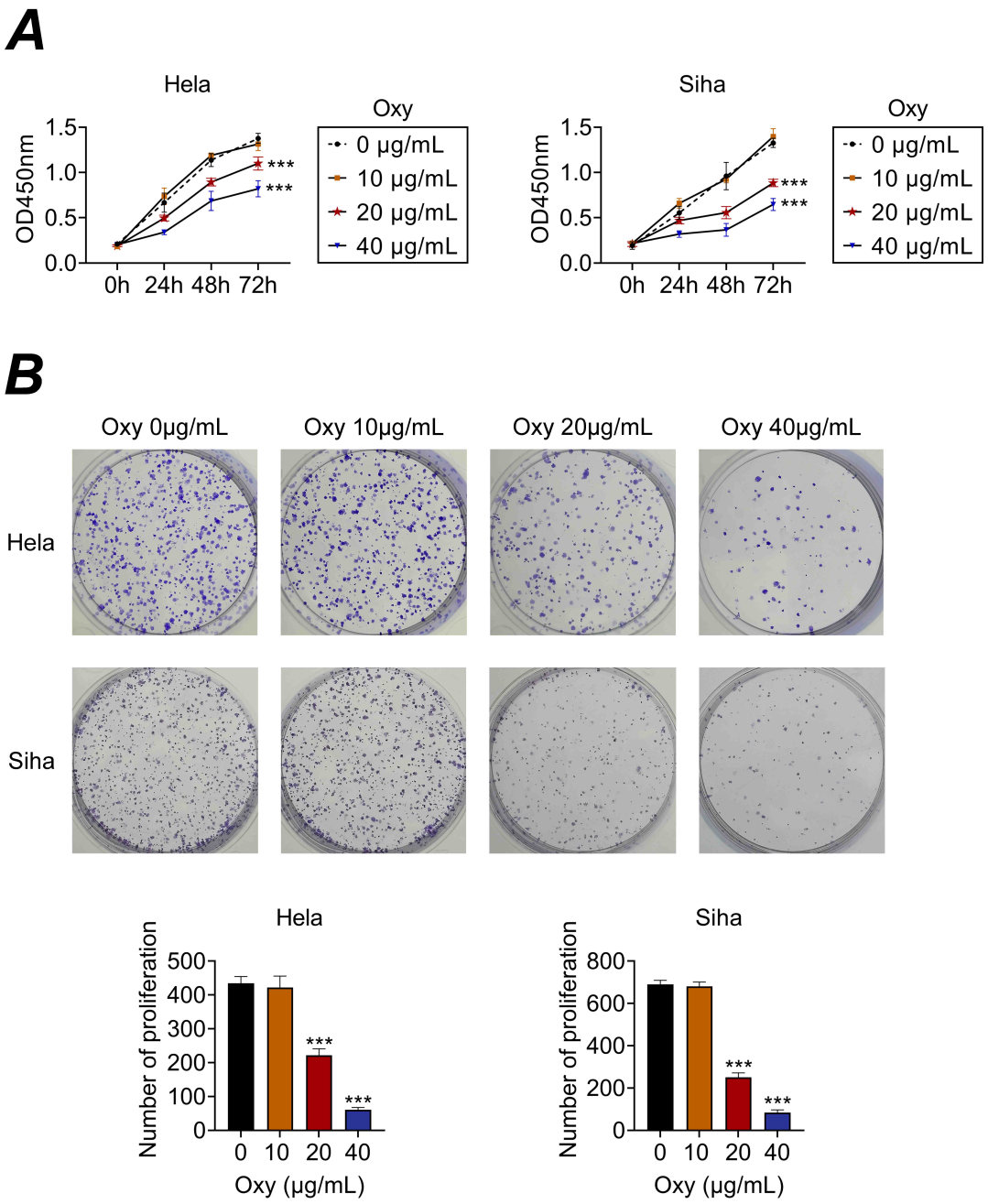

FIGURE 1. Oxycodone inhibits cervical cancer cell growth. (A) CCK-8 assays showed Hela and Siha cervical cancer cell viability treated with varying concentrations of oxycodone (0, 10, 20, 40 μg/mL) for 24 h. OD450 value was measured. (B) Colony formation assays showed Hela and Siha cervical cancer cell viability treated with varying concentrations of oxycodone (0, 10, 20, 40 μg/mL) for 24 h. Colony number was measured. Data were presented as mean ± SD. ***$p < 0.001$. Oxy, oxycodone; OD450, optical density450.

3.4 Oxycodone regulates VEGFA expression to inhibit CC cell migration and angiogenesis

To further elucidate the mechanism underlying oxycodone's inhibitory effects on migration and angiogenesis, rescue experiments were conducted using exogenous VEGFA. Immunoblot confirmed the increase in VEGFA expression upon VEGFA plasmid transfection into HeLa cells upon oxycodone treatment (Fig. 4A). In the presence of exogenous VEGFA, oxycodone's inhibitory effects on growth and motility were significantly reversed, confirmed by CCK-8 and wound healing assays (Fig. 4B,C). Similarly, for angiogenesis, oxycodone's inhibitory effects were significantly reversed in the presence of exogenous VEGFA (Fig. 4D). Oxycodone suppresses cervical cancer cell motility and angiogenesis by modulating VEGFA expression.

4. Discussion

Cervical cancer (CC) poses a significant challenge worldwide [12]. HPV is a major etiology for CC, which can cause precancerous lesions to progress to invasive cancer if untreated [13]. Standard CC treatments include surgery, radiation therapy, and chemotherapy [12]. Despite advances, advanced

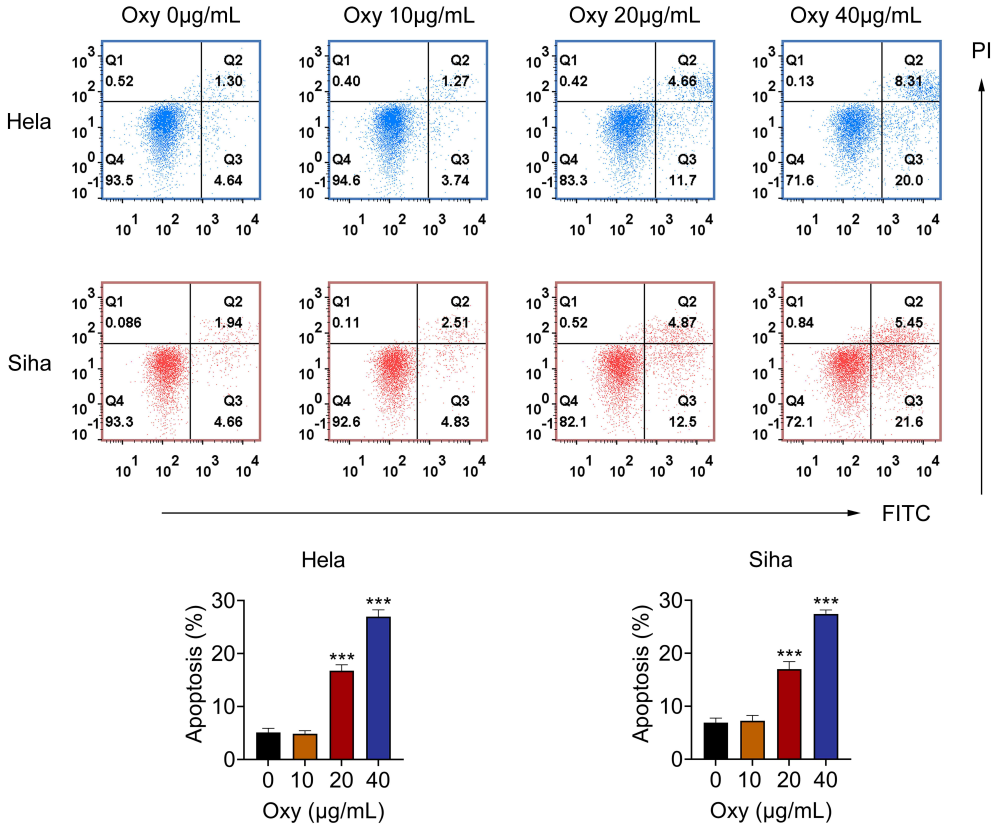

FIGURE 2. Oxycodone induces cervical cancer cell apoptosis. FCM assays showed Hela and Siha cervical cancer cell apoptosis treated with varying concentrations of oxycodone (0, 10, 20, 40 μg/mL) for 24 h. Cell apoptosis percentage was measured. Data were presented as mean ± SD. ***$p < 0.001$. Oxy, oxycodone; PI, propidium iodide; FITC, fluorescein isothiocyanate.

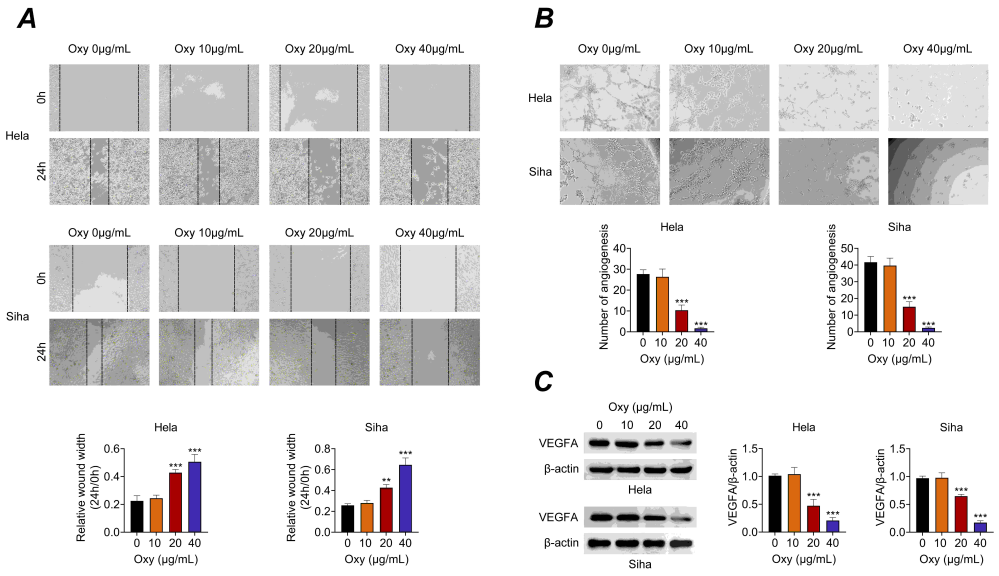

FIGURE 3. Oxycodone suppresses cervical cancer cell migration and angiogenesis. (A) Wound healing assays showed Hela and Siha cervical cancer cells' migration degree treated with varying concentrations of oxycodone (0, 10, 20, 40 μg/mL) for 24 h. Relative wound width was measured. (B) Tube formation assays showed Hela and Siha cervical cancer cells' angiogenesis degree treated with varying concentrations of oxycodone (0, 10, 20, 40 μg/mL) for 24 h. Number of angiogenesis was compared. (C) Immunoblot showed VEGF expression in Hela and Siha cervical cancer cells treated with varying concentrations of oxycodone (0, 10, 20, 40 μg/mL) for 24 h. Data were presented as mean ± SD. **$p < 0.01$, ***$p < 0.001$. Oxy, oxycodone; VEGFA, vascular endothelial growth factor A.

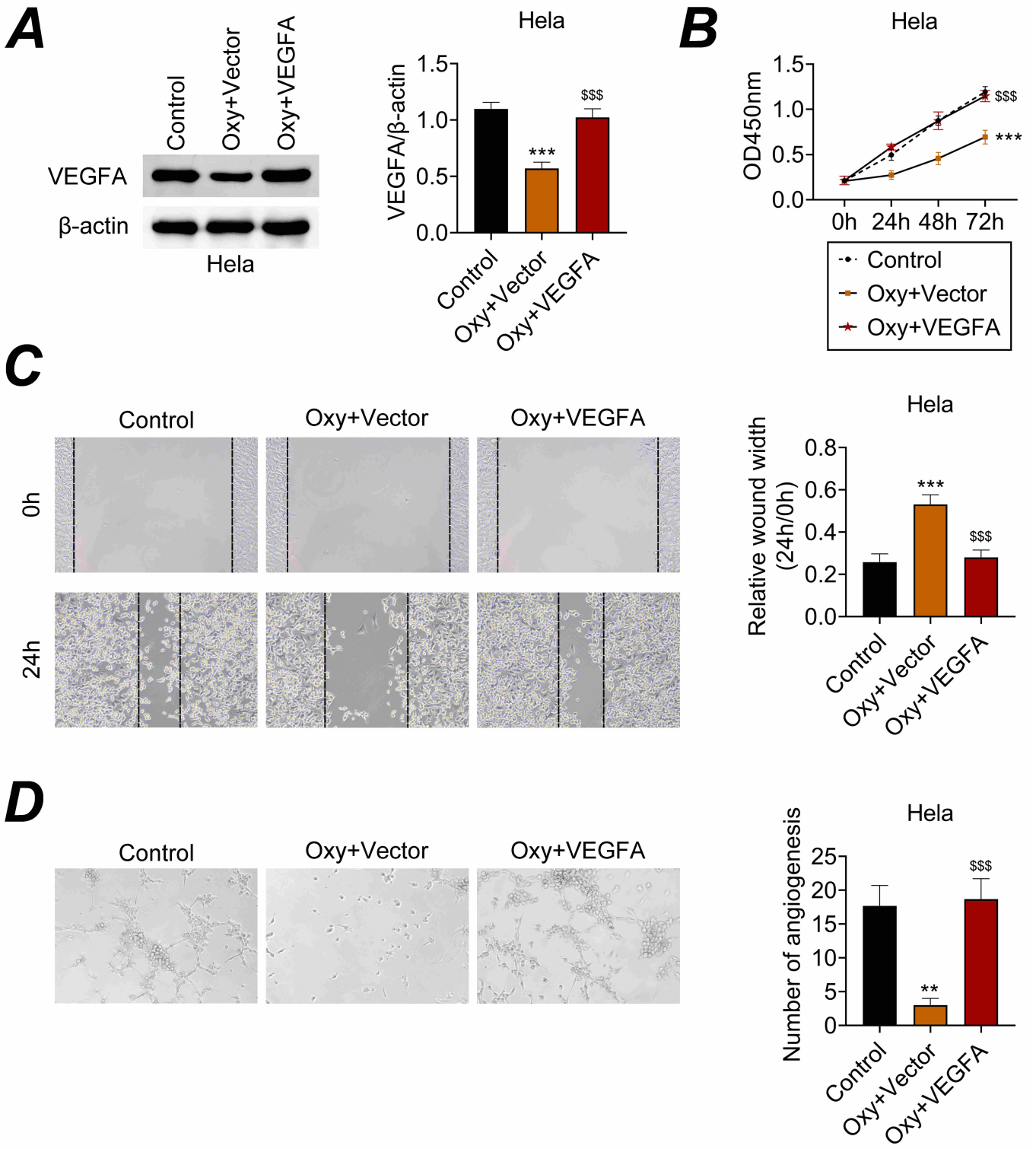

FIGURE 4. Oxycodone regulates VEGFA expression to inhibit cervical cancer cell migration and angiogenesis. (A) Immunoblot showed VEGFA expression in Hela cells upon the indicated treatment and transfection. (B) CCK-8 assays showed Hela and Siha cervical cancer cell viability upon the indicated treatment. (C) Wound healing assays showed Hela and Siha cervical cancer cells' migration degree upon the indicated treatment. (D) Tube formation assays showed Hela and Siha cervical cancer cells' angiogenesis degree upon the indicated treatment. Data were presented as mean ± SD. $^{**}p < 0.01$, $^{***}p < 0.001$, *vs.* control. $^{\$\$\$}p < 0.001$, Oxy + VEGFA *vs.* Oxy + Vector. Oxy, oxycodone; VEGFA, vascular endothelial growth factor A; OD450, optical density450.

CC's prognosis remains poor, necessitating new therapeutic approaches. It has been shown that anesthesia can influence cancer cell behavior and has the potential to be repurposed as therapeutic agents [14]. This study investigates the potential of oxycodone, a semi-synthetic opioid, to inhibit CC growth, motility, and angiogenesis by modulating VEGFA expression.

A growing body of research suggests that anesthetic agents act both as pain relievers and anti-cancer agents. For example, oxycodone inhibits lung cancer cell growth and reduces tumor growth in hepatocellular carcinoma models [15, 16]. Anesthetics are capable of being repurposed for anti-cancer therapy. Herein, oxycodone significantly inhibits CC cell growth and motility, induces apoptosis, and decreases VEGFA expression levels. Oxycodone's anti-cancer effects are also documented in other studies. Angiogenesis is essential for tumor growth and metastasis, providing the necessary nutrients and oxygen to cancer cells. Angiogenesis is regulated by VEGF, which is commonly overexpressed in various cancers, including CC [17, 18]. Angiogenesis and VEGF targeting are promising cancer treatment strategies. Anti-angiogenic therapies, such as bevacizumab, which targets VEGF, have shown efficacy in treating advanced cancers by inhibiting tumor vascularization [19]. Our study indicates that oxycodone inhibits angiogenesis in CC cells by downregulating VEGFA expression. Therefore, oxycodone may be an effective anti-angiogenic therapy for CC.

Oxycodone, well-known for its analgesic properties, primarily functions by binding to opioid receptors and modulating pain signals in the central nervous system [20]. Beyond pain relief, oxycodone affects cellular processes such as growth, migration, and apoptosis [21–23]. The effectiveness of this drug has been demonstrated in various cancer models. By inhibiting cervical cancer cell growth and migration, inducing apoptosis, and downregulating VEGFA expression, our findings add to this growing body of evidence showing that oxycodone is effective against CC.

Oxycodone's role in oncology is gaining interest, particularly in relation to tumor biology. Previous studies have highlighted its potential to inhibit lung cancer cell growth and liver cancer progression, demonstrating its versatile anti-cancer properties. In our research, we show that oxycodone can modulate VEGFA expression and inhibit key processes involved in tumor progression, thereby positioning oxycodone as a promising multi-functional oncology therapeutic agent.

Despite these encouraging findings, our study has several limitations. Oxycodone's efficacy and safety in treating CC remains to be validated *in vivo* due to the *in vitro* nature of this research. Also, oxycodone's effects on VEGFA expression and angiogenesis should be further investigated. Future research should also explore the potential interactions between oxycodone and standard cancer therapies to optimize treatment regimens.

5. Conclusions

This study demonstrates that oxycodone significantly inhibits CC cell growth and motility, induces apoptosis, and downregulates VEGFA expression, thereby reducing angiogenesis. These findings offer both pain management and direct anti-cancer properties. For a clearer understanding of its therapeutic potential and clinical applications, further research is needed.

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