Original Article

Expression of Talin1 in tissues of ovarian cancer and its role in invasion and migration of ovarian cancer cells

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Abstract: Objective: To investigate the expression of Talin1 in tissues of ovarian cancer and its role in invasion and migration of ovarian cancer cells as well as the related mechanisms. Method: The expression of Talin1 in the tissues of ovarian cancer and normal ovarian tissues was detected by immunohistochemical methods; lentivirus silencing Talin1, GFP fluorescence and Western blotting were used to detect the LV3-Talin1 silencing efficiency and efficacy; the effect of Talin1 expression on invasion of ovarian cancer cells was detected by Transwell invasion assay; and the effect of Talin1 expression on migration of ovarian cancer cells was detected by wound scratch test; and expression of the proteins E-Cadherin, Vimentin and Twist was detected by Western blotting. Results: Compared with normal ovarian tissues, the Talin1 expression in tissues of ovarian cancer was significantly increased; and the Talin1 expression in tissues of advanced, poorly differentiated ovarian cancer with lymph node metastasis was significantly higher than that in tissues of early, well-differentiated ovarian cancer without lymph node metastasis; LV3-Talin1 lentivirus could effectively inhibit the expression of Talin1; Talin1 silencing could inhibit invasion and migration of the A2780 cells; Talin1 silencing up-regulated the expression of E-Cadherin and down-regulated the expression of Vimentin and Twist. Conclusion: Talin1 expression is significantly increased in ovarian cancer, and Talin1 is closely associated with staging and grading of ovarian cancer; in addition, Talin1 silencing promotes invasion and migration of ovarian cancer cells by inhibiting epithelial-mesenchymal transition (EMT).

Keywords: Talin1, ovarian cancer, EMT, E-Cadherin, transwell

Introduction

Ovarian cancer is one of the three most commonly seen gynecological malignancies, and its incidence is only second to cervical cancer and endometrial cancer, with a 5-year survival rate of 25%~30%. It is the gynecological malignancy with the highest mortality [1]. Since the ovaries are deep in the pelvis and ovarian cancer in the early stage is lack of specific symptoms, most patients are in the advanced stage and have abdominal cavity implantation metastasis at diagnosis [2]. Therefore, studying the mechanism of invasion and metastasis of ovarian cancer and searching the ovarian cancer-related molecular targets have become the focus in ovarian cancer research.

Tumor metastasis is a complex multi-step, multi-stage process, mainly including local infiltration, invasion in blood vessels, metastasis with and survival in the blood circulation system, emigration from blood vessels, and settlement and proliferation in new sites, and the cytoskeletal changes, cell adhesion and changes in kinetic characteristics, epithelial-mesenchymal transition and activation of various signaling pathways are involved. Tumor microenvironment also plays an important role in tumor metastasis process. The microenvironment includes not only the tumor cells, but also the stromal cells, inflammatory cells, vascular system, and extracellular matrix (ECM). The ECM, which contains the growth factors and cytokines required for tumor growth, plays a very important role in the biological behaviors of tumor [3].

Talin is a homodimer composed of two 270 kDa subunits, and it is an important cytoskeletal protein. It plays an important role in the coupling of membrane protein and cytoskeletal structure and in the signal transduction pathway [4]. Besides, Talin is also a key molecule in
interactions among ECM, integrin and cytoskeleton and plays an important role in tumor invasion and metastasis [5]. Many studies have shown [6, 7] that Talin1 expression is increased significantly in tumor tissues and its expression is associated with invasion and migration of tumors. However, expression of Talin1 in the tissues of ovarian cancer and its role in invasion and migration of the ovarian cancer cells, and the relevant mechanisms are still unclear. In this study, we investigated the expression of Talin1 in the tissues of ovarian cancer, its role in invasion and migration of the ovarian cancer cells and the relevant mechanisms.

Materials and methods

Collection and treatment of clinical specimens

The ovarian cancer tissues of 65 patients confirmed pathologically after surgical excision were collected in Taizhou Hospital of Zhejiang from January 2015 to January 2016, and the normal ovarian tissues of 65 patients who received hysterectomy due to uterine fibroids or other benign uterine lesions and resection of uterine appendages were collected. The patients were aged 52.45 ± 4.79 years. The patients with ovarian cancer did not receive chemotherapy and radiotherapy before surgery. The ovarian cancer staging was in accordance with FIGO2000 criteria: 4 cases in stage I, 10 cases in stage II, 36 cases in stage III, 15 cases in stage IV; 43 poorly differentiated cases, 16 moderately differentiated cases and 6 well differentiated cases. 39 cases received pelvic lymphnodectomy, 26 cases with lymph node metastasis, and 13 cases without lymph node metastasis. When the tumor tissues were removed from the body, they were placed into 4% paraformaldehyde for fixation after blood stain was removed.

Cell line and main reagents

The human ovarian cancer cell line HO8910 was obtained from Wuhan University cell collection center, and C30, A2780, COC1, SKOV3 were from ATCC. Cell culture conditions: cultured in RPMI 1640 containing 10% fetal calf serum at 37°C, 5% CO₂. The fetal calf serum and RPMI 1640 medium were purchased from HyClone. The primary antibodies of Talin1, E-Cadherin, Vimentin and Twist were purchased from Abcam (ab71333, ab40772, ab92547 and ab50887). Transwell chambers were obtained from Millipore (US). Talin1-silencing and control lentiviruses were purchased from Shanghai GenePharma Co., Ltd.

Immunohistochemistry

The tissues were embedded in paraffin and cut into sections of 4 μm in thickness. The immunohistochemical operations were performed according to the immunohistochemical S-P kit (Beijing Zhongshan Golden Bridge Biotech Co., Ltd.); dewaxing of the tissues and then hydrating. Antibody retrieval was performed in microwave with citrate buffer solution for 30 min, cooled down to room temperature, then washed 3 times with PBS, 3 min each time, incubated with 3% H₂O₂ at 37°C for 15 min, then washed 3 times with PBS, 3 min each time, then the primary antibody was placed overnight, washed 3 times with PBS, 3 min each time; the horse-radish peroxidase-labeled donkey anti-rabbit IgG (1:200, Beijing BIOSS Bio-tech Co., Ltd.), washed 3 times with PBS, 3 min each time; then hors eradish peroxidase labeled streptavidin avidin working solution (Beijing BIOSS Bio-tech Co., Ltd.) was placed in 37°C water bath for 20 min, then washed 3 times with PBS, 3 min each time, and then stained with diaminobenzidine (DAB). In the negative control group, the primary antibody was replaced by PBS. All sections were reviewed independently by two pathologists, and 22 representative high power fields (10×40 fold) were selected by each pathologist. The percentage of positive cells in each specimen was counted and the positive results were judged and scored according to the method described by De Falco M et al. [8]: 0 (positive cells less than 1%); 1 (between 1% and 20%), 2 (between 21% and 40%); 3 (between 41% and 60%); and 4 (more than 61%).

Lentivirus transfected into the A2780 cells

One day before the experiment, 5×10³ A2780 cells were inoculated in 96-well plates, to make the cell fusion degree at 40%~60%. According to the GenePharma lentivirus operating manual, the appropriate MOI (multiplicity of infection) of Talin1 lentivirus was determined using the gradients of 0, 10 and 100.

Two sterile EP tubes were prepared. 10 μl of virusesat 1×10⁸ TU/ml were drawn to the first tube and mixed gently, producing no foams. Similarly, 10 μl of viruses were drawn from the
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The experiment consisted of two groups: the silencing group, transfected with Talin1 silencing lentivirus (LV3-Talin1), and the control group, transfected with the negative control lentivirus (LV3-NC). 100-fold diluted virus stock solution was added to the LV3-Talin1 group, and 100-fold diluted negative control virus solution was added to the LV3-NC group. The expression of GFP fluorescence was observed 24 h later.

Effects of Talin1 expression on invasion ability of the ovarian cancer cells detected by transwell invasion assay

All reagents and equipment were pre-cooled on ice. The Transwell chambers were placed in a 24-well plate. 50 μl (0.2 μg/μl) Matrigel gel was evenly applied to inner membrane of Transwell chamber, incubated for 15 min at 37°C to solidify the gel; when digested, centrifuged and counted, the cells were diluted with 2.5×10⁴/mL serum-free medium to prepare cell suspension; the cell suspension was added to the upper Transwell chamber at 200 μL each well, and 500 μL of 10% FBS and medium were added to the lower Transwell chamber, placed in a 37°C incubator for culture; fixed with formalin, stained by crystal violet for 15 min, and then the cells on the inner membrane were wiped with a cotton swab, counted under a microscope, to count the cells that passed through the membrane under 4 high power fields (x40). The experiment was performed in triplicate.

Effects of Talin1 expression on migration ability of the ovarian cancer cells detected by wound scratch assay

Wound scratch assay: The A2780 cells were inoculated to a 6-well plate, and when cell confluence reached 90%, scratch from up to bottom using a 200 μl sterile pipette tip, observe under a microscope, to measure the initial distance of scratch (0 time); at 24 h, 48 h and 72 h, the distances of scratch were measured respectively and photographed, to calculate the cell migration rate. The experiment was performed in triplicate.

Table 1. Comparison of the expression of Talin1 between ovarian cancer tissues and normal ovarian tissues

<table>
<thead>
<tr>
<th>Tissue specimen</th>
<th>Quantity</th>
<th>Talin1 positive N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian cancer</td>
<td>65</td>
<td>45</td>
<td>69.2%*</td>
</tr>
<tr>
<td>Normal ovary</td>
<td>65</td>
<td>15</td>
<td>23.1%</td>
</tr>
</tbody>
</table>

Compared to the normal ovarian tissues, *P<0.05.

Table 2. Relationship between Talin1 protein expression and clinicopathological features of ovarian cancer

<table>
<thead>
<tr>
<th>Clinical pathological parameters</th>
<th>Quantity</th>
<th>Talin1 positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathological stages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>14</td>
<td>7*</td>
</tr>
<tr>
<td>III-IV</td>
<td>51</td>
<td>38</td>
</tr>
<tr>
<td>Degree of differentiation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate-well</td>
<td>22</td>
<td>12*</td>
</tr>
<tr>
<td>Poor</td>
<td>43</td>
<td>33</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26</td>
<td>23*</td>
</tr>
<tr>
<td>No</td>
<td>13</td>
<td>5</td>
</tr>
</tbody>
</table>

*P<0.05.

first tube to the second and mixed well, to get virus solutions at three different concentrations: stock solution, 10x dilution, and 100x dilution.

10 μl of virus solutions at three different gradients were added to three wells in each group, to calculate the MOI of three wells, which were 100, 10 and 1 respectively. The most appropriate MOI was determined to be 100.

Figure 1. The expression of Talin1 in ovarian cancer tissues and normal ovarian tissues detected by immunohistochemical assay (10×).
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Expression of the proteins Talin1, E-Cadherin, Vimentin and Twist detected by western blotting

The proteins were extracted from different ovarian cancer cells, and the protein concentrations were determined by BCA method, and then loading buffer was added for protein denaturation. 8% and 10% SDS-PAGE was prepared, and 20 μg protein sample was added into each hole, then transferred to a PVDF membrane using the electric wet transfer method, sealed 2 h with 5% skim milk, and the primary antibody (Talin1) was diluted by 1:1000 TBST, overnight at 4°C; then 1:5000 dilution of goat anti-rabbit secondary antibody was added, incubated at room temperature for 2 h; and ECL was performed. The experiment was performed in triplicate.

72 h after cell transfection, the total proteins were extracted from the cells in the experimental and control groups, and the protein concentrations were determined by BCA method, and then loading buffer was added for protein denaturation. 8% and 10% SDS-PAGE was prepared, and 20 μg protein sample was added into each hole, then transferred to a PVDF membrane using the electric wet transfer method, sealed 2 h with 5% skim milk, and the primary antibody (E-Cadherin, Vimentin and Twist) was diluted by 1:1000 TBST, overnight at 4°C; then 1:5000 dilution of goat anti-rabbit secondary antibody was added, incubated at room temperature for 2 h; and ECL was performed. The experiment was performed in triplicate.

Statistical analysis

The SPSS 19.0 software was used for statistical analysis, measurement data were expressed in (X ± s), t-test was employed for comparison of means between groups, and P<0.05 indicated statistically significant difference.

Results

Increased expression of Talin1 in ovarian cancer tissues

The immunohistochemical results showed that Talin1 was localized in cytomembrane and cytoplasm. In 65 ovarian cancer tissues, 45 were positive for Talin1 expression; in 65 normal ovarian tissues, 15 were positive for Talin1 expression (Figure 1; Table 1). It suggested that the expression of Talin1 in ovarian cancer tissues was significantly higher than that in normal ovarian tissues, with statistically significant differences (P<0.05).

Correlation between Talin1 expression and clinical-pathological characteristics of ovarian cancer

The expression of Talin1 increased with the increased pathological staging of ovarian cancer (Table 2, P<0.05); with decrease in degree of differentiation, the expression of Talin1 increased gradually (Table 2, P<0.05); in the ovarian cancer tissues with lymph node metastasis, the expression of Talin1 increased significantly (Table 2, P<0.05).

Talin1 expression in different ovarian cancer cell lines

The Western blotting results (Figure 2) showed that compared with other ovarian cancer cell lines, the expression level of Talin1 in the A2780 cells was highest, with statistically significant difference (P<0.05). Therefore, the A2780 cells were chosen as the object for further functional study on Talin1 silencing.

Figure 2. The expression of Talin1 in different ovarian cancer cells, *P<0.05.
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Figure 3. A. Efficiency of transfection of Talin1 knocked down lentivirus into the ovarian cancer A2780 cells detected by GFP fluorescence. B. Expression of Talin1 detected by Western blotting. Error bars represent standard error. *P<0.05.

Figure 4. Effect of silencing Talin1 expression on invasion ability of the A2780 cells detected by Transwell invasion assay. Error bars represent standard error. *P<0.05.

Low expression of Talin1 after LV3-Talin1 was transfected into the A2780 cells

24 h after LV3-Talin1 was transfected into the A2780 cells, the GFP fluorescence results (Figure 3A) showed that compared with the LV3-NC group, expression of green fluorescence in the LV3-Talin1 transfected cells was significantly increased [(90.94 ± 1.46)% vs (20.02 ± 2.03)%, P<0.05]. It suggested that LV3-Talin1 could be well incorporated into the A2780 cells.

The Western blotting results (Figure 3B) showed that compared with the LV3-NC group, the Talin1 expression level in the LV3-Talin1 group was significantly decreased [(86.80 ±
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Silencing Talin1 expression could inhibit invasion ability of the A2780 cells

The Transwell results (Figure 4A and 4B) showed that in the LV3-Talin1 group, the number of cells passing the Matrigel gel was 81.54 ± 6.76, significantly less than that in the LV3-NC group (223.92 ± 12.88), with statistically significant differences (P<0.05). It indicated that silencing Talin1 expression could inhibit invasion ability of the A2780 cells.

Silencing Talin1 expression inhibits migration ability of the A2780 cells

The width of scratches in any three parts of cells in each group was measured under a microscope at the time points of 0 h, 24 h, 48 h and 72 h. The migration rate was calculated according to the formula: Migration rate=[D( h=24,48,72 h)−D( h=0 h)]/D( h=0 h). Results of the wound scratch assay (Figure 5) suggested that compared to the LV3-NC group, migration rate in the LV3-Talin1 group was significantly reduced at 24 h, 48 h and 72 h [24 h (0.17 ± 0.03)% vs (0.29 ± 0.04)%, P<0.05; 48 h (0.28 ± 0.04)% vs (0.57 ± 0.05)%, P<0.05; 72 h (0.61 ± 0.04)% vs (0.87 ± 0.06)%, P<0.05], with statistically significant differences. It was revealed in the wound scratch assay that silencing Talin1 expression could inhibit migration ability of the A2780 cells.

Silencing Talin1 expression could inhibit the expression of E-Cadherin, Vimentin and Twist

Many studies have demonstrated that EMT is activated in invasion and migration of epithelial tumors, which is the critical molecular event enabling the epithelial tumor cells to acquire invasion ability and plays an important role in invasion and migration of malignant tumors [9]. The E-Cadherin protein is the epithelial marker in the EMT process, while Twist and Vimentin are the mesenchymal markers in the process.

Results of Western blotting (Figure 6) showed that compared to the LV3-NC group, the expression levels of Vimentin and Twist in the LV3-Talin1 group decreased significantly [Vimentin (0.14 ± 0.01)% vs (0.81 ± 0.06)%; Twist (0.26 ± 0.02)% vs (0.88 ± 0.06)%, P<0.05]; compared to the LV3-NC group, the expression level of E-Cadherin in the LV3-Talin1 group increased significantly (0.88±0.06) vs (0.38±0.02)%, P<0.05). It suggested that silencing the expression of Talin1 could up-regulate that of
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E-Cadherin, as well as down-regulate that of Vimentin and Twist. It indicated that Talin1 could promote EMT of ovarian cancer cells.

Discussion

Tumor infiltration and metastasis is a complicated process with multiple steps. The first step is to reduce the adhesive capacity of tumor cells, enhance cell mobility and infiltrate the peripheral tissues for distant metastasis [3, 10]. Integrin is involved in all of the processes. The integrin signals are involved in tumor invasion and migration mainly through activating FAK, which is an important linking molecule causing different signal proteins to gather on the adhesion plaques, thereby mediating different biological behaviors.

The Talin protein contains an N-terminal globular head region and C-terminal rod-shaped region, and its head region can bind with FAK. After involved in the formation of adhesion plaque complex, Talin and FAK can bind with integrin intracellular domain to activate integrin and regulate invasion and migration of the tumor cells. It has been shown in study [11] that Talin activates integrin and the highly-expressed Talin head fragments lead to activation of integrin β3, thus demonstrating that Talin plays an important role in the integrin activation process.

In this study, by investigating the expression of Talin1 in ovarian cancer tissues, it has been shown that Talin1 expression in ovarian cancer tissues is significantly higher than that in the normal ovarian tissue, and Talin1 expression in the advanced, poorly-differentiated ovarian cancer tissues with lymph node metastasis is significantly higher than that in the early, well-differentiated ovarian cancer tissues without lymph node metastasis. After silencing Talin1 with lentivirus, the observations on invasion and migration of the ovarian cancer cells have shown that invasion and migration of the ovarian cancer cells are reduced after Talin1 silencing. The study by Fang Kunpeng et al. [12] has found out that Talin1 is associated with the invasion and migration of hepatocellular carcinoma (HCC). The study by Sakamoto et al. [13] has shown that Talin regulates the level of activity of β1 integrin, thereby prompting invasion and migration of the human prostate cancer cells, and Talin silencing results in decreased metastasis of the prostate cancer cells, consistent with the results in this study.

EMT plays an important role in tumor invasion and metastasis. The precondition for metastasis of tumor cells is that the adjacent tissues are infiltrated, the tumor adhesion is weakened to form highly invasive tumor and at the same time, expression of E-Cadherin is down-regulated. A number of studies [14] have shown that expression of E-Cadherin is down-regulated in many malignant tumors, and the malignant tumors with low expression of E-Cadherin have stronger invasion and migration abilities. The
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This study suggests that Talin1 expression is significantly increased in the ovarian cancer tissues and is closely related with tumor staging and grading and lymph node metastasis; in addition, Talin1 promotes invasion and migration of the ovarian cancer cells by inducing EMT. It indicates that Talin1 is likely to be involved in the progression of ovarian cancer and it may evolve into the marker for predicting the progression and prognosis of ovarian cancer and for monitoring the therapeutic effect.

Disclosure of conflict of interest

None.

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