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Expression and clinical significance of long non-coding RNA *HOTAIR* in endometrial carcinoma.

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Abstract

This study aimed to investigate the expression and clinical significance of HOX Transcript Antisense RNA (*HOTAIR*) in endometrial carcinoma, and discuss its correlation with transforming growth factor-β1 (TGF-β1) in EC tissue. Sixty patients with EC were enrolled. The EC tissue and cancer adjacent tissue (control) were collected. The expressions of *HOTAIR* and TGF-β1 mRNA in EC and adjacent tissue were determined using real-time quantitative PCR. Results showed that, the relative expression levels of *HOTAIR* and TGF-β1 mRNA in EC tissue were significantly higher than those in adjacent tissue (P<0.05). The expression of *HOTAIR* mRNA was significantly correlated with pathological grade, muscular invasion depth and clinical stage of EC (P<0.05), and that of TGF-β1 mRNA was significantly correlated with muscular invasion depth and clinical stage of EC (P<0.05). In EC tissue the expressions of *HOTAIR* and TGF-β1 mRNA were positively correlated (r=0.676, P<0.05). There was significant difference of overall survival of patients between high and low *HOTAIR* expression groups (χ^2=4.346, P<0.05). The abnormal expression of *HOTAIR* may be involved in the occurrence and development of EC. The expression of *HOTAIR* is positively correlated with TGF-β1 expression in EC tissue.

Keywords: Endometrial carcinoma, *HOTAIR*, TGF-β1, Correlation.

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Introduction

Long non-coding RNAs (lncRNAs) are a class of RNAs of which the transcript length is more than 200 nt. lncRNAs do not encode the protein, but they regulate the gene expression from multiple levels (epigenetics, transcription, post-transcription, etc.). They play an important role in the occurrence and development of tumor [1,2]. HOX Transcript Antisense RNA (*HOTAIR*) is one of the firstly discovered lncRNAs, and has the regulation function of transcription [3]. Recent studies suggest that, there is abnormal expression of *HOTAIR* in a variety of tumors (liver cancer [4], colon cancer [5], breast cancer [6], lung cancer [7], pancreatic cancer [8], etc.). In addition, the amount of *HOTAIR* expression has important relation with the progression, metastasis and prognosis of tumor [9]. The *in vitro* study shows that, *HOTAIR* can promote the tumor cell proliferation, invasion and migration, and reduce the sensitivity of tumor to chemotherapy drugs [10]. Therefore, *HOTAIR* is expected to become a powerful prediction index and therapeutic target in diagnosis of tumor.

Endometrial Carcinoma (EC) is one of the three most common malignant tumors in female reproductive system. In some Europe and America countries, it has ranked the first in the tumors in obstetrics and gynecology [11]. In China, the incidence of EC is gradually increasing in recent years, which seriously harms the health of women [12]. The occurrence and development of EC is a complex pathological process which involves the participation of a large number of lncRNAs [13]. This study investigated the expression and clinical significance of long non-coding RNA *HOTAIR* in EC, and discussed its correlation with Transforming Growth Factor-β1 (TGF-β1) in EC tissue. The objective was to provide a basis for further investigating the role of *HOTAIR* in the diagnosis, treatment and prognosis of EC.

Subjects and Methods

Subjects

Sixty patients with EC confirmed in the Maternal and Child Health Care Hospital of Gansu Province from September 2015 to December 2016 were enrolled in this study. One EC tissue sample was collected from each patient, and the tissue 2 cm adjacent to EC tissue was used as the control. All specimens were stored in liquid nitrogen immediately after obtaining. The age of the patients ranged from 32-66 years old, with average of 50.7 ± 8.3 years. According to the pathological type, there were 42 cases of adenocarcinoma and 18 cases of other types. According to the pathological grade, there were 18, 25 and 17 cases with grade G1, G2 and G3, respectively. According to the muscular invasion depth, there were 39 cases with

muscular invasion depth ≤ 1/2 and 21 cases with muscular invasion depth>1/2. According to the clinical stage of tumor, invasion depth, there were 36 cases with stage I and 24 cases with stage II. In addition, there were 48 cases with no lymph node metastasis and 12 cases with lymph node metastasis. All patients had not received chemotherapy or radiotherapy before surgery. This study was approved by the ethics committee of Maternal and Child Health Care Hospital of Gansu Province. Written informed consent was obtained from all participants.

Extraction of total RNA

EC or adjacent tissue sample (50-100 mg) was taken. The total RNA of sample was extracted using Trizol (Life Technologies Inc., CA, USA) in accordance with the instruction of kits. The OD260/OD280 value of RNA was measured using F-7000 ultraviolet spectrophotometer (Hitachi High-Technologies Corp., Tokyo, Japan), and the purity was analysed. The total RNA with OD260/OD280 value from 1.8 to 2.0 was considered as qualified. In addition, The 10 g/L agarose gel electrophoresis (Bio-Rad Laboratories, Inc., PA, USA) was performed on the extracted RNA sample. The 28 S and 18 S bands of ribosomal RNA were compared. The color intensity of 2: 1 (28 S: 18 S) by EB staining indicated that the extracted RNA was complete. Finally, the RNA sample was kept at -70°C in refrigerator for use. The entire total RNA extraction process was performed under RNase-free environment.

cDNA synthesis

The reverse transcription synthesis of cDNA was performed in 20 μL reaction system as follows: PrimeScript Buffer (5X), 4 μL; PrimeScript RT Enzyme Mix I, 1 μL; Oligo Dt Primer (50 μmol/L), 1 μL; Random 6 mers (100 μmol/L), 1 μL; total RNA, 13 μL. The operation was in accordance with the instruction of reverse transcription kits. The reaction condition was 37°C for 15 min, followed by 85°C for 5 s.

Real-time quantitative PCR

Real-time quantitative PCR was performed according the instruction of kits. The PCR system (15 μL for each sample) was as follows: Premix Ex Taq (2X), 7.5 μL; forward primer (10 μmol/L), 0.25 μL; reverse primer (10 μmol/L), 0.25 μL; cDNA (5 ng/μL), 3 μL; dH2O, 4 μL. Primers were designed and synthesized by Shanghai Invitrogen Biotechnology Co., Ltd. (Shanghai, China). The primers were as follows: *HOTAIR*: forward 5'-GGTAGAAAAAGCAACCACGAAGC-3'; reverse 5'-ACATAAACCTCTGTCTGTGAGTGCC-3'; TGF-β1: forward: 5'-CCAGA TTGAGACCCTCCTCA-3', reverse: 5'-ATGCAATGCTGTTCTTGCAG-3'; GAPDH (internal reference): forward 5'-CCGGGAAACTGTGGCGTGATGG-3', reverse 5'-AGGTGGAGGTATGGGTGTCGCTGTT-3', After initial denaturation of 15 min at 95°C, the PCR condition was as follows: *HOTAIR*: 50 cycles of 95°C for 15 s, 61°C for 15 s, and 72°C for 15 s; TGF-β1: 40 cycles of 95°C for 10 s, 58°C for 15 s, and 72°C for 10 s; GAPDH: 28 cycles of 94°C for 45 s, 59 °C for 30 s, and 72°C for 20 s. The relative expression level was determined using the $2^{-\Delta\Delta Ct}$ analysis method [14].

Statistical analysis

All statistical analysis was carried out using SPSS19.0 software (SPSS Inc., Chicago, IL, USA). The measurement data were presented as mean ± SD, and were compared using t test. Pearson correlation analysis was performed on the correlation between *HOTAIR* and TGF-β1 expression in EC tissue. P<0.05 was considered as statistically significant.

Results

Expressions of HOTAIR mRNA in EC tissue and adjacent tissue

Real-time quantitative PCR showed that, the relative expression level of *HOTAIR* mRNA in EC tissue was 2.02 ± 0.42, which was significantly higher than 0.88 ± 0.18 in adjacent tissue (P<0.05) (Figure 1).

Expressions of TGF-β1 mRNA in EC tissue and adjacent tissue

Figure 2 showed that, the relative expression amount of TGF-β1 mRNA in EC tissue was 1.85 ± 0.71, which was also significantly higher than 1.32 ± 0.29 in adjacent tissue (P<0.05).

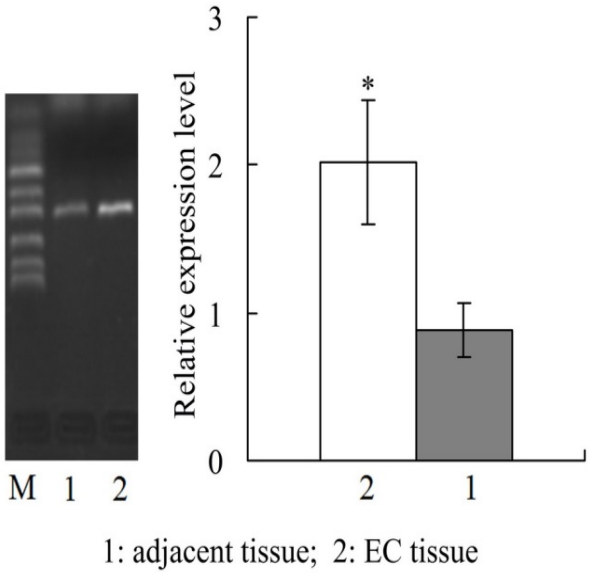

Figure 1. *Expressions of HOTAIR mRNA in EC tissue and adjacent tissue. *P<0.05 compared with adjacent tissue. HOTAIR: HOX Transcript antisense RNA; EC: Endometrial Carcinoma.*

Correlation between HOTAIR mRNA expression and clinicopathological parameters in EC

Table 1 showed that, the expression level of *HOTAIR* mRNA was significantly correlated with pathological grade, muscular invasion depth and clinical stage of EC, respectively (P<0.05), with no significant correlation with patients age (>50 or ≤ 50 years), EC pathological type and lymph node metastasis, respectively (P>0.05).

Correlation between TGF-β1 mRNA expression and clinicopathological parameters in EC

As shown in Table 2, the expression level of TGF-β1 mRNA was significantly correlated with muscular invasion depth and clinical stage of EC, respectively (P<0.05), with no significant correlation with patients age (>50 or ≤ 50 years), EC pathological type, pathological grade and lymph node metastasis, respectively (P>0.05).

Correlation between HOTAIR mRNA and TGF-β1 mRNA expression in EC tissue

Pearson analysis showed that, in EC tissue the expression of *HOTAIR* mRNA and TGF-β1 mRNA were positively correlated (r=0.676, P<0.05).

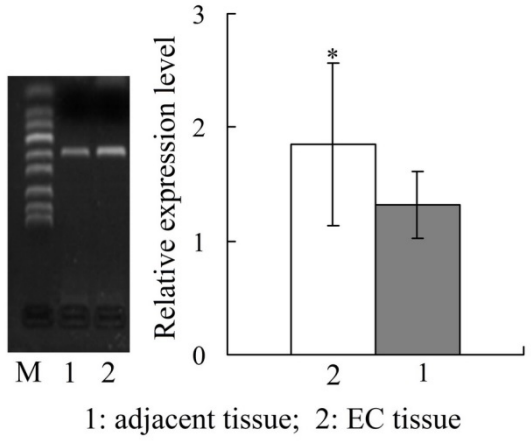

Figure 2. *Expressions of TGF-β1 mRNA in EC tissue and adjacent tissue. *P< 0.05 compared with adjacent tissue. TGF-β1: Transforming Growth Factor-β1; EC: Endometrial Carcinoma.*

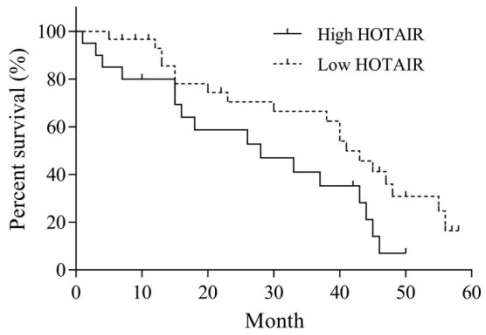

Figure 3. *Relationship between HOTAIR expression and prognosis of patients with EC. HOTAIR: HOX Transcript antisense RNA; EC: Endometrial Carcinoma.*

Relationship between HOTAIR expression and prognosis of patients with EC

According to the expression level of *HOTAIR* mRNA in EC tissue, the patients were divided into high *HOTAIR* and low *HOTAIR* groups, in which the *HOTAIR* mRNA level was higher and lower than the average value, respectively. The overall survival time of high *HOTAIR* group was 28 months (95% CI 0.35-1.32). The overall survival time of low *HOTAIR* was 41 months (95% CI 0.75-2.85). The Log-rank (Mantel-Cox) test in Kaplan-Meier method showed that, there was significant difference of overall survival between two groups (χ^2=4.346, P<0.05, Figure 3). This indicated that, the high expression of *HOTAIR* was closely related with the prognosis of EC.

Table 1. *Correlation between HOTAIR mRNA expression and clinicopathological parameters in EC.*

Clinicopathological parameter	**n**	***HOTAIR* mRNA expression**	**P**
Age (years)			>0.05
>50	43	2.13 ± 0.91	
≤ 50	17	1.96 ± 0.73	
Pathological type			
Adenocarcinoma	42	1.92 ± 0.55	>0.05
Others	18	2.19 ± 0.67	
Pathological grade			<0.05
G1	18	1.09 ± 0.52	
G2	25	2.24 ± 0.88	
G3	17	4.32 ± 0.93	
Muscular invasion depth			<0.05
≤ 1/2	39	1.73 ± 0.58	
>1/2	21	2.86 ± 0.61	
Clinical stage			<0.05
I	36	1.61 ± 0.83	
I+III	24	3.26 ± 1.32	
Lymph node metastasis			>0.05
No	48	1.94 ± 0.87	
Yes	12	2.19 ± 0.59	

HOTAIR: HOX Transcript Antisense RNA; EC: Endometrial Carcinoma.

Table 2. *Correlation between TGF-β1 mRNA expression and clinicopathological parameters in EC.*

Clinicopathological parameter	**n**	**TGF-β1 mRNA expression**	**P**
Age (years)			>0.05
>50	43	1.89 ± 0.65	
≤ 50	17	1.68 ± 0.57	
Pathological type			
Adenocarcinoma	42	1.92 ± 0.54	>0.05
Others	18	1.79 ± 0.65	
Pathological grade			>0.05

G1	18	1.98 ± 0.61	
G2	25	1.72 ± 0.71	
G3	17	1.54 ± 0.57	
Muscular invasion depth			<0.05
≤ 1/2	39	1.94 ± 0.64	
>1/2	21	3.76 ± 0.94	
Clinical stage			<0.05
I	36	1.53 ± 0.63	
I+III	24	2.95 ± 0.78	
Lymph node metastasis			>0.05
No	48	1.66 ± 0.73	
Yes	12	2.13 ± 0.64	

TGF-β1: Transforming Growth Factor-β1; EC: Endometrial Carcinoma.

Discussion

EC is a kind of malignant epithelial tumor that occurs in the endometrium. The incidence of EC is increasing year by year. The diagnosis of EC is mainly based on the disease history, clinical symptoms and pathological outcome. However, the early symptoms of EC are not obvious, and it is easy to be misdiagnosed [15], so the survival rate of EC is not high [16]. The early diagnosis is helpful for the standardized treatment, and improvement of survival rate. At present, EC is still one of the gynecological malignant tumors which cause the death of patients. Therefore, in-depth understanding the pathogenesis of EC and early predicting its invasion and metastasis have important clinical importance for the early diagnosis of disease condition, formulation of treatment scheme, and comprehensive assessment of the prognosis [17].

HOTAIR is an important member of lncRNAs, and it is found to play an important role in the occurrence and development of tumors. Gupta et al. [3] found that, the expression of *HOTAIR* in primary loci and metastases loci of breast cancer tissue is significantly higher than the corresponding normal breast tissue. The high expression of *HOTAIR* is significantly related to the tumor size, clinical stage and survival time. *In vitro* studies have shown that, the up-regulation of *HOTAIR* can enhance the invasion and metastasis of breast cancer cells. Ishibashi et al. [18] found that, the expression of *HOTAIR* in hepatocellular carcinoma tissue is significantly higher than that in adjacent tissue, and the high expression of *HOTAIR* is significantly correlated with the tumor metastasis, recurrence and prognosis. Similarly, the up-regulated *HOTAIR* expression is found in pancreatic cancer [8], gastrointestinal stromal tumor [19], colorectal cancer [20], etc. Therefore, *HOTAIR* may serve as a cancer gene, which promotes the invasion and metastasis of tumor cells. However, the mechanism is not clear. Liu et al. [21] have found the up-regulated *HOTAIR* expression in cisplatin-resistant lung adenocarcinoma tissue. The possible mechanism is that, *HOTAIR* can regulate the expression of *P21* in tumor tissue.

This study observed the expression of *HOTAIR* in EC tissue and adjacent tissue. Results found that, the expression level of *HOTAIR* mRNA in EC tissue was significantly higher than that in adjacent tissue ($P<0.05$). In addition, the expression of *HOTAIR* mRNA was significantly correlated with pathological grade, muscular invasion depth and clinical stage of EC, respectively ($P<0.05$). The study on overall survival showed that, the survival time in high *HOTAIR* group was significantly shorter than that in low *HOTAIR* group ($P<0.05$). It can be concluded that, *HOTAIR* plays an important role in the occurrence and development of EC and the high expression of *HOTAIR* was closely related with the prognosis of EC.

TGF-β1 is a kind of multifunctional polypeptide cytokines. It plays an important role in cell proliferation and differentiation, extracellular matrix formation, vascular cell growth, cell apoptosis, immune suppression and development of tumor [22]. TGF-β1 is highly expressed in a variety of tumors such as prostate cancer, colorectal cancer, gastric cancer, glioma and melanoma [23]. Results of this study found that, the relative expression amount of TGF-β1 mRNA in EC tissue was 1.85 ± 0.71, which was also significantly higher than 1.32 ± 0.29 in adjacent tissue ($P<0.05$). In addition, the expression level of TGF-β1 mRNA was significantly correlated with muscular invasion depth and clinical stage of EC, respectively ($P<0.05$).

Poly-comb repressive complex 2 (*PRC2*) is a member of genes in poly-comb protein family. It can maintain the gene transcription inhibition through modifying the chromatin structure. It is found that, *HOTAIR* can silence the transcription of HOXD site by linking PRC2 and histone methyltransferase LSD1 [24]. The cell cycle regulation-related genes, *PRC2* target genes, are regulated by TGF-β1 [25]. Therefore, it is speculated that *HOTAIR* may has some correlation with TGF-β1. Results of this study found that, in EC tissue the expression of *HOTAIR* mRNA and TGF-β1 mRNA were positively correlated ($r=0.676$, $P<0.05$).

In conclusion, the abnormal expression of *HOTAIR* may be involved in the occurrence and development of EC. In addition, the expression of *HOTAIR* is positively correlated with TGF-β1 expression in EC tissue. This study has provided a basis for further investigating the role of *HOTAIR* in EC. This study still has some limitations. The sample size of this study is relatively small. Larger sample size will make the results more convincing. In our next studies, the sample size should be further increased for obtaining more satisfactory outcomes.

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