

TOP2A Promotes Cell Migration, Invasion and Epithelial–Mesenchymal Transition in Cervical Cancer via Activating the PI3K/AKT Signaling

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Bi Wang,^{1,2,*} Yaping Shen,^{3,*}
Yin Zou,⁴ Zhengjun Qi,⁴
Guijia Huang,⁴ Shan Xia,⁵
Rui Gao,⁶ Fenghu Li,⁵ Zhi
Huang⁷ 

¹Key Laboratory of Endemic and Ethnic Diseases, Ministry of Education, Guizhou Medical University, Guiyang, Guizhou, People's Republic of China; ²Department of Paediatrics, Maternal and Child Health Hospital of Guiyang City, Guiyang, Guizhou, People's Republic of China; ³Department of Interventional Radiology, The Affiliated Baiyun Hospital of Guizhou Medical University, Guiyang, Guizhou, People's Republic of China; ⁴Department of Oncology, The Second Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, People's Republic of China; ⁵Department of Gynecologic Oncology, The Affiliated Cancer Hospital of Guizhou Medical University, Guiyang, Guizhou, People's Republic of China; ⁶Guiyang Customs Guizhou International Travel Healthcare Center, Guiyang, Guizhou, People's Republic of China; ⁷Department of Interventional Radiology, The Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, People's Republic of China

*These authors contributed equally to this work

Correspondence: Fenghu Li
Department of Gynecologic Oncology,
The Affiliated Cancer Hospital of Guizhou
Medical University, Guiyang City, Guizhou
Province, People's Republic of China
Email fenghuli19@163.com

Zhi Huang
Department of Interventional Radiology,
The Affiliated Hospital of Guizhou
Medical University, Guiyang City, Guizhou
Province, People's Republic of China
Email doctor@huangzhi.com

Background/Objective: Topoisomerases type IIA (TOP2A) was identified to present with a high-expression pattern in cervical cancer. However, TOP2A role in the progression of cervical cancer remains unknown. Here, we aimed to explore the effect and reveal the underlying mechanism of TOP2A in the migration, invasion and epithelial–mesenchymal transition (EMT) of cervical cancer.

Materials and Methods: The expression profiles of TOP2A in 20 paired cervical cancer tissues and the paracancerous normal tissues were detected by using Western blotting assay. Transwell chambers were used to test cell migration and invasion abilities. Cell morphology and the expressions of E-cadherin and N-cadherin were detected to assess cell EMT. LY294002 was used to inhibit the activation of PI3K/AKT signaling.

Results: Compared with the paracancerous normal tissues, TOP2A was overexpressed in 85% (17/20) cervical cancer tissues. Repression of TOP2A expression in SiHa cells significantly weakened cell migration and invasion abilities, reduced cell numbers in shuttle shape and increased E-cadherin expression while decreased E-cadherin expression. To the opposite, overexpression of TOP2A in Hela cells induced opposite results. In addition, the expression of p-AKT was increased when TOP2A was overexpressed in Hela cells, and p-AKT expression was decreased when TOP2A was silenced in SiHa cells. Moreover, suppression of the PI3K/AKT signaling with LY294002 treatment apparently rescued TOP2A-mediated promotions in cell migration, invasion and EMT in Hela cells.

Conclusion: This study reveals that TOP2A is abnormally overexpressed in cervical cancer tissues, and TOP2A overexpression leads to cell migration, invasion and EMT via activating PI3K/AKT signaling.

Keywords: topoisomerases type IIA, epithelial–mesenchymal transition, cell morphology, migration, PI3K/AKT signaling

Introduction

Cervical cancer ranks the second common cancer in developing countries and is the third most common reason for cancer-related deaths in women, with 570,000 new cases and 311,000 deaths per year worldwide.¹ The persistent infection of human papillomavirus (HPV) is the major pathogenesis responsible for cervical cancer, and HPV16 and HPV18 accounts for about 73% of all the cervical cancer cases among more than 100 HPV genotypes.^{2,3} Although improvements have been achieved in the diagnostic and therapeutical techniques, majority patients with cervical cancer showed metastasis present with high incidence in cervical cancer, which is one of

the main reasons for the mortality in patients with cervical cancer.⁴ In consequence, it is necessary to explore the molecular mechanisms underlying the metastasis of cervical cancer.

Epithelial–mesenchymal transition (EMT) is a biological process involving the polarization of epithelial cells that enable cells to acquire mesenchymal cell phenotypes.⁵ Importantly, EMT confers cancer cell several properties, such as the enhanced migratory and invasive capacities and the increased resistance to apoptosis.⁶ EMT has been verified to play an important role in the metastasis and invasion of majority kinds of cancers, including cervical cancer,^{7,8} indicating a vital value of EMT possesses in cancer treatment.

Topoisomerases type IIA (TOP2A), as one of the two members of topoisomerase II (TOP2) family, with another is TOP2B, is expressed only in cycling cells and responsible for enzymatic uncoupling during the replication of DNA strands.⁹ TOP2A catalyzes double-strand DNA breaking and induces gene transcription during mitosis.^{10,11} Noticeably, TOP2A has been reported to be overexpressed in colon cancer tissues when compared with the adjacent normal tissues, and TOP2A depletion induced significant decreases in cell proliferation and invasion abilities and promoted cell apoptosis in both colon cancer HCT116 and SW480 cells.¹² In cervical cancer, TOP2A was shown to overexpress during the transformation of cervical intraepithelial neoplasia 2 (CIN2)-CIN3 to cervical cancer.^{13,14} However, the role and mechanisms of TOP2A in the development and progression of cervical cancer still remain unknown.

The PI3K/AKT signaling is an important intracellular signaling pathway which can promote migration and induce EMT in many types of tumors including cervical.¹⁵ Wang et al¹⁶ reported that TOP2A was associated with the activation of PI3K/AKT and Wnt/ β -catenin signaling pathways in testicular seminoma. Therefore, we speculated that TOP2A might play a role in the migration, invasion and EMT of cervical cancer via regulating the activation of PI3K/AKT signaling.

In this study, we aimed to reveal the roles of TOP2A in the EMT, migration and invasion of cervical cancer, and to explore whether PI3K/AKT signaling was involved in. Our results showed that TOP2A was highly expressed in cervical cancer tissues, and TOP2A overexpression significantly facilitated cervical cancer cell migration, invasion and EMT, but these results were significantly repressed when the PI3K/AKT signaling was repressed by LY294002.

Materials and Methods

Tissue Samples and Ethic Statements

Twenty paired cervical cancer tissues and the paracancerous normal tissues were obtained from patients with cervical cancer (mean age 48.5 ± 7.3 years old). All samples were collected between January 2015 and January 2018 from the Affiliated Cancer Hospital of Guizhou Medical University. The written informed consents were obtained from all patients before this study. The protocols involving human samples were conducted in accordance with the Declaration of Helsinki and were approved by the Ethic Committee of the Affiliated Cancer Hospital of Guizhou Medical University.

Cell Lines and Culture

Six human cervical cancer cell lines, including SiHa, CC-10A, CaSki, CAC-1, CaLo, and HeLa were acquired from the American Type Culture Collection (ATCC, Manassas, VA, USA). All of the cell lines were grown in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% (v/v) penicillin and streptomycin, and maintained in a humidified atmosphere at 37°C with 5% CO₂. All of the above reagents were obtained from Thermo Fisher Scientific (MA, USA).

Inhibition of PI3K/AKT Signaling

To repress the activation of PI3K/AKT signaling, the cervical cancer cells were treated with 25 μ M LY294002 (MedChemExpress, Shanghai, China), a selective inhibitor of PI3K/AKT signaling for 24 hours.

Cell Transfection

To upregulate or downregulate TOP2A expression, the cervical cancer cells were instantaneously transfected with the small interfering RNAs (siRNAs, si-TOP2A, cat. no. SR322074, OriGene, Beijing, China) or the overexpressing plasmid of TOP2A (OE-TOP2A, cat. no. SC127832, OriGene). Cell transfection was carried out by using the Lipofectamine[®] 2000 reagent (Thermo Fisher Scientific) based on the manufacturer's instructions, when cell confluence reached at 50–60%. In addition, the negative controls of si-TOP2A and OE-TOP2A vectors were also obtained from OriGene, which were named as si-NC and OE-NC.

Western Blotting

With the help of RIPA lysis buffer (Solarbio, Beijing, China) and protease inhibitor cocktail (Roche Diagnostics,

Indianapolis, IN), total proteins were extracted from cervical tissues and cells. Following protein concentrations being measured by using the Bradford assay (BCA, Thermo Fisher Scientific), same amount of protein samples (25 μ g) from various groups were separated by the SDS-PAGE and blotted onto the nitrocellulose filter membranes (Millipore, MA, USA). Then, the membranes were sealed with 5% non-fat skim milk for 30 min at room temperature. After that, the membranes were probed with the primary antibodies at 4°C overnight and then incubated with the corresponding HRP-conjugated second antibodies (Santa Cruz Biotechnology, Shanghai, China) at room temperature for 1 hour. The primary antibodies used in this experiment included TOP2A (cat. no. ab52934; 1:1000 dilution), p-AKT (cat. no. ab38449; 1:1000 dilution), AKT (cat. no. ab8805; 1:2000 dilution), and GAPDH (cat. no. ab181602; 1:5000 dilution), all obtained from Abcam (Cambridge, MA, USA). After that, the protein levels in the bands were quantified by using an enhanced chemiluminescence (ECL) detection system (Millipore).

Transwell Chamber Assay

To evaluate the effect of TOP2A/AKT on the invasion and migration of cervical cancer cells, transwell chambers with/without basement membrane matrix (8- μ m pore; Corning, Life Science, Lowell, MA, USA) were applied, respectively. In brief, the transfected cells (5×10^4 /well) were suspended in serum-free medium and inoculated in the upper chambers, while the lower chambers were

supplemented with 600 μ L cell culture medium with 15% FBS. Then, the cells were maintained at 37 °C with 5% CO₂ for 24 hour and 48 hours to detect cell migration and invasion abilities, severally. After that, cells in the lower of the upper chambers were stained with 1% crystal violet for 20 min at room temperature. Cell invasion and migration abilities were assessed by counting cell numbers with positive staining in six randomly selected regions.

Statistical Analysis

Statistical analyses were performed using the SPSS software (version 22.0). Data in this study were expressed as mean \pm SD. Comparisons between 2 groups were executed by using the student's *t*-test, including TOP2A expression levels between tumor tissues and normal tissues in [Figures 1A](#) and [Supplementary Figure 1](#), and TOP2A expression in [Table 1](#). And, comparisons between multiple groups were executed by using the one-way ANOVA followed by Tukey post hoc, including protein expression, cell invasion and cell migration abilities between 3 groups ([Figures 2, 4](#) and [5](#)). If a *p* value is less than 0.05, the differences between groups were considered statistically significant.

Results

TOP2A is Overexpressed in Cervical Cancer Tissues

To reveal the role of TOP2A plays in the development and progression of cervical cancer, we first compared the expression patterns of TOP2A in 20 cases of cervical cancer tissues

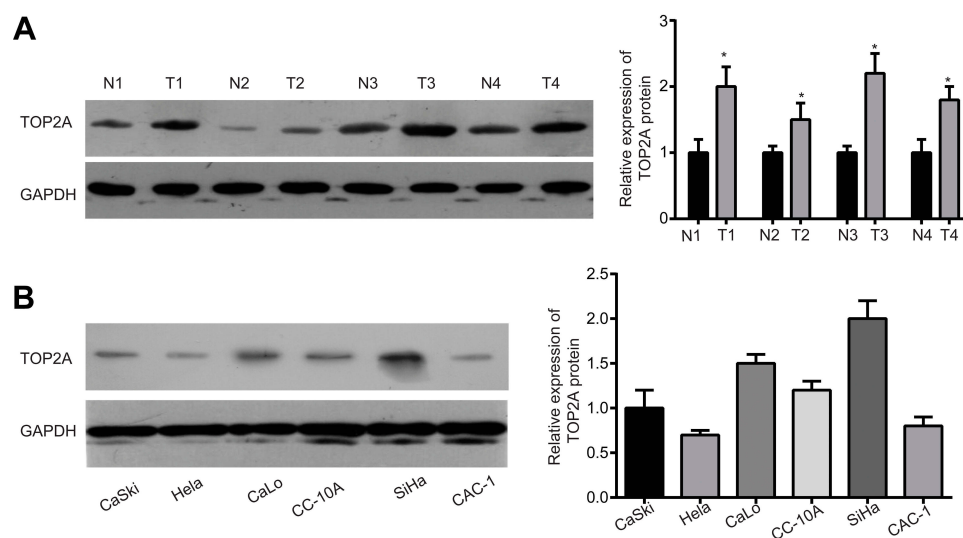


Figure 1 TOP2A expression was increased in cervical cancer tissues. **(A)** The expression of TOP2A protein in 4 paired cervical cancer tissues and the adjacent normal tissues was determined by using the Western blotting assay. **(B)** Western blotting analysis of the protein levels of TOP2A in six cervical cancer cell lines. (**p*<0.05).

Abbreviations: TOP2A, Topoisomerase type IIA; N, Normal tissue; T, Tumor tissue; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Table 1 Relationship Between TOP2A Expression and Clinicopathological Features in 20 Cervical Cancer Cases (* $p < 0.05$)

Variance	Number of Patients (%)	TOP2A (Relative Expression To Normal Group, Mean \pm SD)	p value
Stage			
I B1	3 (15.0)	1.3 \pm 0.3	*
I B2	10 (50.0)	2.0 \pm 0.4	
II A	7 (35.0)	2.6 \pm 0.4	
Differentiation			
Poorly	7 (35.0)	2.6 \pm 0.5	*
Well+moderately	13 (65.0)	1.9 \pm 0.4	
Tumor Size			
<3 cm	10 (50.0)	1.9 \pm 0.3	*
\geq 3cm	10 (50.0)	2.2 \pm 0.4	
Lymph Node Metastasis			
Negative	11 (55.0)	2.2 \pm 0.4	*
Positive	9 (45.0)	3.1 \pm 0.6	

Abbreviations: TOP2A, Topoisomerases type IIA; Stage I B1, the maximum diameter of the tumor < 2 cm; Stage I B2, 2 cm < the maximum diameter of the tumor < 4 cm.

and the paired adjacent normal tissues. Compared with the normal tissues, TOP2A protein level was significantly increased in 85% (17/20) of cervical cancer tissues

(Figures 1 and Supplementary Figure 1), and 4 representative images selected from the 17 paired cancer tissues with TOP2A high expression were shown in Figure 1A. In addition, TOP2A expression was significantly increased in cervical cancer tissues with advanced TNM stage, poor differentiation, large tumor size and lymph node metastasis (Table 1). Moreover, we randomly selected six cervical cancer cell lines (SiHa, CC-10A, CaSki, CAC-1, CaLo, and HeLa) and detected the expression levels of TOP2A in these cell lines. The results demonstrated that TOP2A protein showed different expression patterns of TOP2A in SiHa, CC-10A, CaSki, CAC-1, CaLo, and HeLa cells, with HeLa the lowest and SiHa the highest (Figure 1B). Therefore, we chose HeLa and SiHa cell lines for the next experiments.

TOP2A Promotes the Migration and Invasion of Cervical Cancer Cells

Then, both of the loss-of-function assays in SiHa cells and the gain-of-function assays in HeLa cells were performed to investigate the effect of TOP2A on the migration and invasion of cervical cancer cells. The expression of TOP2A protein was significantly decreased when SiHa cells were transfected with the si-TOP2A as compared with the si-NC group, and si-NC transfection induced no

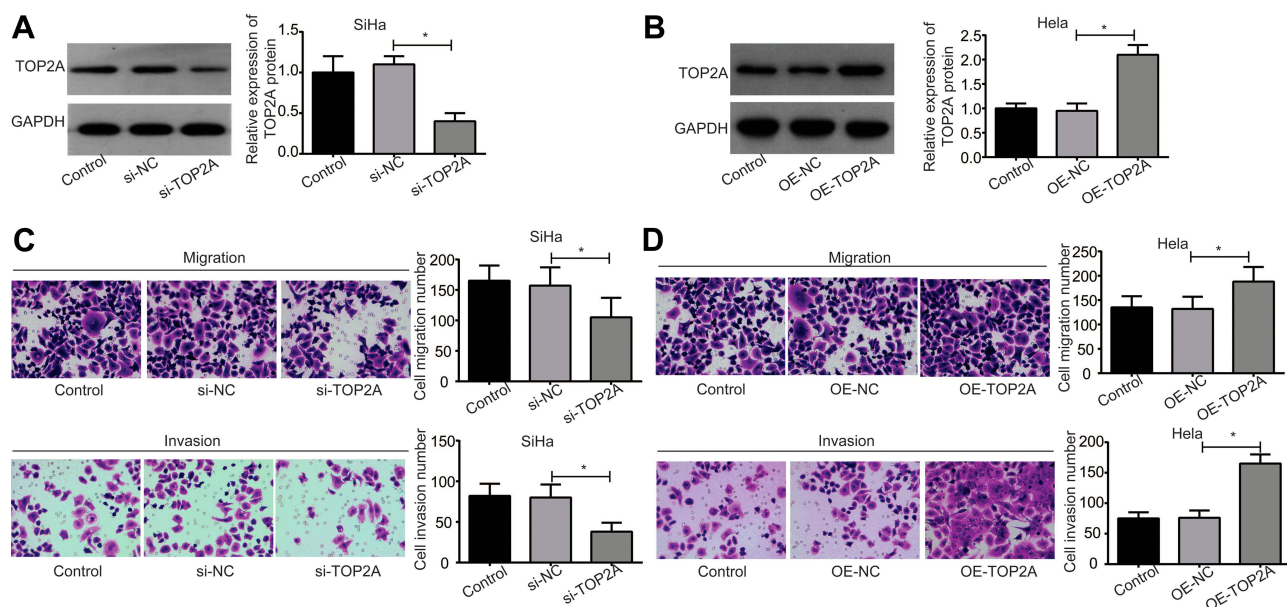


Figure 2 Evaluation of the effect of TOP2A on the migration and invasion of cervical cancer cells. **(A)** The expression of TOP2A protein was detected by using the Western blotting assay after SiHa cells were transfected with the si-NC or si-TOP2A. **(B)** The expression of TOP2A protein was detected by using the Western blotting assay after HeLa cells were transfected with the OE-NC or OE-TOP2A. **(C)** The migration and invasion abilities of SiHa cells were determined by using the transwell chambers after the cells were transfected with si-NC or si-TOP2A. **(D)** The effect of TOP2A overexpression on the migration and invasion of HeLa cells were measured by using the transwell chambers. (* $p < 0.05$).

Abbreviations: TOP2A, Topoisomerases type IIA; si, small interfering RNA; NC, negative control; OE, overexpressing; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

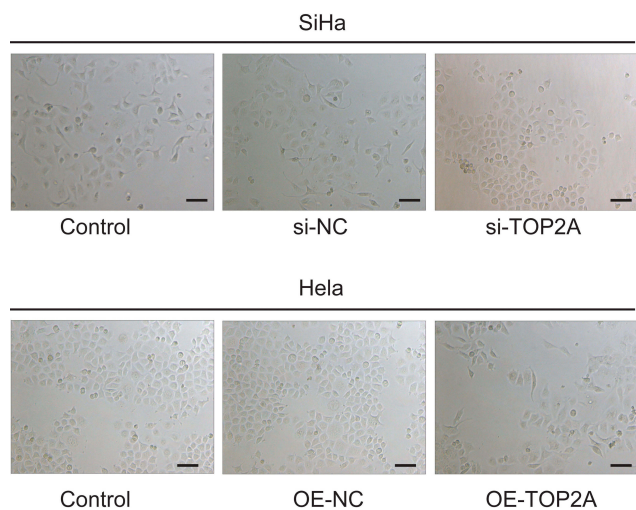


Figure 3 Assessment of TOP2A roles in cervical cell EMT. The cell morphology of HeLa and SiHa cells in different groups was recorded using the inverted microscope. (Scale bar=100 μ m).

Abbreviations: TOP2A, Topoisomerases type IIA; si, small interfering RNA; NC, negative control; OE, overexpressing; EMT, epithelial–mesenchymal transition.

obvious change in the expression of TOP2A compared to control group (Figure 2A). However, OE-TOP2A transfection led to an obvious increase in the protein level of TOP2A in HeLa cells as compared with the OE-NC group (Figure 2B). Knockdown of TOP2A significantly weakened SiHa cell migration and invasion abilities (Figure 2C), and overexpression of TOP2A in HeLa cells significantly enhanced cell migration and invasion abilities (Figure 2D). These results demonstrate that TOP2A facilitates cell migration and invasion in cervical cancer.

TOP2A Induces EMT and PI3K/AKT Activation in Cervical Cancer Cells

To reveal the underlying mechanism of TOP2A in promoting cervical cancer migration and invasion, we also assessed the effects of TOP2A on EMT process and the activation of PI3K/AKT signaling. The results showed that the cell morphology of SiHa was transformed from shuttle shape to roundness when TOP2A was downregulated (Figure 3), whereas the morphology of HeLa cells was transformed to shuttle shape when TOP2A was overexpressed (Figure 3). In addition, the expression levels of N-cadherin and p-AKT were significantly decreased, and E-cadherin expression was increased when SiHa cells were transfected with si-TOP2A (Figure 4). To the contrary, the expression levels of N-cadherin and p-AKT were increased and E-cadherin expression was decreased when HeLa cells were transfected with OE-TOP2A (Figure 4). These results illustrate that TOP2A induces EMT and PI3K/AKT activation in cervical cancer cells.

TOP2A Promotes Cervical Cancer Cell Migration, Invasion and EMT via Activating the PI3K/AKT Signaling

Finally, we explored the effect of PI3K/AKT pathway on TOP2A-mediated cervical cancer cell migration, invasion and EMT. LY294002, an inhibitor of PI3K/AKT signaling was recruited to repress the activation of PI3K/AKT signaling in HeLa cells. Compared with the OE-TOP2A group, the expression of p-AKT was significantly reduced when HeLa

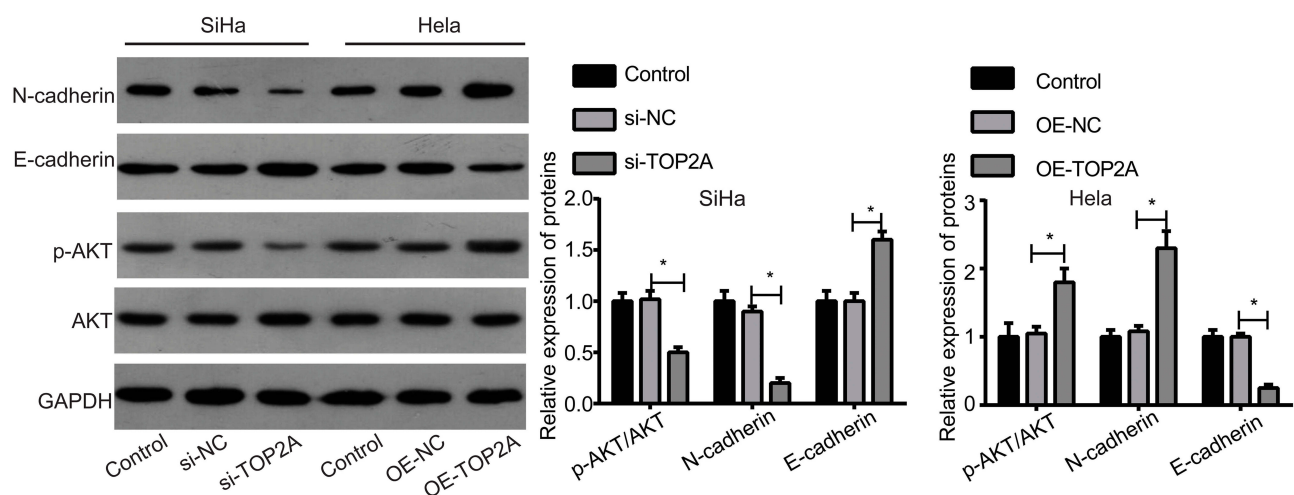


Figure 4 Assessment of the effects of TOP2A on the expressions of E-cadherin, N-cadherin, p-AKT and AKT. The protein levels of p-AKT, AKT, N-cadherin and E-cadherin were tested by Western blotting in different groups of HeLa and SiHa cell lines. (* $p < 0.05$).

Abbreviations: TOP2A, Topoisomerases type IIA; si, small interfering RNA; NC, negative control; OE, overexpressing; AKT, serine/threonine kinase; p-AKT, phosphorylated-serine/threonine kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

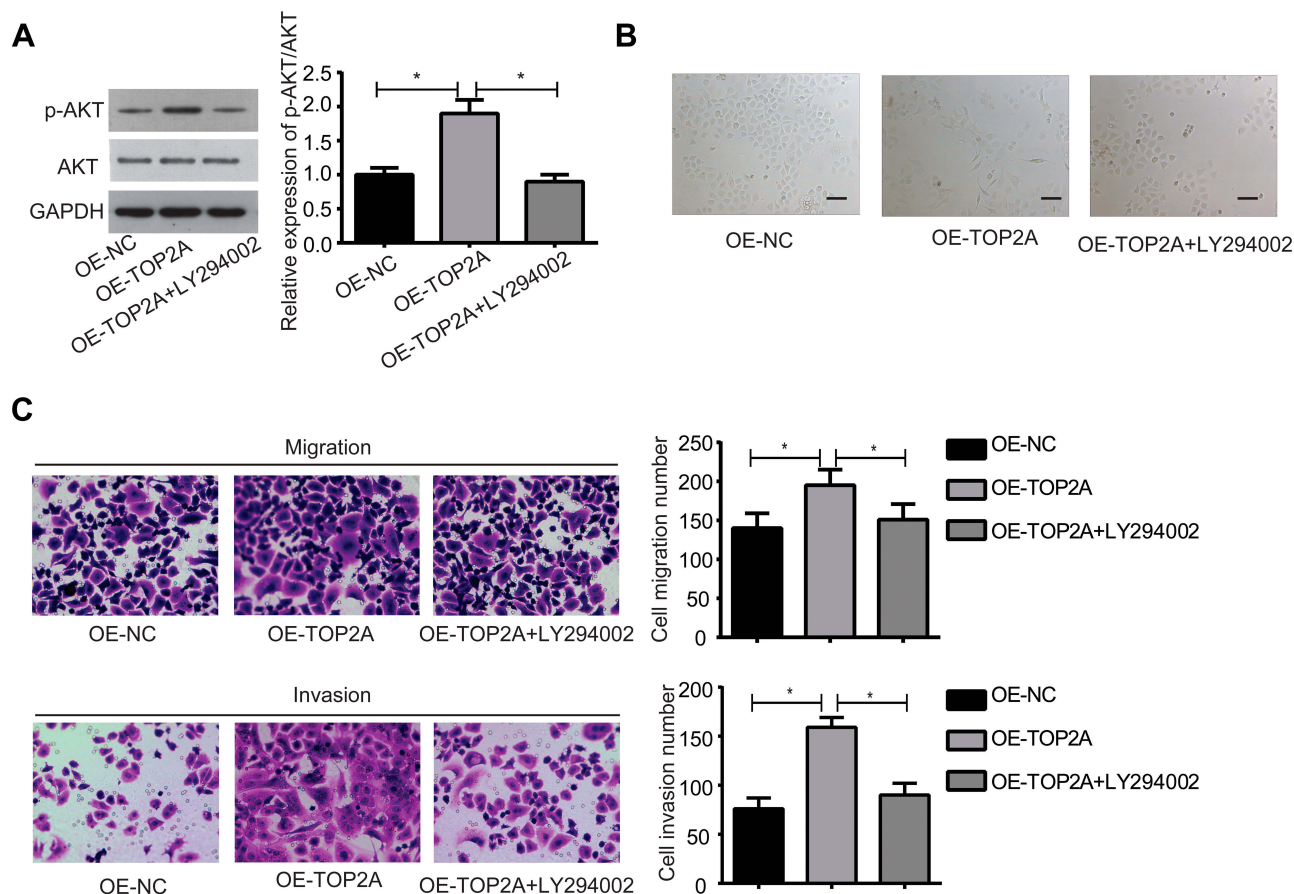


Figure 5 Inhibition of the PI3K/AKT signaling rescued TOP2A roles in the promotions of cell EMT, migration and invasion in HeLa cells. HeLa cells were divided into the OE-NC, OE-TOP2A and OE-TOP2A+LY294002 groups and were submitted to the following experiments. **(A)** Western blotting assay was used to detect the expressions of p-AKT and AKT proteins. **(B)** Cell morphology was recorded using the inverted microscope (Scale bar=100 μ m). **(C)** Cell migration and invasion were assessed by using the transwell chambers. (* p <0.05).

Abbreviations: TOP2A, Topoisomerases type IIA; NC, negative control; OE, overexpressing; p-AKT, phosphorylated-serine/threonine kinase; AKT, serine/threonine kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; EMT, epithelial-mesenchymal transition; PI3K, phosphatidylinositol 3-kinase.

cells were treated with LY294002 (Figure 5A). In addition, LY294002 administration dramatically abolished the morphology change of HeLa cells induced by TOP2A overexpression (Figure 5B). Moreover, LY294002 treatment obviously rescued the promotions of cell migration and invasion caused by TOP2A overexpression in HeLa cells (Figure 5C). These results show that TOP2A promotes cervical cancer cell migration, invasion and EMT via activating the PI3K/AKT signaling.

Discussion

It's well known that the malignant progression of cervical cancer is closely associated with the enhanced motility and invasiveness of cancer cells.¹⁷⁻¹⁹ In this study, we investigated the effects of TOP2A on the migration, invasion and EMT of cervical cancer cells. Our results demonstrated that upregulation of TOP2A significantly enhanced

the invasion, migration and EMT of cervical cancer cells via facilitating the activation of PI3K/AKT signaling.

TOP2A, as a member of TOP2 family, is essential for DNA replication, chromosome condensation, and the separation of sister chromatids during mitosis, as well as plays an important role in the transcriptional initiation.²⁰ Up to now, TOP2A was shown to be implicated in the progression of many kinds of cancers. A most well-known cancer associated with TOP2A is breast cancer, in which TOP2A is usually simultaneously deleted or overexpressed with ERBB2 (erb-b2 receptor tyrosine kinase 2), thus the two genes are usually co-tested to guide the usage of Herceptin, an anticancer agent.^{21,22} With the help of bioinformatic analysis and immunohistochemistry (IHC) technology, Ma et al.²³ revealed that TOP2A was overexpressed in non-small cell lung cancer (NSCLC) comparing to normal lung tissues, which associated with the worse overall survival in NSCLC patients. Song et al.²⁴ demonstrated that TOP2A

expression was dramatically increased following human cytomegalovirus (HCMV) infection in glioma cells, and miR-144-3p upregulation significantly reduced TOP2A expression to inhibit cell viability and invasion of HCMV-positive glioma cells. Labbe et al²⁵ found that the high expression of TOP2A and EZH2 in prostate cancer patients closely linked to aggressive disease progression. In addition, they also clarified that TOP2A and EZH2 acted as key driving oncogenes in prostate cancer, which was concluded from a fact of sensitivity to combination-targeted therapy.²⁵ In cervical cancer, TOP2A expression was not only identified to be increased, but also associated with HPV infection,^{13,14,26} indicating that TOP2A might exert a role in the progression of cervical cancer. To further elucidate the specific role of TOP2A in cervical cancer progression, we first investigated its expression pattern in cervical cancer tissues and cells. The results showed that TOP2A expression was significantly upregulated in cervical cancer tissues, especially in cervical cancer tissues with advanced TNM stage, poor differentiation, large tumor size and lymph node metastasis, as compared with the normal tissues. In addition, we observed that TOP2A was differently expressed in various cervical cancer cell lines, including SiHa, CC-10A, CaSki, CAC-1, CaLo, and HeLa, among which SiHa showed the highest expression and HeLa showed the lowest expression. Moreover, we clarified that TOP2A overexpression in HeLa cells markedly enhanced cell migration and invasion abilities, while knockdown of TOP2A in SiHa cells caused the opposite results.

EMT is of importance to induce cancer cell migration and invasion.⁸ In this process, epithelial cells transform into mesenchymal cells with decreased expression of epithelial cells markers, such as E-cadherin, and increased expression of mesenchymal cell markers, such as N-cadherin.²⁷ To further reveal the role and mechanism underlying TOP2A in promoting the migration of cervical cancer, we also explored TOP2A function in the EMT of cervical cancer cells. Consistent with cell migration and invasion, TOP2A upregulation dramatically accelerated the EMT process in cervical cancer cells with a decreased expression of E-cadherin and an increased expression of N-cadherin, as well as induced cell morphology to shuttle shape.

The hyper-activation of PI3K/AKT signaling can significantly accelerate cancer cell proliferation, migration and EMT and inhibit cell apoptosis in majority kinds of cancers, including cervical cancer.^{28,29} A work by Wang et al¹⁶ in testicular seminoma using bioinformatics analysis demonstrated that TOP2A expression was associated with the

activation of PI3K/AKT and Wnt/ β -catenin signaling pathways. Here, we found that TOP2A overexpression significantly promoted the activation of PI3K/AKT signal with increased expression of p-AKT in cervical cancer HeLa cells, suggesting that PI3K/AKT signal might be involved in TOP2A-mediated cervical cancer progression. To this end, LY294002 was applied to suppress the activation of PI3K/AKT signaling in HeLa cells. We observed that suppression of PI3K/AKT signaling apparently whittled TOP2A-mediated enhancements in cell migration, invasion and EMT.

In conclusion, our findings demonstrate that TOP2A is overexpressed in cervical cancer tissues, which then obviously promotes cancer cell migration, invasion and EMT via activating the PI3K/AKT signaling. This study might provide an efficient target for the treatment and prevention of cervical cancer migration.

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Disclosure

The authors declare that they have no competing interests.

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