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Overexpression of HOTAIR in Tumor Tissues of Patients with Colon Cancer Correlates with Tumor Metastasis and Differentiation

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Abstract

Background: Aberrant expression level of Hox transcript antisense intergenic RNA (HOTAIR) has been associated with the etiopathogenesis of numerous cancers. Studies on epidemiological data have demonstrated that the risk of susceptibility to colon cancer varies among different populations due to several reasons. In this study, we aimed to assess the expression level of HOTAIR in tumoral tissues of patients with colon cancer and compare it with normal marginal tissues.

Methods: In this case-control study, we recruited a total of 50 patients with colon cancer and collected tumoral and matched marginal tumor free tissues during surgery. Afterwards, we isolated the total RNA from each sample, synthesized cDNA, and performed quantitative analysis by Real-time PCR using the SYBR Green PCR Master Mix in order to measure the transcript level of HOTAIR in samples.

Results: The expression level of HOTAIR was upregulated in tumor tissues compared with normal tumor-free marginal tissues belonging to colon cancer patients (P= 0.0023). Moreover, the expression level of HOTAIR and the clinicopathological specifications of the patients had statistically significant correlations.

Conclusions: HOTAIR may play a role in the development of colon cancer and have the potential for application as a biomarker for colon cancer prognosis.

Keywords: Colorectal cancer, HOTAIR, Transcription, Cancer biomarker

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Introduction

Colon cancer is one of the most prevalent cancers with a high level of mortality worldwide.[1-3] Despite the breakthroughs in colon cancer treatment, particularly in

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chemotherapy, colorectal cancer-related death rate is gradually rising.[4, 5] Over the recent years, most studies have focused on molecular based markers in tumor cells for a better understanding of cancer mechanisms and selecting the most useful chemotherapy adjuvant regarding each cancer case.[6]

Long non-coding RNAs (lncRNAs) are non-protein coding RNAs which are approximately 200 nucleotide long. They function as most important cell mechanisms to regulate other genes.[7] One of the most famous members of lncRNA family is Hox transcript antisense intergenic RNA (HOTAIR), a molecule acting like an oncogene in various types of cancers such as gastric, breast and liver cancers.[8-11] Previous studies on colorectal cancer show significant changes in HOTAIR expression levels in tumor tissues compared with normal colon mucosa in patients with colorectal cancer. A relationship was further established between HOTAIR expression levels and metastases circumstances in colon cancer.[12,13]

To clarify and better fathom the HOTAIR roles in colorectal cancer, we aimed to evaluate the transcript levels of HOTAIR in tumoral tissues of colorectal cancer and compare them with normal marginal tissues in an Iranian Azari population. In addition, we determined the correlation between HOTAIR expression level and clinicopathological features of patients with colorectal cancer.

Patients and Methods

Study subjects and sampling

In this case-control study, we studied 50 patients with colorectal cancer (cases) and 50 of their marginal tissues (controls). The samples were collected from colorectal cancer patients who had referred to Imam Reza hospital of Tabriz University of Medical Sciences. To obtain a pure sample population, all the recruited patients were native to East Azerbaijan, northwest of Iran. Through sample gathering, we excluded patients whom undergone chemotherapy and radiation therapy. We collected all samples during surgery, transferred them to RNase inhibitor solation (Qiagen, Cat No. 76104), and stored them at -80 till RNA extraction. We further gathered the clinical data of the patients (Table 1). The Human Research Ethics Committees from the Tabriz University of Medical Sciences (IR.TBZMED.REC.1398.801) approved the protocol of this study. Also, all patients signed written informed consent.

Table 1. Clinicopathological characteristics of the patients with colon cancer

Characteristic	Value (N=50)
Age	
<60	26 (52%)
>60	24 (48%)
Sex	
Male	32 (64%)
Female	18 (36%)
Smoking	
Yes	31 (62%)
No	19 (38%)
Tumor metastasis	
pM0	42 (84%)
pM1	8 (16%)
Tumor location	
Rectum	13 (26%)
Right colon	21 (42%)
Left colon	16 (32%)
Differentiation pattern	
Poor	11 (22%)
Moderate	26 (52%)
Well	13 (26%)

RNA extraction

We extracted the total RNA from tumoral and marginal tissues by Tripure isolation reagent (Roche, Cat No.11667165001) according to the manufacture's manuals. Yield and purity of RNA were specified via a NanoDrop spectrophotometer at 260/280 nm (Nano Drop ND-2000C Spectrophotometer, Thermo Fisher Scientific, USA). Additionally, for quality assessment, we examined the samples by gel electrophoreses on 1% agarose. Afterwards, RNA samples were stored at -80 till cDNA synthesis.

Complementary DNA (cDNA) syntheses and Real-time PCR quantification

TAKARA cDNA syntheses kit (TAKARA, Cat No. 6130) synthesized cDNA. The

Table2. Primer sequence and PCR conditions

Gene	Forward primer seq	Reverse primer seq	Annealing temperature
HOTAIR	5'-CAAACGTGGCAGAGGGCAAGA-3'	5'-TCTCTGGGCGTTCATGTGGCGA-3'	59 °C
GAPDH	5'-CAAGATCATCAGCAATGCCTCC-3'	5'-GCCATCACGCCACAGTTTCC-3'	59 °C

StepOnePlus Real-time PCR (Applied Biosystems, Foster City, USA) and the SYBR Green gene expression Master mix (Takara, Korea, Cat No. RR820W) performed the quantitative analysis. We further used glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an endogenous control to normalize the amount of total RNA in each sample.[14] Table 2 summarizes the primer sequences and PCR conditions. Melting curve confirmed the purity of each amplified product. We further analyzed the products to ensure the identity of the specific PCR product. The comparative cycle threshold (Ct) method calculated the relative transcript level of HOTAIR as previously described by Schmittgen and Livak.[15]

Statistical analysis

We performed the statistical analysis via Graph Pad Prism 6 (Graph Pad Software Inc. San Diego, CA, USA). Kolmogorov-Smirnov's normality test assessed the normal data distribution. Two sample t-tests compared the target gene expression level between colon cancer tissues and their paired marginal tissues. Pearson's correlation test assessed the correlation between the expression of target genes and patient's clinical parameters. All results were expressed as mean±standard deviation (SD). Statistical significance level was less than 0.05 for all *P* values.

Results

There existed a significant upregulation in HOTAIR transcription in the tumor tissues of colon cancer patients in comparison with marginal matched normal tissues (*P*=0.0023). Moreover, there was no HOTAIR expression in the five marginal normal tissues of the colon cancer

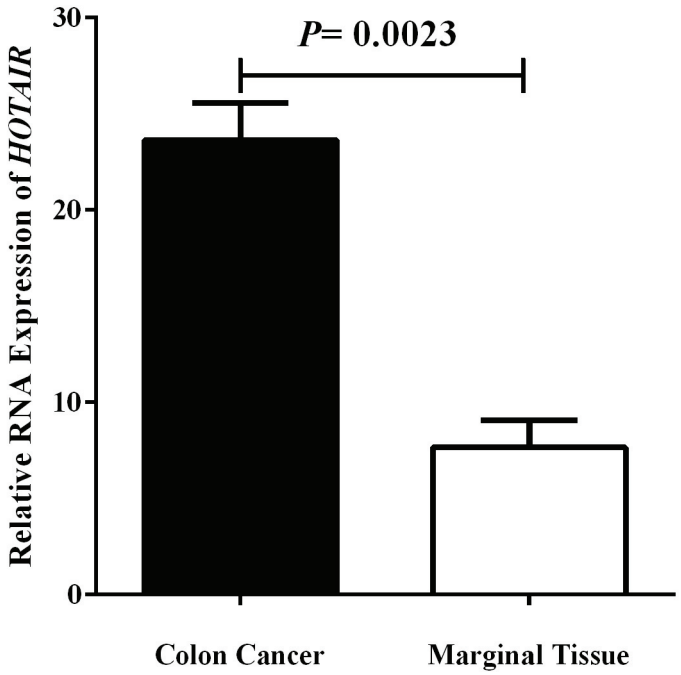

Figure 1. Bar graph illustrates HOTAIR RNA level in two compared groups.
HOTAIR: Hox transcript antisense intergenic RNA

Table3. HOTAIR expression level and clinical features

Characteristic	Relative mRNA expression (Mean ± SEM)	*P* value
Age		
<60	25.21±1.12	0.451
>60	23.02±4.21	
Sex		
Male	24.21±3.11	0.571
Female	26.25±2.278	
Smoking		
Yes	26.12±3.51	0.613
No	25.1±2.85	
Tumor metastasis		
pM0	27.23±3.41	0.042
pM1	23.12±4.11	
Tumor location		
Rectum	24.12±3.25	0.376
Right colon	25.45±6.32	
Left colon	24.12±5.11	
Differentiation pattern		
Poor	21.22±3.21	0.0197
Moderate	26.25±1.14	
Well	27.12±6..14	

patients; however, all the tumoral tissues expressed HOTAIR (Figure 1).

Lymph node metastases, differentiation, and tumor stage were three pathological features which had significant relationships with HOTAIR expression level ($P<0.05$). Furthermore, in cases with lymph node metastases, HOTAIR expression level was significantly higher than cases without metastasis. Alternately, we detected low levels of HOTAIR expression in cells with a better differentiation in comparison to poorly differentiated cells. However, age, sex, and tumor location of the colon cancer patients did not significantly correlate with HOTAIR expression level (Table 3).

Discussion

In the present research, in accordance with previous studies, we identified the overexpression of HOTAIR in colon cancer tissues. Furthermore, we found a meaningful relationship between the expression level of HOTAIR and tumor tissue differentiation and metastasis status. Recently, Gupta and colleagues showed that HOTAIR expression was associated with breast cancer metastasis.[16] Moreover, using in vitro data, Kogo and colleagues showed that HOTAIR overexpression increased the invasiveness of colorectal cancer cells. These results indicate that HOTAIR might also play a role in promoting the metastasis of colorectal cancer.[17]

Upregulation of HOTAIR has been corroborated in various types of cancers such as gastric, liver, and breast cancer. The overexpression of HOTAIR has further been revealed in metastatic tissues in comparison to non-metastatic tissues.[18-20] Therefore, it seems necessary to perform more studies with large sample sizes to obtain more valid and reliable conclusions as to the precise role of HOTAIR in colon cancer.

In conclusion, long non-coding RNAs such as HOTAIR, might be involved in regulating genes that are critical to cancer development. In this study, the transcription levels of HOTAIR were higher in tumor tissues, modulating the clinicopathological picture of patients. However, we did not evaluate the consequence of this upregulation in regard to molecular pathways. Further studies in the future will hopefully open new horizons to cancer therapy and diagnosis

through evaluating the mechanobiology of lncRNAs.

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Conflicts of Interest

None declared.

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