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Effect of Twist, Snail and YB−1 gene expression in cervical cancer tissue on cell invasion and epithelial−mesenchymal transition

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ABSTRACT

Objective: To study the effect of Twist, Snail and YB-1 gene expression in cervical cancer tissue on cell invasion and epithelial-mesenchymal transition. **Methods:** Cervical cancer tissue samples and tissue samples adjacent to carcinoma were collected from 138 patients with radical operation for cervical cancer, fluorescence quantitative PCR method was used to detect the mRNA expression of Twist, Snail and YB-1 genes, cell invasion-related genes and epithelial-mesenchymal transition marker genes, the Pearson test was used to analyze the correlation of Twist, Snail and YB-1 gene mRNA expression in cervical cancer tissue with cell invasion and epithelial-mesenchymal transition. **Results:** Twist, Snail and YB-1 gene mRNA expression in cervical cancer tissue were higher than those in tissue adjacent to carcinoma, the invasion genes STAT3, YAP1, TUG1, FoxM1 and Rab11 mRNA expression were higher than those in tissue adjacent to carcinoma, and the epithelial-mesenchymal transition markers E-cadherin and β-catenin gene mRNA expression were lower than those in tissue adjacent to carcinoma while vimentin gene mRNA expression was higher than that in tissue adjacent to carcinoma. Pearson test showed that Twist, Snail and YB-1 gene mRNA expression in cervical cancer tissue were directly correlated with cell invasion and epithelial-mesenchymal transition. **Conclusion:** Twist, Snail and YB-1 genes are highly expressed in cervical cancer tissue, and their abnormal expression directly leads to the increased tumor cell invasion activity and the aggravated epithelial-mesenchymal transition.

1. Introduction

Cervical cancer is the female malignant tumor with the highest incidence, it is without significant early clinical manifestation, and therefore, the clinical diagnosis rate is not high, which directly leads to the missing of optimal timing of treatment and poor treatment outcome. The occurrence of cervical cancer is directly related to abnormal gene expression, and detecting the specific gene expression is expected to become the reliable means for cervical cancer judgment, and guide the choice of clinical treatment[1]. It has been found in different researches that Twist, Snail and YB-1 gene are closely related to the occurrence and development of cervical cancer, the differences in above gene expression in cervical cancer tissue and the tissue adjacent to carcinoma were compared in this study, and the inner link of their expression with tumor invasion and epithelial-mesenchymal transition was further explored so as to provide reference for long-term cervical cancer monitoring.

2. Materials and methods

2.1. General information

A total of 138 patients who received radical operation for cervical cancer in our hospital between June 2012 and May 2016 were selected, they were 41–70 years old, and the tumor staging was as follows: 65 cases with Ib stage, 48 cases with IIa stage and 25 cases with IIa stage. Cervical cancer tissue samples and tissue samples adjacent to carcinoma were collected, and the research got the

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informed consent from patients themselves and was approved by the hospital ethics committee. Inclusion criteria: (1) pathologically diagnosed with cervical cancer; (2) with primary cervical cancer; (3) not receiving preoperative chemoradiotherapy. Exclusion criteria: (1) associated with primary and malignant tumor diseases of other viscera; (2) associated with systemic infectious diseases; (3) with previous history of cervical surgery.

2.2. Fluorescence quantitative PCR

Cervical cancer tissue specimens and tissue specimens adjacent to carcinoma were collected and added in Trizol lysis buffer (Beijing Chreagen Biotechnology Co., LTD., the article number 15596) to decompose cells, 0.2 mL chloroform (Shanghai Binzhi Biotechnology Co., LTD., the article number 0757-500ML) was added, aqueous phase was collected after high-speed centrifuge, and isopropanol (Shanghai Yanhui Biotechnology Co., LTD., the article number 0918) was added to precipitate total RNA and air dry it at room temperature. Reverse transcription kits (Bio-Techne China branch, the article number MYS928) were used to synthesize the sample cDNA of target genes, fluorescence quantitative PCR kits (Bio-Techne China branch, the article number UYQ891) and gene-specific primers were used for target gene amplification, and the primers are shown in Table 1. The corresponding PCR amplification curve was obtained in the computer, and target gene mRNA expression was calculated.

Table 1

Gene primers for target gene amplification.

Gene	Primer sequence
Twist	Forward 5'-ATG TGA CCG AAC ATG GCA G-3'
	Reverse 5'-TGC CGT AGC AAG TCA ACA-3'
Snail	Forward 5'-TGA CCA TGC AAC TGG ACT-3'
	Reverse 5'-AAC CTG ACC AAT GAC AGT-3'
YB-1	Forward 5'-GGC TTG ACC ATG ACA ACT GAT-3'
	Reverse 5'-AGT AGC ACG TTG ACA CGA T-3'
STAT3	Forward 5'-GTC ATG TAA CAT GGC TAA GT-3'
	Reverse 5'-TGA TCC ATG CAA CGT AAC ATG-3'
YAP1	Forward 5'-GCT GAC CAT GCA ACT TAG C-3'
	Reverse 5'-TGT GCC GTA GCA CAC AGT A-3'
TUG1	Forward 5'-TGA CCT GAA CCG TAG TAG A-3'
	Reverse 5'-TGC GAA CTG AAC GAT GAC-3'
	Reverse 5'-TGT CAA CGA TGA AGA CGA-3'
FoxM1	Forward 5'-ACC TTA CCA TGA CAC ACG TA-3'
	Reverse 5'-TGC TTG AAC GTT GAC AGT ACT A-3'
	Reverse 5'-TGT CGT AGC AAA CTG ATA-3'
Rab11	Forward 5'-TGA TGC CCT GAT GAA CTG AT-3'
	Reverse 5'-TGT CAA CAT GCA AAC TGT G-3'
E-cadherin	Forward 5'-GTC GTA CCG TAA AGT CGT AGA-3'
	Reverse 5'-TGT CCA TGG TAC ATG AAT CGA-3'
vimentin	Forward 5'-TGA TCC TGA CCA ATG CGG ATA-3'
	Reverse 5'-TGA CCT AGG CGT AAC GTA G-3'
β-catenin	Forward 5'-TGA TCA ACT GAT CAG TGA A-3'
	Reverse 5'-TGA CGT AAC GTG TAA CGT AG-3'

2.3. Statistical analysis

The personnel involved in statistics all received professional training, and the statistical software used in the study was SPSS20.0. Gene mRNA expression and other measurement data were in terms of mean ± standard deviation, comparison between two groups was by grouping t test and correlation analysis was by Pearson test. $P<0.05$ was the standard of statistical significance in differences between groups.

3. Results

3.1. Twist, Snail and YB-1 gene

Comparison of Twist, Snail and YB-1 gene mRNA expression in cervical cancer tissue and tissue adjacent to carcinoma was as follows: Twist, Snail and YB-1 gene mRNA expression in cervical cancer tissue were significantly higher than those in tissue adjacent to carcinoma. Differences were statistically significant in Twist, Snail and YB-1 gene mRNA expression in cervical cancer tissue and tissue adjacent to carcinoma (all $P<0.05$), shown in Table 2.

Table 2

Comparison of Twist, Snail and YB-1 gene mRNA expression in cervical tissue (n=138, $\bar{x}\pm s$).

Groups	Twist	Snail	YB-1
Tissue adjacent to carcinoma	99.12±10.43	93.26±10.19	95.73±11.73
Cervical cancer tissue	173.85±21.66	204.55±26.97	172.46±23.58
t	14.282	21.946	15.821
P	<0.05	<0.05	<0.05

3.2. Invasion gene mRNA expression

Comparison of invasion genes STAT3, YAP1, TUG1, FoxM1 and Rab11 mRNA expression in cervical cancer tissue and tissue adjacent to carcinoma was as follows: STAT3, YAP1, TUG1, FoxM1 and Rab11 mRNA expression in cervical cancer tissue were higher than those in tissue adjacent to carcinoma. Differences were statistically significant in invasion genes STAT3, YAP1, TUG1, FoxM1 and Rab11 mRNA expression in cervical cancer tissue and tissue adjacent to carcinoma (all $P<0.05$), shown in Table 3.

3.3. Epithelial-mesenchymal transition marker genes

Comparison of epithelial-mesenchymal transition markers E-cadherin, vimentin and β-catenin gene mRNA expression in cervical cancer tissue and tissue adjacent to carcinoma was as follows: E-cadherin and β-catenin gene mRNA expression in cervical cancer tissue were significantly lower than those in tissue

Table 3

Invasion gene mRNA expression in cervical tissue (n=138, $\bar{x}$±s).

Groups	STAT3	YAP1	TUG1	FoxM1	Rab11
Tissue adjacent to carcinoma	99.23±11.62	98.74±10.65	101.53±13.62	97.32±10.64	102.28±13.51
Cervical cancer tissue	173.38±22.84	203.95±24.63	185.45±21.93	154.27±19.61	171.04±20.63
t	13.291	21.381	18.623	14.382	19.723
P	＜0.05	＜0.05	＜0.05	＜0.05	＜0.05

adjacent to carcinoma while vimentin gene mRNA expression was higher than that in tissue adjacent to carcinoma. The differences were statistically significant in the epithelial-mesenchymal transition markers E-cadherin, vimentin and β-catenin gene mRNA expression in cervical cancer tissue and tissue adjacent to carcinoma (all P＜0.05), shown in Table 4.

Table 4

Epithelial-mesenchymal transition marker gene mRNA expression in cervical tissue (n=138, $\bar{x}$±s).

Groups	E-cadherin	vimentin	β-catenin
Tissue adjacent to carcinoma	99.12±10.84	101.27±12.31	95.36±9.93
Cervical cancer tissue	41.28±6.39	182.49±24.36	39.42±5.18
t	11.291	15.482	12.472
P	＜0.05	＜0.05	＜0.05

3.4. Correlation analysis

Pearson test showed that Twist, Snail and YB-1 gene mRNA expression in cervical cancer tissue were positively correlated with invasion genes STAT3, YAP1, TUG1, FoxM1 and Rab11 mRNA expression (r_{Twist}=0.672, 0.598, 0.718, 0.583 and 0.682; r_{Snail}=0.597, 0.638, 0.693, 0.731 and 0.705; r_{YB-1}=0.597, 0.662, 0.584, 0.624 and 0.674, all P＜0.05); they were negatively correlated with epithelial-mesenchymal transition markers E-cadherin and β-catenin gene mRNA expression (r_{Twist}=-0.711 and -0.641; r_{Snail}=-0.673 and -0.599; r_{YB-1}=-0.715 and -0.653, all P＜0.05), and positively correlated vimentin mRNA expression (r_{Twist}=0.715, rSnail=0.673 and r_{YB-1}=0.593, all P＜0.05).

4. Discussion

Early diagnosis and severity judgment of cervical cancer have been the clinical difficulties, and looking for the specific genes directly related to biological behavior of tumor is a hotspot of current research. Twist, Snail and YB-1 are the genes directly related to epithelial-mesenchymal transition (EMT), and it has been found that they are highly expressed in pancreatic cancer, ovarian cancer, breast cancer and other malignant solid tumors and are closely related to the invasion and metastasis of tumor[2,3]. In the study, the above gene mRNA expression levels in different cervical tissue were detected, and it was found that Twist, Snail and YB-1 gene mRNA expression in cervical cancer tissue were significantly higher than those in tissue adjacent to carcinoma, confirming that there are the unusually high expression of Twist, Snail and YB-1 gene in cervical cancer tissue. The abnormal expression of Twist, Snail and YB-1 gene is the important factor leading to cervical cancer development, but the specific impact on cervical cancer cells remains to be further confirmed in following study.

The invasive activity of tumor cells directly decides the malignant degree of solid tumor, the migration and movement ability of the cells are strengthened during the malignant transformation of cells, and the performance in genetics is the abnormal expression of invasion-related genes[4,5]. STAT3 is cancer gene commonly studied in recent years, it is closely associated with the tumor proliferation and differentiation, angiogenesis, immune escape and so on, and research has shown that silencing STAT3 gene expression can down-regulate the planting and invasive ability of cervical cancer cell[6]. YAP1 is the downstream transcription molecule of Hippo-YAP2 signaling pathways, it is generally highly expressed in colorectal cancer, ovarian cancer and other cancers, and it is considered to be a reliable indicator for judging the prognosis of patients with cervical cancer at present[7]. TUG1 can regulate tumor cell apoptosis and invasion, and silencing its expression can directly reduce the tumor cell invasion activity[8]. The study of Xiao *et al*[9] shows that that FoxM1 plays an important role in cervical cancer cell proliferation and invasion, down-regulating its gene expression can reduce the invasion and migration ability of many strains of cervical cancer cells, and it can be used as the new target for cervical cancer treatment. Rab11 gene is directly related to the invasion and metastasis of breast cancer and colorectal cancer, it mainly regulates actin cytoskeleton reorganization and cell cycle, and down-regulating its expression is a new target for cancer treatment[10,11]. In the study, the above gene mRNA expression levels in different cervical tissue were compared, and it was found that STAT3, YAP1, TUG1, FoxM1 and Rab11 mRNA expression in cervical cancer tissue were significantly higher than those in tissue adjacent to carcinoma (P＜0.05), indicating that the unusually high expression of pro-proliferation genes is involved in the occurrence of cervical cancer. Further Pearson test showed that Twist, Snail and YB-1 gene expression in cervical cancer tissue were positively correlated with pro-invasion gene STAT3, YAP1, TUG1, FoxM1 and Rab11 mRNA expression, indicating that Twist, Snail and YB-1 gene can induce abnormal expression of invasion genes and play a role in the evolution of cervical cancer.

Many studies have confirmed that EMT plays a key role in the

invasion and metastasis of cervical cancer. EMT refers to the transition of epithelial cells with polarity into mesenchymal cells with activity ability, and epithelial marker protein expression changes during the process. E-cadherin belongs to adhesion molecules and can maintain normal epithelial cell morphology and structural integrity, its expression reduction and even deletion can decrease intercellular adhesion ability, also prompt vimentin into cytoplasm, further activate the EMT signal transduction, and accelerate the invasion and metastasis of tumor cells[12,13]. Vimentin is the member of the cell intermediate filament family, it is usually not expressed in the epithelial tissue, Vimentin expression increases after tumor cell canceration, and its expression level is positively correlated with invasion and metastasis ability of tumor cells[14,15]. β-catenin is the factor exerting synergetic effect with E-cadherin, it is mostly exprssed in normal or non-invasive cell membrane, β-catenin expression is suppressed in the process of EMT, and the expression loss is associated with tumor stage[16,17]. In the study, E-cadherin and β-catenin gene mRNA expression in cervical cancer tissue were significantly lower than those in tissue adjacent to carcinoma ($P<0.05$) while vimentin gene mRNA expression was higher than that in tissue adjacent to carcinoma ($P<0.05$), confirming that the EMT marker molecules E-cadherin and β-catenin expression deletion and the vimentin expression increase are directly involved in the occurrence of cervical cancer. And Pearson test showed that Twist, Snail and YB-1 gene expression in cervical cancer tissue were negatively correlated with E-cadherin and β-catenin gene expression, and positively correlated with vimentin mRNA expression, indicating that the abnormally expressed Twist, Snail and YB-1 gene influence the EMT process to be involved in tumor progression.

There is high expression of Twist, Snail and YB-1 gene in cervical cancer tissue, and their expression is directly related to the tumor cell invasion activity and epithelial-mesenchymal transition degree. Detecting Twist, Snail and YB-1 gene expression in cervical tissue can become the new means for early diagnosis of cervical cancer, and inhibiting their gene expression is expected to become the new target for disease treatment, which is to be confirmed in follow-up research.

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