RESEARCH ARTICLE

Expression and Significance of Twist and E-cadherin in Ovarian Cancer Tissues

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Abstract

Objective: To investigate the expression of Twist and E-cadherin in ovarian cancer tissues as well as the role of epithelial-mesenchymal transformation (EMT) in ovarian cancer metastasis. Method: The expressions of Twist and E-cadherin in 54 cases of ovarian cancer and paracancerous tissues were detected by Western blottin g and reverse transcriptase polymerase chain reaction. We used RNA interference to silence Twist expression in human ovarian cancer cell line, and detected E-cadherin expression using Western blotting. Results: There was an increase in the relative abundance of Twist proteins and a decrease in E-cadherin in ovarian cancer compared with normal ovary tissues ($P < 0.05$). The expression levels of Twist and E-cadherin mRNA were 1.49 ± 0.53 and 0.82 ± 0.24 in ovarian cancer, and 1.14 ± 0.38 and 1.08 ± 0.19 in paracancerous tissues, respectively. The difference between the indicators in ovarian cancer and in paracancerous tissues was statistically significant ($P < 0.05$). When the Twist expression was silenced in an ovarian cancer cell line, the expression of the E-cadherin protein increased ($P<0.05$). Conclusion: The expression of Twist is upregulated, whereas that of E-cadherin is downregulated in ovarian cancer. EMT, mediated by Twist, may be correlated with ovarian cancer metastasis.

Keywords: Twist - E-cadherin - ovarian cancer - epithelial-mesenchymal transition

Asian Pacific J Cancer Prev, **14 (2)**, 669-672

Introduction

Ovarian cancer is among the top diseases in the list of malignant gynaecologic tumours, and in recent years, mortality related to ovarian cancer has witnessed an upward trend. Primary ovarian cancer is likely to spread to the surface of the pelvic and abdominal organs. However, the occurrence, development and infiltration, and proliferation mechanism of ovarian cancer remain unclear.

Epithelials cell adhere to the neighbouring cells with adherent junction, tight junction, desmosome, or semidesmosome. During tumour development, cancer cells separate themselves from the junctions and invade the neighbouring interstitial components. Epithelial-mesenchymal transformation (EMT) facilitates the transformation of epithelial cells from epithelial phenotypes with polarity to fibroblast-like mesenchymal phenotypes with high-mobility. Developmental biologists have found that EMT plays an important role in the early embryonic development. EMT is closely related with the metastasis of the tumours, particularly in cancer cell invasion and diffusion, and also plays a pivotal role in the invasion and metastasis of epithelial cancer (Puisieux, 2009). Interruption or reversion of EMT inhibits the invasion of cancer cells and reduces the rate of cancer metastasis. Therefore, EMT has become a hot topic among researchers studying epithelial cancer metastasis. EMT is characterized by the procedures of losing epithelial markers (e.g., E-cadherin) and gaining interstitial markers (e.g., N-cadherin).

Twist is a new oncogene, whose expression is increased in many kinds of neoplasms (Kwok et al., 2005; Niu et al., 2007; Zhang et al., 2007). Yoshida et al. (2009) has found that the expression of the Twist protein is higher in ovarian cancer tissues than in ovarian benign and borderline tumours. Meanwhile, Twist is also a crucial regulatory factor in the EMT process (Kang and Massague, 2004). To date, only a few studies have focused on Twist-mediated EMT in ovarian cancer. In the current work, we have detected the expressions of Twist and E-cadherin in ovarian cancer tissues, and used RNA interference to silence the Twist expression in human ovarian cancer cell line, then detected E-Cadherin expression using Western blot. We also investigated the possible mechanism of Twist-mediated EMT in the occurrence, development, and metastasis of ovarian cancer.

Materials and Methods

Samples

We received and cured 54 cases of ovarian cancer

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patients diagnosed through pathological examination from March 2009 to May 2011. All the patients did not receive neoadjuvant chemotherapy prior to their operation. In addition, 54 cases of normal ovary tissues were set as contrast. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Yantai Yuhuangding Hospital. Written informed consent was obtained from all participants.

Reverse transcriptase polymerase chain reaction (RT-PCR)

First, 1 ml Trizol was added to 50 mg tissue homogenised in liquid nitrogen; this was left to stand for 5 min to 10 min at room temperature. Then, 0.2 ml chloroform was added to the sample, which was blended for 15 s before centrifugation. The supernatant was removed and placed in a 1.5 ml Eppendorf tube, to which an equal amount of isometrical dimethycarbinol was added. After centrifugation, the RNA sediment was washed with 70% ethyl alcohol and dried at room temperature for about 10 mins before finally resolving it in 22 μl DEPC water.

cDNA was synthesised before carrying out PCR. The following primers were used: Twist forward primer 5'-CGGAGACCTAGATGTCATTGTT -3', reverse primer 5'-CTTCTATCAGAATGCAGAGGTG-3', amplified fragment length 244 bp N-cadherin forward primer 5'-ACCAGCCTCCAACTGGTAT -3', reverse primer 5'-TACCTCAACATCCCATTGA -3', amplified fragment length 310 bp E-cadherin forward primer 5'-GTCTGTCATGGAAGGTGCT -3', reverse primer 5'-TACGACGTTAGCCTCGTTC -3', and amplified fragment length 199 bp. The PCR conditions were as follows: 34 cycles at 941 °C for 1 min, 501 °C for 1 min and 721 °C for 1 min, and a final extension at 721 °C for 5 min. Preliminary experiments were conducted to ensure that the PCR conditions were at the logarithmic phase of the PCR reaction. cDNA of β-actin was amplified as a control for the amount of cDNA present in each sample. Semi-quantitative method was used to analyse the expressions of the three genes. PCR products were electrophoresed on 2% agarose gels and then analysed using a gel documentation system (Ultra-Violet Product Limited, CA, USA). The relative expression levels were generated by comparing the density to the controls, which were indicated underneath each gel.

Western blot analysis

Western blot analysis was performed in accordance with a previously described procedure (Kajiyama et al., 2003). Here, 30 μg of cell lysates were prepared by suspending the Twist protein in anticancer drug-resistant cell pellets in the lysis buffer (50 mM Tris-HCl, pH 8.0; 150 mM NaCl; 1% NP40; 0.5% DOC; and 0.1% SDS), which included proteinase inhibitors (1 mg/ml aprotinin, 1 mg/ml leupeptin, and 1mM PMSF). Protein concentration was measured using the BCA Protein Assay kit (Sigma, USA). Equal amounts of protein (30 mg) were loaded to an SDS-polyacrylamide gel for electrophoresis, through which the proteins were transferred to a PVDF membrane (Millipore, USA). The membrane was then incubated with primary antibodies for 1 h at room temperature with primary antibodies against the Twist protein (Santa Cruz), E-cadherin, and N-cadherin (Fuzhou Maxim Company). The membrane was washed thrice with Tween/PBS for 15 min each time, and then incubated with the appropriate secondary antibody for 1 h. The expression of actin was also measured as an internal loading control using a specific antibody (Santa Cruz Biotechnology). The respective ratios of the relative amounts of each protein to actin were indicated underneath each gel. Results represented three independent experiments.

RNA interference

Ovarian cancer cell line A2780 cells were transfected with pGenesil Twist shRNA plasmid and pGenesil si-control plasmid. After cultivation for 24, 48, and 72 h, the cells were collected. Transfection was observed under a fluorescence microscope. RT-PCR was used to detect the expression of the Twist mRNA in the transfected cells, and then Western-blot was used to detect the expression of E-cadherin pretein.

Statistical analysis

SPSS13.0 statistical software was used. For the data comparison, we adopted the inspection, x^2 inspection, and Fisher's exact test. The difference, $P < 0.05$, has statistical significance.

Results

RT-PCR

RT-PCR was employed to test the expressions of Twist and E-cadherin mRNA in 54 cases of ovarian cancer tissues and 54 normal ovary tissues. The results revealed that, in the ovarian cancer tissue, the expressions of the Twist protein (Figure 1A) whereas that of E-cadherin decreased (Figure 1B) compared with the normal ovary tissue. The mRNA level of Twist/β-actin was significantly higher in ovarian cancer tissue (1.49 ± 0.53) compared with the normal ovary tissue (1.14 ± 0.38) ($P < 0.05$), that of E-cadherin/β-actin was significantly lower in ovarian cancer tissue (0.82 ± 0.24) than in normal ovary tissue (1.08 ± 0.19) ($P < 0.05$) (Figure 2, Table 1).

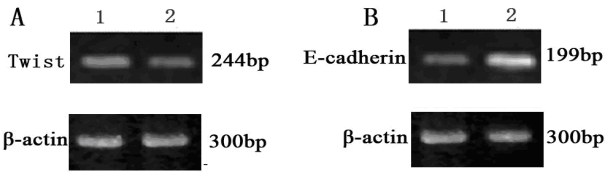

Figure 1. RT-PCR Results of the Twist, E-cadherin and N-cadherin. 1. Ovarian cancer tissues; 2. Normal ovary tissues

Table 1. Table 1 Comparison of the Expressions of the Twist, E-cadherin mRNA Between Ovarian Cancer and Normal Ovary Tissues

Tissue	n	Twist mRNA	E-cadherin mRNA
Ovarian cancer	54	1.49±0.53*	0.82±0.24*
Control	54	1.14±0.38	1.08±0.19

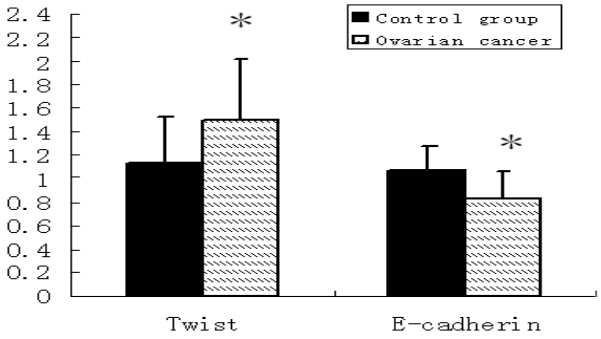

Figure 2. mRNA Expression Result

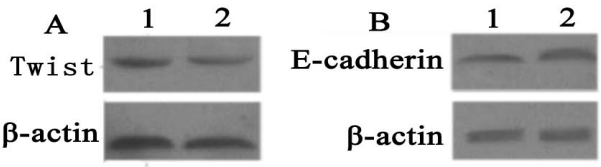

Figure 3. Westen Blot Analysis

Western blot analysis

Western blot analysis results revealed that the expressions of the Twist protein in ovarian cancer tissue were higher than in the normal ovary tissue, whereas that of the E-cadherin protein was reduced. Relative gray value showed that P values were all less than 0.05 (Figure 3).

RNA interference

Western blot analysis results revealed that the expression of E-cadherin in A2780-Twist-shRNA group increased significantly compared with that A2780-non-transfection group ($P < 0.05$).

Discussion

Ovarian cancer is among the top diseases in the list of malignant gynaecologic tumours. The possible reason is that the invasion of ovarian cancer in most of the patients has already proliferated intra-abdominally, and such invasion and metastasis have led to the failure of the treatments being applied. Therefore, it is necessary to study the relevant molecular mechanism in the occurrence, development, and metastasis of ovarian cancer.

Tumour metastasis is a multi-step process involving the cell adhesion in the primary tumour, penetration of partial mesenchyma, entrance of partial vessel or lymphatic vessel, aggregation with the blood platelet, adhesion and interaction with the distant endothelium, movement out of the vessel, and finally, settling down and further growth (Geho et al., 2005; Thompson and Newgreen, 2005). EMT plays a major role in embryonic development, organ fibrosis, and intrusion and metastasis of neoplasms (Radisky, 2005). Epithelial cells lose polarity during EMT, and the motor ability phenotype is gained by remoulding some molecules' cytoskeleton and down-regulating or up-regulating them through tight junction, adhesion junction and mesenchyma markers, thus reinforcing metastatic ability.

EMT is a critical event in the process of tumour metastasis. An increasing number of studies (Thiery, 2002; Larue L and Bellacosa, 2005) have demonstrated that EMT is essential in the intrusion and diffusion of cancer cells, and plays a crucial role in the early stage of infiltration and metastasis of the epithelial tumour. Moreover, the main feature of EMT is the expression of E-cadherin, which is the down-regulated epithelial cell marker, together with the increased expression of N-cadherin, which is the interstitial cell marker (Yang et al., 2004; Lee et al., 2006). E-cadherin is the main intercellular adhesion molecule, which maintains the mutual adhesion of the epithelial cell and restrains cell migration. E-cadherin down-regulation reduces the adhesion strength of the cell, resulting in the enhancement of the cell movement; in turn, this process allows the cancer cells to invade the surrounding tissue across the basement membrane, thereby becoming the foundation of subsequent intrusion and metastasis (Brasch et al., 2012; De Beco et al., 2012). The current research indicates that the expression of E-cadherin is reduced in various tumours, and is relevant to tumour metastasis (Kim et al., 2008; Myong, 2012). The expression rates of E-cadherin in ovarian borderline tumour and ovarian cancer are lower than that in benign ovary tumour (Dara et al., 1997). This expression is relevant to the poor differentiation, peritoneal implantation, and low overall survival rate (Cho et al., 2006). Meanwhile, over-expression can promote the locomotive ability of epithelial cells (De Wever et al., 2004).

Twist, which is over-expressed in numerous tumour cells, is a newly discovered cancer gene. Twist encodes the inhibitor of the apoptosis protein and plays an important role in apoptosis and drug resistance of the tumour, as well as in EMT, generation of vessels, and tumour intrusion and metastasis. Twist is also the key regulation factor during EMT (Kang and Massague, 2004). Twist has also been considered as a prognostic biomarker in certain human cancers (Qin et al., 2012). Although no study has yet to report on Twist-mediated EMT in ovarian cancer, an earlier work has discovered that the Twist protein is relevant to the relapse, metastasis, and poor prognosis of ovarian cancer (Hosono et al., 2007). In the current study, we found that the expression of the Twist protein and its mRNA level in ovarian cancer tissue are obviously higher than in paracancerous tissue ($P < 0.01$), suggesting that Twist plays a role in the generation of ovarian cancer. Meanwhile, we also detected the expressions of E-cadherin in the ovary tissue, and we used RNA interference to silence the Twist expression in human ovarian cancer cell line, then detected E-Cadherin expression using Western blot. We found that there is high expression of Twist, while there is low expression of E-cadherin in ovarian cancer tissue. When the Twist expression was silenced in ovarian cancer cell line, the expression of the E-cadherin protein increased. The expression reduction of E-cadherin lessens the adhesion strength among ovarian cancer cells. Given the lack of natural barriers of the ovary tissue, the deciduous cancer cells can disseminate easily in the pelvic and abdominal cavity, which facilitates the implantation metastasis. The decreased E-cadherin expression signify that the EMT mechanism plays a role in the generation of ovarian cancer.

Twist acts directly or indirectly on the E-box of the E- cadherin promoter, which restrains the expression of

E-cadherin in the transcription level. Meanwhile, Twist expression has a positive correlation with the hepatocyte cancer metastasis and a negative correlation with E-cadherin expression (Lee et al., 2006). In the current work, we found that the expression of Twist protein in ovarian cancer tissue is higher than those of normal ovary tissue, along with its mRNA level. Moreover, we using RNAi to silence the Twist expression in ovarian cancer cell line, we found that the expression of the E-cadherin protein increased. In view of the above information and our research results, we deduce that when the expression of Twist, a known cancer gene, is increased in ovarian cancer tissue, it restrains the expression of E-cadherin in the transcription level and promotes the occurrence of EMT; thereby playing an important role in ovarian cancer generation, development, and metastasis. Recently, it has suggested that Twist is regulated by the F-box protein FBXL14 (Lander et al., 2011). This result demonstrates that tumour generation, development, and metastasis comprise a complex process with multiple genes, links, and steps. Thus, the function of the EMT mechanism, as mediated by Twist, during ovarian cancer generation and development, requires further intensive study.

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