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Original Research

Interleukin-17 promotes ovarian carcinoma SKOV3 cells via MTA1-induced epithelial-to-mesenchymal transition

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Summary

Objectives: Interleukin-17 (IL-17) induced chronic inflammation has been associated with development, invasion, and metastasis of tumors, which has been demonstrated to promote development of ovarian cancer, prostate cancer, colon cancer, skin cancer, breast cancer, lung cancer and pancreatic cancer. The present authors found that IL-17 promoted ovarian cancer developed, in addition, with a concurrent increase of metastasis-associated genes-1 (MTA1). Whether IL-17 mediates MTA1's action and the underlying mechanisms remain unknown. *Material and Methods:* Cell invasion was detected by wound-healing assay and transwell assay after treatment with IL-17 at 20 ng/ml concentrations for 24 hours. The apoptotic rates of cells were detected by a flow cytometer (FCM) after IL-17 treatment at 20 ng/ml concentrations for 24 hours. The expression of MTA1, Vimentin, Twist, Snail, Slug, N-cadherin, and E-cadherin was detected by Western blot analysis and reverse transcription polymerase chain reaction (RT-PCR) after treatment with 20 ng/ml of IL-17 for 8, 16, 24, and 36 hours, respectively. *Results:* Wound-healing assay and transwell assay demonstrated IL-17 increases ovarian carcinoma cell invasion, and the FCM showed that the apoptotic rates in the IL-17 group were lower than those in the control group ($p < 0.01$). Western blot analysis detected that MTA1, Vimentin, Twist, Snail, slug, and N-cadherin in the IL-17 group were higher than those in the control group, E-cadherin in the IL-17 group were lower than those in the control group, and RT-PCR detected that MTA1 mRNA levels were positively correlated with the time of IL-17 affected on ovarian carcinoma cells ($p < 0.05$). *Conclusions:* IL-17 induces MTA1 expression to enhancing epithelial-to-mesenchymal transition (EMT) and tumor cell invasion, which indicates IL-17-MTA1-EMT axis as potential targets for developing new strategies in the prevention and treatment of ovarian cancer.

Key words: IL-17; MTA1; Epithelial-to-mesenchymal transition; Ovarian cancer.

Introduction

Among female-specific cancers worldwide, ovarian cancer is one of the three female reproductive malignant tumors, and it has the highest mortality; About 80% of patients are diagnosed at advanced stage [1], despite radical surgery and initial high response rates to first-line chemotherapy; up to 70% of patients experience relapses with a median progression-free survival of 12–18 months [2]. There remains an urgent need for novel targeted therapies to improve clinical outcomes in ovarian cancer. Chronic inflammation has been associated with a variety of human cancers. Approximately 15% of all human cancers have been suggested to result from infection and chronic inflammation [3]. Inflammation is a complex response involving many immune cells, chemokines, and cytokines as well as matrix-degrading enzymes.

Interleukin-17 (IL-17) is a key pro-inflammatory cytokine that plays critical roles in many inflammation and autoimmune disease [4]. IL-17 is secreted by T helper 17 cells, natural killer cells, and other immune cells [5], it acts on IL-17RA/IL-17RC receptor complex to activates IL-17-downstream factors promote cancer formation through increased cellular proliferation, attenuated apoptosis, and sustained angiogenesis, as well as creation of an immunotolerant microenvironment [3]. IL-17 induced chronic inflammation has been associated with development, invasion, and metastasis of tumors, which has been demonstrated to promote development of ovarian cancer [6], prostate cancer [7], colon cancer [8], skin cancer [9], breast cancer [10], lung cancer [11], and pancreatic cancer [12].

Metastasis-associated genes-1 (MTA1) has been identified as a metastasis relevant gene in 1994, and emerged as one of the highly deregulated oncogenes in human cancer, and its elevated levels correlate well with tumor aggressiveness and unfavorable outcomes for cancer patients in general [13]. MTA1 protein control a spectrum of cancer promoting processes by modulating the expression of target genes and the activity of MTA-interacting protein, which regulated the transcription factors linked to epithelial-to-mesenchymal transition (EMT) process during metastasis [14]. MTA1 interacts with the E-cadherin chromatin to repress its transcription, leading to increased invasion [15]. The mechanism of MTA1 regulation of E-cadherin is dependent on MTA1 interaction with Snail and Slug proteins, the two upstream regulators of E-cadherin gene ex-

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pression. EMT has been associated with cellular invasiveness and cancer metastasis [16], thereby, provides a novel mechanism for cancer invasion [13].

The present study aims to observe the growth of SKOV3 cells after added with IL-17 and to investigate the mechanism of the IL-17 promoted occurrence and development in ovarian carcinoma.

Materials and Methods

IL-17 was obtained from R&D Systems, MTA1 was obtained from Santa Cruz Biotechnology, E-cadherin, N-cadherin, Fibronectin, Vimentin, Snail, slug, and Twis was obtained from Cell Signaling Technology. IL-17 was dissolved in phosphate-buffered saline (PBS). Invasion assay was performed using Corning BioCoat Matrigel Invasion Chambers following the manufacturer's instructions, and RevertAid First Strand cDNA Synthesis Kit were obtained from Santa Cruz Chemicals, ZSGB-BIO Chemicals, and MBI Chemicals, respectively. TRIzol and UltraSYBR mixture were also purchased from CWBIO Chemicals. All cells were grown in RPMI-1640 culture medium containing 10% fetal bovine serum, L-glutamine (2 mmol/l), penicillin (100 U/ml), and streptomycin (100 U/ml) at 37°C in a humidified 5% CO_2 atmosphere.

SKOV3 cells (0.2×10^6) were seeded in six-well plates. Cells were allowed to grow, until 100% confluence was achieved. Scratch was made in the plate using P200 pipette tip. After treatment with IL-17 at 20 ng/ml, images were collected at 0, 12, 24, 36, and 48 hours under an inverted microscope. Cell migration was analyzed using NIH ImageJ software. The experiment was conducted in triplicate.

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 24 hours, non-invaded cells were removed from the upper chamber with a cotton swab; the cells invaded through the Matrigel-coated porous membrane were fixed with methanol, stained with 0.5% crystal violet, and counted under a microscope.

The apoptotic rates of cells were detected by flow cytometry (FCM) after treatment with IL-17 at 20ng/ml for 24 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.

The expression of MTA1 in cells 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 expression of Vimentin, Twist, Snail, Slug, N-cadherin, and E-cadherin in cells was detected after treatment with 20 ng/ml in the IL-17 group for 24 hours. The cells were harvested and lysed in 100 µl of lysis buffer through incubation on ice for 30 minutes; subsequently, the ex-tracts were centrifuged at 18,000 × g for 15 minutes to remove cell debris. Protein concentrations were determined using the Bio-Rad 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 anti-MTA1, anti-Vimentin, anti-Twist, anti-Snail, anti-Slug, anti-N-cadherin, and anti-E-cadherin antibodies. 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 with Tris-buffered saline three times, the antibody binding was visualized using ECL and autoradiography. Quantification of the bands was conducted using the quantity one densitometric analysis software.

The total mRNA was extracted, and the mRNA expression of MTA1 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. 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 were extracted with the Feature Extraction software. This software utilizes the robust multi-array average algorithm to adjust the background signals. Normalized data were 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. These data were ordered by p value.

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

Since MTA1 is known to promote cancer cell [17], the authors assessed whether IL-17 can promote ovarian caner invasion. Effect on cell migration was assessed by scratch wound assay and Transwell migration assay. In IL-17 group, healed wound area was found to be smaller than the control (Figure 1A). There was statistically significant (p <0.01 or 0.001, Figures 1B and C) difference in IL-17 group between control group and with a time-dependent manner. Similar trends were observed in transwell migration assay, and the authors found that SKOV3-IL-17 cells invaded through Matrigel-coated porous membrane in significantly larger numbers than SKOV3 control cells ($p <$ 0.01, Figure 2).

To further understand the role of IL-17 underlying the reduced ovarian cancer apoptosis in SKOV3 cells, the authors examined the apoptotic rates of SKOV3 cells after treatment with IL-17 at 20 ng/ml for 24 hours, the authors found that the apoptotic rates decreased in the IL-17 group compared with the control group ($p < 0.01$, Figure 3).

Since MTA1 can promote cancer invasion [18], the authors assessed if IL-17-induced MTA1 could promote ovarian cancer invasion. When treated with 20 ng/ml in the IL-17 group for 8, 16, 24, and 36 hours, Western blot anal-

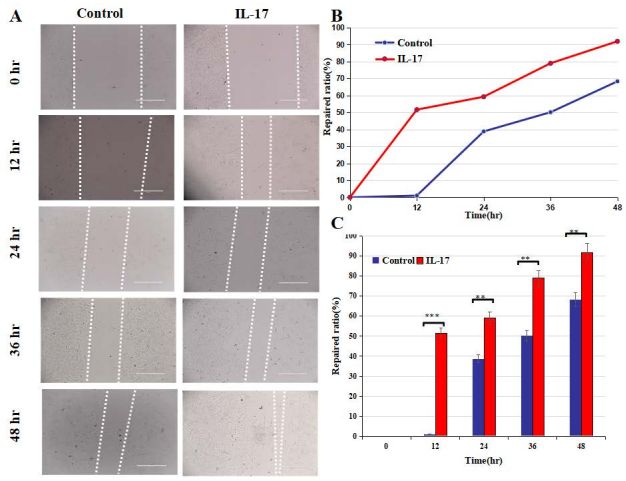

Figure 1. — IL-17 increasing ovarian cancer cell invasion is detected by wound healing assay at 20 ng/ml concentrations for 24 hours A) Representative wound healing images at 0, 12, 24, 36, and 48 hOURS. Wounds are made with a pipette tip in confluent monolayers. B) Curve of wound healing repaired rates. C) Quantification of wound healing repaired rates. Data are mean ± SD. $^{**}p < 0.01$, $^{***}p < 0.001$. The experiments were repeated three times.

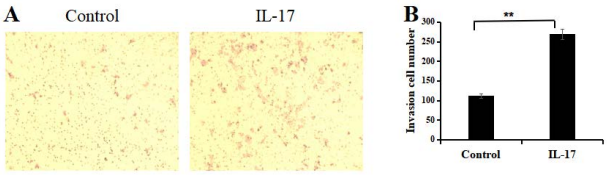

Figure 2. — IL-17 increasing ovarian cancer cell invasion is detected by Transwell assay at 20 ng/ml concentrations for 24 hours. A) Representative photomicrographs of ovarian epithelial cancer cells invading through the Matrigel®-coated porous membrane. B. The number of invasion cells. Data are represented as mean ± SEM, n = 3 wells per group, $^{**}p < 0.01$. The experiments were repeated three times.

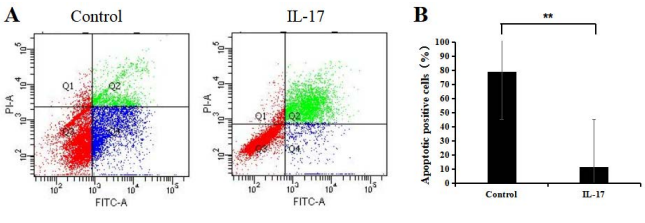

Figure 3. — Apoptotic rates of cells are detected by flow cytometry after added IL-17 at 20 ng/ml concentrations for 24 hours. A) Representative photograph of flow cytometry analysis. B) Flow cytometry analysis comparison of apoptotic cells, demonstrated that cell apoptosis was decreased in the IL-17 treated group compared with the control group. Data are represented as mean ± SD, $^{**}p < 0.01$. The experiments were repeated three times.

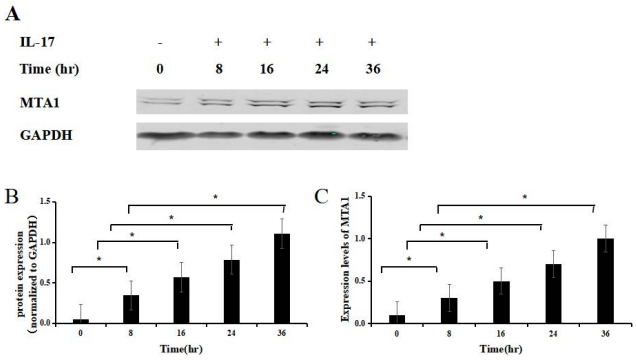

Figure 4. — Western blot and RT-PCR analysis of MTA1 in SKOV3 cells after adding IL-17 for 8, 16, 24, and 36 hours, respectively. A) Western blot analysis of MTA1 in ovarian cancer cells. B) Western blot analysis protein expression of MTA1. Data are represented as mean ± SD, $^{*}p < 0.05$. The experiments were repeated three times. C) RT-PCR analysis the mRNA levels of MTA1. Data are represented as mean ± SD, $^{*}p < 0.05$. The experiments were three times.

ysis detected that the proteins of MTA1 were more increased with a time-dependent manner (Figure 4), and the expression of MTA1 was statistically significant ($p < 0.05$, Figure 4B). These results suggested that IL-17 can induce MTA1 expression.

In order to further verify if IL-17 induces MTA1 expression, RT-PCR detected that the mRNA relative quantity of MTA1 were increased in ovarian cancer correlated with the time of IL-17 affected on the cells ($p < 0.05$, Figure 4C),

MTA1 can interact with the E-cadherin chromatin to repress its transcription, leading to increased invasion [15]. Thus, the authors tested if IL-17 could down-regulate E-cadherin to enhance EMT. The authors generated IL-17 overexpressing cell SKOV3-IL-17, and Western blot analysis showed that MTA1, Vimentin, Twist, Snail, slug, and N-cadherin in the IL-17 group were higher than those in the control group, E-cadherin in the IL-17 group were lower than those in the control group ($P < 0.01$, Figures 5). These results suggested that IL-17 induced MTA1 expression, leading up-regulated Vimentin, Twist, Snail, slug, and N-cadherin, and down-regulated N-cadherin, hence inducing EMT.

Discussion

Cancer is a disorder of hyperproliferation involving morphological cell transformation, uncontrolled proliferation, dysregulated apoptosis, invasion, metastasis, and angiogenesis [19]. The link between inflammation and cancer has been targeted for the prevention or treatment of malignant tumours. The role of IL-17 in carcinogenesis was quite controversial [20], IL-17 was proposed to have both pro-tumorigenic role [21] and anti-tumorigenic role [22]. IL-17 induced chronic inflammation has been associated with de-

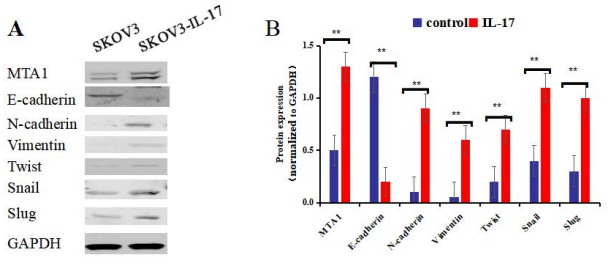

Figure 5. — IL-17 induces EMT in SKOV3 cells. A) Western blot analysis of EMT markers in SKOV3 cells. B) Western blot analysis protein expression of EMT markers. Data are represented as mean ± SD, $^{**}p < 0.01$. The experiments were repeated three times.

velopment, invasion, and metastasis of tumors, which has been demonstrated to promote development of ovarian cancer [6], prostate cancer [7], colon cancer [8], skin cancer [9], breast cancer [10], lung cancer [11], and pancreas cancer [12]. IL-17 has been shown to cause marked epithelial hyperproliferative responses and inflammatory infiltrates in a colon cancer model [8], and also a recent study has demonstrated a hematopoietic-to-epithelial IL-17 signaling axis as another important driver of pancreatic carcinogenesis [12].

IL-17 induced chronic inflammation has been associated with development, invasion and metastasis of tumors, which has been demonstrated to promote development of ovarian cancer [6]. In the present study, the authors found that IL-17 promoted ovarian cancer developed, in addition, with a concurrent increase of metastasis-associated genes-1(MTA1). Whether IL-17 mediates MTA1's action and the underlying mechanisms remain unknown. The study showed that MTA1 could regulate the transcription factors linked to epithelial-to-mesenchymal transition (EMT) process during metastasis [14], which interacts with the E-cadherin chromatin to repress its transcription, leading to increased invasion [15]. The mechanism of MTA1 regulation of E-cadherin is dependent on MTA1 interaction with Snail and Slug proteins, the two upstream regulators of E-cadherin gene expression. It is not clear whether MTA1 expression plays any role in regulating EMT markers levels in ovarian cancer. The present study clearly shows that MTA1 can regulate EMT markers levels, resulting in regulation invasiveness and metastasis of ovarian cancer. The present study provides several lines of evidence to support the existence of IL-17-MTA1-EMT axis in ovarian carcinogenesis. Firstly, IL-17 increased ovarian epithelial cancer SKOV3 cell invasion and flow cytometry analysis demonstrated that cell apoptosis was decreased in the IL-17 treated group compared with the control group. Secondly, IL-17 directly induced MTA1 expression in ovarian cancer cell. Thirdly, MTA1 mRNA levels were positively correlated with the time of IL-17 affected on ovarian carcinoma cells, in a time-dependent manner. Finally, IL-17 induced expression of EMT markers in ovarian cancer cell, which strongly supports that IL-17 mediates EMT action in induction of MTA1. Therefore, it is possible that IL-17-induced MTA1 expression triggers EMT, resulting in promoting cellular proliferation and inhibiting apoptosis.

In summary, then present finding demonstrate that IL-17 induces MTA1 expression to enhancing EMT and tumor cell invasion, which indicates IL-17-MTA1-EMT axis as potential targets for developing new strategies in the prevention and treatment of ovarian cancer, this reveals the role and mechanisms of interleukin-17 in the occurrence and development of ovarian epithelial cancer. The authors also found that MTA1 mRNA levels were positively correlated with the time of IL-17 affected on ovarian carcinoma cells, thus providing novel targets for ovarian epithelial cancer therapy. However, further studies are warranted to assess the prognostic potential IL-17 in human ovarian cancer.

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