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Original Article

Long-chain non-coding RNA HOTAIR expression in tissue samples correlates with gastric cancer survival

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Abstract: To determine whether tissue expression of long non-coding RNA (lncRNA) HOTAIR in gastric cancer is correlated with patient survival, gastric cancer tissues from 100 individuals were selected as the case group; 50 normal adjacent tissues were selected as the control group. Relative expression of HOTAIR was determined from specimens using qRT-PCR. Clinical characteristics, overall survival (OS), and progression free survival (PFS) were assessed for potential correlation with expression of HOTAIR. Relative expression of HOTAIR lncRNA in gastric cancer tissue was significantly higher than that in normal gastric tissue ($t=16.838$, $P<0.05$). Additionally, expression levels were correlated with differences in tumor size, differentiation degree, lymph-vascular invasion, depth of invasion, lymph node metastasis, presence of distant metastasis, and clinical stage ($P<0.05$). Survival analysis showed that OS and PFS of patients in the low HOTAIR expression group (below the mean for the total population) were significantly higher than those in the high HOTAIR expression group ($P<0.05$). Cox multiple regression analysis showed that OS of patients was correlated with HOTAIR expression level, clinical stage, lymph-vascular invasion, lymph node metastasis, and distant metastasis ($P<0.05$); PFS was correlated with HOTAIR expression level, clinical stage, lymph-vascular invasion, tumor differentiation, and distant metastasis ($P<0.05$). In brief, HOTAIR lncRNA is overexpressed in gastric cancer tissue, and its expression level is correlated with clinical and pathological characteristics of the tumor. These features have a significant impact on the prognosis of patients, suggesting HOTAIR may be a useful prognostic biomarker for gastric cancer.

Keywords: Gastric cancer, long non-coding RNA HOTAIR, prognosis, survival analysis

Introduction

Although gastric cancer is the most common gastrointestinal cancer, most patients lack typical clinical manifestations at an early stage. Thus, a considerable number of patients with gastric cancer have progressed to the middle and advanced stages before diagnosis, missing the optimal time for surgical resection and worsening the prognosis [1]. Traditionally, the prognosis of gastric cancer was associated with patient and clinical characteristics such as gender, age, tumor location or size, pathologic stage, lymph node metastasis, and treatment modality. However, recent advances in molecular and genetic approaches have implicated oncogene activation, tumor suppressor inactivation, tumor immune function, cellular DNA content and cell cycle changes in tumor prognosis [1, 2]. Indeed, p53, PCNA, RAS, Bmi-1, CD44V6, nm23, p16, PTEN, and KAI1 are just a few of the proteins that regulate metastasis, recurrence, and other biological behaviors of gastric cancer. The identification of prognostic biomarkers could promote better outcomes in gastric cancer patients [2].

More recently, distinct classes of RNA molecules have been implicated in roles in a variety of diseases, including cancer. Medium-and long-chain non-coding RNAs (lncRNA) are endogenous transcripts consisting of more than 200 nucleotides. These non-protein coding transcripts regulate gene expression and function to participate in physiological processes such as embryonic development, metabolism, genomic imprinting, and evolution of species. Further, lncRNA may act as tumor promoters or suppressors [3].

Table 1. Correlation between relative tumor expression of HOTAIR lncRNA and clinical features of gastric cancer

Characteristics	Number of cases	High HOTAIR expression (n=44)	Low HOTAIR expression (n=56)	χ^2	P
Gender					
Male	73	33	40	0.159	>0.05
Female	27	11	16		
Age (year)					
≤60	57	23	34	0.716	>0.05
>60	43	21	22		
Tumor size (cm)					
≤5	78	26	52	16.372	<0.05
>5	22	18	4		
Pathological type					
Tubular adenoma	85	37	48	0.128	>0.05
Signet ring cell carcinoma	8	4	4		
Others	7	3	4		
Differentiation					
Poor	56	36	20	21.256	<0.05
Moderate/Well	44	8	36		
Lymph-vascular invasion					
Absent	28	2	26	21.440	<0.05
Present	72	42	30		
Depth of invasion					
T1/T2	25	4	21	10.606	<0.05
T3/T4	75	40	35		
Lymph metastasis					
N0	30	3	27	20.107	<0.05
N1/N2/N3	70	41	29		
Distant metastasis					
Negative	91	35	56	12.587	<0.05
Positive	9	9	0		
TNM stage					
I~II	57	11	46	32.826	<0.05
III~IV	43	33	10		
Chemotherapy					
No	60	28	32	0.433	>0.05
Yes	40	16	24		

HOX transcription antisense RNA (HOTAIR) is the first lncRNA demonstrated to act *in trans*: located within the HOX gene cluster on chromosome 12, HOTAIR is shuttled to chromosome 2, where it regulates gene transcription [4]. Overexpression of HOTAIR can promote metastasis and infiltration of breast cancer, hepatocellular cancer, esophageal, and colon cancers, influencing patient prognosis [5-8]. To gain insight to whether HOTAIR may serve as a prognostic marker in gastric cancer, its expression was assessed in a small set of gastric cancer tissues and studied with respect to patient survival time. The findings suggest that HOTAIR warrants further investigation for its prognostic potential.

Materials and methods

Participants and specimens

Gastric cancer cases comprised 100 paraffin-embedded specimens of gastric cancer tissues

Table 2. Survival outcomes of gastric cancer patients

Group	Outcome	Estimate (months)	Std. Error	95% CI	
				Lower Bound	Upper Bound
Total	OS	39.300	3.002	33.416	45.184
Population	PFS	33.100	2.707	27.794	38.406
High HOTAIR	OS	24.600	3.328	18.076	31.124
Expression	PFS	19.500	4.379	10.917	28.083
Low HOTAIR	OS	58.306	4.904	48.695	67.917
Expression	PFS	49.700	15.332	19.649	79.751

that were surgically removed from June 2007 to June 2010. All specimens derived from radical gastrectomy and were histopathologically confirmed as gastric cancer after surgery. None of the patients had received radiotherapy, chemotherapy, or other anti-tumor therapy before surgery; all received postoperative radiotherapy as appropriate. Exclusion criteria were: patients <18 years or >90 years old; those complicated with malignancies at other sites; those complicated with other diseases that might influence degree of tumor progression. Patients included 73 males and 27 females, ages 24 to 82 years, with a mean age of 59.6 ± 10. 9 years. The control group included 50 specimens of normal adjacent tissues that were simultaneously removed during gastrectomy. This study was approved by the Ethics Committee of Hongqi Hospital affiliated to Mudanjiang Medical College, and written informed consent was obtained from all subjects.

Patients were followed via telephone and home visit to aid in the determination of overall survival (OS) and progression-free survival (PFS).

lncRNA expression

Real-time fluorescence quantification PCR (qRT-PCR) was used to quantitatively detect relative expression of HOTAIR lncRNA in specimens. TRIzol reagent (ThermoFisher Scientific, Waltham, MA, USA) was used to extract total RNA from paraffin-embedded tissues. PrimeScript RT Reagent Kit with gDNA Eraser kit (TaKaRa Biotech, Code No. DRR047A) was used to reverse-transcribe RNA into cDNA. Quantitative real-time fluorescence PCR (qRT PCR) was used to detect relative expression of lncRNA HOTAIR using 18 s rRNA as an internal reference. The forward primer was 5'-GGCGGATGCAAGTTAATAAAAC-3' and reverse primer was 5'-TACGCCTGAGTGTTCACGAG-3'. Reactions consisted of 12.5 μL qPCR Super Mix, 0.5 μL forward primer (10 mM), 0.5 μL reverse primer (10 mM), 0.5 μL cDNA, and 11 μL DEPC water. The reaction was performed as follows: 50°C for 2 min; an initial denaturation step of 95°C for 10 min; 40 cycles of 95°C (15 s) and 60°C (1 min); and a final extension step at 72°C for 5 min. qRT-PCR results were measured as cycle threshold (Ct) values. Relative expression of targeted lncRNA was indicated by the ratio of Ct value of lncRNA to Ct value of reference genes and was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

SPSS 22.0 statistical software was used for data analysis. Measurement data are expressed as mean ± standard deviation. Pairwise comparison was performed with independent-samples *t* test. Numerical data are expressed as percentage, and were compared by χ^2 test. OS and PFS were compared using Kaplan-Meier survival analysis. Comparisons between the two groups were made using Log-rank test. Variables affecting OS and PFS were analyzed using Cox hazard regression model. For all analyses, $P<0.05$ was considered as statistically significant.

Results

Relative expression of lncRNA HOTAIR in gastric tissues

qRT-PCR was used to compare expression of HOTAIR in gastric tumors and adjacent normal tissues. Mean relative expression in gastric tumors was 17.8 ± 8.7 (n=100); mean expression in adjacent normal tissues was 2.7 ± 1.5 (n=50). These differences were statistically significant (t=16.838, $P<0.05$).

Relative expression of HOTAIR correlates with gastric cancer characteristics

The mean relative expression of HOTAIR in gastric cancer tissues was used as a cut-off value to divide the cancer cases into two groups: high HOTAIR expression (44 cases) and low HOTAIR expression (56 cases). Patients in these two

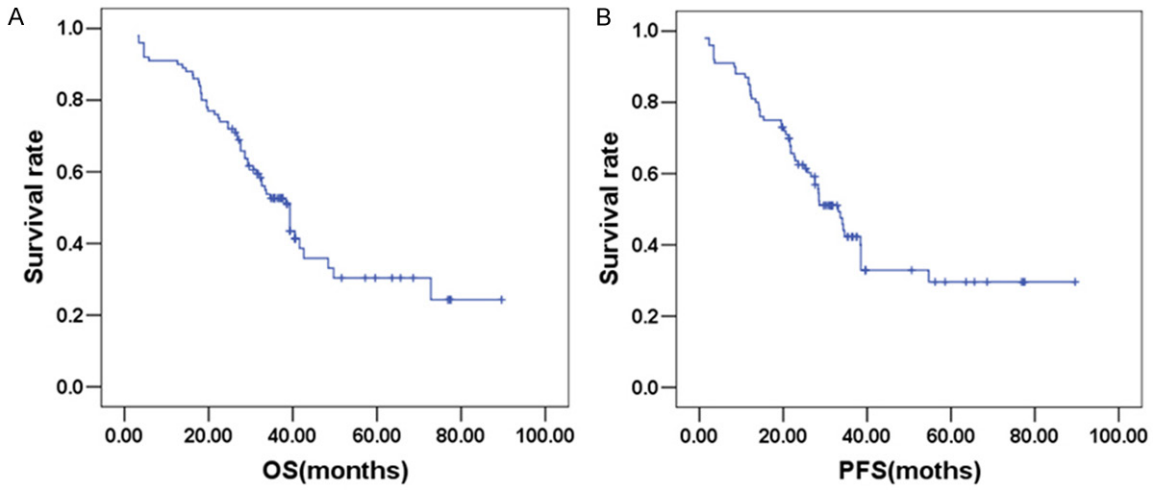

Figure 1. Kaplan-Meier survival analysis curve of OS (A), PFS (B) of all gastric cancer patients.

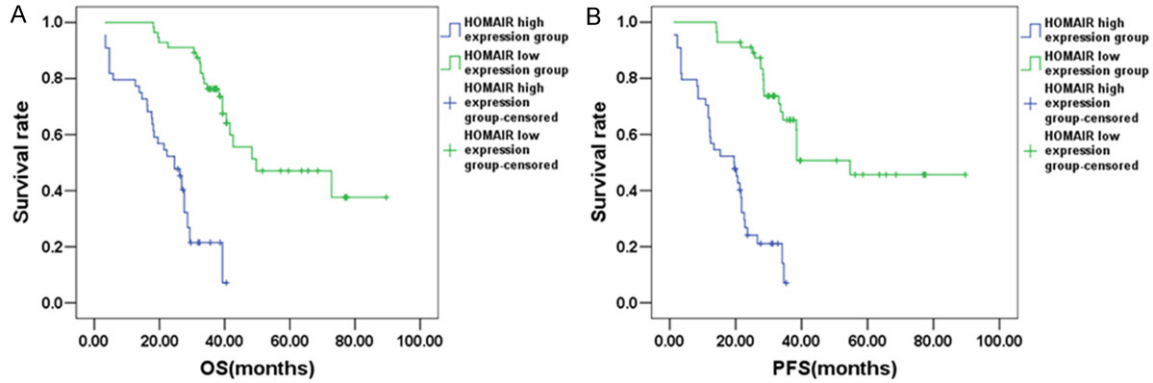

Figure 2. Kaplan-Meier survival analysis curve of OS (A) and PFS (B) of patients with different HOTAIR expression levels.

subgroups had statistically significant differences in tumor size, degree of differentiation, lymphatic vessel infiltration status (yes or no), infiltration depth, degree of lymph node metastasis, distant metastasis status (yes or no), and clinical stage (χ^2=16.372, 21.256, 21.440, 10.606, 20.107, 12.587, 32.826, respectively; P<0.05), as shown in **Table 1**.

Relative expression of HOTAIR correlates with gastric cancer survival

Survival analysis showed that overall survival (OS) and progression free survival (PFS) of patients in the low HOTAIR expression group were significantly better compared to those in the high expression group (χ^2=47.956 and 47.833, respectively; P<0.05). Estimates of OS and PFS (with 95% confidence intervals) are shown in **Table 2**; the survival analysis curves are shown in **Figures 1** and **2**.

Variables influencing survival of gastric cancer patients

The multivariate Cox regression analysis showed that OS was associated with lncRNA HOTAIR expression, clinical stage, lymphatic vessel infiltration, lymph node metastasis, and distant metastasis (χ^2=12.045, 8.176, 7.502, 8.136, 9.168, respectively; P<0.05), and PFS was associated with lncRNA HOTAIR expression, clinical stage, lymphatic vessel infiltration, degree of tumor differentiation, and distant metastasis (χ^2=5.694, 6.754, 8.922, 6.053, 7.138, respectively; P<0.05), as shown in **Table 3**.

Table 3. Variables that influence survival outcomes of gastric cancer patients

Outcome	Variables	β	χ^2	HR	P
OS	TNM stage (Stage III~IV)	0.616	12.045	2.122	<0.05
	High HOTAIR expression	0.527	8.176	1.828	<0.05
	Lymph-vascular invasion (Present)	0.432	7.502	1.727	<0.05
	Lymph metastasis (N1/N2/N3)	0.116	8.136	1.367	<0.05
	Distant metastasis (Positive)	1.068	9.168	2.848	<0.05
PFS	TNM stage (Stage III~IV)	0.563	5.694	1.671	<0.05
	High HOTAIR expression	0.715	6.754	1.964	<0.05
	Differentiation (Poor)	0.268	8.922	1.383	<0.05
	Lymph-vascular invasion (Present)	0.639	6.053	1.694	<0.05
	Distant metastasis (Positive)	0.953	7.138	2.067	<0.05

Discussion

As demonstrated for other tumor types, HOTAIR lncRNA expression was up-regulated in gastric cancer tissues, and the higher expression was correlated with more advanced tumors, suggesting that overexpression of HOTAIR may be an important oncogenic factor in gastric cancer. Several studies have found over-expressed lncRNAs in a variety of malignant tumors of the digestive tract, particularly HOTAIR, ncRuPAR, CCAT1, and H19, making them candidates for a new class of biomarkers for tumor diagnosis [8-11].

Up-regulation of HOTAIR has been correlated with clinicopathological features of other malignant tumors. For example, in liver, intestinal, pancreatic, laryngeal, nasopharyngeal, and lung tumors, HOTAIR expression levels are correlated with clinical stage and lymph node metastasis [12-17]. Thus, in addition to its potential to be a diagnostic marker, HOTAIR is proposed as a prognostic marker in these cancers. Our findings are consistent with a link between HOTAIR expression and disease characteristics.

HOTAIR inhibits expression of *HOXD* through a synergistic effect with PRC2 at the translational level. *HOXD* is a cluster in the *HOX* gene family, participating in gene regulation related to normal cell differentiation and development, as well as regulating proliferation and differentiation of embryonic cells [18]. Abnormal expression of *HOX* genes can cause cell transformation toward malignancy and eventually tumor formation. *IRF1*, *OPN*, and *c-MYC* can regulate HOTAIR expression levels, while HOTAIR can regulate expression and function of multiple genes, especially *HOXD10* [19, 20]. Although most studies have reported HOTAIR's tumor promoting effect, some studies have reported that HOTAIR expression is significantly reduced in lung cancer tissues and promotes tumor metastasis [21]. Therefore, further research is required to confirm the role of HOTAIR in the onset and progression of tumors.

The results of this study have shown that patients with higher HOTAIR expression level have shorter OS and PFS, and poorer prognosis, and that HOTAIR expression level, together with biological behavior of tumors and pathological features, can influence the prognosis of gastric cancer patients. These results implicate up-regulated HOTAIR expression in pathological processes [22-26], making it an important factor in predicting patient prognosis. HOTAIR promotes tumor progression by activating Wnt signaling to promote proliferation and migration of tumor cells; increasing methylation of the *PTEN* promoter, thereby silencing *PTEN* expression and releasing its inhibitory effect on Akt signaling. Akt signaling up-regulates MMP9 to promote tumor invasiveness and inhibit apoptosis of tumor cells. HOTAIR can also up-regulate VEGF and other pro-angiogenic factors through interacting with p53, and down-regulate TGF-β and other anti-angiogenic factors, to induce tumor angiogenesis, thereby advancing disease [22]. Finally, HOTAIR can form a regulatory network with miRNA, so as to regulate progression of tumor [23-25]. Further studies are needed to confirm the exact mechanism of abnormal HOTAIR expression in progression and prognosis of gastric cancer.

In summary, in gastric cancer tissues, HOTAIR lncRNA expression level is up-regulated and correlates with biological behaviors and pathological features of tumors. Further, HOTAIR is associated with survival outcomes, suggesting it may be useful as a prognostic biomarker.

Disclosure of conflict of interest

None.

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