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Expressions and significances of TTF-1 and PTEN in early endometrial cancer

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Abstract. – OBJECTIVE: To analyze the expressions and significances of TTF-1 (Thyroid transcription factor-1) and PTEN (Phosphatase and tensin homolog) in early endometrial cancer.

PATIENTS AND METHODS: 41 patients with endometrial cancer, 38 patients with proliferative endometrium and 13 patients with normal endometrium, were selected. The fluorescence quantitative PCR (polymerase chain reaction) was used to detect the expression levels of TFF-1 and PTEN mRNAs (Messenger ribonucleic acids) in the above three endometria, and their relations with clinical pathological characteristics were analyzed. The RT-PCR (reverse transcription-polymerase chain reaction) was employed to detect the expression levels of miR-135b, miR-125b and Snail mRNAs in the three endometria, and their correlations with the expressions of TTF-1 and PTEN mRNAs, were analyzed.

RESULTS: The expression levels of TFF-1 and PTEN mRNAs in endometrial cancer tissues were significantly lower than those in the other two groups, while those in normal endometrium tissues were the highest, and the differences were statistically significant ($p < 0.05$). There were no significant differences in the menstruation status and the expression levels of TFF-1 and PTEN mRNAs between adenocarcinoma and squamous carcinoma ($p > 0.05$). With the increase of FIGO (International Federation of Gynecology and Obstetrics) stage and depth of invasion, as well as the metastasis of pelvic lymph nodes, the expression levels of TFF-1 and PTEN mRNAs were significantly decreased, and the differences were statistically significant ($p < 0.05$). The expression level of miR-135b mRNA in endometrial cancer was significantly higher than that in the other two groups, while that in normal endometrium was the lowest. The expression levels of miR-125b and Snail mRNAs were significantly lower than those in the other two groups, while those in normal endometrium were the highest; the differences were statistically significant ($p < 0.05$). Expression levels of TTF-1 and PTEN mRNAs were negatively correlated with the expression level of miR-135b mRNA, and positively associated with the expression levels of miR-125b and Snail mRNAs ($p < 0.05$). The results from the ROC (Receiver Operating Characteristic) model showed that, for the diagnosis of endometrial cancer with TTF-1 mRNA, the sensitivity was 86.5%, the specificity was 84.2%, the accuracy (area under curve - AUC) was 0.823, 95% CI (confidence intervals) = 0.762-0.921, $p = 0.012$. For the diagnosis of endometrial cancer with PTEN mRNA, the sensitivity was 85.3%, the specificity was 83.6%, the accuracy was 0.842, 95% CI = 0.785-0.936, $p = 0.010$.

CONCLUSIONS: TTF-1 and PTEN can be used as molecular markers for the early diagnosis of endometrial cancer, which are closely related to clinical features and may affect tumor progression by regulating the proliferation activity of tumor cells.

Key Words:
Endometrial cancer, TTF-1, PTEN, miR-135b, miR-125b, Snail.

Introduction

Endometrial cancer is the most common malignant tumor of the female genital tract. The tumor stage is closely related to the outcome of the final treatment, the patients with early stage endometrial cancer have good prognosis, while the patients with middle/advanced stage endometrial cancer only have a 5-year survival rate of about 10% to 30%[1]. About 70% of the patients with endometrial cancer get medical

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attention due to irregular vaginal bleeding and other symptoms, more than 50% of the patients with endometrial cancer are in the middle and advanced stages at the time of clinical diagnosis and have no operative treatment chances[2]. Although early clinical symptoms are not typical, and logical image examinations, such as transabdominal sonography, transvaginal ultrasonography, CT (Computed Tomography) or MRI (Magnetic Resonance Imaging) are sensitive, the changes of form and structure often lag behind the dysfunction of tumors[3]. Therefore, an important means to improve the early diagnosis and the outcome of clinical treatment is to seek molecular markers that are closely related to the biological behaviors of tumors and have good sensitivity and specificity[4]. The expressions of proliferation-related genes such as miR-135b, miR-125b and Snail play important roles in the development and progression of multiple tumors[5,6], but lack the specificity for the early diagnosis of endometrial cancer. Phosphatase and tensin homolog (PTEN) is considered as a specific molecule of endometrial cancer, and the loss of its expression is an important mechanism leading to tumorigenesis[7]. There are many studies of TTF-1 (thyroid transcription factor-1) in non-small cell lung cancer. Specific expression of TTF-1 is found in adenocarcinoma, and the expression level is closely related to tumor prognosis[8]. The study has confirmed that the positive expression of TTF-1 is found in endometrial cancer[9]. This study aims to determine whether TTF-1 and PTEN can be used as sensitive molecules for the early diagnosis of endometrial cancer.

Patients and Methods

Patients

A total of 92 patients who were admitted to our hospital from January 2013 to January 2016 and underwent gynecological operations for the first time, were continuously selected. This study was approved by the Ethics Committee of Affiliated Hospital of Jining Medical University (Jining, Shandong Province, China). Signed written informed consents were obtained from all participants before the study. Including 41 patients with endometrial cancer (33 patients with adenocarcinoma, 8 patients with squamous carcinoma), 38 patients with proliferative endometrium and 13 patients with normal endometrium. Patients with endometrial cancer aged 39 to 76 years, with a mean age of (52.6 ± 13.3) years; 12 patients were menopausal. Patients with proliferative endometrium aged 35 to 73 years, with a mean age of (51.5 ± 14.6) years; 10 patients were menopausal. Patients with normal endometrium aged 37 to 77 years, with a mean age of (53.7 ± 15.5) years; 5 patients were menopausal. These patients did not undergo systematic chemotherapy and radiotherapy before the operation.

Fluorescence Quantitative PCR (Polymerase Chain Reaction)

The obtained endometrial cancer, proliferative endometrium and normal endometrium tissue specimens were mixed and homogenized with RNAios; then the homogenates were centrifuged at high speed (for 25 min at 12,000 g) and supernatants were taken. Then, supernatans were added with chloroform and isopropyl alcohol in sequence and were centrifuged for 30 min at 1,000 g to get RNA plaques. After that, reverse transcription kits (Promega Corporation, Madison, WI, USA) were used to synthesize cDNA; fluorescent quantitative PCR kits and primer design (Tiangen Biotech Co., Ltd, Beijing, China) were employed to amplify target genes such as TTF-1, PTEN, miR-135b, miR-125b and Snail. The reaction system: 2 μl of cDNA, 10 μl of Mastermix containing fluorescent dyes, 0.8 μl of 5 μmol/L upstream primers and 0.8 μl of 5 μmol/L downstream primers, 6.4 μl of deionized water; the reaction procedure: 95°C for 15 s, specific annealing temperature for 20 s, 72°C for 25 s, with a total of 40 cycles. The data were normalized by using internal reference genes (β-action was set to 100).

Statistical Analysis

SPSS23.0 software (Version X; IBM, Armonk, NY, USA) was used to enter and analyze data. Normal data were expressed as mean ± standard deviation, the single-factor ANOVA (Analysis of Variance) was utilized to compare the differences among groups, and the LSD (least-significant difference) test was used for pairwise comparisons. Qualitative data were indicated as ratio and the χ^2-test was employed to compare the differences among groups; the univariate linear Pearson method was applied to analyze the correlations among the expression levels of gene mRNAs; the receiver operating characteristic (ROC) curve was utilized to analyze the sensitivity and accuracy of the diagnosis. $p < 0.05$ indicated that the differences were statistically significant.

Results

Comparisons of Expression Levels of TFF-1 and PTEN mRNAs Among Groups

The expression levels of TFF-1 and PTEN mRNAs in endometrial cancer tissues were significantly lower than those in the other two groups, while those in normal endometrium tissues were the highest, and the differences were statistically significant ($p < 0.05$) (Figure 1).

The Relationship Between the Expressions of TTF-1 and PTEN mRNAs in Endometrial Cancer and the Clinical Pathological Features

There were no statistically significant differences in the menstruation status and the expression levels of TFF-1 and PTEN mRNAs between the adenocarcinoma tissue and the squamous carcinoma tissue ($p > 0.05$). With the increase of FIGO (International Federation of Gynecology and Obstetrics) stage and depth of invasion as well as the metastasis of pelvic lymph nodes, the expression levels of TFF-1 and PTEN mRNAs were significantly decreased, and the differences were statistically significant ($p < 0.05$) (Table I).

Comparison of Expression Levels of Proliferation-Associated Molecule mRNAs Among Groups

The expression level of miR-125b mRNA in endometrial cancer was significantly higher than that in the other two groups, while that in normal endometrium was the lowest; the expression levels of miR-125b and Snail mRNAs in endometrial cancer were significantly lower than those in the other two groups, while those in normal endometrium were the highest; the differences were statistically significant ($p < 0.05$) (Figure 2).

Correlations of TTF-1 and PTEN mRNAs with the Proliferation-Associated Molecule mRNAs

The expression levels of TTF-1 and PTEN mRNAs were negatively correlated with the expression level of miR-135b mRNA, and

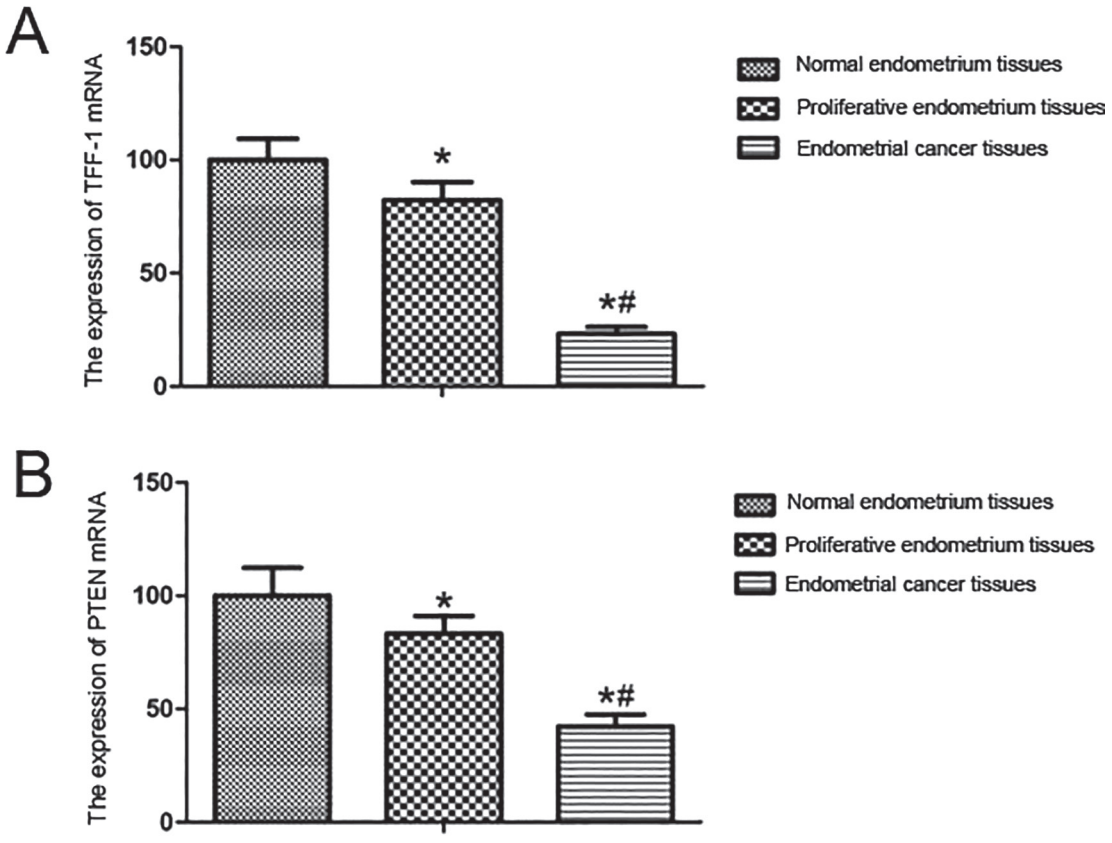

Figure 1. Comparison of expression levels of TFF-1 and PTEN mRNAs among groups (*: compared with normal endometrium tissues, $p < 0.05$; #: compared with proliferative endometrium tissues, $p < 0.5$)

Table I. The relationship between the expressions of TTF-1 and PTEN mRNAs in endometrial cancer and the clinical pathological features.

		Case	TTF-1	PTEN
Menopausal	Yes	12	35.62 ± 6.20	42.18 ± 4.52
	No	29	32.14 ± 5.13	40.36 ± 4.49
Tissue type	Adenocarcinoma	33	32.82 ± 4.19	43.39 ± 5.11
	Squamous carcinoma	8	29.74 ± 3.32	41.68 ± 4.12
FIGO stage	Stage I	14	69.37 ± 7.12	63.12 ± 8.58
	Stage II	20	53.29 ± 5.84	52.67± 9.97
	Stage III	7	21.27 ± 2.7	26.91 ± 8.63
Depth of invasion	< 1/2	24	45.38 ± 5.12	49.83 ± 5.26
	≥ 1/2	17	27.64 ± 3.06	23.46 ± 2.85
Pelvic lymph node metastasis	Yes	16	20.37 ± 2.41	32.17 ± 3.46
	No	25	63.28 ± 7.03	58.34 ± 6.03

positively associated with the expression levels of miR-125b and Snail mRNAs ($p < 0.05$) (Table II).

Analysis of the Sensitivity and Accuracy of TTF-1 and PTEN mRNAs in the Diagnosis of Endometrial Cancer

The expression levels of TTF-1 and PTEN mRNAs were utilized as the reference indexes for the diagnosis of endometrial cancer and were included in the ROC model. The results showed that, for the diagnosis of endometrial cancer with TTF-1 mRNA, the sensitivity was 86.5%, the specificity was 84.2%, and the accuracy (area under curve – AUC) was 0.823, 95% CI (confidence interval) = 0.762-0.921, $p = 0.012$. For the diagnosis of endometrial cancer with PTEN mRNA, the sensitivity was 85.3%, the specificity was 83.6%, the accuracy was 0.842, 95% CI = 0.785-0.936, $p = 0.010$ (Figure 3).

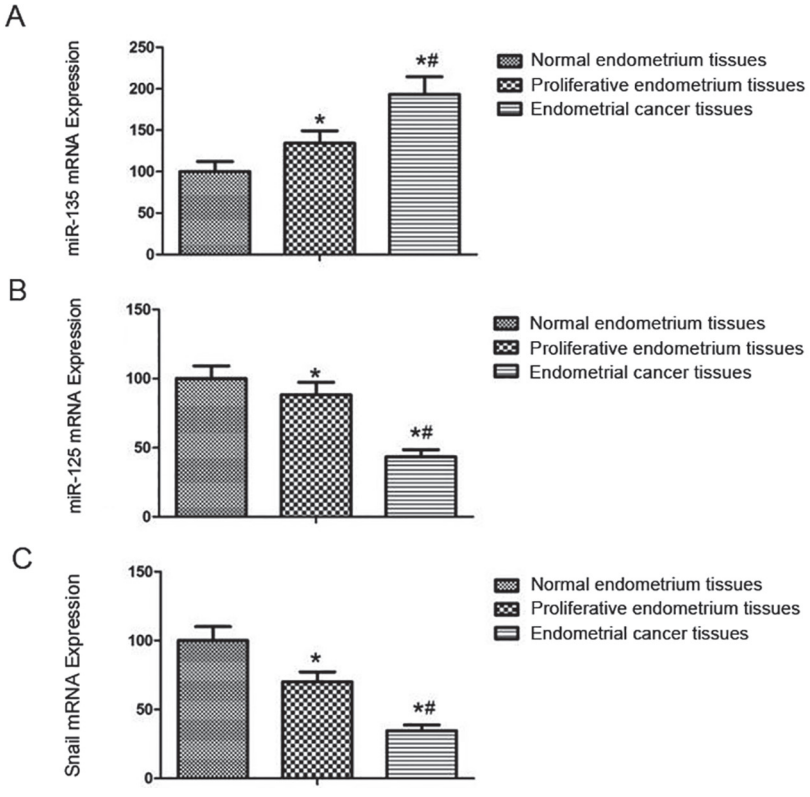

Figure 2. Comparison of expression levels of proliferation-associated molecule mRNAs among groups (*: compared with normal endometrium tissues, $p < 0.05$; #: compared with proliferative endometrium tissues, $p < 0.5$).

Table II. Correlation of TTF-1and PTEN mRNAs with the proliferation-associated molecule mRNAs.

	TTF-1		PTEN	
	Coefficient of determination r	*p*	Coefficient of determination r	*p*
miR-135b	-0.323	0.021	-0.438	0.025
miR-125b	0.492	0.023	0.372	0.018
Snail	0.512	0.012	0.502	0.015

Discussion

The mutation rate of PTEN gene is high in endometria, about 30-45%[10]. The loss of PTEN expression is associated with endometrial hyperplasia and malignant transformation[11]. The loss of PTEN expression is found both in atypical endometrial hyperplasia and endometrial cancer, but is relatively rare in the normal endometrium. It is, therefore, speculated that the loss of PTEN gene expression is one of the important mechanisms of endometrial cancer development. It has been shown that the expression of thyroid transcription factor-1 (TTF-1) can be detected in thyroid cancer, lung adenocarcinoma, ovarian cancer, endometrial cancer and other tumors[12]. It is found that, the median survival time of the small cell lung cancer patient who has positive TTF-1 expression is extended[13]. Endometrial cancer is mostly the adenocarcinoma, and Fujiwara et al[14] suggest that TTF-1 is also involved in the development and progression of endometrial cancer. Results of this study showed that the expression levels of TFF-1 and PTEN mRNAs in endometrial cancer tissues were significantly lower than those in the other two groups, while the expression levels of TFF-1 and PTEN mRNAs in normal endometrium tissues were the highest. It is suggested that, TTF-1 and PTEN can act as tumor suppressors, and the reduction or loss of their expressions may be the important control factors in the development of endometrial cancer. The low expressions of TTF-1 and PTEN were closely related to the increase of FIGO stage and depth of invasion as well as the pelvic lymph node metastasis, but were irrelevant to the menstruation status and the pathological type of tumors. This work concluded that the expression levels of TTF-1 and PTEN were not associated with the pathologic type of tumors, which was in contradiction with the result of the previous study[15]. Causes of the above contradiction may be the race and sample size. The low expressions of TTF-1 and PTEN were closely associated with the biological behaviors of endometrial cancer, such as tumor proliferation, differentiation and invasion. The expression levels of TTF-1 and PTEN can be used as the important indicators for the diagnosis of endometrial cancer and the determination of disease progression[16]. MiR-135b, miR-125b and Snail have proved to be clearly associated with the cell proliferation in endometrial cancer[17]. MiR-135b can target to promote the cell proliferation in endometrial cancer[18]; the overexpression of miR-125b can inhibit the proliferation of tumor cells and the cell cycle progression and promote the apoptosis of tumor cells[19]. Snail can inhibit the cell proliferation in endometrial cancer and its expression in endometrial cancer is low[20,21]. This study concluded that, the expression level of miR-135b mRNA in endometrial cancer tissues was significantly higher than that in the other two groups, while that in normal endometrium tissues was the lowest. The expression levels of miR-125b and Snail mRNAs

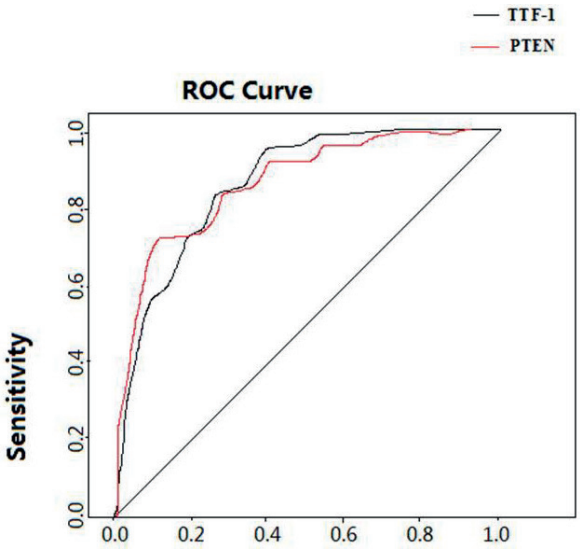

Figure 3. ROC analysis of the sensitivity and accuracy of TTF-1 and PTEN mRNAs in the diagnosis of endometrial cancer.

in endometrial cancer tissues were significantly lower than those in the other two groups, while those in normal endometrium tissues were the highest. The expression levels of TTF-1 and PTEN mRNAs were negatively correlated with the expression level of miR-135b mRNA, and positively associated with the expression levels of miR-125b and Snail mRNAs. It is suggested that TTF-1 and PTEN may affect the development and progression of tumors by controlling the expressions of miR-135b, miR-125b and Snail genes. Further analysis showed that, as the reference indexes for the diagnosis of endometrial cancer, the expression levels of TTF-1 and PTEN mRNAs had good sensitivity, specificity and accuracy.

Conclusions

TTF-1 and PTEN can be used as molecular markers for the early diagnosis of endometrial cancer, and they are closely related to clinical features and may influence tumor progression by controlling the proliferation activity of tumor cells.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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