Long non-coding RNA *HOTAIR* is a powerful predictor of metastasis and poor prognosis and is associated with epithelial-mesenchymal transition in colon cancer

ZE-HUA WU[1*], XIAO-LIANG WANG[1*], HUA-MEI TANG[2], TAO JIANG[1], JIAN CHEN[1], SU LU[2], GUO-QIANG QIU[1], ZHI-HAI PENG[1] and DONG-WANG YAN[1]

Departments of [1]General Surgery, and [2]Pathology, Shanghai Jiao Tong University Affiliated First People's Hospital, Shanghai 200080, P.R. China

Received January 25, 2014; Accepted April 15, 2014

DOI: 10.3892/or.2014.3186

Abstract. Colon cancer is one of the most frequently diagnosed cancer and the third most fatal malignancy worldwide. HOTAIR, a cancer-associated long non-coding RNA (lncRNA), is a powerful biomarker of metastasis and poor prognosis in a diverse group of cancers. Nevertheless, an understanding of how *HOTAIR* is involved in colon cancer progression is limited. In the present study, we hypothesized that HOTAIR plays a crucial role in colon cancer development. We evaluated the expression of HOTAIR in 120 colon cancer samples, matched adjacent non-tumor mucosa and 32 lymph node metastasis tissues by real-time PCR. Increased HOTAIR expression was significantly correlated with the depth of tumor invasion, lymph node metastasis, organ metastasis, histological differentiation, vascular invasion and advanced tumor stage. Patients with high HOTAIR expression had higher recurrence rates and reduced metastasis-free and overall survival than patients with low HOTAIR expression. Moreover, our findings revealed that HOTAIR had a limited effect on cell proliferation but significantly promoted colon cancer cell migration and invasion *in vitro*. Depletion of HOTAIR increased the expression of E-cadherin while concomitantly decreasing expression of vimentin and MMP9. Hence, HOTAIR may be another pleiotropic modulator participating in epithelial-mesenchymal transition (EMT). These results indicate that HOTAIR may also be a valuable predictor for colon cancer management; furthermore, this lncRNA may be a potential target for cancer prevention and treatment.

Correspondence to: Dr Dong-Wang Yan or Dr Zhi-Hai Peng, Department of General Surgery, Shanghai Jiaotong University Affiliated First People's Hospital, 85 Wujin Road, Shanghai 200080, P.R. China
E-mail: yandw70@163.com
E-mail: pengzhihai1212@aliyun.com

[*]Contributed equally

Key words: HOTAIR, lncRNA, colon cancer, metastasis, epithelial-mesenchymal transition

Introduction

Colon cancer is one of the most frequently diagnosed cancer and the third most fatal malignancy worldwide (1). Growing urbanization and industrialization have led to the steady rise in colon cancer incidence in China during the last 20 years (2). In certain high incidence areas, colon cancer has become the second leading cause of cancer-related mortality. Surgical resection is widely considered as the most useful therapy for colon cancer. Tumor metastasis is the major cause for the recurrence of colon cancer and leads to the failure of colon cancer treatment following radical operation. At present, clinicopathologic staging is the main risk assessment for colon cancer metastasis. Clinical outcomes, however, are quite variable, even among patients at the same stage. Therefore, research focusing on the molecular basis of colon tumor metastasis with the aim of implementing individualized therapeutic regimens for patients with colon cancer is urgently required.

The complicated process of tumor metastasis involves a number of molecular biological changes, including genetic and epigenetic mutations (3). Recently, the contribution of long non-coding RNAs in cancer progression has attracted increased attention. Long non-coding RNAs (lncRNAs) (typically >200 nt), a type of regulator non-coding RNAs, are increasingly reported to play key roles in numerous biological processes such as development, differentiation, diseases via integrity of nuclear structure, chromatin remodeling and post-transcription regulation (4). Gupta *et al* (5) first discovered that *HOTAIR* (Hox transcript antisense intergenic RNA) is one of the cancer metastasis-associated lncRNAs, and is a powerful biomarker of breast cancer metastasis and poor prognosis. Many other groups successively reported that *HOTAIR* overexpression is linked to the malignant features of many other cancer types (6-13). Moreover, Rinn *et al* (14) and Tsai *et al* (15) suggested that *HOTAIR* lncRNA serves as a scaffold for at least two histone modification complexes in

order to induce transcriptional silencing of hundreds of genes including tumor-suppressor genes. Nevertheless, the mechanism of how *HOTAIR* is involved in colon cancer progression remains unknown.

In the present study, we evaluated the expression of *HOTAIR* in colon cancer tissue and paired normal mucosa and metastasized lymph node, and then determined the correlation between *HOTAIR* expression and clinicopathologic characteristics. Using RNA interference experiments, we ascertained whether *HOTAIR* has effects on colon cancer cell biological properties. Furthermore, we revealed how *HOTAIR* initiates epithelial-mesenchymal transition of colon cancer, the main mechanistic step resulting in metastasis.

Materials and methods

Tissue samples and cell lines. Samples from 120 patients undergoing colectomy between October 2007 and December 2010 by the same surgical team at the Shanghai Jiao Tong University Affiliated First People's Hospital were collected and archived for the study. Fresh colon cancer samples and matched adjacent non-tumor tissues were obtained from 52 males and 68 females with ages ranging from 35 to 88 years. The samples were stored at -80˚C immediately following removal. None of the patients had received any preoperative therapy, and patients with stage II, III and IV disease underwent standard adjuvant chemotherapy according to The National Comprehensive Cancer Network Practice Guidelines for Colon Cancer (1). Follow-up was carried out completely (median follow-up time, 55.5 months; range, 10-72 months). The study was approved by the Ethics Committee of Shanghai Jiaotong University Affiliated First People's Hospital. Informed written consent was obtained from each patient enrolled in our study according to the Declaration of Helsinki. Colon cancer cell lines were obtained from the American Type Culture Collection and cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS) in a humidified atmosphere containing 5% CO_2 at 37˚C.

RNA isolation and quantitative RT-PCR for assessment of mRNA expression. Total RNAs from all samples were isolated with AllPrep DNA/RNA Mini kit (Qiagen, Hilden, Germany). After the RNA integrity and purity were checked, the first single-stranded cDNAs were synthesized from 3 μg of RNA, according to the manufacturer's instructions (Fermentas, Shenzhen, China). One microliter of the cDNAs from each sample was used as a template for qRT-PCR analysis with the ABsolute qPCR SYBR-Green Mix (Fermentas), employing the ABI Prism 7900 system (Applied Biosystems Inc., Foster City, CA, USA).

The following primers were used for qRT-PCR: *HOTAIR* sense 5'-GGTAGAAAAAGCAACCACGAAGC-3' and antisense 5'-ACATAAACCTCTGTCTGTGAGTGCC-3'; GAPDH sense 5'-GGAGCGAGATCCCTCCAAAAT-3' and antisense 5'-GGCTGTTGTCATACTTCTCATGG-3'.

The expression level of *HOTAIR* was normalized to the transcription level of GAPDH. The quantitative PCR reaction for each sample was repeated in triplicate. The relative *HOTAIR* expression was calculated using the $2^{-\Delta\Delta Ct}$ comparative method.

RNA interference. Two colon cancer cell lines were transfected with 50 nM positive siRNAs (si-*HOTAIR* 5'-GAACGGGAG UACAGAGAGAUU-3') or negative control siRNA (Mock 5'-CUACAACAGCCACAACGUCdTdT-3') employing Lipofectamine RNAiMAX (Life Technologies, Carlsbad, CA, USA) following the manufacturer's instructions. Total RNA isolation was performed 72 h later for real-time PCR analysis, while other functional assays were carried out 48 h post-transfection. All RNA interference assays were carried out in triplicates independently.

Cell proliferation assay. For cell proliferation assay, cells were seeded in 96-well plates ($2x10^3$ cells/well) in triplicate. At the appropriate time (24, 48, 72, 96 and 120 h), the cells were incubated with MTT (5 mg/ml; Sigma, St. Louis, MO, USA) for 4 h at 37˚C. MTT solution was then carefully removed and replaced with dimethyl sulfoxide (DMSO; Sigma). Absorbance was measured at a wavelength of 570 nm.

Cell migration and invasion assays. Cell migration and invasion assays were carried out using Transwell chambers (micropore size, 8 μm, 24-well; BD Biosciences, Franklin Lakes, NJ, USA) without Matrigel (for migration assay) or with Matrigel (for invasion assay). Both were performed according to the manufacturer's protocol. Briefly, the treated cells were plated in the upper chamber at a concentration of $5x10^4$ in 500 μl FBS-free media. The bottom chambers maintained suitable media with 10% FBS for 48 h. Then, the bottom of the chamber insert was fixed and stained with Giemsa solution. The stained cells were counted under a microscope at x200 magnification; meanwhile, images were captured and saved. The experiments were carried out in triplicate.

Western blot analysis of protein expression. The total protein was extracted from cells using Thermo Scientific Pierce RIPA buffer combined with Thermo Scientific Halt proteinase inhibitor cocktail and then quantified using Thermo Scientific BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA). The equivalent amounts of protein (20 μg) for each sample were electrophoresed on 10% sodium dodecyl sulfate-polyacrylamide gels and transferred onto PVDF membranes (Millipore, Billerica, MA, USA). Membranes were blocked using 5% non-fat milk solution for 1 h, and then probed with primary polyclonal antibodies at 4˚C overnight, followed by secondary antibodies (1:5,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at room temperature. β-actin was used as a loading control. After washing with TBST (Tris-buffered saline with 0.01% Tween-20), the proteins were detected by enhanced electrochemiluminescence (Millipore) and radiography. The primary antibodies used above were anti-E-cadherin (1:1,000; Abcam, Cambridge, UK) and anti-Vimentin (1:1,000; Abcam), anti-MMP9 (1:500; Cell Signaling Technology, Boston, MA, USA) and anti-β-actin antibody (1:1,000; Cell Signaling Technology).

Statistical analysis. All statistical analyses were set with a significance level of $P \leq 0.05$ and carried out using SPSS 19.0 statistical software (SPSS Inc., Chicago, IL, USA). The differences between 2 groups were analyzed with the Student's t-test and χ^2 test, as appropriate. The relationship between

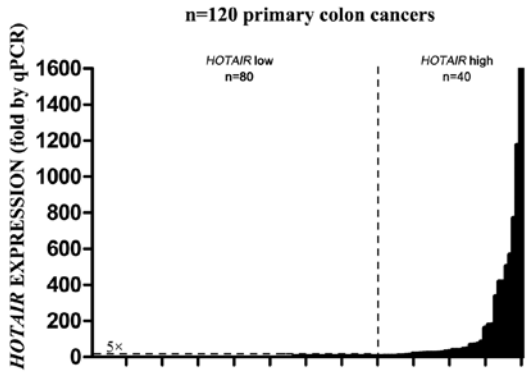


Figure 1. Quantitative PCR analysis of *HOTAIR* expression in 120 primary colon cancer tumors. *HOTAIR* was overexpressed from 2- to nearly 1,600-fold in the cancer tissues. Forty of the 120 tumors had >5-fold *HOTAIR* overexpression compared to adjacent normal tissues (*HOTAIR* high), whereas the other 80 samples did not exhibit >5-fold overexpression (*HOTAIR* low).

Table I. Expression of *HOTAIR* in colon cancer tissues and lymph node metastatic tissues.

Tissue sample	n	Expression of *HOTAIR*		
		Low n (%)	High n (%)	P-value
Cancer tissue	120	80 (66.7)	40 (33.3)	<0.001[a]
LNM tissue	32	5 (15.6)	27 (84.4)	

LNM, lymph node metastasis. [a]Significant difference in the expression of *HOTAIR* between primary colon cancer and LNM tissues.

HOTAIR expression and clinicopathological characteristics was estimated with the Mann-Whitney U test and the Kruskal-Wallis test appropriately. Survival curves were calculated by Kaplan-Meier method, with the log-rank test employed for the comparison of differences. Variables estimated with significant means by univariate analysis were subjected to the multivariate Cox proportional hazards regression model.

Results

Overexpression of HOTAIR in colon cancer tissues. qPCR was applied to examine the expression level of *HOTAIR* in 120 pairs of cancer and matched normal tissues, and 32 metastasized lymph node tissues. As shown in Fig. 1, *HOTAIR* was overexpressed from 2- to nearly 1,600-fold in the cancer tissues. According to a *HOTAIR* expression level of >5-fold compared to normal tissues, 120 patients were divided into a high expression group (n=40) and a low expression group (n=80). In addition, 27 of 32 (84.4%) lymph node metastasis tissues showed high expression of *HOTAIR*, with significant means between primary cancer tissues and metastatic lymph node tissues (Table I).

Correlation between HOTAIR expression and clinicopathological characteristics. The correlation between *HOTAIR* expression and clinicopathological characteristics is summarized in Tables II and III. Increased *HOTAIR* expression was significantly associated with AJCC stage (P<0.001), lymph node metastasis (pN stage, P=0.002), organ metastasis (pM stage, P<0.001), vascular invasion (P=0.019) and histologic differentiation (P<0.001). No significant correlations were found between *HOTAIR* expression and age, gender or tumor location. The Kaplan-Meier curves (Fig. 2) revealed that patients in the high expression group had a significant poorer clinical prognosis than those in the low expression group, both for metastasis-free survival and overall survival (log-rank test P<0.001). With regard to Cox univariate proportional hazards regression model, the level of *HOTAIR* expression, lymph node metastasis, organ metastasis, AJCC stage, degree of differentiation and vascular invasion were prognostic factors.

Table II. Correlation between *HOTAIR* expression and clinicopathological characteristics in the colon cancer cases.

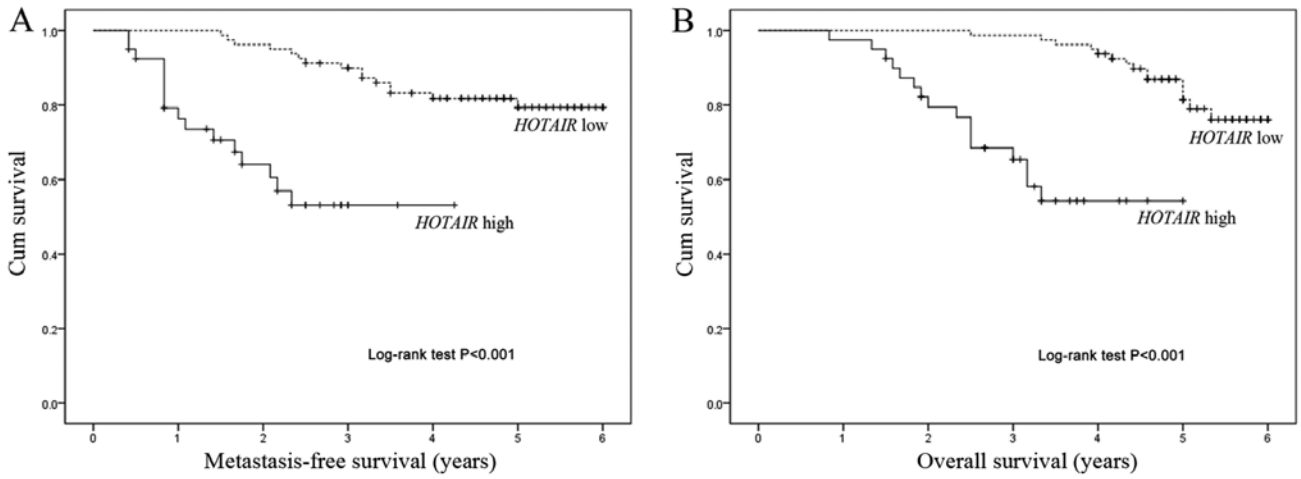
	HOTAIR expression		
	Low (n=80)	High (n=40)	P-value
Age (years)			
<65	28	17	0.424
≥65	52	23	
Gender			
Male	40	24	0.301
Female	40	16	
Location			
Right	20	11	0.768
Others	60	29	
AJCC stage			
I	12	1	<0.001[a]
II	37	7	
III	26	23	
IV	5	9	
pT stage			
T1	3	0	0.226
T2	17	4	
T3	41	26	
T4	19	10	
pN stage			
N0	48	12	0.002[a]
N1	25	17	
N2	7	11	
pM stage			
M0	78	28	<0.001[a]
M1	2	12	
Differentiation			
Well	11	3	0.001[a]
Moderate	61	21	
Poor	8	16	
Vascular invasion			
Yes	6	9	0.019[a]
No	74	31	

[a]P<0.05 indicates a significant difference among the variables.

Table III. Association between clinical characteristics and metastasis-free and overall survival by Cox regression model analysis.

	Metastasis-free survival				Overall survival			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
HOTAIR expression								
Low	-		-		-			
High	6.330 (2.610-15.348)	<0.001[a]	3.878 (1.369-10.981)	0.011[a]	8.147 (3.466-9.152)	<0.001[a]	3.915 (1.226-12.499)	0.021[a]
Age (years)								
<65	-				-			
≥65	0.803 (0.385-1.675)	0.559			0.977 (0.487-1.961)	0.948		
Gender								
Male	-				-			
Female	1.492 (0.693-3.213)	0.307			1.048 (0.514-2.135)	0.898		
Location								
Right	-				-			
Other	0.596 (0.280-1.266)	0.178			0.696 (0.338-1.432)	0.325		
pT stage								
T1	-				-			
T2	1.146 (0.101-12.970)	0.912			1.169 (0.104-13.150)	0.900		
T3	1.137 (0.319-9.333)	0.905			1.085 (0.132-8.939)	0.940		
T4	4.167 (0.560-30.992)	0.163			4.383 (0.588-32.663)	0.149		
pN stage								
N0	-		-		-		-	
N1	3.172 (1.288-7.812)	0.012[a]	2.885 (1.147-7.255)	0.024[a]	3.270 (1.315-8.130)	0.011[a]	2.626 (0.995-6.931)	0.051
N2	10.485 (4.046-27.170)	<0.001[a]	7.878 (2.903-21.384)	<0.001[a]	11.135 (4.227-29.331)	<0.001[a]	6.982 (2.365-20.614)	<0.001[a]
pM stage								
N0	-		-		-		-	
M1	8.386 (12.739-25.670)	<0.001[a]	3.580 (1.121-11.437)	0.031[a]	6.935 (2.365-10.864)	<0.001[a]	2.856 (0.745-10.945)	0.126
AJCC								
N0	-		-		-		-	
III-IV	4.644 (2.060-10.466)	<0.001[a]	5.331 (0.959-29.636)	0.056	4.782 (2.105-10.864)	<0.001[a]	7.571 (1.330-43.103)	0.023[a]
Differentiation								
N0	-		-		-		-	
Moderate	1.930 (0.814-4.576)	0.135	1.570 (0.667-3.699)	0.302	1.681 (0.709-3.985)	0.239	1.489 (0.591-3.751)	0.399
Poor	4.094 (1.565-10.715)	0.004[a]	1.889 (0.677-5.273)	0.225	4.106 (1.568-10.751)	0.004[a]	2.234 (0.761-6.556)	0.143
Vascular invasion								
No	-				-			
Yes	2.282 (0.531-9.809)	0.267			2.124 (0.501-9.010)	0.307		

HR, hazard ratio; CI, confidence interval. P<0.05 indicates that the 95% CI of HR did not include 1.



		Time (years)							Median Time
		0	1	2	3	4	5	6	
Low	No. at Risk	80	80	76	66	46	20	2.5	3.00
	No. of events	0	3	5	5	1	1	0	
High	No. at Risk	38	26	13	2.5	0.5	0	0	1.44
	No. of events	8	5	3	0	0	0	0	

		Time (years)							Median Time
		0	1	2	3	4	5	6	
Low	No. at Risk	80	80	80	79	65	29	2.5	3.00
	No. of events	0	0	1	3	6	5	0	
High	No. at Risk	40	38	29	15	2.5	0.5	0	1.99
	No. of events	1	6	5	4	0	0	0	

Figure 2. Kaplan-Meier curves and life tables based on *HOTAIR* expression level. (A) Metastasis-free survival curves and (B) overall survival curves of 120 primary colon tumors. The survival of the *HOTAIR* high group (n=40) was significantly shorter than that of the the low expression group (n=80; log-rank test, P<0.001). The relevant life tables are provided below the curves, respectively.

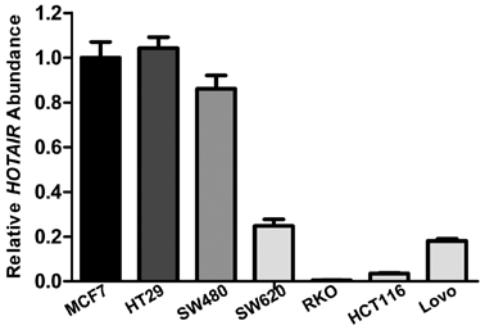


Figure 3. qRT-PCR analysis of *HOTAIR* expression in 7 cancer cell lines. The expression levels in 6 colon cancer cell lines were normalized to the level in MCF-7, a breast cancer cell line. *HOTAIR* expression levels in SW480 and HT29 were higher than the levels in the other 4 cell lines.

Additionally, multivariate analysis, including the variables above with P<0.05, indicated that *HOTAIR* expression was an independent clinical risk indicator for both metastasis-free survival (HR, 3.878; 95% CI, 1.369-10.981; P=0.011) and overall survival (HR, 3.915; 95% CI, 1.226-12.499; P=0.021).

HOTAIR promotes migration and invasion of colon cancer cells. As reported by other research groups, *HOTAIR* may play a pro-oncogenic role in certain types of cancers such as breast (5), hepatocellular (6), gastrointestinal (8), pancreatic (9) and lung cancer (11). To examine the effect of *HOTAIR* on colon cancer, a series of functional experiments concerning cell proliferation and cell metastasis was performed. We quantified the expression levels of *HOTAIR* in the colon cancer cell lines by qRT-PCR, and compared the levels to the level in MCF-7, a breast cancer cell line that overexpresses endogenous *HOTAIR* (Fig. 3). *HOTAIR* expression levels in colon cancer cell lines SW480 and HT29 were higher than that in the other 4 cell lines. *HOTAIR* knockdown was carried out by using small interfering RNA transfection in the cell lines. The efficiency of siRNA-mediated knockdown in the SW480 or HT29 cells was >60% (data not shown). As illustrated by Fig. 4, results from the MTT assay showed that the proliferation rate of cells depleted of *HOTAIR* was slightly slower than the rate of cells treated with mock siRNA throughout 5 continuous days.

We next ascertained whether manipulation of the *HOTAIR* level affects cell metastasis *in vitro*. Migration and invasiveness of tumor cells are key aspects of metastasis. In the Transwell migration assay, fewer SW480 and HT29 colon cancer cells treated by si-*HOTAIR* migrated to the 8-μm pores of the Transwell chambers when compared to the cells treated with Mock (Fig. 5A and B). Furthermore, similar results were obtained in the invasion assay using a Matrigel environment study (Fig. 5C and D). All of these studies were repeated three times and the differences were statistically significant (P<0.05). Based on these outcomes, it was suggested that *HOTAIR* is involved in the progression of colon cancer mainly by promoting cancer cell migration and invasiveness.

HOTAIR regulates expression of E-cadherin, vimentin and MMP9. Epithelial-mesenchymal transition (EMT) with alterations in gene expression profiles of cells initiates the

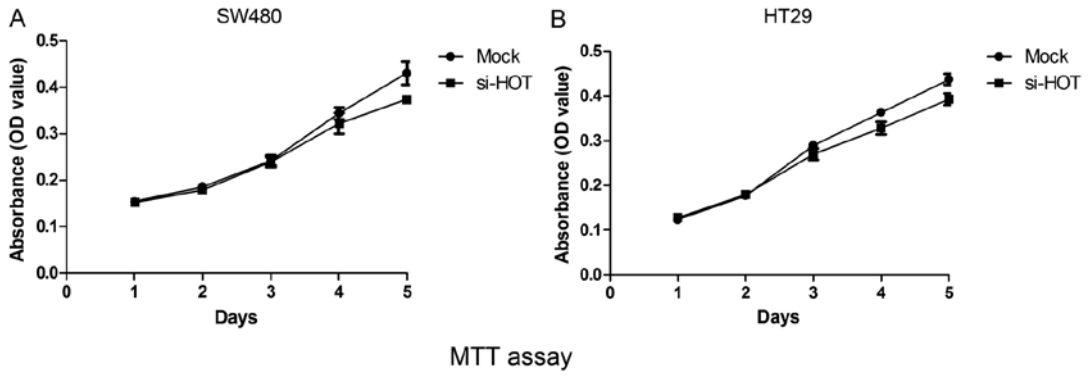


Figure 4. Cell proliferation assay of colon cancer cells. (A) Growth curves show SW480 cell proliferation following treatment with Mock or si-HOTAIR as described in Materials and methods. Cell viability was tested by MTT assay. (B) Growth curves show HT29 cell proliferation following treatment with Mock or si-HOTAIR as described in Materials and methods. Cell viability was tested by MTT assay. Error bars represent standard deviation (n=3).

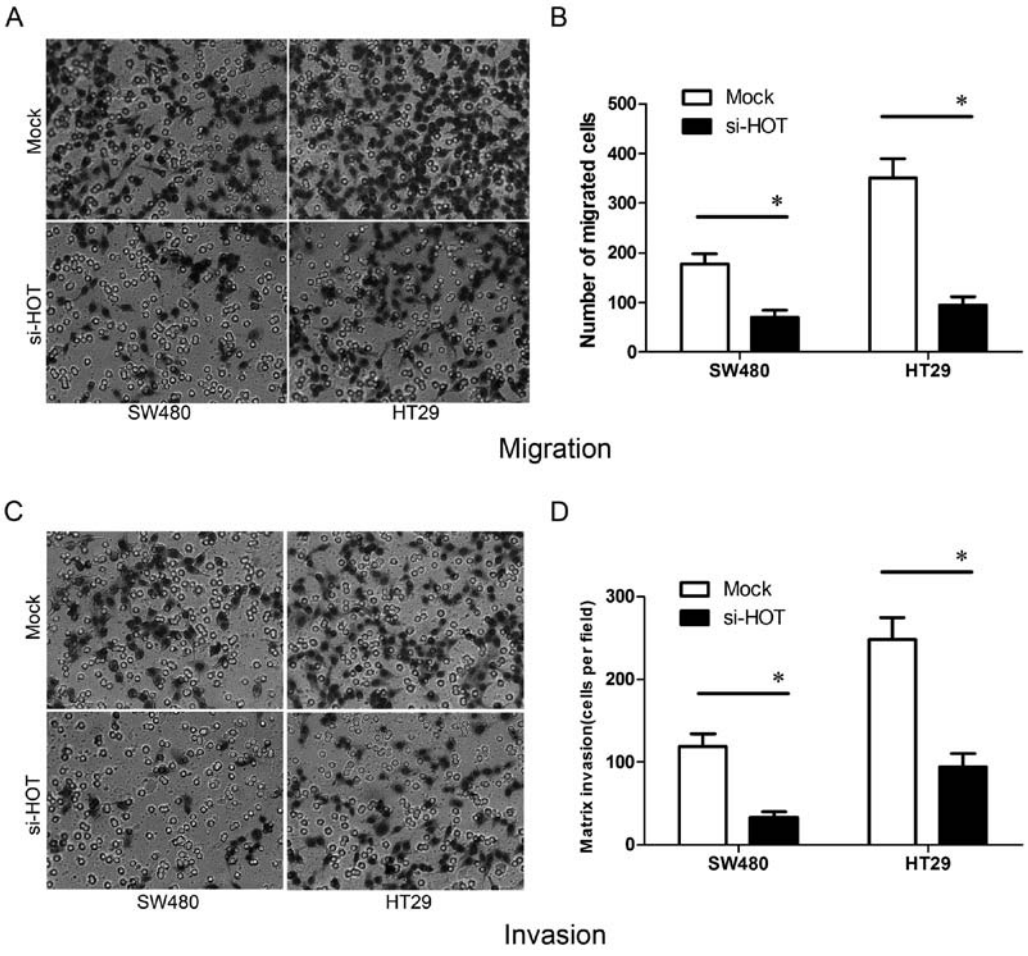


Figure 5. Cell migration and invasion assays of colon cancer cells *in vitro*. (A) Image shows SW480 and HT29 cell migration following treatment with Mock or si-HOTAIR as described in Materials and methods. (B) Histogram represents the number of migrated cells. (C) Image shows SW480 and HT29 cell invasion following treatment with Mock or si-HOTAIR as described in Materials and methods. (D) Histogram represents the number of invaded cells. Error bars represent standard deviation (n=3). *Significant difference between Mock group and si-HOTAIR group.

invasion-metastasis cascade. Therefore, we ascertained whether *HOTAIR* promotes EMT to regulate tumor progression. Several key genes involved in EMT of colon cancer were selected for further western blot analysis (Fig. 6). When SW480 and HT29 cells were treated by *HOTAIR* knockdown, expression of E-cadherin, a hallmark of epithelial cell was increased, while expression of vimentin, a mesenchymal cell marker, was decreased. Additionally, in the si-*HOTAIR*-treated cells, expression of matrix metalloproteinase 9 (MMP9), which functions as an important proteinase to degrade the extracel-

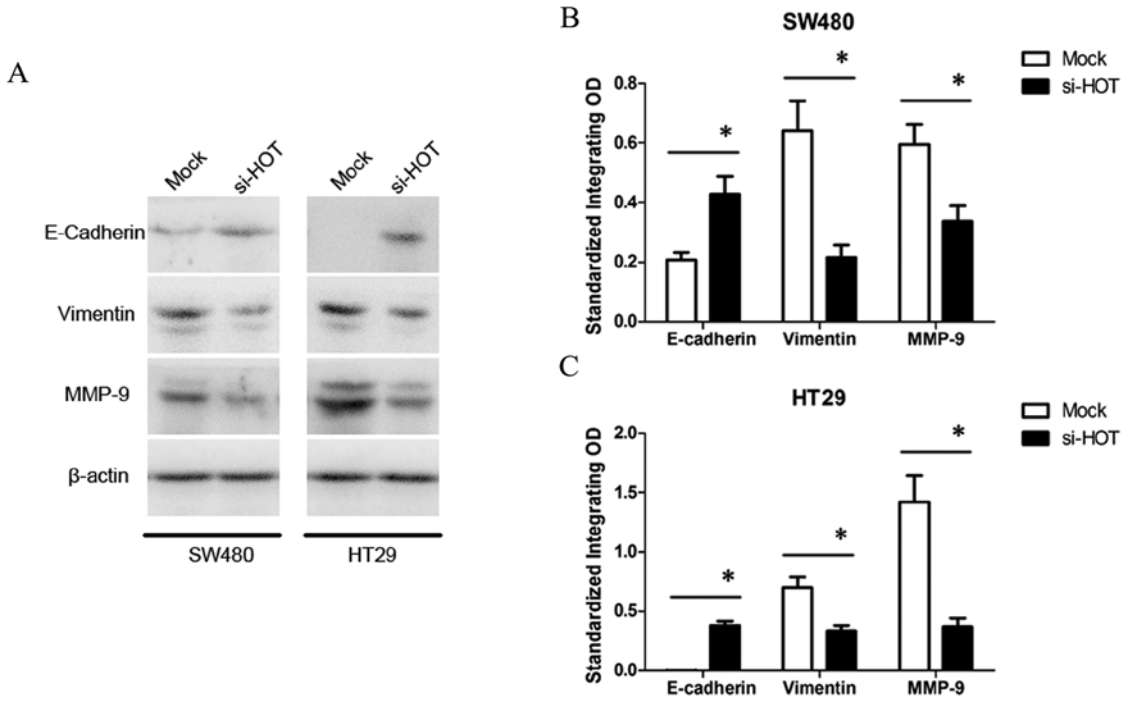


Figure 6. Western blot analysis of colon cancer cell lysates. (A) Cell lysates were subjected to western blot analysis following treatment with negative control or si-HOTAIR as described in Materials and methods. (B and C) Histograms were constructed by analyzing the standard integrating optical density. Knockdown of HOTAIR increased E-cadherin expression, whereas decreased expression of vimentin and MMP9 was noted. Error bars represent standard deviation (n=3).

lular matrix for facilitating cell motility, was inhibited as well. These data indicate that the regulation of EMT is partly involved in the mechanism of *HOTAIR*-induced metastasis.

Discussion

HOTAIR lncRNA, with a 2158-nucleotide transcript, is transcribed from the HOXC locus in an antisense manner, which is also the reason why it is termed *HOXC* antisense intergenic RNA (14). *HOTAIR* has very high nucleotide conservation in vertebrates and an anatomic conservative expression pattern from embryo to adulthood. Moreover, the lncRNA is selectively required to physically interact with polycomb repressive complex 2 (PRC2) to regulate the chromatin methylation state. PRC2, comprising H3K27 histone methyl transferase EZH2, SUZ2 and EED, is involved in stem cell pluripotency and cancer progression (16-18). In 2010, *HOTAIR* was reported as a powerful predictor of breast cancer metastasis and poor prognosis. Gupta and his coworkers (5) found a positive association among high expression pattern of *HOTAIR*, breast cancer malignant biologic characteristics and reduced survival. The present study showed that the level of *HOTAIR* lncRNA expression in tumor tissues was distinctly higher than the level in paired normal mucosa. Additionally, *HOTAIR* was more highly expressed in invaded lymph nodes than in primary cancer tissues. Increased *HOTAIR* expression was significantly correlated with the depth of tumor invasion, lymph node metastasis, organ metastasis, histological differentiation, vascular invasion and advanced tumor stage. Patients with high *HOTAIR* expression had higher recurrence rates and poorer metastasis-free and overall survival rates than patients with low *HOTAIR* expression. Multivariate analyses revealed that *HOTAIR* expression was an independent factor for colon cancer prognosis after surgery. These results indicated that *HOTAIR* also could be a meaningful predictor for colon cancer management.

The 5' domain of *HOTAIR* binds to PRC2 and is required for histone H3 lysine 27 trimethylation in order to silence HOXD and other target genes (5,14). On the other hand, the 3' domain of *HOTAIR* maintains LSD1-binding activity (15). LSD1, the first identified demethylase that specifically catalyzes the demethylation of histone H3 lysine 4, is believed to be linked to the transcriptional silencing of tumor-suppressor genes (19-22). Tsai *et al* (15) suggested that *HOTAIR*, as a modular scaffold, is required to target both PRC2 and LSD1 to many promoter elements of genes in order to coordinate chromatin modification for gene repression. Loss of *HOTAIR* notably weakens the activities of PRC2 and LSD1 in modifying the histone state. Therefore, it is reasonable to expect that *HOTAIR* plays a crucial role in cancer development other than a merely novel cancer biomarker. At present, the study results revealed that *HOTAIR* had a limited effect on cell proliferation. As *HOTAIR* knockdown using siRNA significantly reduced the ability of cancer cells to invade the Transwell membranes, this lncRNA may promote colon cancer cell migration and invasion, which is mainly consistent with previous studies in other cancer types.

We further addressed how *HOTAIR*-dependent gene regulation in colon tumors is involved in the enhancement of an aggressive biological behavior. The early step leading to metastasis is migration and invasion of cancer cells from original tissues into the surrounding stroma. In order to acquire such abilities, carcinoma cells must inherit a drastic phenotypic alteration - the epithelial-mesenchymal transition (EMT).

EMT is a biological process that allows polarized epithelial cells to experience multiple biochemical changes that enable them to assume a mesenchymal cell phenotype (23). Upon the phenotype change, cancer cells lose intercellular junction, breach the basement membrane and acquire enhanced motility. The complexity of EMT means that only small numbers of centrally pleiotropic regulators orchestrate the complement, such as Twist, Slug and Snail and the miR200 family. The present study demonstrated that depletion of *HOTAIR* increased expression of E-cadherin while concomitantly decreasing expression of vimentin and MMP9. Together with previous studies using global gene expression analysis, the emerging evidence indicates that increased expression of *HOTAIR* in cancer cells appears to reprogram the chromatin profile of epithelial cells to that of mesenchymal cells, cooperating with PRC2 and LSD1 at least. Hence, *HOTAIR* may be another pleiotropic modulator participating in EMT.

Collectively, our data indicate that *HOTAIR* is a valuable factor for colon cancer prognosis. Moreover, *HOTAIR* can promote colon cancer cell migration and invasiveness and may participate in epithelial-mesenchymal transition. Further studies are warranted to advance our understanding of the involvement of *HOTAIR* in cancer development, since this lncRNA is a potential target for cancer prevention and treatment.

Acknowledgements

The present study was supported by funds from the National Natural Science Foundation of China (81172328), the Important International Cooperation grants from the National Natural Science Foundation of China (81220108021), the Medical Guidance Project of Shanghai Science and Technology Commission (124119a1700), the Science and Technology Innovation Plan of Shanghai Science and Technology Commission (11431921000), the Frontier Technology Union Research Project of Shanghai Municipal Hospitals (SHDC12012105), and the Medical Climbing Project from Songjiang Health Bureau of Shanghai (2011PD03).

References

1. NCCN Clinical Practice Guidelines in Oncology for Colon Cancer (Version 3). 2013. [EB/OL] Available at http://www.nccn.org/professionals/physician_gls/f_guidelines.asp
2. Li M and Gu J: Changing patterns of colorectal cancer in China over a period of 20 years. World J Gastroenterol 11: 4685-4688, 2005.
3. Fearon ER: Molecular genetics of colorectal cancer. Annu Rev Pathol 6: 479-507, 2011.
4. Ulitsky I and Bartel DP: lincRNAs: genomics, evolution, and mechanisms. Cell 154: 26-46, 2013.
5. Gupta RA, Shah N, Wang KC, *et al*: Long non-coding RNA *HOTAIR* reprograms chromatin state to promote cancer metastasis. Nature 464: 1071-1076, 2010.
6. Yang Z, Zhou L, Wu LM, *et al*: Overexpression of long non-coding RNA HOTAIR predicts tumor recurrence in hepatocellular carcinoma patients following liver transplantation. Ann Surg Oncol 18: 1243-1250, 2011.
7. Lu L, Zhu G, Zhang C, *et al*: Association of large noncoding RNA HOTAIR expression and its downstream intergenic CpG island methylation with survival in breast cancer. Breast Cancer Res Treat 136: 875-883, 2012.
8. Niinuma T, Suzuki H, Nojima M, *et al*: Upregulation of miR-196a and *HOTAIR* drive malignant character in gastrointestinal stromal tumors. Cancer Res 72: 1126-1136, 2012.
9. Kim K, Jutooru I, Chadalapaka G, *et al*: HOTAIR is a negative prognostic factor and exhibits pro-oncogenic activity in pancreatic cancer. Oncogene 32: 1616-1625, 2013.
10. Li D, Feng J, Wu T, *et al*: Long intergenic noncoding RNA HOTAIR is overexpressed and regulates *PTEN* methylation in laryngeal squamous cell carcinoma. Am J Pathol 182: 64-70, 2013.
11. Nakagawa T, Endo H, Yokoyama M, *et al*: Large noncoding RNA HOTAIR enhances aggressive biological behavior and is associated with short disease-free survival in human non-small cell lung cancer. Biochem Biophys Res Commun 436: 319-324, 2013.
12. Zhuang Y, Wang X, Nguyen HT, *et al*: Induction of long intergenic non-coding RNA HOTAIR in lung cancer cells by type I collagen. J Hematol Oncol 6: 35, 2013.
13. Kogo R, Shimamura T, Mimori K, *et al*: Long noncoding RNA *HOTAIR* regulates polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers. Cancer Res 71: 6320-6326, 2011.
14. Rinn JL, Kertesz M, Wang JK, *et al*: Functional demarcation of active and silent chromatin domains in human *HOX* loci by noncoding RNAs. Cell 129: 1311-1323, 2007.
15. Tsai MC, Manor O, Wan Y, *et al*: Long noncoding RNA as modular scaffold of histone modification complexes. Science 329: 689-693, 2010.
16. Sparmann A and van Lohuizen M: Polycomb silencers control cell fate, development and cancer. Nat Rev Cancer 6: 846-856, 2006.
17. Richly H, Aloia L and Di Croce L: Roles of the Polycomb group proteins in stem cells and cancer. Cell Death Dis 2: e204, 2011.
18. Crea F, Fornaro L, Bocci G, *et al*: EZH2 inhibition: targeting the crossroad of tumor invasion and angiogenesis. Cancer Metastasis Rev 31: 753-761, 2012.
19. Ding J, Zhang ZM, Xia Y, *et al*: LSD1-mediated epigenetic modification contributes to proliferation and metastasis of colon cancer. Br J Cancer 109: 994-1003, 2013.
20. Huang Z, Li S, Song W, *et al*: Lysine-specific demethylase 1 (LSD1/KDM1A) contributes to colorectal tumorigenesis via activation of the Wnt/β-catenin pathway by down-regulating Dickkopf-1 (DKK1). PLoS One 8: e70077, 2013.
21. Tang M, Shen H, Jin Y, *et al*: The malignant brain tumor (MBT) domain protein SFMBT1 is an integral histone reader subunit of the LSD1 demethylase complex for chromatin association and epithelial-to-mesenchymal transition. J Biol Chem 288: 27680-27691, 2013.
22. Abdel-Wahab O and Dey A: The ASXL-BAP1 axis: new factors in myelopoiesis, cancer and epigenetics. Leukemia 27: 10-15, 2013.
23. Kalluri R and Weinberg RA: The basics of epithelial-mesenchymal transition. J Clin Invest 119: 1420-1428, 2009.