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WWOX regulates the Elf5/Snail1 pathway to affect epithelial-mesenchymal transition of ovarian carcinoma cells *in vitro*

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Abstract. – OBJECTIVE: Ovarian cancer is a highly invasive type of cancer. A previous study demonstrated that E-cadherin expression was upregulated in a human ovarian cancer cell line with a high expression of WW domain-containing oxidoreductase (WWOX), which is a tumor suppressor. Also, the migration and invasion ability of these cells was reduced. Snail family members are involved in the epithelial-to-mesenchymal transition (EMT) of ovarian cancer cells, and the expression of Snail family members is regulated by the transcription factor Elf5. The aim of the present research was to elucidate the role of WWOX in EMT of ovarian carcinoma cells through the Elf5/Snail pathway by gain and loss of function approaches in *in vitro* experiments.

MATERIALS AND METHODS: First, a WWOX gene expressing plasmid was transfected into CD133+CD117+ HO8910 ovarian carcinoma cells, and an Elf5 shRNA plasmid was transfected into these cells to assess the changes in EMT-related factors, including Snail1, and the invasive ability of tumor cells ability. Second, the human ovarian carcinoma cell lines HO8910 and SKOV3 were divided into six groups to detect the same indicators.

RESULTS: The results demonstrated that the high expression of WWOX resulted in an increased E-cadherin expression, decreased Snail1 activity, and decreased invasion ability in CD133+CD117+ HO8910 cells. Elf5 shRNA transfection did not affect the WWOX expression; however, it decreased the expression of E-cadherin and Elf5 activity, while increasing Snail1 activity and invasion ability in CD133+CD117+ HO8910 cells. It was also observed that WWOX overexpression in HO8910 and SKOV3 cells inhibited the expression of EMT-related proteins and inhibited cell migration and invasion.

CONCLUSIONS: Taken together, the results of the present report suggest that WWOX can decrease Snail1 activity by enhancing the activity of Elf5, thus upregulating E-cadherin expression and eventually inhibiting EMT of ovarian carcinoma.

Key Words:
Ovarian carcinoma, WWOX, Elf5, Epithelial-to-mesenchymal transition.

Abbreviations
ANOVA, analysis of variance; bHLH, basic helix-loop-helix; EMT, epithelial-to-mesenchymal transition; FBS, fetal bovine serum; JNK1, Jun N-terminal protein kinase 1; TRADD, tumor necrosis factor receptor associated death domain; TRAF2, tumor necrosis factor receptor-associated factor 2; WWOX, WW domain-containing oxidoreductase.

Introduction

Due to its hidden location and complicated mechanism of oncogenesis, ovarian carcinoma is difficult to diagnose at the early stages of the disease and responds poorly to treatment. Therefore, it has the highest mortality rate among gynecological malignant neoplasms and poses a significant threat to the health of women worldwide[1]. WW domain-containing oxidoreductase (WWOX) gene is an oxidoreductase containing WW domain and it exerts a pivotal function in metabolism, oncogenesis, and tumor development[2]. In a previous study[3], our group demonstrated that the WWOX gene can inhibit the growth of HO8910 cells (a human poorly differentiated serous cystadenocarcinoma cell line) and promote their apoptosis. The WWOX gene is also regulated by epigenetic mechanisms and is hypermethylated in ovarian carcinoma. Abnormal CpG island methylation may be an important mechanism leading to low or loss of WWOX gene expression and ovarian carcinoma oncogenesis[4-6].

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During ovarian carcinoma oncogenesis and development, epithelial-to-mesenchymal transition (EMT) is a key process. EMT is defined as a process by which epithelial cells gain certain properties of mesenchymal cells under certain physiological or pathological conditions, including the loss of epithelial surface markers, cell-cell adhesion and cell polarity, the rebuilding of the cytoskeleton, and the gain of mesenchymal phenotypes. These changes increase not only the invasion ability of tumor cells, but also the malignant grade[7,8]. In a previous research, our group investigated the human ovarian carcinoma cell line HO8910 and selected a population of $CD133^+CD117^+$ tumor cells harboring features of so-called cancer stem cells. When compared with normal HO8910 cells, these cells exhibited increased invasive ability, decreased E-cadherin and β-catenin expression, and increased expression of N-cadherin, vimentin, and fibronectin, with apparent EMT. In addition, this study identified a lower WWOX expression in $CD133^+CD117^+$ tumor cells[9].

Our group also examined Elf5 activity in $CD133^+CD117^+$ cells and in normal HO8910 cells and identified a lower Elf5 expression in the former group. Following transfection with the WWOX gene, $CD133^+CD117^+$ cells exhibited increased Elf5 activity, indicating an association between WWOX expression and Elf5 activity[10].

The aim of the present study was to investigate through which pathway WWOX affects EMT of ovarian carcinoma cells. The purpose of the current paper is to address this question using a series of *in vitro* experiments.

Materials and Methods

Cell Lines, Antibodies, and Reagents

The ovarian carcinoma cell lines HO8910 and SKOV3 were purchased from Shanghai Cell Bank, Chinese Academy of Sciences; $CD133^+CD117^+$ HO8910 cells were selected and stored at the Gynecologic Oncology Laboratory, in the Affiliated Hospital of Xuzhou Medical University; pcDNA3.1-WWOX plasmid and Elf5 shRNA plasmid were constructed and stored at the Gynecologic Oncology Laboratory, affiliated with the Hospital of Xuzhou Medical University; Roswell Park Memorial Institute-1640 (RPMI-1640) tissue culture media and fetal bovine serum (FBS) were purchased from Gibco (Thermo Fisher Scientific, Waltham, MA, USA); Lipofectamine 2000 was purchased from Invitrogen (Carlsbad, CA, USA); pcDNA3.1 empty plasmid, WWOX-siRNA, WWOX overexpression plasmid, and primers were purchased from GenePharma Co., Ltd (Shanghai, China); E-cadherin, N-cadherin, vimentin, Snail1, Elf5, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibodies were purchased from Proteintech (Chicago, IL, USA); Matrigel Basement Membrane Matrix was purchased from BD Biosciences (Franklin Lakes, NJ, USA); a transwell chamber was purchased from Chemicon; TRIzol RNA extraction kits were purchased from Life Technologies (Thermo Fisher Scientific, Waltham, MA, USA); Real Time fluorescence quantitative PCR kits were purchased from Cowin Biotech (Beijing, China).

Cell Culture

Human ovarian carcinoma cell lines HO8910 and SKOV3 were cultured in RPMI-1640 media supplemented with 10% FBS and 1% penicillin-streptomycin. Cells were grown at 37°C in 5% CO_2. Transient transfection was performed with Lipofectamine 2000 to cells in the exponential growth phase.

Experimental Groups

(i) $CD133^+CD117^+$ cells were divided into WWOX group transfected with WWOX-expressing plasmid, empty plasmid group, and control group; (ii) $CD133^+CD117^+$ HO8910 cells with high expression level of WWOX were divided into sh Elf5 group transfected with Elf5 shRNA plasmid, and shCtrl group; (iii) the human ovarian carcinoma cell line SKOV3 was divided into SKOV3-WWOX group transfected with WWOX-expressing plasmid, an empty plasmid group and SKOV3 group; (iv) the human ovarian carcinoma cell lines HO8910 and SKOV3 were divided into six different treatment groups, including WWOX-siRNA transfected experimental group; scrambled siRNA transfected control group; WWOX-overexpressing plasmid transfected group (oeWWOX); empty vector control group; WWOX-siRNA and WWOX-overexpressing-plasmid co-transfected experimental group and negative control group.

Western Blot Analysis

The protein concentration in total cell lysates from the different experimental groups was determined using the bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Normalized total cell lysates were mixed with 5×loading buffer, electrophoresed in sodium dodecyl sulphate-polyacrylamide gel

electrophoresis (SDS-PAGE) gels, and transferred to polyvinylidene difluoride (PVDF) membranes using Western blot methods. Following blocking in 10% non-fat milk for 2 h, different targets of protein bands were probed with corresponding antibodies overnight at 4°C. The membranes were given three 5-min washes with Tris-Buffered Saline and Tween-20 (TBST) and then incubated for 2 h at room temperature. The membranes were then washed three times for 10 min with TBST and developed with chemiluminescence reagents. The images were scanned and stored using an Odyssey infrared imaging system.

RT-qPCR

Total cellular RNA was extracted using TRIzol reagent by adding 1 ml of TRIzol reagent per 10 cm^2 dish according to the manufacturer's instructions. cDNA was synthesized with a reverse transcription kit and used as the template to perform fluorescence-based quantitative real-time PCR analysis with the primers listed in Table I according to the manufacturer's protocol. The PCR reaction conditions were as follows: 95°C for 10 min; 95°C for 15 s; 60°C 1 min for 40 cycles. Relative mRNA levels were calculated using the $2^{-\Delta\Delta Ct}$ method with GADPH for normalization. Statistical analysis was performed (Table I). All experiments were performed in triplicate.

In Vitro Cell Scratch Assay

Cells were inoculated in 6-well plates, with approximately $5x10^5$ cells per well. The cells were cultured to approximately 90% confluence after different treatments. A straight line was made with a 100 µl plastic pipette tip by scraping the cells perpendicular to the base of the tissue culture well to create a cell-free gap. The cell surface was washed once slowly with phosphate-buffered saline (PBS) to remove the cell debris. Cells were continually cultured in a serum-free media for 48 h. Images were taken at 0 and 48 h to record the position of the scratch and to calculate the distance of cell migration.

Transwell Migration Assay

Cell migration experiments were performed in 24-transwell chambers. Briefly, wells were kept in starvation for 24 h prior to the assay. Cells were suspended in serum-free media, diluted, and counted. Cells (100,000/200 µl HO8910 and 50,000/200 µl SKOV3, respectively) were added to the upper chambers in serum-free RPMI-1640. A total of 800 µl of RPMI-1640 with 20% FBS was added to the lower chamber. The plates were cultured at 37°C for 18 h. The interior of each insert was wiped with a cotton swab to remove the cells that did not migrate. The membranes were fixed with 90% methanol for 30 min and stained with crystal violet for 20 min. The membranes were rinsed twice with PBS, the chambers were air-dried, and the cells that had migrated through the filter were counted under a microscope. A total of five microscopic fields were randomly selected, and the average number of the migrated cells was calculated.

Transwell Invasion Assay

Matrigel was plated in the upper chamber at room temperature overnight. Cell migration experiments were performed in 24-transwell chambers. Briefly, cells were kept in starvation for 24 h prior to performing the assay. The cells were then suspended in serum-free media, diluted, and counted. Cells (100,000/200 µl HO8910 and 50,000/200 µl SKOV3, respectively) were added to the upper chambers in serum-free RPMI-1640. A total of 800 µl of RPMI-1640 with 20% FBS was added to the lower chamber. The plates were cultured at 37°C for 36 h. The interior of each insert was wiped with a cotton swab to remove the cells that did not migrate. The membranes were fixed with 95% methanol for 30 min and stained with crystal violet for 20 min. The membranes were then rinsed twice with PBS, the chambers were air-dried, and the cells that had migrated through the filter were counted under a microscope. A total of five microscopic fields were randomly selected and the average number of the migrated cells was calculated.

Table I. Primer sequences of real-time PCR.

Gene name	Primer sequence
GAPDH-F	CGCTGAGTACGTGGAGTC
GAPDH-R	GCTGATGATCTTGAGGTGTTGTC
WWOX-F	TCATTGTGGTCTCCTCAGAGTCCC
WWOX-R	AGCCAGCATCGCCCAATAGTC
Snail1-F	CTTCTCCTCTACTTCAGTCTCTTCC
Snail1-R	TGAGGTATTCCTTGTTGCAGTATTT
Elf5-F	CTTGTTCCCTATCTTCCCATT
Elf5-R	AAGCCTCCAAAGTTCTCATCT
E-cadherin-F	CTTCCATGACAGACCCCTTAA
E-cadherin-R	AGAACGCATTTGCCACTACAC
N-cadherin-F	TGTTGGGTGAAGGGGTGCTTG
N-cadherin-R	CACCAGGTTCATCTGTTGC
V-cadherin-F	AGGGGAGTCITCATCATCTGRRGC
V-cadherin-R	ACGGACACGGGACTCATCA

Statistical Analysis

The data were analyzed using SPSS 17.0 software (IBM, Armonk, NY, USA). All data are presented as the mean ± standard deviation ($\bar{x} \pm s$). The Student's *t*-test was used to compare significant differences between two groups. Analysis of variance (ANOVA) was used to compare three or more groups. An alpha of 0.05 is used as the cut off for significance. $p<0.05$ was considered to indicate a statistically significant difference. GraphPad Prism 6 software (GraphPad Software, Inc., La Jolla, CA, USA) was used to produce the graphs.

Results

Changes of EMT-Related Factors and Cell Invasiveness of $CD133^+$ $CD117^+$HO8910 Cells Transfected with WWOX Gene

Following transfection of the WWOX gene, $CD133^+CD117^+$ HO8910 cells exhibited an increased expression level of E-cadherin, decreased Snail1 activity, and no change in Snail2 and Snail3 activity (Figure 1A). Following transfection of the WWOX gene, $CD133^+CD117^+$ HO8910 cells exhibited a decreased invasive ability (Figure 1B).

Changes of EMT-Related Factors and Cell Invasiveness in $CD133^+$ $CD117^+$HO8910 Cells with a High Expression of the WWOX Gene Following Transfection into Elf5shRNA

Following transfection of Elf5 shRNA, no change was observed in WWOX expression in CD133+CD117+HO8910 cells. However, a lower expression of E-cadherin, deceased Elf5 activity, increased Snail1 activity, and changes in Snail2 or Snail3 activity were observed (Figure 2A). Following transfection of Elf5 shRNA, $CD133^+CD117^+$ HO8910 cells exhibited an increased invasive ability (Figure 2B).

Changes of EMT-Related Factors and Invasive Ability of SKOV3 Cells Following Transfection of WWOX Gene

Following transfection of the WWOX gene, SKOV3 cells exhibited increased expression of E-cadherin and a decreased expression of N-cadherin (Figure 3A). Following the transfection of the WWOX gene, SKOV3 cells exhibited a decreased invasive ability (Figure 3B), SKOV3 cells also exhibited an increased Elf5 activity and expression of E-cadherin; however, the activity of Snail1 was decreased (Figure 3C).

Following the Upregulation and Downregulation of WWOX Gene Expression, the Changes of EMT-Related Factors, Elf5, Snail1, Cell Migration and Invasive Ability of HO8910 Cells and SKOV3 Cells Were Observed

Human ovarian carcinoma cell lines HO8910 and SKOV3 were divided into six treatment groups. These groups included WWOX-siRNA transfection group; interfering sequence negative control transfection group; WWOX overexpression plasmid transfection group; plasmid negative control group; WWOX-siRNA and WWOX overexpression plasmid co-transfection group, and negative control group.

The Effects of WWOX on Ovarian Carcinoma Cell Migration Ability

The effects of the WWOX gene on ovarian carcinoma cell migration ability were evaluated by a cell scratch assay. The width of the scratch was measured at 3 random locations under a microscope at 0 and 48 h, and the migration rate was calculated as follows: Migration rate = [D(t = 0 h) − D(t = 48 h)] / D(t = 0 h). The upregulation of WWOX expression was observed to induce a decreased migration rate, while the downregulation of WWOX expression increased the migration rate. Overexpressing WWOX after the downregulation of WWOX also decreased the migration rate. The differences among each group were statistically significant ($p<0.01$, Figure 4A-4C). These results indicate that WWOX can decrease the migration ability of ovarian carcinoma cells, while attenuating WWOX can promote the migration of these cells.

The Effects of WWOX on Ovarian Carcinoma Cell Migration Ability by a Transwell Assay

The numbers of migrated cells in experimental and control groups were measured. There were fewer migrating cells in the WWOX overexpression group than in control group. When WWOX was silenced, the number of cells that migrated was significantly increased. The number of migrated cells was significantly lower following the transfection of the WWOX plasmid into silencing group ($p<0.01$; Figure 5A and 5B). Results demonstrate that WWOX can inhibit the migration ability of ovarian carcinoma cells.

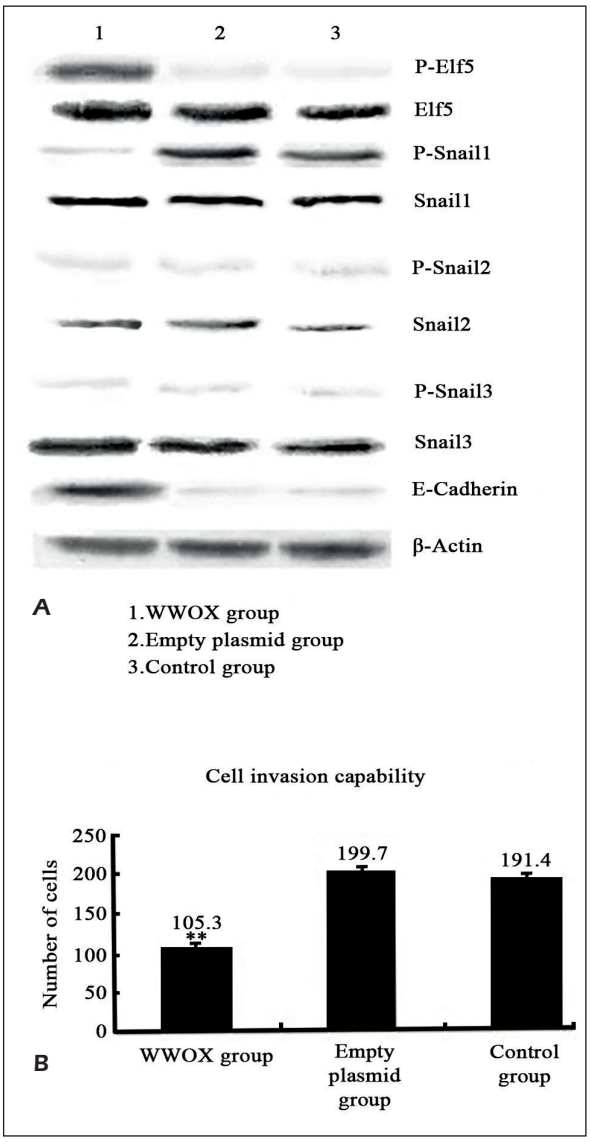

Figure 1. A, Following transfection of the WWOX gene, CD133+CD117+ HO8910 cells exhibited an increased expression level of E-cadherin, decreased Snail1 activity, and no change in Snail2 and Snail3 activity. **B**, Following transfection of the WWOX gene, CD133+CD117+ HO8910 cells exhibited a decreased invasion ability.

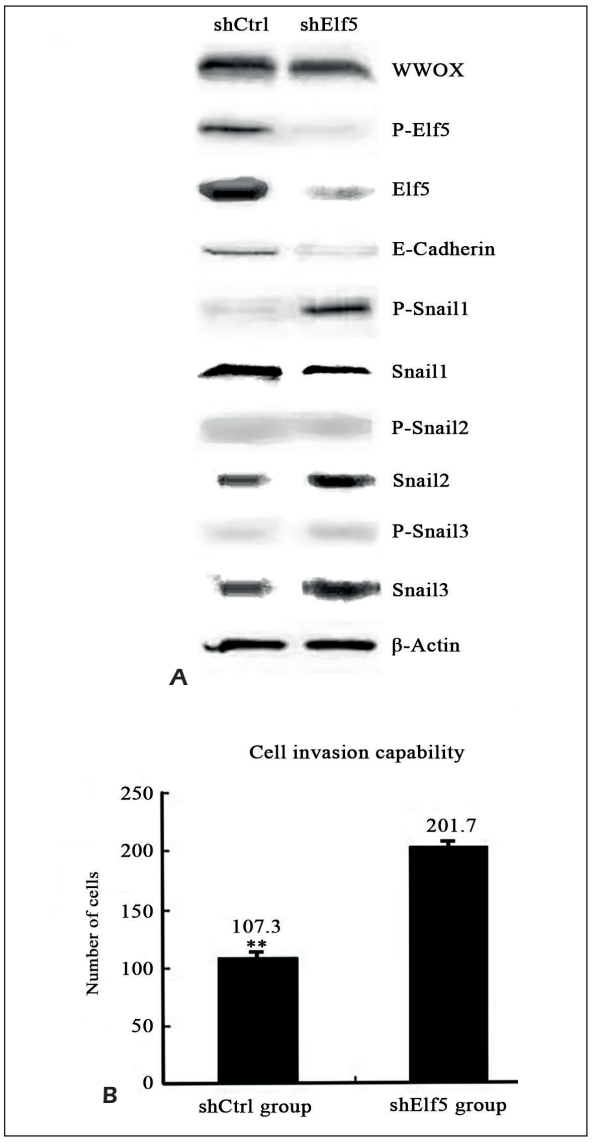

Figure 2. A, Following transfection of Elf5 shRNA, there was no change in the expression of WWOX. However, there was a lower expression of E-cadherin, deceased Elf5 activity, increased Snail1 activity, and a change in Snail2 or Snail3 activity. **B**, Following transfection of Elf5 shRNA, CD133+CD117+ HO8910 cells exhibited increased invasive ability.

The Effects of WWOX on Ovarian Carcinoma Cell Invasion Ability

The ability of tumor cells to invade through Matrigel can reflect their invasive ability. The number of cells passing through the microporous membrane in each group was assessed by a transwell assay. When compared with control group, WWOX overexpression can inhibit the ability of ovarian carcinoma cells to migrate through the filter, while silencing WWOX can promote the ability of ovarian carcinoma cells to migrate through the filter. The transfection of WWOX following interference decreased the number of migrated cells. The differences among each group were statistically significant (p<0.01; Figure 6A and 6B). These results revealed that the invasive ability was decreased following the overexpression of WWOX, and the downregulation of WWOX in ovarian carcinoma cells could promote their invasive ability.

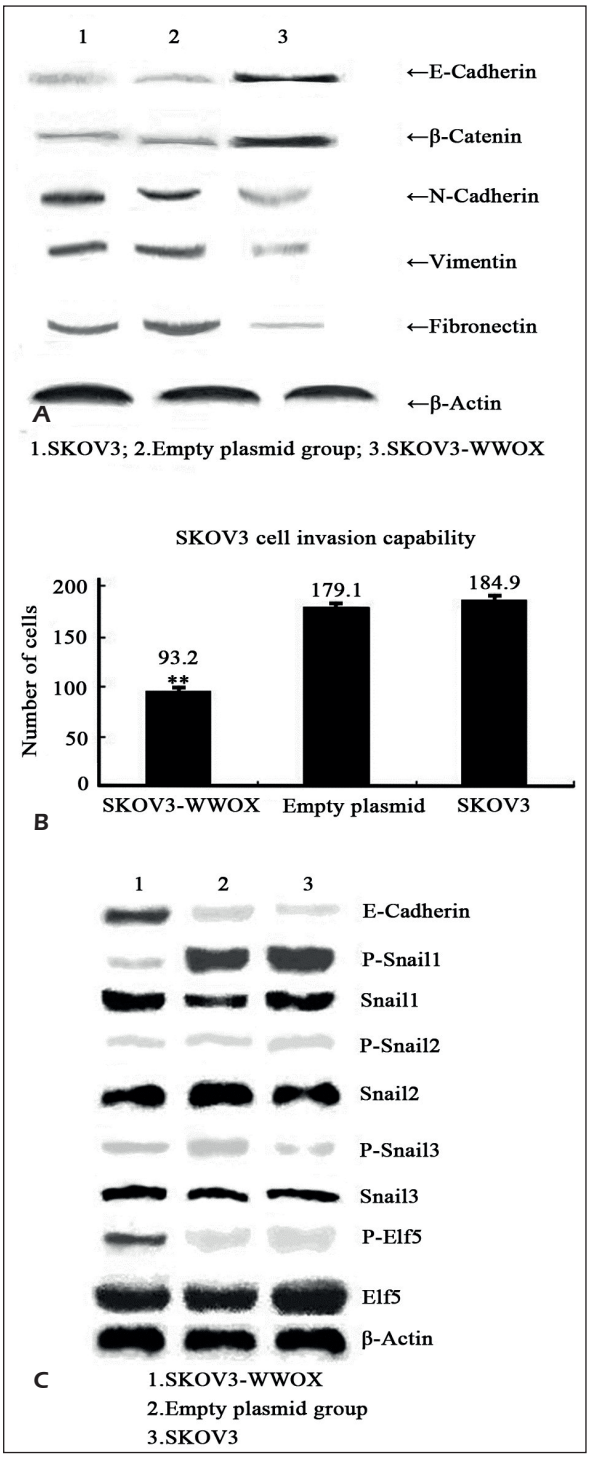

Figure 3. **A**, Following transfection of the WWOX gene, SKOV3 cells exhibited an increased expression of E-cadherin and a decreased expression of N-cadherin. **B**, Following transfection of the WWOX gene, SKOV3 cells exhibited a decreased invasive ability. **C**, Following transfection of the WWOX gene, SKOV3 cells exhibited increased Elf5 activity and the expression of E-cadherin was increased. Whereas Snail1 activity was decreased.

WWOX Induces EMT of Ovarian Carcinoma Cells

RT-qPCR and Western blot analysis revealed that increased E-cadherin mRNA and protein expression levels decreased N-cadherin, Vimentin mRNA, and protein expression levels with WWOX overexpression. When WWOX was downregulated by siRNA, the mRNA and protein expression levels of E-cadherin were decreased, while the mRNA and protein expression levels of N-cadherin and Vimentin were increased ($p<0.05$; Figure 7A-7E), indicating that WWOX can regulate the expression of EMT-related genes.

The Effects of WWOX on the Expression of EMT Regulating Molecules Elf5 and Snail1

To examine the molecular mechanisms by which WWOX regulates ovarian carcinoma EMT, the changes in Elf5, and Snail following the downregulation of WWOX were examined by RT-qPCR and Western blot analysis. Results revealed an increase in Elf5 mRNA and protein expression levels and a decrease in Snail1 levels following transfection of the WWOX overexpression plasmid. In addition, post-transfection of WWOX-siRNA, the mRNA, and protein expression level of Elf5 decreased, and that of Snail1 increased. The differences among groups were statistically significant ($p<0.05$, Figure 8A-8E). These results demonstrated that WWOX can inhibit EMT by regulating the expression of EMT upstream transcription factors Elf5 and Snail1.

Discussion

Invasion and metastasis of ovarian carcinoma and postoperative recurrence are the principal causes of mortality in the majority of patients with ovarian carcinoma. At present, the mechanism underlying tumor invasion and metastasis has yet to be completely elucidated. While there are common mechanisms of tumor metastasis, there are also specific signaling pathways in different types of tumors. Therefore, the aim of the present work was to explore and elucidate the specific mechanism underlying invasion and metastasis of ovarian carcinoma, and to design a corresponding treatment. It is imperative to improve the treatment response and survival rate of patients with ovarian carcinoma. The WWOX gene is located on the FRA16D common chromosomal fragile site and spans the 16q23.3-24.1 region. WWOX

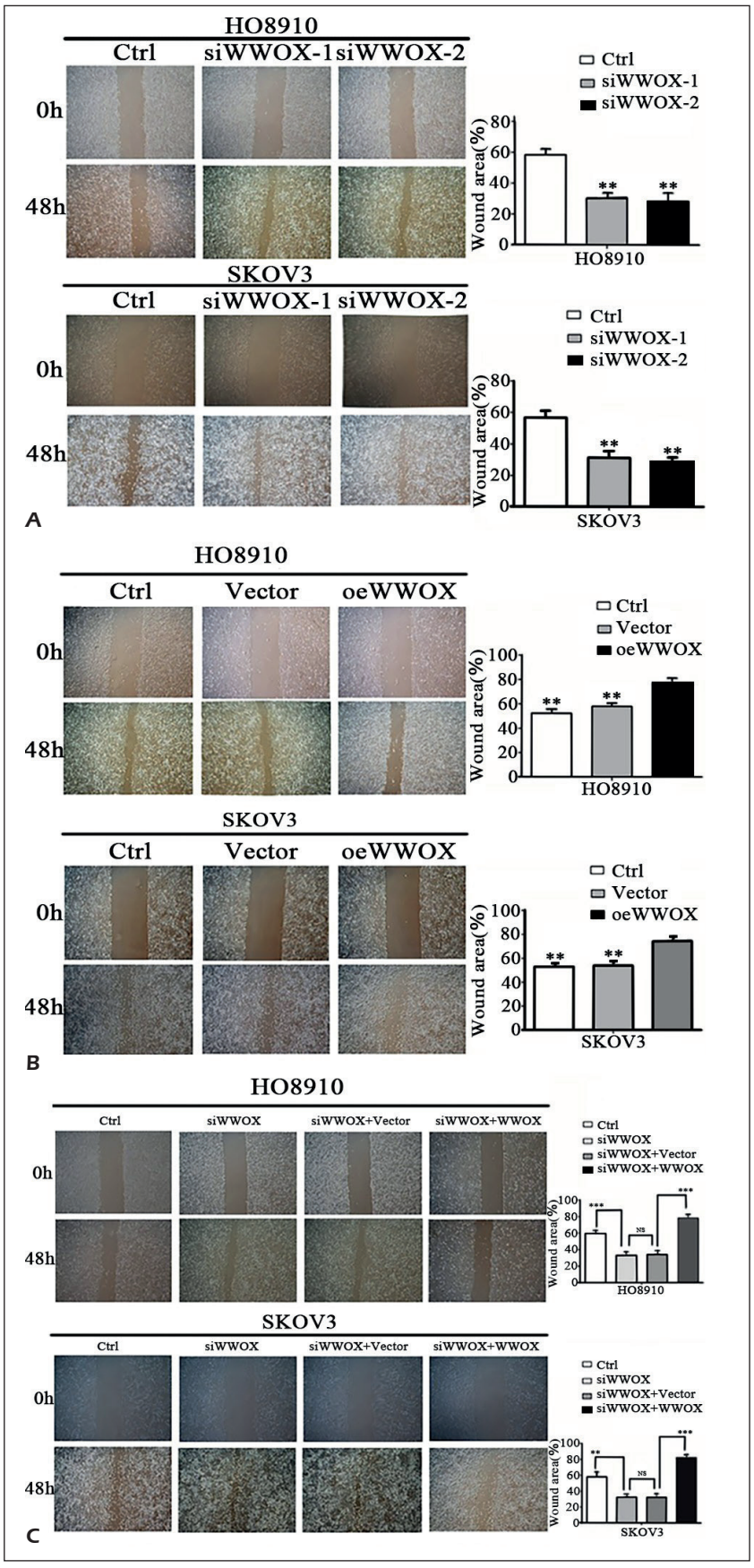

Figure 4. A, In two ovarian carcinoma cell lines, the cells presented with increased migration ability after silencing the WWOX gene. **B**, In the two examined ovarian carcinoma cell lines, the cells exhibited a decrease in migration ability following overexpression of the WWOX gene. **C**, In two ovarian carcinoma cell lines, the cells exhibited decreased migration ability when WWOX was overexpressed after silencing the WWOX gene. $^{**}p<0.01$, $^{*}p<0.05$. 100×.

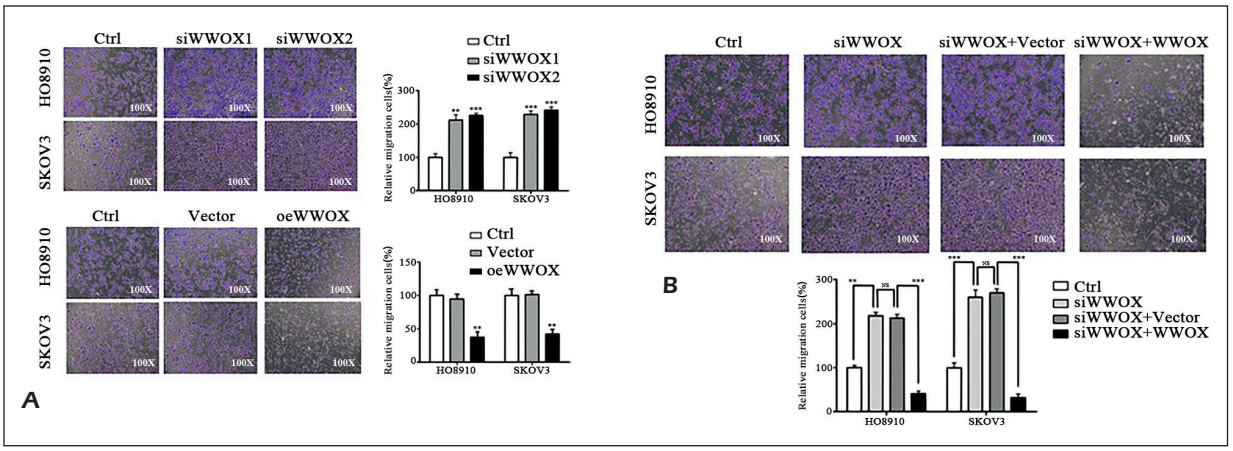

Figure 5. A, In two ovarian carcinoma cell lines, the cells exhibited increased migration ability after silencing the WWOX gene, and had a diminished migration ability following overexpression of the WWOX gene. B, In two ovarian carcinoma cell lines, the cells exhibited a decreased migration ability in response to overexpression of WWOX, after silencing of the WWOX gene. **p<0.01, *p<0.05. 100×.

is composed of 414 amino acids and contains two WW domains at the N-terminal. WWOX serves an important role in multiple signal transduction pathways[11]. WWOX is located in the nucleus and it has been hypothesized to participate in multiple signal transduction pathways, including the apoptotic pathway mediated by tumor necrosis factor receptor-associated death domain (TRADD) and tumor necrosis factor receptor-associated factor 2 (TRAF2), as well as the cell stress pathway mediated by Jun N-terminal protein kinase 1 (JNK1). This leads to the inhibition of tumor proliferation and the promotion of tumor cell apoptosis[12,13].

WWOX is an important tumor suppressor gene, and several investigations have demonstrated that WWOX is closely associated with the oncogenesis and development of breast carcinoma, prostatic carcinoma, lung carcinoma, lymphoma, hepatocellular carcinoma, and pancreatic carcinoma in different animal models[11]. Research results from different laboratories identified that the mRNA level of WWOX was lower in ovarian carcinoma tissue when compared with that in benign neoplasm of ovary or normal ovarian tissue[14,15]. Zhang et al[16] demonstrated that transfection of the WWOX gene into ovarian carcinoma cells can lower their invasion ability. In an *in vitro*

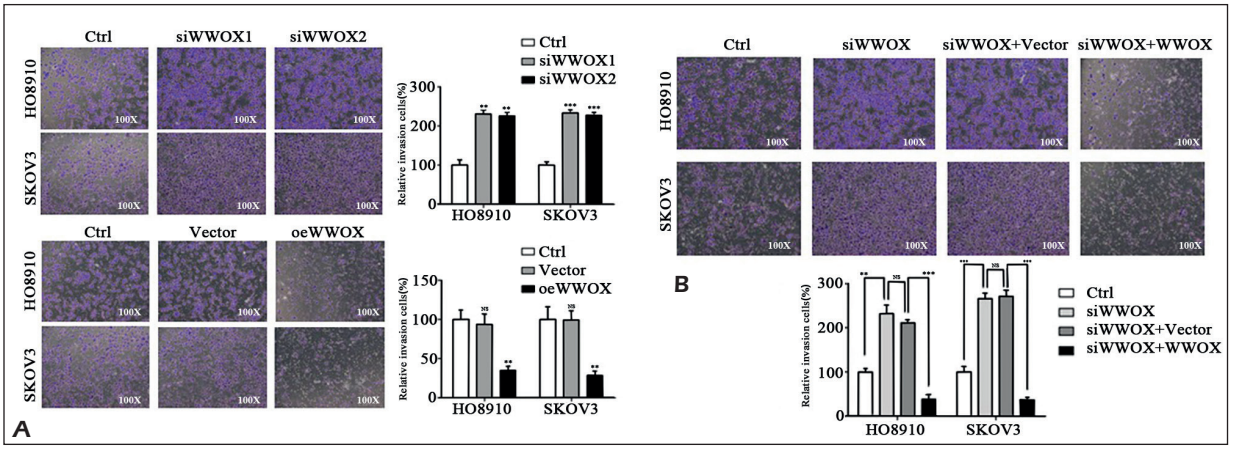

Figure 6. A, In two ovarian carcinoma cell lines, the cells exhibited an increased invasive ability after silencing the WWOX gene, and show decreased invasion ability following the over expression of the WWOX gene. **B**, In two ovarian carcinoma cell lines, the cells exhibited a decreased invasion ability when overexpressing the WWOX gene after silencing the WWOX gene. **p<0.01, *p<0.05. 100×.

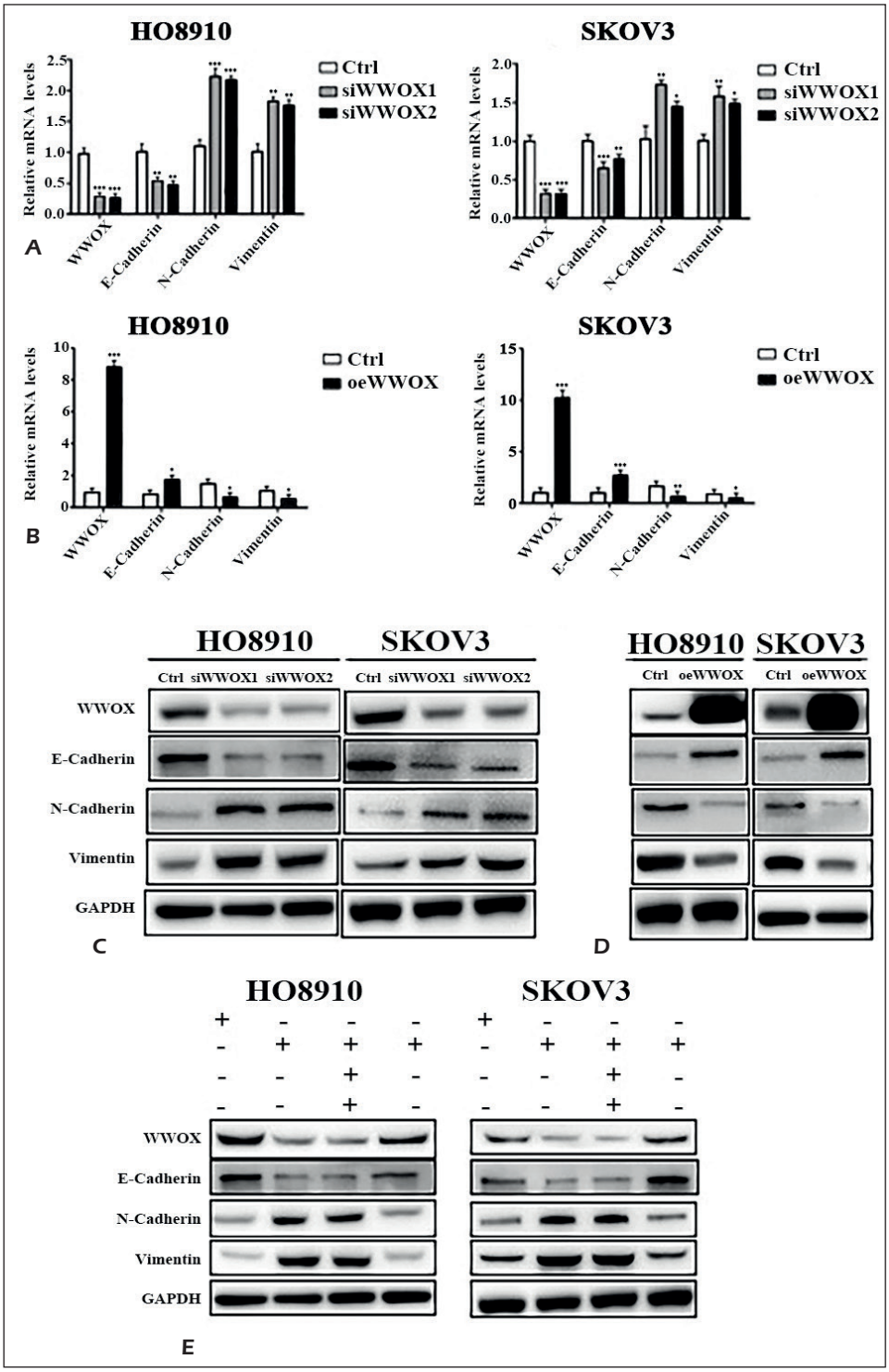

Figure 7. A-B, Relationship of the WWOX gene to EMT-related protein at the mRNA level was here examined using RT-qPCR; C-E, Relationship of the WWOX gene to EMT-related protein at the protein level was here examined using Western blot. $^{**}p<0.01$, $^{*}p<0.05$.

study using an ovarian carcinoma cell line, Gourley et al[17] demonstrated that WWOX overexpression can lower their oncogenesis and enhance cell-cell adhesion. Xiong et al[18] reported that WWOX overexpression can efficiently inhibit the growth of A2780 ovarian carcinoma cells. Some studies[19,20] further proved that WWOX served an important role in the oncogenesis and development of ovarian carcinoma, and have been the focus of an increasing amount of attention. However, the detailed function and molecular mechanisms governing this process have yet to be completely elucidated.

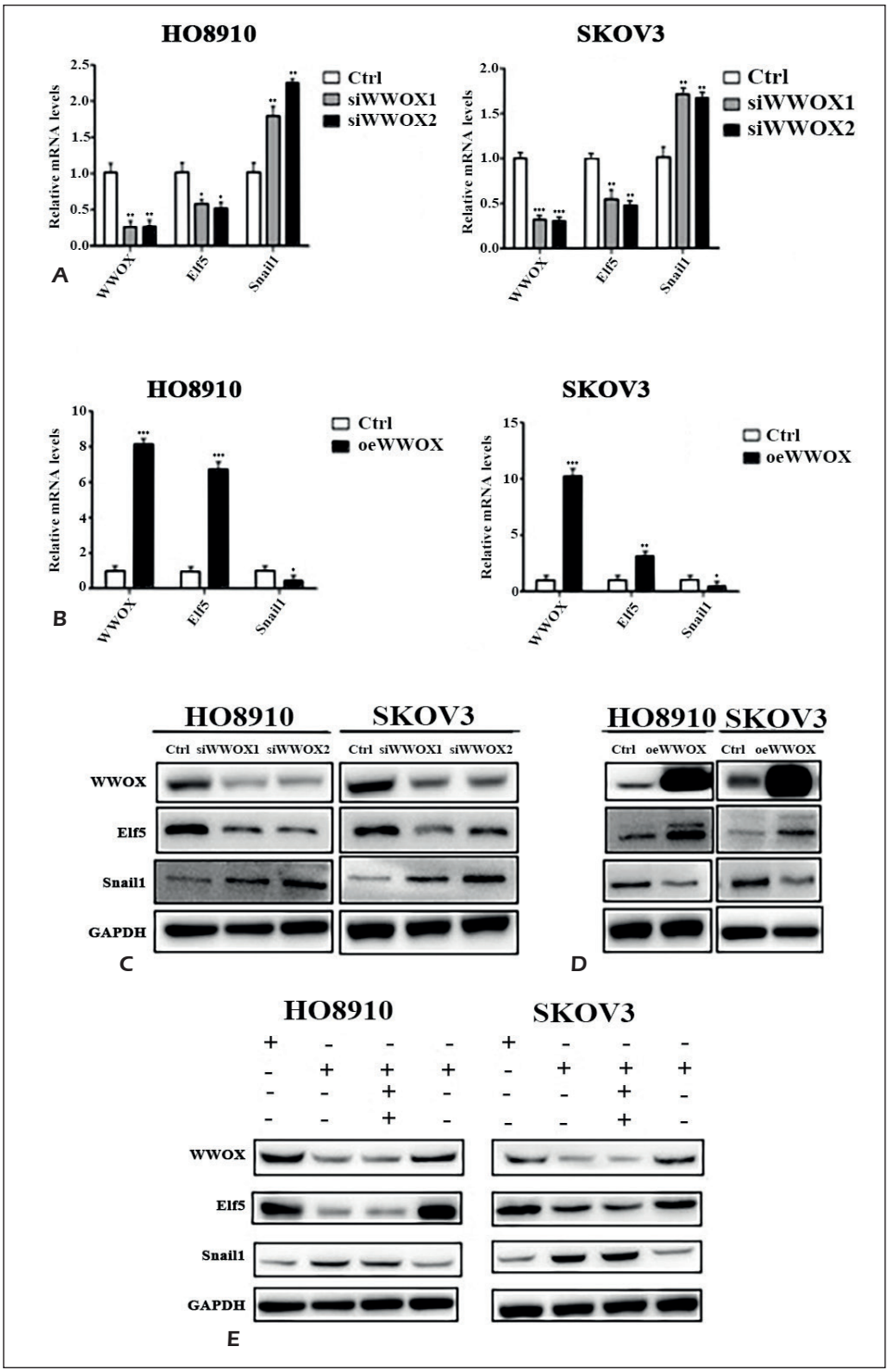

Figure 8. A-B, Relationship of WWOX gene to Elf5 and Snail in mRNA level was examined using RT-qPCR. **C-E**, Relationship of the WWOX gene to Elf5 and Snail at the protein level was examined using Western blot analysis. $^{**}p<0.01$, $^{*}p<0.05$.

The major molecular traits of EMT are the downregulation of the expression of epithelial markers, including E-cadherin and β-catenin, and the upregulation of the expression of mesenchymal markers, including fibronectin and N-cadherin. The downregulation of E-cadherin expression is the characteristic change of EMT and is a prerequisite condition for carcinoma cell invasion[21,22].

As the most important marker of EMT, E-cadherin is primarily regulated by the transcription factor Snail1. Snail1 can bind to the E-BOX in the E-cadherin promoter, inhibit its transcription, and

lead to EMT[23,24]. Snail1 is a basic helix-loop-helix (bHLH) containing transcription factor which is present in Drosophila, mice, and humans. It is a member of the Snail family [including Snail1 (Snail), Snail2 (Slug), Snail3 (Smuc)]. Snail1 contains four Type I zinc-finger domains and one SNAG domain, which has the ability to undergo protein binding[25-27]. Thus far, the aforementioned studies[28,29] investigating EMT have demonstrated the involvement of a Snail family member. Chakrabarti et al[30] identified Snail2 (Slug), one of Snail transcription factor family members, as the main control factor of EMT of breast carcinoma cells. As a transcription factor regulating the EMT of breast carcinoma cells, Snail2 is located at the central aspect of the signal cascade and regulated by its upstream transcription factor Elf5. Elf5 may regulate the EMT of breast carcinoma cells by directly inhibiting Snail2 activity and decreasing the ability of breast carcinoma cells to invade.

Elf5 (E74-like factor 5) is an epithelial-specific member in Ets (E twenty-six) transcription factor family, and it is also called ESE2. Elf5 is located at the 13-15 region of the short arm of human chromosome 11, a region subject to LOH in human carcinoma, it contains two recognizable domains (E1 and E2), one basic sequence contained in all ETS transcription factors which can bind to the TTCC core sequence, and an E3 domain participating in protein-protein interactions. Elf5 plays an important role in cell development, differentiation and apoptosis, and regulates cell proliferation and tumorigenesis[31-33].

These findings and our previous research indicated that the low expression of WWOX may be associated with EMT. Therefore, the present research constructed a plasmid for WWOX expression and transfected it into $CD133^{+}CD117^{+}$ HO8910 cells, which are considered to be cancer stem cells in ovarian carcinoma. In $CD133^{+}CD117^{+}$ in HO8910 cells with a high WWOX expression, an increase in the expression of E-cadherin was observed. Also, there was a decreased Snail1 activity, and these cells had a decreased invasive ability, which demonstrated that WWOX can control the EMT of ovarian carcinoma cells.

To further validate these findings, the present study designed a downregulation experiment by constructing Elf5 shRNA plasmid and transfected it into $CD133^{+}CD117^{+}$ HO8910 cells with high WWOX expression. Following transfection of Elf5 shRNA, the results revealed no change in the expression of WWOX, a lower expression of E-cadherin, reduced Elf5 activity, and increase in Snail1 activity, no change in Snail2 and Snail3 activity, as well as a greater invasive ability of these cells. Following transfection of the WWOX plasmid into ovarian carcinoma cell line SKOV3, there was an increased E-cadherin expression, a lowered N-cadherin expression, and a decreased migration ability. These findings demonstrated that WWOX can regulate EMT of SKOV3. Following transfection with WWOX, SKOV3 cells also exhibited increased Elf5 activity, increased E-cadherin expression levels, and decreased Snail activity.

The present work also observed the following: i) Upregulation of WWOX led to a decreased migration rate, while downregulation of WWOX led to an increased migration rate in ovarian carcinoma cells. These findings indicate that WWOX overexpression can lower the migration ability of ovarian carcinoma cells, and interfering with WWOX can promote their migration ability; ii) WWOX overexpression can decrease the number of migrated ovarian carcinoma cells, while the number of migrated ovarian carcinoma cells significantly increased after the silencing of WWOX. Transfection of the WWOX overexpressing plasmid after the silencing of WWOX also decreased the number of migrating cells. These findings indicate that the invasion ability of ovarian carcinoma cells was attenuated following the overexpression of WWOX, while the invasion ability of ovarian carcinoma cells was increased when the expression of WWOX was downregulated.

Our reports further indicated the following: i) When WWOX was overexpressed, there were increased mRNA and protein expression levels of the EMT-related protein E-cadherin, decreased mRNA and protein expression levels of N-cadherin, and Vimentin. When WWOX was interfered by siRNA, the mRNA and protein expression level of E-cadherin were decreased, while the mRNA and protein expression level of N-cadherin and Vimentin were increased. These findings indicate that WWOX can regulate the expression of EMT-related genes; ii) Following transfection of the WWOX overexpression plasmid, Elf5 mRNA and protein expression levels increased, while Snail1 levels decreased. Following the transfection of WWOX-siRNA, Elf5 mRNA and protein expression levels decreased, while the Snail levels increased. These findings demonstrated that WWOX could inhibit EMT by regulating the expression of EMT upstream transcription factors Elf5 and Snail1.

Conclusions

We demonstrated that, *in vitro*, the WWOX gene can decrease Snail1 activity by enhancing the activity of transcription factor Elf5, thus increasing E-cadherin expression levels and eventually inhibiting the EMT of ovarian carcinoma. Our work offers a clear and solid theoretical basis for clinical treatment of ovarian carcinoma, and it has important value for basic research and clinical practice. In following investigations, our conclusion should be examined in animal models and in clinical practice, which may offer a solid theoretical basis for clinical treatment of ovarian carcinoma.

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Conflict of Interests

The Authors declared that they have no conflict of interests.

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