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Original Article

Interleukin-17 promotes the development of ovarian cancer through upregulation of MTA1 expression

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Abstract: Interleukin-17 (IL-17) has been demonstrated to promote the development of cancer cells in most organs. The purpose of this study was to determine if IL-17 promotes the development of ovarian cancer cells via upregulation of metastasis associated 1 (MTA1) expression. Human ovarian cancer cell lines SKOV-3, CAOV-3, and OVCAR-3 were used to test if IL-17 regulates MTA1 mRNA and protein expression. Flow cytometer assays (FCM), Tunel assays, wound healing assays, and transwell assays were used to investigate cell apoptosis, migration, and invasion. Nude mouse ovarian cancer tissues were stained for IL-17 and MTA1 using immunohistochemical staining, RT-PCR, and Western blot, respectively. Twenty human serum and tissues, including 10 cases of ovarian cancer patients and 10 cases of control patients, were tested for IL-17 and MTA1 using the enzyme linked immunosorbent assay (ELISA), RT-PCR, and Western blot analysis. In addition, we found that IL-17 upregulated MTA1 mRNA and protein expression in both vitro and in vivo. ELISA demonstrated the high expression of IL-17 in the serum of ovarian cancer patients. Human ovarian cancer tissues had an increased number of IL-17-positive and MTA1 expressions compared to normal ovarian tissues. These findings demonstrate that IL-17 upregulates MTA1 mRNA and protein expression to promote ovarian cancer migration and invasion. It may be a new target for the treatment of ovarian cancer from the field of biological immunotherapy, but it may also be an important supplement to the study of IL-17 target sites and target pathways.

Keywords: Interleukin-17, MTA1, ovarian cancer, migration, invasion

Introduction

Ovarian cancer is one of the three malignant tumors found in the female reproductive system, and it has the highest mortality rate. The treatment of ovarian cancer is through surgery combined with radiotherapy and chemotherapy. This procedure can only remove some solid tumor cells but has little effect on residual and disseminated tumor cells [1]. Inflammation is known as the eighth biological feature of malignant tumors, which plays an important role in the processes of tumor occurrence, development, invasion, and metastasis [2]. There are a large number of inflammatory cytokines in the tumor microenvironment. They can not only recruit inflammatory cells to the tumor site and amplify the inflammatory effect, but also promote the growth and metastasis of tumor cells and the formation of tumor blood vessels and lymphatic vessels [3]. Inflammation plays an important role in the occurrence and development of tumors, and its research progress and prospects have been explored as a target of tumor therapy.

Interleukin-17 (IL-17) is mainly produced by T helper cells (Th17). It can induce a variety of cytokines, chemokines, inflammatory factors, and antimicrobial proteins to identify target genes mediating autoimmunity and chronic infection [4]. Studies have shown that IL-17 is closely related to tumorigenesis [5]. It is a proinflammatory cytokine that participates in innate and adaptive immune responses and is associated with a variety of diseases, such as infectious diseases, autoimmune diseases, and cancer [6, 7]. The latest research results show that IL-17 can not only regulate tumor angiogenesis and enhance tumor immune escape but also

activate natural killer (NK) cells and cytotoxic T lymphocytes (CTL) and recruit neutrophils, NK cells, CD4+ and CD8+ T cells to tumor tissues [8]. IL-17 has abnormal expression in breast cancer, gastric cancer, lung cancer and other tumor cell lines, but its role in the occurrence and development of ovarian cancer is not clear [9-11].

Tumor metastasis associated gene-1 (MTA1) is one of the most concerning tumor metastasis genes. It plays an important role in the process of cancer cell invasion and metastasis by regulating a series of proteins related to invasion and metastasis [12]. MTA1 is a metastasis associated protein that is expressed in many tumor tissues, regulating the malignant potential of tumor cell proliferation, invasion, and migration [13]. At present, studies have shown that MTA1 is abnormally expressed in gynecological diseases such as ovarian cancer, endometrial cancer, cervical cancer, and trophoblastic tumors, but its specific regulation mechanism is not clear [14, 15]. The purpose of the present study was to determine if IL-17 regulates MTA1 expression and its biological consequences in ovarian cancer.

Materials and methods

Cell culture

IL-17 was obtained from Ruixin Biotech (Cat# 10343, Fujian, China), MTA1 was obtained from Abcam (Cat# ab71153, England), CAOV-3 was obtained from iCell Biotech (Shanghai, China), OVCAR-3 was obtained from EallBio (Beijing, China), and SKOV-3 was obtained from Otwo Biotech (Guangzhou, China). The cells were routinely cultured in RPMI-1640 (Cat# WH190-730CE, EallBio, Beijing, China) in a humidified 5% CO_2 incubator at 37°C. The medium contained 10% fetal bovine serum (FBS; Cat# NT9670B, EallBio, Beijing, China) without any antibiotics. Cells were allowed to grow until 100% confluence was achieved. For induction of MTA1 expression, the cells were cultured in serum-free medium in 60-mm culture dishes and treated without or with 20 ng/ml recombinant human IL-17 for 8, 16, 24, and 36 h.

Wound healing assay

CAOV-3 and OVCAR-3 cells were cultured in RPMI-1640 supplemented with 10% FBS in 60-mm tissue culture dishes until 100% confluence was achieved. Scratch was made with a sterile 1,000-μl pipette tip across the center of each dish. After scratching, the dishes were gently washed twice with RPMI-1640 to remove the detached cells. The dishes were replenished with serum-free medium. The control group was treated with 0.1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), while 20 ng/ml IL-17 (dissolved in 0.1% BSA in PBS) was added into the medium of the treatment group. Images were collected at 12, 24, 36, and 48 hours under an ImageJ software microscope. Horizontal gap distances were measured at 5 points along each wound and averaged to represent the width of the wound. The wound healing rate was calculated as (wound width of time zero - that of each time point) ÷ wound width of time zero × 100%; the data represents the mean ± standard deviation (SD, error bars) of 3 independent experiments.

Invasion assay

Invasion assay was performed using Corning® BioCoat™ Matrigel® Invasion Chambers following the manufacturer's instructions. Approximately 0.2×10^6 cells were seeded in the upper chamber in serum-free medium in triplicate wells per group, while the lower chamber contained medium with 10% FBS. After treatment with IL-17 at 20 ng/ml for 12, 24, 36, and 48 hours, non-invaded cells were removed from the upper chamber with a cotton swab; the cells that invaded through the Matrigel®-coated porous membrane were fixed with methanol, stained with 0.5% crystal violet, and counted under a microscope.

Apoptosis assay

The apoptotic rates of cells were detected by flow cytometry (FCM) after treatment with IL-17 at 20 ng/ml for 12, 24, 36, and 48 hours. The instrument was equipped with four lasers with emission wavelengths ranging from 350 nm to 647 nm. PI fluorescence was detected through 488 nm diachronic long/band pass filters. The droplet cell-sorting function was used to separate fluorescence-positive cells from fluorescence-negative cells. Each assay was performed at least three times.

TUNEL assay

TUNEL assay was performed to detect apoptosis in the nude mice being transplanted with ovarian cancer SKOV-3 cells. The mice were

randomly divided into three groups (n = 10): control (0.2 ml saline), Secukinumab (10 mg/kg), and Pterostilbene (10 mg/kg). The mice in all groups were intraperitoneally injected once a week for four weeks. All the mice were killed by cervical dislocation on the sixth week. Tissue specimens were placed in PBS, then the solution was removed from glass slides, and 20 μL of TdT solution containing 5 μL of terminal deoxynucleotidyl transferase and 15 μL of reaction buffer was mounted on cells at 37°C. The solution is then removed from the glass slides, and the slides are soaked in buffer consisting of 0.5 mL of STOP/Wash buffer and 17 mL of water. 20 μL of Anti-Digoxigenin-Peroxidase was then mounted on the cells at room temperature. Glass slides are then soaked in peroxidase substrate solution consisting of 30 mL of 50 mM Tris-HCl, pH 7.5, containing a full micro spatula of 3,3'-diaminobenzidine tetrahydrichloride (DAB), and 0.002% (v/v) H_2O_2. Cells are finally enclosed in water for observation under a microscope.

Immunohistochemistry (IHC) staining

A total of 30 BALB/C (nu/nu) female nude mice (aged 4-6 weeks; body weight of 17 g-19 g) were used. SKOV3 cells were prepared in 5 × 10^7/ml of phosphate-buffered saline (PBS) cell suspension and subcutaneously injected into the right forearm axillary (0.1 ml) of nude mice. The mice were randomly divided into three groups (n = 10): control (0.2 ml saline), Secukinumab (10 mg/kg), and Pterostilbene (10 mg/kg) [16, 17]. The mice in all groups were intraperitoneally injected once a week for four weeks. The specimens were fixed with 10% formalin, embedded in paraffin blocks, and cut into 4-μm thick tissue sections. IHC staining followed a previously published protocol [17, 18]. The primary antibodies used were: rabbit anti-goat anti-MTA1 monoclonal antibody (1:25 dilution, Cat# ab71153, Abcam, England); goat anti-rabbit Vimentin monoclonal antibody (1:25 dilution, Cat# 10366-1-AP, Sanying Biotech, Wuhan, China); goat anti-rabbit Twist1 monoclonal antibody (1:25 dilution, Cat# 25465-1-AP, Sanying Biotech, Wuhan, China); goat anti-rabbit anti-Snail monoclonal antibody (1:25 dilution, Cat# ab53519, Abcam, England); goat anti-rabbit Slug monoclonal antibody (1:25 dilution, Cat# 12129-1-AP, Sanying Biotech, Wuhan, China); goat anti-rabbit N-cadherin monoclonal antibody (1:25 dilution, Cat# 22018-1-AP, Sanying Biotech, Wuhan, China); and goat anti-rabbit E-cadherin monoclonal antibody (1:25 dilution, Cat# 20874-1-AP, Sanying Biotech, Wuhan, China). VECTASTAIN ABC Kits and DAB Peroxidase Substrate Kit (Vector Laboratories, Inc., Burlingame, CA) were used according to the manufacturer's instructions.

Western blot analysis

The expression of MTA1, Vimentin, Twist, Snail, SNAI2, N-cadherin, and E-cadherin in nude mice was detected after treatment with Secukinumab (10 mg/kg) and Pterostilbene (10 mg/kg). Human ovarian cancer tissues had an increased number of IL-17-positive and MTA1 expression compared to normal ovarian tissues. They were previously collected from 2020 to 2021 and archived at West China Second University Hospital of Sichuan University, Chengdu, Sichuan Province, China. The inclusion criteria were: (1) patients with a clear pathological diagnosis of the ovarian lesions; and (2) patients who had signed informed consent prior to the surgical procedures. Exclusion criteria were: (1) patients with concurrent autoimmune disease, active or chronic infection, cardiovascular disease, or connective tissue disease; (2) patients with a history of other malignant tumors; or (3) patients who received immunosuppressive treatment, radiotherapy, or chemotherapy prior to the surgical procedures.

The cells were harvested and lysed in 100 μl of lysis buffer through incubation on ice for 30 minutes; subsequently, the extracts were centrifuged at 18,000 × g for 15 minutes to remove cell debris. Protein concentrations were determined using the BioRad protein assay. After the addition of the × 3 loading buffer, the samples were incubated at 95°C for five minutes and resolved using SDS-PAGE. Proteins were transferred onto nitrocellulose membranes and probed with antibodies. The primary antibodies used were: rabbit anti-goat anti-MTA1 monoclonal antibody (1:25 dilution, Cat# ab71153, Abcam, England); goat anti-rabbit Vimentin monoclonal antibody (1:25 dilution, Cat# 10366-1-AP, Sanying Biotech, Wuhan, China); goat anti-rabbit Twist1 monoclonal antibody (1:25 dilution, Cat# 25465-1-AP, Sanying Biotech, Wuhan, China); goat anti-rabbit anti-

Snail monoclonal antibody (1:25 dilution, Cat# ab53519, Abcam, England); goat anti-rabbit SNAI2 monoclonal antibody (1:25 dilution, Cat# 12129-1-AP, Sanying Biotech, Wuhan, China); goat anti-rabbit N-cadherin monoclonal antibody (1:25 dilution, Cat# 22018-1-AP, Sanying Biotech, Wuhan, China); and goat anti-rabbit E-cadherin monoclonal antibody (1:25 dilution, Cat# 20874-1-AP, Sanying Biotech, Wuhan, China). The antigen-antibody complexes were incubated for one hour at room temperature with HRP-conjugated secondary antibodies at a final dilution of 1:1500. After washing the mixture three times with Tris-buffered saline, the antibody binding was visualized using ECL and autoradiography.

Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) analysis

The total mRNA was extracted, and the mRNA expression of MTA1,Vimentin, Twist, Snail, Slug, N-cadherin, and E-cadherinin was detected by reverse transcription-polymerase chain reaction (RT-PCR) after treatment with 20 ng/ml in the IL-17 group for 8, 16, 24, and 36 hours. In the same way, the expression of MTA1, Vimentin, Twist, Snail, SNAI2, N-cadherin, and E-cadherin in nude mice was detected after treatment with Secukinumab (10 mg/kg) and Pterostilbene (10 mg/kg). Human ovarian cancer tissues had increased levels of IL-17-positive and MTA1 expression compared to normal ovarian tissues. For PCR amplification, the total RNA was fragmented and labeled. After purification, the labeled RNA was hybridized to probe the hybridization chamber gasket slides. After washing, the slides were scanned using an Agilent microarray scanner. The raw data was extracted with the Feature Extraction software. This software utilizes the robust multi-array average algorithm to adjust the background signals. Normalized data was obtained using the quantile method of intramicroarray normalization and the median method of baseline transformation among the microarrays. Differentially expressed genes with a raw expression level of over 400 in more than four of the 12 samples used for profiling were extracted. The PCR primer sequences were: MTA1 forward: 5'-AGCCGTGCTTCGGTATCTTGAGA-3'; MTA1 reverse: 5'-CGCTTCTTCATGTTGCCGTCCA-3'; Vimentin forward: 5'-CCTTGACATTGAGATTGCCACCTACA-3'; Vimentin reverse: 5'-TCGTGATGCTGAGAAGTTTCGTTGAT-3'; Twist forward: 5'-GACTTCCTCTACCAGGTCCTCCAG-3'; Twist reverse: 5'-CCTCCATCCTCCAGACCGAGAA-3'; Snail forward: 5'-ATGCACATCCGAAGCCACACG-3'; Snail reverse: 5'-GGCACTGGTACTTCTTGACATCTGAG-3'; Slug forward: 5'-GCTTCAAGGACACATTAGAACTCACA-3'; Slug reverse: 5'-ACAGCAGCCAGATTCCTCATGTT-3'; N-cadherin forward: 5'-CCTGACACTGTGGAGCCTGATG-3'; N-cadherin reverse: 5'-TGGAGCCACTGCCTTCATAGTC-3'; E-cadherin forward: 5'-TTCGGAGGAGAGCGGTGGTCAA-3'; E-cadherin reverse: 5'-GCAACGTCGTTACGAGTCACTTCAG-3'; GAPDH forward: 5'-CCAGGTGGTCTCCTCTGACTTCA-3'; GAPDH reverse: 5'-CTGTTGCTGTAGCCAAATTCGTTGT-3'. These datasets were ordered by *p* value.

ELISA analysis

Twenty cases of serum from patients, including 10 cases of normal patients and 10 cases of ovarian cancer patients, were collected. The blood was collected with a sterile tube, naturally coagulated at room temperature for 10-20 minutes, centrifuged at 2-8°C for about 20 minutes (2000-3000 RPM), carefully collected the supernatant, and centrifuged again in case of precipitation during storage. Then, 100 μL/well IL-17 termination solution is added to the enzyme label plate. Gently shake the enzyme label plate until the color is uniform. Reading value: within 20 minutes, read the 450 nm light absorption value.

Statistical analyses

All data were expressed as the mean ± SD and analyzed using SPSS 13.0. The linear *t*-test was used for statistical analysis, and $P<0.05$ was considered statistically significant.

Results

IL-17 promotes migration of ovarian cancer cells

Our previous studies showed that IL-17 is closely related to ovarian cancer [1-3] and confirmed that IL-17 can promote the invasion and metastasis of the ovarian cancer SKOV-3 cell line [17], and the upregulation of MTA1 expression in ovarian cancer SKOV-3 cells suggests that IL-17 is involved in the regulation of MTA1 expression and its role may be to induce MTA1

expression. In this study, we further confirmed the role of IL-17 in the regulation of MTA1 expression with two other ovarian cancer cells, CAOV-3 and OVCAR-3. The wound healing assay was performed to investigate if IL-17 could promote ovarian cancer cell migration. CAOV-3 and OVCAR-3 cells were grown to complete confluence in 60-mm culture dishes, and a wound was made by scratching the monolayer cells with a sterile pipette tip. The cells were either untreated or treated with 20 ng/ml of IL-17. At 12, 24, 36, and 48 hours, photomicrographs were taken. We found that IL-17A treatment significantly accelerated the wound healing of CAOV-3 and OVCAR-3 cells compared to the control group ($P<0.05$, **Figure 1A** and **1B**).

IL-17 promotes invasion of ovarian cancer cells

Next, a transwell assay was performed to investigate if IL-17 could promote ovarian cancer cell invasion. Corning® BioCoat™ Matrigel® Invasion Chambers were used. CAOV-3 and OVCAR-3 cells were plated in the upper chamber in serum-free medium, while the lower chamber contained medium with 10% fetal bovine serum (FBS) with or treated with 20 ng/ml IL-17. After 12, 24, 36, and 48 hours, the cells that invaded through the Matrigel®-coated porous membrane were stained with 0.5% crystal violet and counted. We found that IL-17 increased invasion of CAOV-3 and OVCAR-3 cells that were treated with IL-17 ($P<0.05$, **Figure 2A** and **2B**).

IL-17 promotes apoptosis of ovarian cancer cells

To further understand the role of IL-17 in promoting ovarian cancer cell apoptosis in ovarian cancer cells, the apoptotic rates of cells were detected by flow cytometry (FCM) after treatment with IL-17 at 20 ng/ml for 12, 24, 36, and 48 hours. We found that the apoptotic rates decreased in the IL-17 group compared with the control group ($P<0.05$, **Figure 3C**).

A tunel test was also performed to detect apoptosis in the nude mice being transplanted with ovarian cancer SKOV-3 cells. The mice were randomly divided into three groups (n = 10): control (0.2 ml saline), Secukinumab (10 mg/kg), and Pterostilbene (10 mg/kg). Tissue specimens were processed according to the Tunel assay protocol, enclosing cells in water for observation under a microscope. We found that the positive cells increased in the Secukinumab and Pterostilbene groups compared with the control group (**Figure 3A**).

We also observed changes in tumor size in different nude mouse groups. A total of 30 BALB/C (nu/nu) female nude mice (aged 4-6 weeks; body weight, 17 g-19 g) were used. SKOV3 cells were prepared in 5×10^7/ml of phosphate-buffered saline (PBS) cell suspension and subcutaneously injected into the right forearm axillary (0.1 ml/mouse) of nude mice. The tumor growth was observed every week. The mice were randomly divided into three groups (n = 10): control (0.2 ml saline), Secukinumab (10 mg/kg), and Pterostilbene (10 mg/kg). The mice in all groups were intraperitoneally injected once a week for four weeks. The tumors and weights of nude mice were observed and determined weekly, with the administration day as the first day. The formula $V = ab^2/2$ was used to calculate tumor volume, where a is the diameter and b is the short diameter. All the mice were killed by cervical dislocation on the sixth week. We found that the tumor volume increased in the Secukinumab and Pterostilbene groups compared with the control group after the first week of injection; there was no significant change after two weeks of injection for the Secukinumab group; and there was no significant change after the fourth week of injection for the Pterostilbene group (**Figure 3B**).

IL-17 induces the expression of MTA1

We previously found that IL-17 induces MTA1 expression to enhance EMT and tumor cell invasion, which indicates the IL-17-MTA1-EMT axis as a potential mechanism of interleukin-17 in the occurrence and development of ovarian cancer [19]. In this study, we further confirmed the IL-17-MTA1-EMT axis with two other ovarian cancer cells, CAOV-3 and OVCAR-3. The specimens were fixed with 10% formalin, embedded in paraffin blocks, and cut into 4-μm thick tissue sections. IHC staining followed a previously published protocol [18, 19]. The expression of MTA1, Vimentin, Twist, Snail, SNAI2, and N-cadherin decreased in the Secukinumab and Pterostilbene groups compared with the control group, while E-cadherin increased (**Figure 4A**).

The protein expression of MTA1, Vimentin, Twist, Snail, SNAI2, N-cadherin, and E-cadherinin

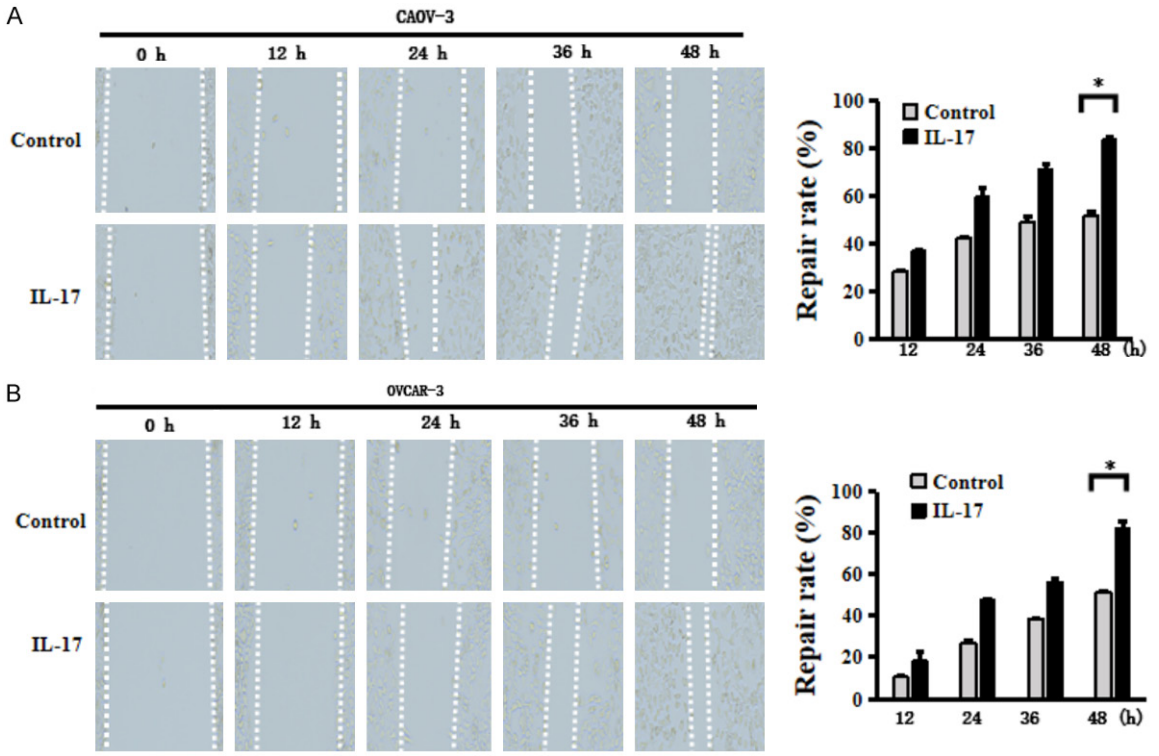

Figure 1. IL-17 increased ovarian cancer CAOV-3 and OVCAR-3 cell invasion is detected by a wound healing assay at 20 ng/ml IL-17 after 12, 24, 36, and 48 hours later. A. Wounds are made with a pipette tip in confluent monolayers. B. Quantification of wound healing repair rates. Datasets are mean ± SD. **P*<0.05.

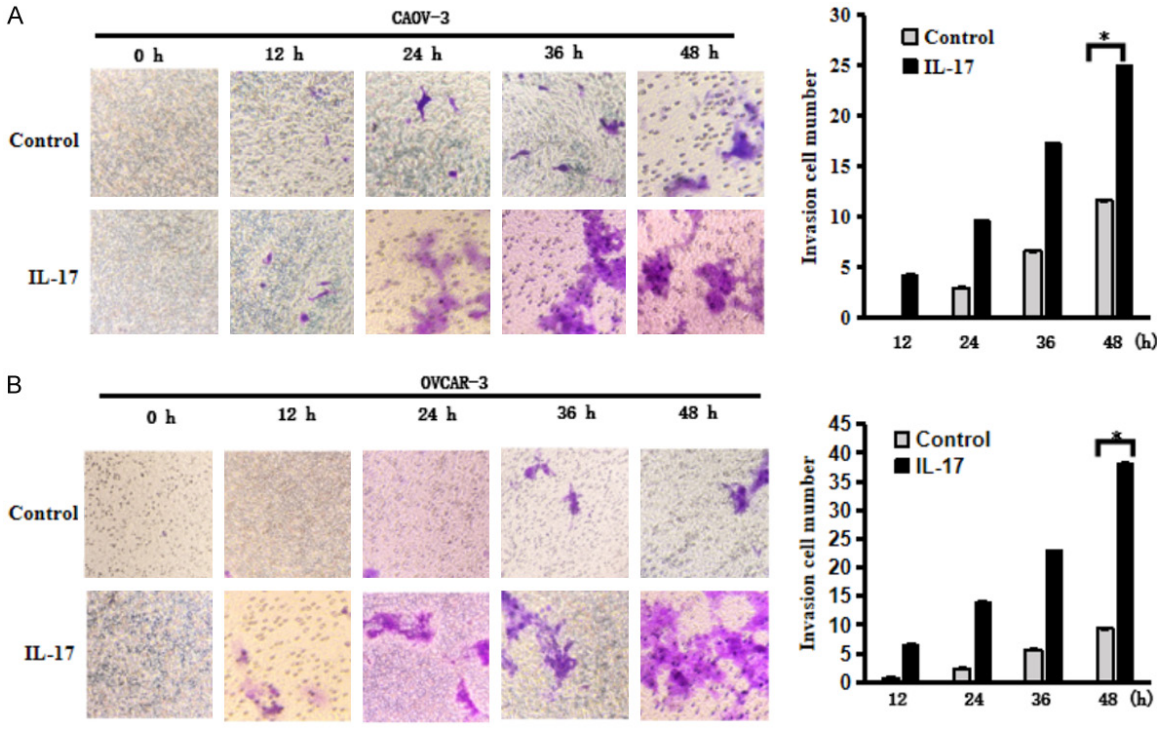

Figure 2. IL-17 increased ovarian cancer CAOV-3 and OVCAR-3 cell invasion is detected by transwell assay at 20 ng/ml IL-17 for 12, 24, 36, and 48 hours. A. Representative photomicrographs through the Matrigel®-coated porous membrane. B. The number of invasion cells. The data are presented as mean SEM, with a **P*<0.05 significance level.

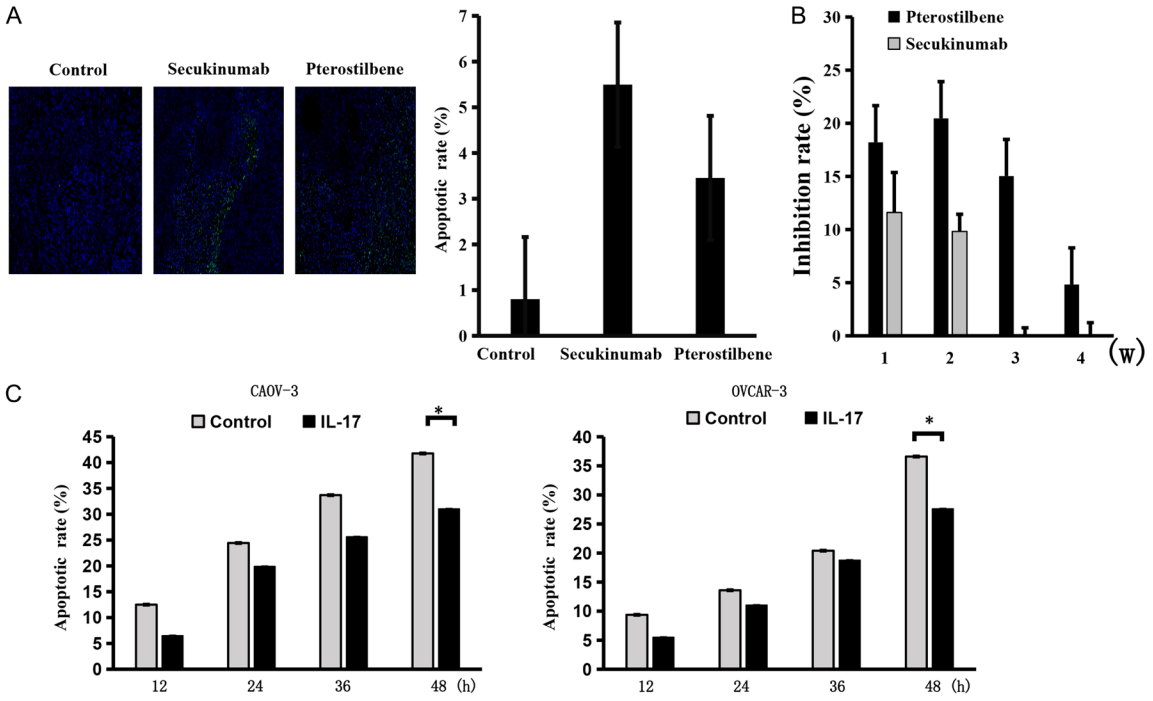

Figure 3. IL-17 promotes ovarian cancer apoptosis. A. In nude mice, the tunel test was performed, and positive cells increased in the Secukinumab and Pterostilbene groups; B. The tumor volume increased in the Secukinumab and Pterostilbene groups compared with the control group after the first week of injection; C. Apoptotic rates decreased in the IL-17 group, **P*<0.05.

was detected by Western blot analysis after treatment with 20 ng/ml in the IL-17 group for 8, 16, 24, and 36 hours. In the same way, the protein expression of MTA1, Vimentin, Twist, Snail, SNAI2, N-cadherin, and E-cadherin in nude mice was detected after treatment with Secukinumab (10 mg/kg) and Pterostilbene (10 mg/kg). We discovered that IL-17 induced MTA1 protein expression in CAOV-3 and SKOV-3 at 8 and 16 hours, and in OVCAR-3 cells at 24 hours, and that nude mice treated with Secukinumab, an interleukin-17 blocker, significantly reduced MTA1 protein expression. We also found that Vimentin, Twist, Snail, SNAI2, and N-cadherin were highly expressed in the IL-17 group at 16 and 24 h in CAOV-3, OVCAR-3, and SKOV-3 cells. E-cadherin was low expressed at 36 h in CAOV-3 cells and at 8 h in OVCAR-3 and SKOV-3 cells. For nude mice, treatment with Secukinumab and Pterostilbene groups, which are interleukin-17 blockers and MTA1 blockers, significantly reduced Vimentin, Twist, Snail, SNAI2, and N-cadherin protein expression and induced E-cadherin protein expression (**Figures 5B** and **6B**).

To check if IL-17 induces MTA1, Vimentin, Twist, Snail, SNAI2, N-cadherin, and E-cadherin mRNA expression, we similarly treated cells with 20 ng/ml in the IL-17 group for 8, 16, 24, and 36 hours. In the same way, the mRNA expression of MTA1, Vimentin, Twist, Snail, SNAI2, N-cadherin, and E-cadherin in nude mice was detected after treatment with Secukinumab (10 mg/kg) and Pterostilbene (10 mg/kg). A quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) was performed for the analysis of mRNA expression. We found that IL-17 induced MTA1 mRNA expression in both CAOV-3 and OVCAR-3 cells. However, we observed that the peak levels of MTA1 mRNA expression were at 36 h in CAOV-3 cells, whereas the peak levels of MTA1 mRNA expression were at 24 h in OVCAR-3 cells. We also discovered that treating nude mice with Secukinumab, an interleukin-17 inhibitor, significantly reduced MTA1 mRNA expression. IL-17 also induced Vimentin, Twist, Snail, SNAI2, and N-cadherin mRNA expression, induced E-cadherin mRNA expression, Vimentin and N-cadherin at 16 h in both CAOV-3 and OVCAR-

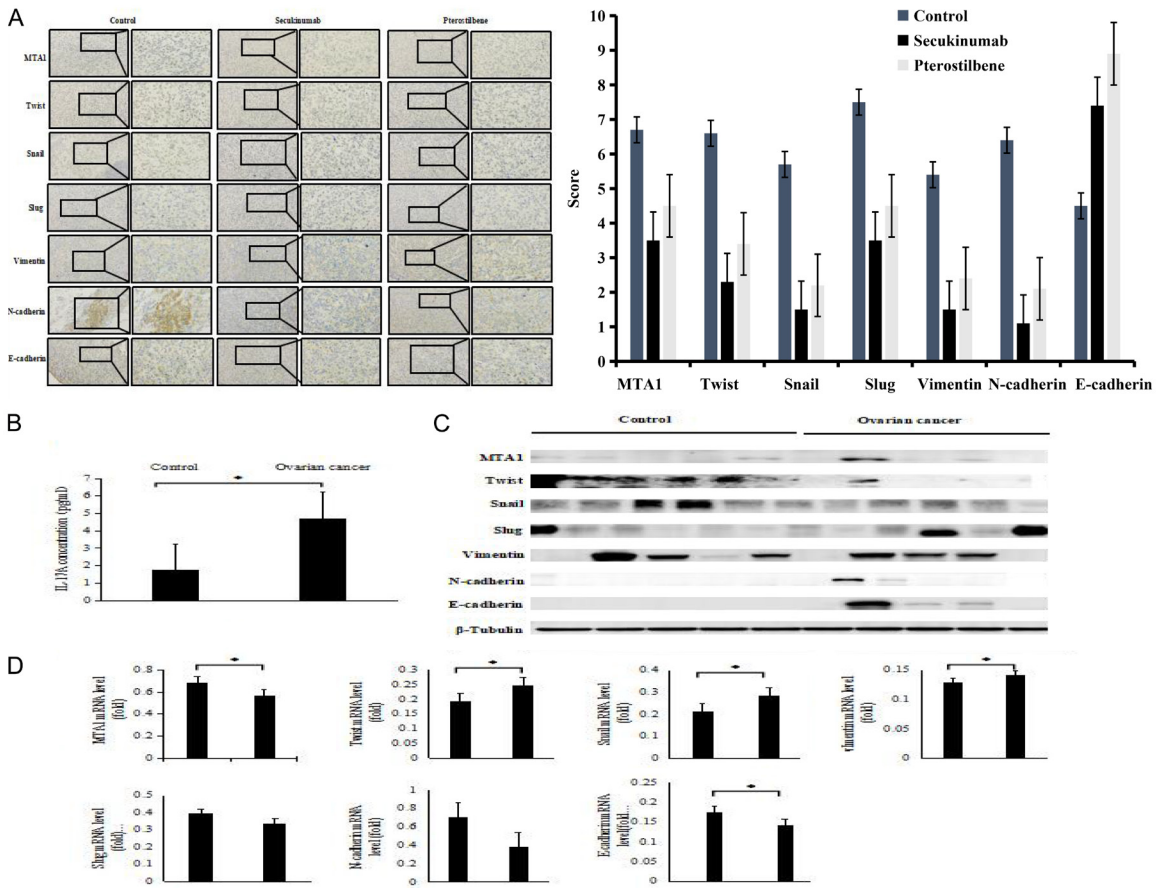

Figure 4. IL-17 upregulates MTA1 expression to promote ovarian cancer. A. IHC showed that the expression of MTA1, Vimentin, Twist, Snail, SNAI2, and N-cadherin were decreased in the Secukinumab and Pterostilbene groups compared with the control group, E-cadherin was increased; B. ELISA assays demonstrated the high expression of IL-17 in the serum of ovarian cancer patients; C. Western blot protein expression, using β-Tubulin levels as loading controls; D. Relative mRNA expression normalized by GAPDH levels; data represent mean ± standard deviation (SD, error bars) **P*<0.05.

3 cells, Twist at 36 h in CAOV-3 cell, at 24 h in OVCAR-3 cell, Snail at 24 h in both CAOV-3 and OVCAR-3 cells, SNAI2 at 16 h in CAOV-3 cell, at 24 h in OVCAR-3 cell, and E-cadherin was low expression at 16 h in both CAOV-3 and OVCAR-3 cells. For nude mice, treatment with Secukinumab and Pterostilbene groups, which are interleukin-17 blockers and MTA1 blockers, significantly reduced Vimentin, Twist, Snail, SNAI2, and N-cadherin protein expression and induced E-cadherin protein expression (**Figures 5A** and **6A**). These results suggest that IL-17 induced MTA1 expression, leading to up-regulated Vimentin, Twist, Snail, SNAI2, and N-cadherin, and down-regulated N-cadherin.

ELISA assays demonstrated the high expression of IL-17 in the serum of ovarian cancer patients. When compared to normal ovarian tissues, human ovarian cancer tissues had an increased number of IL-17-positive and MTA1 expression (**Figure 4B-D**).

Discussion

IL-17 is like a double-edged sword. In an acute inflammatory reaction, it can be secreted quickly to protect the body from the harm of harmful substances. When the human body has chronic inflammation caused by a variety of genetic and environmental factors, it will accelerate the course of a variety of chronic diseases. Therefore, IL-17 is closely related to human health [20]. Studies have confirmed that IL-17 is involved in the malignant progression of various tumors [21], and IL-17 can promote tumor angiogenesis in animal models [22]. Other studies have confirmed that IL-17 can promote the growth and progression of human cervical cancer cells in nude mice [23].

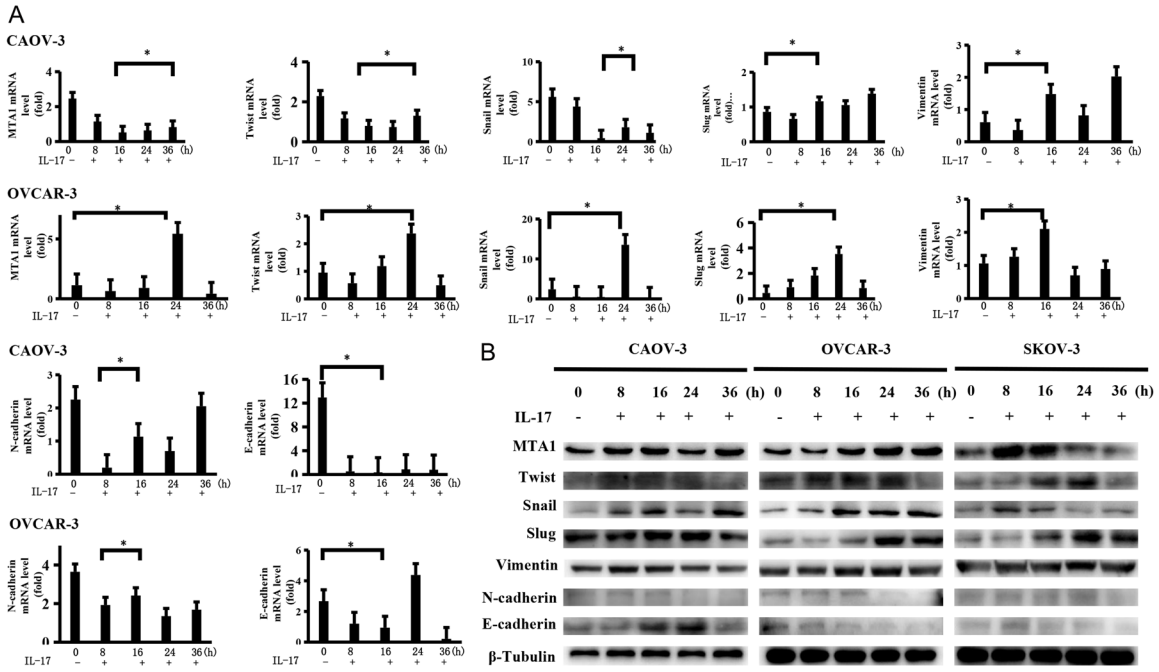

Figure 5. IL-17 induces MTA1, Vimentin, Twist, Snail, SNAI2, N-cadherin, and E-cadherin protein expression when treated with 20 ng/ml IL-17. A. GAPDH-normalized relative mRNA expression; data represent mean standard deviation (SD, error bars) *P<0.05; B. Western blot protein expression, using β-Tubulin levels as loading controls.

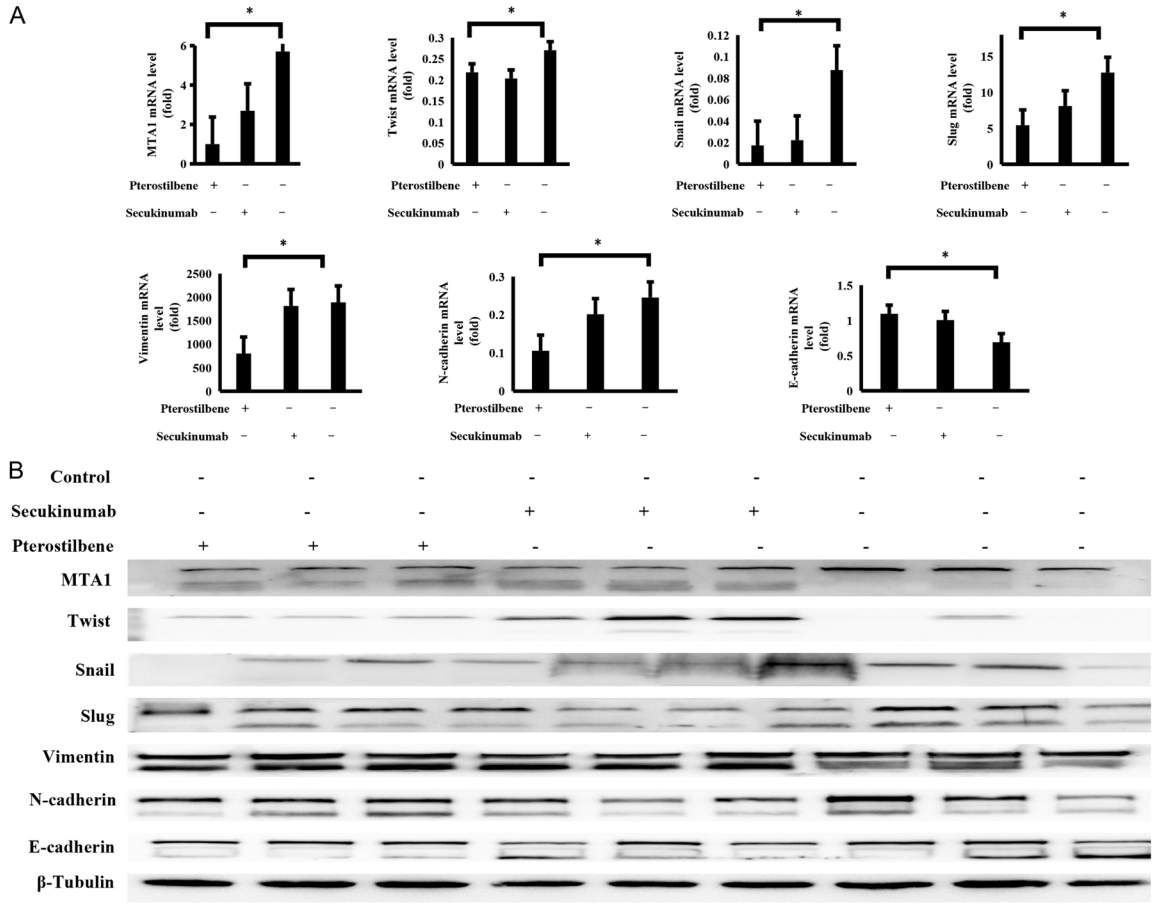

Figure 6. Secukinumab (10 mg/kg) and Pterostilbene (10 mg/kg) groups reduced mRNA expression of MTA1, Vimentin, Twist, Snail, SNAI2, and N-cadherin and induced N-cadherin expression. A. GAPDH-normalized relative mRNA expression; data represent mean standard deviation (SD, error bars) *P<0.05; B. Western blot protein expression, using β-Tubulin levels as loading controls.

Inflammation in the tumor microenvironment can promote the proliferation and survival of malignant cells, promote tumor angiogenesis and metastasis, weaken the acquired immune response of the body, and change the body's response to hormones and chemotherapeutic drugs [24, 25]. IL-17 is found at higher levels in breast cancer, gastric cancer, lung cancer, and other tumor cell lines [9-11], but its role in the development and progression of ovarian cancer is unknown. In previous studies, we found that IL-17 stimulation of ovarian epithelial cancer SKOV-3 cells can significantly enhance cell invasion [19]. The hypothesis of this research is that IL-17 may promote epithelial-mesenchymal transformation by inducing the expression of MTA1, so as to promote the invasion and metastasis of ovarian cancer. In the present study, we found that IL-17-induced migration, invasion, and apoptosis of CAOV-3 and OVCAR-3 cells, and the tumor volume increased in the Secukinumab and Pterostilbene groups compared with the control group after the first week of injection. We also found that IL-17 induced protein and mRNA expression of MTA when nude mice were treated with Secukinumab, which is an interleukin-17 blocker, significantly reduced mRNA and protein expression of MTA1. Recent studies have confirmed that MTA1 directly regulates the epithelial to mesenchymal transition (EMT) of tumor cells [26]. In order to find the possible molecular mechanism of IL-17 in the progression of ovarian cancer, through in vitro and vivo experiments, we found that IL-17 up-regulates MTA1 and activates mRNA and protein expression of Vimentin, Twist, Snail, SNAI2, and N-cadherin, inhibites mRNA and protein expression of E-cadherin. For nude mice, treatment with Secukinumab and Pterostilbene groups, which are interleukin-17 blockers and MTA1 blockers, significantly reduced Vimentin, Twist, Snail, SNAI2, and N-cadherin protein expression, induced E-cadherin protein expression. These results suggest that IL-17 induced MTA1 expression, leading to up-regulated Vimentin, Twist, Snail, SNAI2, and N-cadherin, and down-regulated N-cadherin.

Conclusions

In conclusion, the present study shows that IL-17 upregulates MTA1 mRNA and protein expression to promote ovarian cancer migration and invasion. It may be a new target for the treatment of ovarian cancer from the field of biological immunotherapy, but it may also be an important supplement to the study of IL-17 target sites and target pathways. Future studies should be explored to confirm.

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Disclosure of conflict of interest

None.

Abbreviations

IL-17, Interleukin-17; MTA1, Metastasis Associated 1; RT-PCR, Quantitative Reverse Transcription Polymerase Chain Reaction; FCM, Cytometer Assays.

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