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β-Elemene inhibits TGF-β1-induced EMT and invasion of cervical cancer cells through mediating β-catenin/TCF7/Sox2 signaling pathway

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1. Abstract

1.1 Aims: The aim of this study was to investigate the effect and mechanism of β-elemene on TGF-β1-induced EMT, the migration and invasion in cervical cancer.

1.2. Methods: Firstly, we investigated the expression of TGF-β1 and β-catenin by immunohistochemistry and determined their prognostic values in the cervical cancer. Then EMT model was established by stimulating SiHa and HeLa cells with TGF-β1 and further explore the mechanism of β-elemene on TGF-β1-induced EMT, migration and invasion of cervical cancer cells.

1.3. Results: The results showed that TGF-β1 and β-catenin was overexpressed in cervical cancer tissues, and was positively correlated with FIGO staging, grade, lymph vascular infiltration and nodal metastasis, Besides, TGF-β1 and β-catenin overexpression predicted lower cumulative survival rate. TGF β1 (10 ng/ml) could increase the migration and invasion but β-elemene (40 μg/ml) could significantly inhibit TGF-β1-induced invasion and migration in SiHa cells and HeLa cells. TGF-β1 could increase the expression of β-catenin, Sox2, MMP-2, MMP-9 and Vimentin and reduce the expression of E-cadherin and promote β-catenin entry into the nucleus. However, after the treatment with β-elemene, the effect of TGF-β1 was reversed The results of immunoprecipitation showed that β-catenin could bind to Sox2 and β-elemene could decrease the binding of β-catenin to Sox2 in cervical carcinoma, indicating that β-elemene could inhibit β-catenin/TCF7/Sox2 signaling pathway to play an anti-tumor role.

1.4. Conclusion: In conclusion, we demonstrate that β-elemene inhibits TGF-β1-induced EMT, the migration and invasion of cervical cancer cells through mediating β-catenin/TCF7/Sox2 signaling pathway.

2. Introduction

Cervical cancer is the second most common female malignant tumor due to relatively low cervical cancer screening and HPV vaccination rate in developing countries [1]. According to the World Health Organization (WHO), the mortality of cervical cancer will increase by 25% in the next decade [2]. Invasion represents the initiate step of the migration of malignant cells away from the primary site and the emergence of metastasis is the leading cause of cervical cancer-related death [3]. Therefore, a better understanding of the molecular mechanisms involved in invasion and metastasis of cervical cancer will provide more effective treatment and control for cervical cancer patients.

Transforming growth factor β (TGF-β) is highly expressed in cervical cancer and there are compelling data that TGF-β1 can

promote Epithelial-Mesenchymal Transition (EMT) and tumor metastasis in various human cancers [4-5]. As the key factor of phenotypic transformation of EMT, TGF-β1 plays a major role in EMT through TGF-β/smad pathway and TGF-β/non-smad signaling pathway, such as Wnt/β catenin, ERK1/2, p38 MAPK, JNK, PI3K, AKT/PKB and RhoA signal pathway via increasing the expression of EMT-inducing transcription factors, such as Snail1, Slug (Snail2) and Zinc Finger E-box-binding homeobox 1 and 2 (ZEB1/2) [6,7]. In addition, TGF-β1 can increase β3 integrin expression to enhance cancer cell intravasation and increase Tregs via Cox-2/PGE2 to suppress the proliferation of CD4+ T cells to promote tumor cells to distant metastasis [8]. At present, TGF-β1 is often used as the main inducement for cell culture in vitro to study EMT. Initially, the EMT model was established by using TGF-β1 stimulation for 36 hours with breast epithelial cells. and EMT changes occurred in breast epithelial cells [9]. In this study, firstly, the EMT model of cervical cancer cells induced by TGF-β1 was established, then the effects of TGF-β1 on morphology, migration and invasion of cervical cancer cells were observed. Tang Q et al have confirmed that TM4SF1 promotes EMT and cancer stemness via the Wnt/beta-catenin/SOX2 pathway in colorectal cancer [10]. However, the mechanism of TGF-β1 inducing EMT in cervical cancer is still unclear. In this study, we proposed for the first time that TGF-β1 can promote invasion and metastasis of cervical cancer through β- Catenin/TCF7/ Sox2 signaling pathway.

Curcuma has been clinically used to treat cervical inflammation and HPV infection in the 1940s [11]. β-Elemene (1-methyl-1-vi-nyl-2,4-diisopropylcyclohexane）, the main single component extracted from turmeric oil [12]. β-Elemene has been clinically used in the treatment of malignant tumors in brain, breast gland, liver and other tissues [13-14]. One of its preparations has been approved by the State Food and Drug Administration for the treatment of primary and secondary brain tumors. Zhu et al found that β-elemene reversed the malignant phenotypes of human glioblastoma cells through β-catenin-mediated regulation of stemness-, differentiation- and epithelial-to-mesenchymal transition-related molecules [15]. Our previous studies have confirmed that β-elemene can inhibit the proliferation of SiHa cells via attenuation of the wnt/ β-catenin signaling pathway [16]. However, the specific mechanism of β-elemene inhibiting the invasion and metastasis of cervical cancer remains unclear. In this study, we found that TGF-β1 promote EMT, migration and invasion of cervical cancer but the above responses could be reversed after treatment with β-elemene and we further revealed that β-elemene inhibits TGF-β1-induced EMT and invasion of cervical cancer cells through mediating β-catenin/TCF7/Sox2 signaling pathway. These findings provide a new signaling transduction mechanisms for the invasion and metastasis of cervical cancer and provide new therapeutic strategies for cervical cancer, especially advanced cervical cancer.

3. Methods

3.1 Chemicals and Reagents

SiHa and HeLa cells were obtained from the American Type Culture Collection (Manassas, VA, USA). β-Elemene was obtained from JinGang Pharmaceuticals (Dalian, China) and dissolved in phosphate-buffered saline (PBS) as a 5mg/ml stock solution for experimental use. Recombinant Human TGF-β1 was purchased from Beyotime Institute of Biotechnology (Shanghai, China). Dulbecco's modified Eagle medium (DMEM), PBS, Trypsin/EDTA solution and fetal bovine serum (FBS) were purchased from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). Matrigel was purchased from BD Biosciences (Bedford, MA, USA). Ready-to-use immunohistochemically kit was purchased from Maixinbio Inc (Fuzhou, China). Nuclear slurry separation kit and magnetic bead was purchased from Beyotime Institute of Biotechnology (Shanghai, China). Primary antibodies against β-catenin, Sox2, TCF7, c-Myc, MMP-2, MMP-9, E-cadherin, Vimentin, GAPDH, H3 and the secondary HRP-conjugated goat anti-rabbit-IgG and HRP-conjugated goat anti-mouse-IgG antibodies were purchased from Proteintech (Beijing, China). All the antibodies were diluted at 1:1000.

3.2. Patients and Tissue Samples

104 cervical cancers and 25 normal cervical specimens were obtained during surgery at the Department of Gynecology of the First Affiliated Hospital of China Medical University (Shenyang, Liaoning, China). The tumor specimens were independently confirmed by two pathologists. None of the patients had received auxiliary treatment before biopsy. The tissue specimens were frozen into liquid nitrogen immediately after collection and preserved at-80 °C. The study was approved by the Ethics Committee of the first affiliated Hospital of China Medical University and obtained the written informed consent of the patients. All the experiments were conducted in accordance with the guiding principles and regulations.

3.3. Immunohistochemistry

Immunohistochemical staining was performed by SP method and the test procedure strictly followed kit instructions. Each piece of wax block was divided into 3 pieces with thickness of 4μm. The pathological section was dewaxed, dehydrated and hydrated and then the antigen was repaired by microwave. After that, the first antibody, the second antibody and the Streptomyces anti biotin protein-peroxide solution were added in turn. We used Diaminobenzidine (DAB) to show color, hematoxylin to restain, Gradient ethanol to dehydrate and dry and neutral tree glue seal piece. 0.01 mol/l PBS solution was used instead of first antibody as negative control and reagent company known positive tablets as positive control.

3.4. Cell Cultures

SiHa and HeLa cells were cultured in DMEM containing 10% FBS and placed in an incubator in a saturated humidified atmosphere with 5% CO2 at 37⁰C. Logarithmically growing cells were used in all subsequent experiments.

3.5. Cell Proliferation Assay

The proliferation of cervical cancer cells was detected by MTT assay. Cervical cancer cells were inoculated in 5×10^3 cell / hole 96-well micro drop plate and were treated with TGF-β1 (0, 5 and 10 ng/ml) or β-elemene (0, 10, 20, 40, 50 μg/ml) for 24 h, 48 h and 72 h. After that, 20 μl MTT solution was added to each well and incubation continued at 37°C for further 4 h. Dimethyl sulfoxide (150 μl) was added to each well and incubation was continued at room temperature for 20 min. The optical density value of each well was detected at a wavelength of 490 nm. Cell proliferation (%) = (experimental group-blank group) / (control group-blank group) × 100%). Each assay was performed in triplicate.

3.6. Morphological Observation of Cells

The SiHa and HeLa cells were spread into the petri dish (1×10⁴ cells/dish) of 10cm. TGF-β1 (10 ng/ml) was added on the second day. The morphological changes of the cells were observed by microscope on the 0th day, the first day, the second day, the third day and the 4th day, respectively. Each test was conducted in triplicate.

3.7 Trans well assay

For the motility assay, SiHa and HeLa cells were resuspended at a density of 1×10⁵ cells/ml in serum-free medium. The cell suspension (200 μl) was mixed with different concentrations of TGF-β1 (0, 5 and 10 ng/ml) and β-elemene (0, 40 μg/ml) and placed in the upper part of the Trans well chamber (BD Biosciences). At the same time, 600 μl of conditioned medium containing 20% FBS and the same concentration of β-elemene was added to the bottom of the Trans well chamber. After incubating for another 24 hours at 37 ⁰C, SiHa and HeLa cells that had migrated to the bottom chamber were fixed in methanol for 30 minutes, stained with crystal violet at room temperature and counted under a light microscope. For the invasion assay, 40 μl Matrigel (0.5 mg/ml) (Becton Dickinson Labware, Bedford, MA, USA) was spread onto the upper part of the Trans well chamber and left for 4 h at 37 ⁰C. The other steps were similar to the motility assay; however, the cells were incubated for 72 h at 37 ⁰C in the trans well device and then fixed, stained, and counted as above. Each test was conducted in triplicate.

3.8. Wound-Healing Assay

SiHa and HeLa cells at a density of 1×10^5 cells/well in medium containing 10% FBS were plated into a 6-well plate and incubated until the cell monolayer covered the plate. A sterile 200μl plastic pipette tip was then used to scratch vertically on the cell layer to make a wound, after which the cells were incubated for 48 h with different concentrations of TGF-β1 (0, 5 and 10 ng/ml) under the serum-free conditions. The gap was then measured at 0 and 48

hours under a microscope. Each test was conducted in triplicate.

3.9. Nuclear Plasma Separation

After SiHa and HeLa cells were treated with TGF-β1 (0 and 10 ng/ml) and β-elemene (0 and 40μg/ml) for 48 h. According to the instructions of the nuclear slurry separation kit, the plasma protein and the nuclear protein were extracted respectively. Then a BCA assay kit (KeyGen biotechnology) was used to measure the concentrations of the plasma protein and the nuclear protein. The remaining steps are the same as the following western blotting analysis.

3.10. Preparation of Proteins and Western Blotting Analysis

After SiHa and HeLa cells were treated with TGF-β1 (0 and 10 ng/ml) and β-elemene (0 and 40μg/ml) for 48 hours. Radio immuno-precipitation assay buffer (RIPA buffer) containing phenylmeth-anesulfonyl (PMSF) was then used to extract the total protein and a BCA assay kit (KeyGen biotechnology) was used to measure the protein concentrations. Equal amounts of protein (60 μg) were separated by 10% SDS-PAGE (SDS-polyacrylamide gel electrophoresis) and then transferred onto PVDF (polyvinylidene difluoride) membranes. The membranes were blocked in 5% skimmed milk at room temperature for 2 h and incubated with appropriate primary antibodies against β-catenin, Sox2, TCF7, c-Myc, MMP-2, MMP-9, E-cadherin, Vimentin and GAPDH antibodies at 4⁰C overnight. Thereafter, the membranes were incubated with the secondary antibodies (anti-rabbit or anti-mouse) for 1 h at 37 ⁰C. Finally, the immunoreactive proteins were detected using the chemiluminescence method (GE Healthcare Life Sciences) together with an ECL kit (KeyGen biotechnology). Each test was conducted in triplicate.

3.11. Co-immunoprecipitation

After SiHa and HeLa cells were treated with β-elemene (0 and 40 μg/ml) for 48h, collecting cells and adding 4℃ pre-cooled IP lysate, then fully mixed, followed by ice bath for 30 minutes. After that the mixture was centrifuged for 20 minutes at 4℃ (12,000-16,000 g), The culture fluid was extracted to 1.5ml EP tube (200 μg/tube) and the first antibody (anti-β-catenin) was added. Then the sample was incubated on ice and slowly shook overnight. After 18 hours, 20 microliters of protein A/gagarose were added to each test tube and well mixed. Then each sample was incubated on ice for 4 hours, then centrifuged for 20 minutes at 4℃ (12,000-16,000 g). Washing the sediment and immune complex with pre-cooled PBS for 5 times (500 ml/time), sucking the clean PBS and adding loading buffer to cook, followed by the next western blot. Finally, Applied the anti-Sox2 (1: 500) and luminous.

3.12. Statistical Analysis

SPSS (Version 17.1) was used to analyze the statistical data and the data were shown as the mean (± SD). χ2test was used to compare data. Student's t test was used to measure the differences between the control group and the experimental group and Kaplan– Meier

survival plots were generated and comparisons were constructed with log-rank statistics. P<0.05 implied that the result of the experiment was statistically significant. Treated groups vs. untreated cervical cancer cells (* P<0.05; ** P<0.01; *** P<0.001). The experiment was repeated three times.

4. Results

4.1. Expression of TGF-B1 and B-Catenin Correlates with Pathogenesis and Aggressiveness of Cervical Cancer

The expression levels of TGF-β1 and β-catenin were detected in cervical cancer samples and normal samples by Immunohistochemistry. As shown in Figure 1, TGF-β1 and β-catenin expression levels were stronger in cervical cancer samples (Figure 1A2,1B2) compared with normal samples (Figure 1A1,1B1, P<0.05). TGF-β1 and β-catenin expression was positively correlated with

FIGO staging (I & II vs. III & IV, P = 0.039 and 0.002), grade (1 & 2 vs. 3, P =0.026 and 0.034), lymph vascular infiltration (positive & negative, P = 0.008 and 0.038) and nodal metastasis (positive & negative, P = 0.006 and 0.032). Besides, TGF-β1 and β-catenin overexpression was corrected with poor cumulative survival rate (Figure 1A3 and 1B3, P = 0.022 and 0.018). Details could be found in (Table 1).

4.2. TGF-B1 Promotes the Proliferation of Cervical Cancer Cells.

We used MTT to detect the effect of TGF-β1 on the proliferation of SiHa and HeLa cells treated with TGF-β1 (0, 5, 10, 15, 20 ng/ml) for 24, 48, 72 h. The results showed that TGF-β1 could promote proliferation of SiHa (Figure 2A) and HeLa cells (Figure 2B) in a time and dose-dependent manner.

Table 1: Association of TGF-β1 and β-catenin expression with clinicopathological characteristics in 52 patients with cervical cancer.

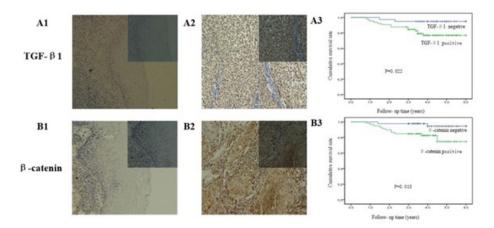| Characteristics	No. of pts.	Negative no (%)	Positive no(%)	χ^2	P-value	Negative no (%)	Positive no(%)	χ^2	P-value
Age				0.863	0.353			1.897	0.168
≤55	42	18	24			22	20		
>55	62	21	41			24	38		
FIGO stage				4.254	0.039			9.226	0.002
I-II	67	30	37			37	30		
III-IV	37	9	28			9	28		
Grade				4.973	0.026			4.497	0.034
2-Jan	70	20	50			36	34		
3	34	19	15			10	24		
Tumor size				0.074	0.786			0.064	0.8
<4cm	71	26	45			32	39		
≥4cm	33	13	20			14	19		
Lymph vascular infiltration				6.982	0.008			4.303	0.038
Positive	41	9	32			13	28		
Negative	63	30	33			33	30		
Nodal metastasis				7.683	0.006			4.584	0.032
Positive	39	8	31			12	27		
Negative	65	31	34			34	31		
Pts., patients; FIGO, International Federation of Gynecology and Obstetrics.									

Figure 1: Expression of TGF-β1 and β-catenin correlates with pathogenesis and aggressiveness of cervical cancer. TGF-β1 and β-catenin expression levels were stronger in cervical cancer samples (1A2,1B2) compared with normal samples (Figure 1A1,1B1). Besides, TGF-β1 and β-catenin overexpression were corrected with poor cumulative and relapse-free survival rate (Figure 1A3 and 1B3).
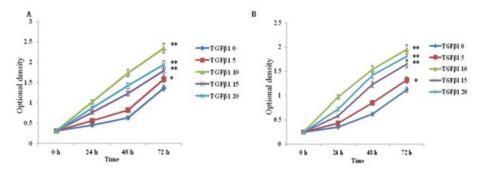
Figure 2: TGF-β1 promotes the proliferation and survival of SiHa and HeLa cells. Following treatment of SiHa (A) and HeLa cells(B) with increasing doses of TGF-β1 (0 20 ng/ml) for 24, 48 and 72 h, the MTT assay was used to detect the proliferation and survival of SiHa and HeLa cells. Each test was conducted in triplicate.

4.3. TGF-B1 Induces EMT in Siha and Hela Cells

The morphological effects of TGF-β1 (10 ng/ml) in SiHa and Hela cells were observed. The results showed that the morphological of cells increased, prolonged and fusiform after treatment with TGF-β1 (10 ng/ml). After 4 days, the morphological changes and cell length increased significantly (Figure 3A). The results of western blot showed that the expression of E-cadherin protein decreased and Vimentin protein increased after 48 hours of treatment with TGF-β1 (10 ng/ml) (Figure 3B).
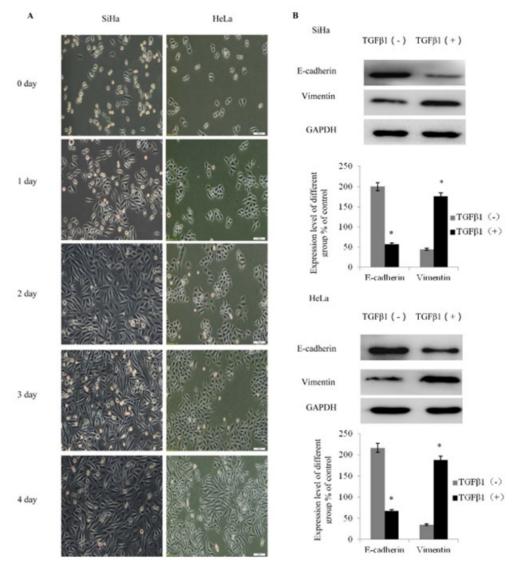

Figure 3: **TGF β1 induces EMT in SiHa and HeLa cells**. (A) Morphological changes of SiHa and HeLa cells following 0, 1, 2, 3 and 4 days of TGF β1 (10 ng/ml) treatment. (B) Western blot analysis of E-cadherin, Vimentin and GAPDH expression with or without TGF β1 treatment after 4 days. Each test was conducted in triplicate.

4.4. TGF B1 Promotes the Migration and Invasion of Cervical Cancer Cells

Trans well assay showed that TGF β1 could promote the migration and invasion of SiHa cells and HeLa cells (Figure 4A) in a dose-dependent manner. The results of scratch test showed that TGF β1 could promote the migration and invasion of SiHa cells and HeLa cells (Figure 4B) in a dose-dependent manner.

4.5. B-Elemene Inhibits the Proliferation of Cervical Cancer Cells

The SiHa and HeLa cells were treated with different concentrations of β-elemene (0, 10, 20, 30, 40, 50 μg/ml) for 24, 48, 72 hours. The results revealed that the viability of SiHa (Figure 5A) and HeLa (Figure 5B) cells in a dose and time dependent manner
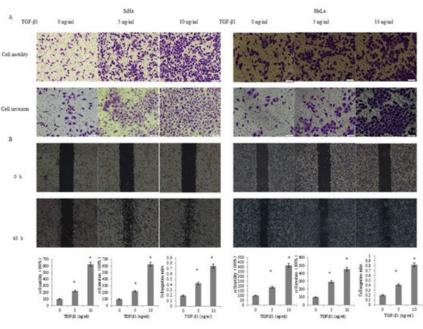

Figure 4: TGF β1 promotes the invasion and migration of SiHa and HeLa cells. (A) After treating SiHa and HeLa cells with increasing doses of TGF β1 (0, 5 and 10 ng/ml) for 24 h and 72 hours, motility and invasion assays were performed. The number of invaded SiHa and HeLa cells was counted. (B) The migration of SiHa and HeLa cells was detected by a wound-healing assay after TGF β1 treatment and the cell migration distances were quantified. Each test was conducted in triplicate.
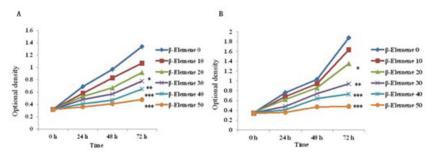

Figure 5: β-Elemene inhibits the proliferation of SiHa cells and HeLa cells. Following treatment of SiHa (A) and HeLa cells(B) with increasing doses of β-elemene (0 50 μg/ml) for 24, 48 and 72 h, the MTT assay was used to detect the proliferation and survival of SiHa and HeLa cells. Each test was conducted in triplicate.

4.6. B-Elemene Inhibits TGF-B1-Induced The Migration and Invasion in Siha and Hela Cells

The results of transwell assay showed that TGF β1 (10 ng/ml) could increase the migration and invasion but β-elemene (40 μg/ml) could significantly inhibit the migration and invasion induced by TGF β1 in SiHa cells and HeLa cells (Figure 6).

4.7. Invasion of Siha and Hela Cells by Inhibiting B-Catenin and Sox2 Signal Transduction

The levels of β-catenin, Sox2, c-Myc, MMP-2, MMP-9, E-cadher-

in and Vimentin protein were detected by western blot after treatment with TGF-β1 (10 ng/ml) or β-elemene (40 μg/ml) for 48 h. The results showed that TGF-β1 could up-regulate the expression of β-catenin, Sox2, MMP-2, MMP-9, Vimentin, E-cadherin and down-regulate the expression of E-cadherin. However, after treatment with β-elemene, the effect of β-elemene was reversed, which indicated that β-elemene could inhibit the migration and invasion of SiHa and HeLa cells (Figure 7) induced by TGF-β1 by inhibiting β-catenin and Sox2 signal transduction.
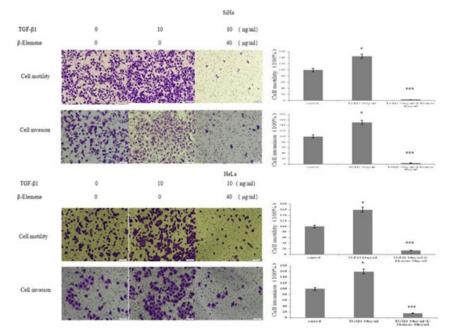
Figure 6: β-Elemene inhibits TGF-β1-induced invasion and migration of SiHa and HeLa cells. After treating SiHa and HeLa cells with increasing doses of TGF β1 (0, 10 ng/ml) and β-elemene (0, 40 μg/ml) for 24 h and 72 hours, motility and invasion assays were performed. The number of invaded SiHa and HeLa cells was counted. Each test was conducted in triplicate.
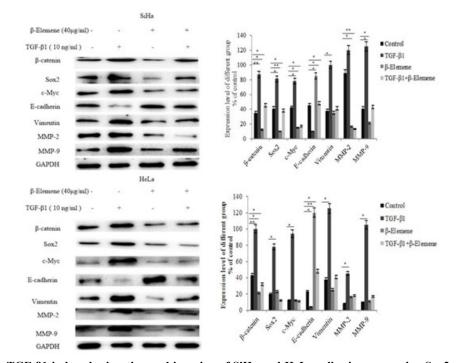

Figure 7: β-Elemene inhibits TGF-β1-induced migration and invasion of SiHa and HeLa cells via suppressing Sox2 and β catenin signaling. Western blot analysis of β-catenin, Sox2, c-Myc, MMP-2, MMP-9, E-cadherin, Vimentin and GAPDH expression with or without TGF-β1 (10 ng/ml) or β-elemene (40 μg/ml) treatment after 48 h in SiHa and HeLa cells. Each test was conducted in triplicate.

4.8. B-Elemene Reverses the Expression of B-Catenin in Nucleus and Cytoplasm Promoted by TGF-B1 and Plays an Anti-Tumor Role by Inhibiting B-Catenin/TCF7/Sox2

In order to study the mechanism of TGF-β1 and β-elemene in cervical cancer cells, cervical cancer cells were collected after treatment with TGF-β1 and β-elemene. The proteins in cytoplasm and nucleus were collected after plasmolysis. The expression of β-catenin protein in cytoplasm and nucleus was detected by west-
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ern blot. The results showed that the expression of β-catenin in nucleus increased after treatment with TGF-β1, but the expression of β-catenin in nucleus decreased significantly after treatment with β-elemene (Figure 8A). The results of immunoprecipitation showed that β-catenin could bind to Sox2 and β-elemene could decrease the binding of β-catenin to Sox2 in cervical carcinoma, indicating that β-elemene could inhibit β-catenin/TCF7/Sox2 signaling pathway to play an anti-tumor role (Figure 8B).
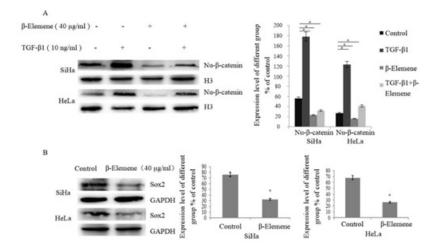
Figure 8: β-Elemene reverses the expression of β-catenin in nucleus and cytoplasm promoted by TGF-β1 and plays an antitumor role by inhibiting β-catenin/TCF7/Sox2 signaling pathway. (A) After SiHa and HeLa cells were treated with TGF-β1 (0 and 10 ng/ml) and β-elemene (0 and 40 μg/ml) for 48 h, the plasma protein and the nuclear protein were extracted and measured respectively. Western blot analysis of β-catenin, H3 in the nuclear. (B) After SiHa and HeLa cells were treated with β-elemene (0 and 40 μg/ml) for 48h, immunoprecipitation was carried out to analysis the effect of β-elemene on the binding of β-catenin and Sox2. Each test was conducted in triplicate.

5. Discussion

Cervical cancer is a local disease in the early stage, which can be transferred to distant organs in the late stage. The invasion and metastasis of the tumor is closely related to EMT [17]. During the development of EMT the changes of the cells is mainly transition from polarized epithelial phenotypes to an elongated fibroblastic phenotype and then the cells degrade ECM, showing aggressive behavior [18]. TGF-β1 can induce EMT through inducing the production of TSLP, RANTES, and TARC [19]. Our results showed that TGF-β1 and β-catenin expression expression was significantly higher in cervical cancer tissues than in normal cervical tissues. In addition, TGF-β1 and β-catenin expression was positively related to FIGO stage, grade, lymph vascular infiltration and nodal metastasis, which together demonstrate a poor prognosis. In this study we used TGF-β1 to stimulate SiHa and HeLa cells and established the EMT model of cervical cancer accompanied by an increasing of vimentin and decreasing of E-cadherin. We further found that TGF β1 could promote the migration and invasion of cervical cancer cells SiHa cells and HeLa cells. Zhao et al showed that TGF-β1 can also promote the invasion and metastasis of breast cancer cells by trans activating EGFR [20]. TGF-β1 has also been reported to be secreted by tumor cells in a form of paracrine to stimulate the fibroblasts activation and Mesenchymal Stem Cells (MSCs) differentiation. In addition, the exosome and cytokines secreted by tumor cells and Cancer-Associated Fibroblast (CAFs) may induce MSCs to synthesize α-SMA and tumor promoting factors, which may promote the proliferation, migration and invasion of colorectal cancer cells [21]. These evidences suggested that TGF-β1 was involved as a significant active factor in the progression and development of cancer. Thus, we hypothesize that downregulating TGF-β1 may hinder the development of cervical cells through regulating a series of related downstream genes.

TGF-β1 can stimulate the Wnt/β-catenin signaling pathway, increased the release of extracellular matrix components and induced tumor progression and fibrosis [22]. The Wnt/β-catenin signaling pathway, also called the canonical Wnt signaling pathway, is one of the key signaling pathways in the regulation of cell proliferation and it serves a significant role in the pathological process of malignant tumors [23]. Abnormal regulation of the transcription factor β-catenin, which is the pivotal component of the Wnt signaling pathway, leads to early events in carcinogenesis [24]. The β-catenin-dependent signaling pathway is triggered by the binding of secreted cysteine-rich glycoprotein ligands Wnts to the LRP-5/6 receptors and FZD receptors. Subsequently, the phosphorylation and inhibition of GSK3β ensure an elevation of cytosolic β-catenin concentration. Un-phosphorylated β-catenin in the cytosol migrates to the nucleus and accumulates, interacting with T Cell-specific Factor (TCF)/Lymphoid Enhancer-binding Factor (LEF) and coactivators, such as Pygopus and Bcl-9, to trigger the Wnt target genes like c-Myc, cyclin D1 and CDKN1A, resulting in the upregulation of TCF/LEF target gene [25]. Our study showed that β-catenin expression expression was significantly higher in cervical cancer tissues and it was positively related to FIGO stage, grade, lymph vascular infiltration and nodal metastasis, which together demonstrate a poor prognosis. Previous studies have shown that rac1, as the upstream molecule of β-catenin, can promote trophoblast invasion, by activating β-catenin to regulate snail and MMP-9, indicating that TGF-β1-induced EMT is mediated by ERK-dependent β-catenin upregulation and nuclear translocation in renal tubular epithelial cells [26]. Previous studies have confirmed that TM4SF1 promotes EMT and cancer stemness via the Wnt/beta-catenin/SOX2 pathway in colorectal cancer [10]. Oxidative stress promotes tumorigenesis and stem-like characteristics by activating Wnt/β-catenin/MYC/Sox2 axis in ALK positive an-

aplastic large cell lymphomas [27]. Tang et al found that knockdown of Sox2 Inhibits osteosarcoma cells invasion and migration via modulating Wnt/β-Catenin signaling pathway [28]. Luo et al found MicroRNA 21 promotes migration and invasion of glioma cells via activation of Sox2 and β catenin signaling [29]. Therefore, there is a requirement for the development of novel antitumor reagents for attenuating TGF-β1 and β-catenin signaling pathway.

There is conclusive evidence that β-elemene has antitumor effect in lung cancer, glioblastoma and breast cancer cell [9,15,30]. Our previous studies have confirmed that β-elemene can inhibit the proliferation of SiHa cells [16]. Cheng et al found that β-elemene synergizes with gefitinib to inhibit stem-like phenotypes and progression of lung cancer via down-regulating EZH2 [30]. Our study found that the expression of β-catenin in nucleus increased after treatment with TGF-β1, but the expression of β-catenin in nucleus decreased significantly after treatment with β-elemene. It is suggested that β-elemene can reverse the nucleation of β-catenin promoted by TGFβ-1. When the tumor progresses, Wnt/ β-catenin signaling pathway is activated, leading to the increase of β-catenin nucleation and up-regulation of the expression of downstream genes [31]. Yang et al found that overexpression of SOX2 promotes migration, invasion, and epithelial-mesenchymal transition through the Wnt/β-catenin pathway in laryngeal cancer Hep-2 cells [32]. Gao et al found that SOX2 promotes the epithelial to mesenchymal transition of esophageal squamous cells by modulating slug expression through the activation of STAT3/HIF-α signaling [33]. Our study showed that β-catenin could bind to Sox2 and β-elemene could decrease the binding of β-catenin to Sox2, indicating that β-Elemene inhibits TGF-β1-induced EMT and invasion of cervical cancer cells through mediating β-catenin/TCF7/Sox2 signaling pathway.

In conclusion, our study provides the first evidence that EMT model of cervical cancer was established by stimulating cervical cancer cells with TGF-β1 and further revealed that β-Elemene inhibits EMT through mediating β-catenin/TCF7/Sox2 signaling pathway in cervical cancer cells. However, this remains to be investigated in an animal experiments setting. The results of the present study suggest β elemene may be a potential novel therapeutic agent for the treatment of recurrence and uncontrolled cervical cancer.

6. Funding

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