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Long non-coding RNA *HOTAIR* is an independent prognostic marker for nasopharyngeal carcinoma progression and survival

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Identification of new nasopharyngeal carcinoma (NPC) biomarkers is of great clinical value for the diagnosis and treatment of NPC. *HOTAIR*, a cancer-related long non-coding RNA, was tested and its prognostic value for NPC was evaluated. As determined using *in situ* hybridization (ISH), 91 of 160 (56.87%) paraffin-embedded NPC biopsies showed high expression levels of *HOTAIR* (staining index score ≥ 6). *HOTAIR* was upregulated in tumors with a large size ($P = 0.021$), more advanced clinical stage ($P = 0.012$) and increased lymph node tumor burden ($P = 0.005$). Quantified using real-time PCR, *HOTAIR* expression levels in fresh tissue and paraffin-embedded samples were 5.2~48.4-fold higher compared with non-cancer tissue samples. Moreover, *HOTAIR* expression levels increased with clinical stage progression, which was consistent with ISH findings in the paraffin-embedded tissue. Most importantly, NPC patients with higher *HOTAIR* levels had a poor prognosis for overall survival using univariate and multivariate analysis. In addition, *HOTAIR* mediated the migration, invasion and proliferation of NPC cells *in vitro*. *HOTAIR* is a potential biomarker for the prognosis of NPC, and dysregulation of *HOTAIR* might play an important role in NPC progression. (*Cancer Sci* 2013; 104: 458–464)

Nasopharyngeal carcinoma (NPC) is a rare tumor that arises from the nasopharynx epithelium. Nasopharyngeal carcinoma occurs around the world;[1] however, it is vastly more common in certain regions of Southeast Asia than elsewhere. The incidence of NPC in South China, especially in the Cantonese region around Guangzhou, is approximately 100-fold higher compared with Europe and North America,[1] with viral, environmental and genetic factors implicated in its etiology.[2–5] Although treatment with radiotherapy temporarily controls the primary tumor, frequent tumor recurrence and distant metastases remain major obstacles for long-term patient survival.[6,7] Therefore, identifying more accurate predictive biomarkers is of great clinical value to further understand NPC cell biology and develop novel therapeutic strategies.

The discovery of numerous non-coding RNA (ncRNA) transcripts in human cells has dramatically altered our understanding of the biology of normal and malignant cells. A large class of small ncRNA, microRNA, has been characterized as oncogenes or tumor suppressors through post-transcriptional regulation of protein expression. However, the findings that long non-coding RNA (lncRNA) more than 200 nt in length are differentially expressed in cancer cells and bind to various regulatory proteins have increased the complexity of ncRNA involvement in tumor biology regulation.[8–10] Dysregulated lncRNA expression levels characterize the entire spectrum of disease,[10] and aberrant lncRNA function drives cancer through the disruption of cell processes, typically by facilitating transcriptional regulation.[11] This process can occur through the targeting of either genomically local (cis-regulation) or genomically distant (trans-regulation) genes. Of the cis-regulatory lncRNA (imprinting lncRNA), H19 has been most extensively studied in cancer.[12–14] In model systems, silencing H19 expression impaired cell growth and clonogenicity in lung cancer cell lines *in vitro*[14] and decreased xenograft tumor growth of Hep3B hepatocellular carcinoma cells *in vivo*.[12] Other cis-regulatory lncRNA, such as HOTAIRMI and HOTTIP, play important roles in differentiation status of cancer cells in leukemias.[15] Similar to most cis-acting lncRNA, trans-regulatory lncRNA typically facilitate the epigenetic regulation of gene expression. In cancer, trans-regulatory lncRNA gained widespread attention through the characterization of *HOTAIR*. *HOTAIR* is located in the HoxC cluster and was found to regulate the HoxD cluster genes through a trans-regulatory mechanism.[16] Recently, upregulated expression of *HOTAIR* was observed in numerous solid tumors, including breast cancer,[17] hepatocelluar carcinoma[18] and colon cancer.[19] In breast cancer, overexpression of *HOTAIR* facilitates aberrant polycomb repressive complex (PRC2) function by increasing PRC2 recruitment to the genomic positions of target genes and mediates the epigenetic repression of PRC2 target genes.[17] Clinically, overexpression of *HOTAIR* is an independent predictor of overall survival and progression-free survival for several cancers.[17,18] Thus, lncRNA represent a novel but poorly characterized aspect of cancer biology. While a biological understanding of *HOTAIR* in breast and hepatocellular carcinomas has progressed, increased understanding of the functional role of *HOTAIR* in other human cancers, such as NPC, is needed.

In the present study, we examined *HOTAIR* expression in normal and malignant human nasopharyngeal tissue and cell lines. Our results demonstrated that high *HOTAIR* expression is associated with NPC progression and predicts a poor patient prognosis. In addition, we studied how *HOTAIR* influences the invasiveness of NPC cells *in vitro*.

Matherials and Methods

Patient and tissue specimens. A total of 160 paraffin-embedded NPC samples from Sun Yat-Sen Memorial Hospital from June 2005 to June 2012 were examined in the present study. Fresh tumor samples of primary NPC and non-NPC tissues were obtained from biopsies from the Department of Otolaryngology/Head and Neck, Sun Yat-Sen Memorial Hospital, as approved by the Research Ethics Board at Sun

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Yat-Sen Memorial Hospital. Written informed consent was obtained from all patients. No patients had received therapy prior to biopsy. The disease stages of the patients were classified according to American Joint Committee on Cancer (AJCC). The clinical information of the samples is described in detail in Supporting Information Table S1.

Quantitative PCR assay. Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA, USA) or a RNeasy FFPE Kit (QIAGEN, Hilden, Germany). cDNA was obtained using reverse transcription of total RNA with a TaqMan Reverse Transcription Kit (Applied Biosystems Inc., Carlsbad, CA, USA). The primer sequences of *HOTAIR* were as follows: forward, 5′-GGTAGAAAAAGCAACCACGAAGC-3′; and reverse, 5′-ACATAAACCTCTGTCTGTGAGTGCC-3′. Amplification and analysis were performed on the Roche LightCycler480 (Roche, Basel, Switzerland).

***In situ* hybridization and data analyses.** *HOTAIR* expression was examined using *in situ* hybridization (ISH) in NPC and non-NPC paraffin-embedded sections. Briefly, after dewaxing and rehydration, the samples were digested with proteinase K, fixed in 4% paraformaldehyde, hybridized with the 5′digoxin-labeled locked nucleic acid (LNA)-modified *HOTAIR* probe (Exiqon, Vedbaek, Denmark) at 55°C overnight, and subsequently incubated overnight at 4°C with anti-digoxin monoclonal antibody (Roche). After staining with nitro blue tetrazolium/5-bromo-4-chloro-3-indolylphosphate, the sections were mounted and observed. Positive expression of *HOTAIR* (in blue) was primarily detected in the cytoplasm. The staining scores were determined based on both the intensity and proportion of *HOTAIR*-positive cells in 10 random fields under a × 40 objective. The proportion of positively stained tumor cells was graded as follows: 0, no positive cells; 1, <10%; 2, 10–50%; and 3, >50%. The cells at each staining intensity were recorded on a scale of 0 (no staining), 1 (light blue), 2 (blue) and 3 (dark blue). The staining index (SI) was calculated as follows: SI = staining intensity × proportion of positively stained cells. Using this method, the expression of *HOTAIR* was evaluated using SI and scored as 0, 1, 2, 3, 4, 6 or 9. A SI score of 6 was used as a cut-off value based on the distribution of frequency of SI score for *HOTAIR* expression and a measurement of heterogeneity with the log-rank test statistic with respect to overall survival, and the expression levels of *HOTAIR* were defined as high ($SI \geq 6$) or low ($SI < 6$). In addition, ISH signals for *HOTAIR* expression were quantified in the form of mean optical density (MOD) using the AxioVision Rel.4.6 computerized image analysis system assisted with the automatic measurement program (Carl Zeiss, Oberkochen, Germany), as previously reported.[20]

Statistical analyses. Statistical analyses were performed using SPSS version 16.0 (SPSS, Chicago, IL, USA). A Chi-squared test was used to analyze the relationship between *HOTAIR* expression levels and the clinicopathological characteristics. A one-way ANOVA was used to compare the *HOTAIR* expression levels between the NPC tumors of different clinical stages. Survival curves were plotted using the Kaplan–Meier method and compared using the log-rank test. The survival data were evaluated using univariate and multivariate Cox regression analyses. Receiver operating characteristic curves (ROC) were used to evaluate the average values of sensitivity and specificity of *HOTAIR*. $P < 0.05$ was considered significant.

Results

Correlation of *HOTAIR* expression with clinicopathological features in nasopharyngeal carcinoma. To correlate *HOTAIR* expression with NPC progression in the clinic, we used ISH to localize *HOTAIR* expression in nasopharyngeal tissue. A total of 160 paired paraffin-embedded non-cancer and NPC tissues were analyzed for *HOTAIR* expression. Specific *HOTAIR* staining was observed scattered in the cytoplasm of carcinoma cells in 149 of 160 cases, whereas no staining was observed in normal nasopharyngeal epithelia. However, staining was occasionally identified in nasopharyngeal cells in 14 of 41 cases of metaplasia with atypical hyperplasia (Fig. 1A). Furthermore, *HOTAIR* expression levels were compared in NPC samples in different stages. As shown in Figure 1B and S1, a quantitative analysis showed that *HOTAIR* staining, determined using MOD, in TNM stage I to IV tumors was significantly higher than in normal nasopharyngeal tissue; the MOD of *HOTAIR* staining were also significantly different between various clinical stages ($P < 0.01$; Fig. S1).

Next we correlated *HOTAIR* expression levels with the clinicopathological status of patients with nasopharyngeal carcinoma (Table 1). The expression levels of *HOTAIR* were upregulated in tumors with a higher tumor burden, as defined by a larger tumor size ($P = 0.021$, Table 1), more advanced clinical staging ($P = 0.012$; Table 1) and increased lymph node tumor burden ($P = 0.005$; Table 1), as well as the status of distant metastasis ($P = 0.023$; Table 1). However, there was no significant correlation between the expression levels of *HOTAIR* and age, gender or histological classification. To further evaluate whether *HOTAIR* upregulation was linked to NPC clinical progression, *HOTAIR* levels were quantified using real-time PCR in 160 paired paraffin-embedded non-cancer and NPC specimens (Fig. 1C) and 20 fresh frozen non-cancer nasopharyngeal tissue, as well as 20 NPC tissues with different TNM stages (Fig. 1D). *HOTAIR* expression levels were 5.2~48.4-fold higher compared with non-cancer paraffin-embedded samples (Fig. 1C) or fresh frozen tissue (Fig. 1D). More importantly, *HOTAIR* expression levels increased with clinical stage progression (Fig. 1C,D), which was consistent with the ISH findings in paraffin-embedded tissue. These data imply that upregulated *HOTAIR* in cancer cells correlates with NPC progression.

High expression levels of *HOTAIR* are correlated with poor prognosis in NPC patients. Tumor recurrence and distant metastases are responsible for the poor survival of NPC patients. Therefore, next we analyzed the prognostic value of *HOTAIR* expression levels in 160 cancer patients using Kaplan–Meier analysis and the log-rank test. Among the 160 patients, 82 of 91 with high *HOTAIR* expression levels developed local recurrence (50 cases) and/or distant recurrence (32 cases), whereas 38 of 61 with low *HOTAIR* expression levels developed local recurrence (28 cases) and/or distant recurrence (10 cases). Using local recurrence as the end-point and a cut-off $SI \geq 6$ for *HOTAIR* expression, a survival curve with a median follow-up period of 69 months demonstrated that patients with low *HOTAIR* expression had a longer local recurrence-free survival (LRFS) than those with high *HOTAIR* expression ($P = 0.024$; Fig. 2A). Similarly, the distant metastasis-free survival (DMFS) of patients with low *HOTAIR* expression was 83.6% at 82 months but only 64.8% in those with high *HOTAIR* expression ($P = 0.008$; Fig. 2B). Overall, patients with high *HOTAIR* expression had worse disease-free survival (DFS) than those with low *HOTAIR* expression ($P = 0.017$; Fig. 2C). More importantly, the high *HOTAIR*-expression group had shorter overall survival than the low-expression group ($P = 0.006$; Fig. 2D).

Using univariate analysis (Cox's proportional hazards model), the variables clinical stage ($P < 0.001$), lymph node classification ($P < 0.001$) and *HOTAIR* expression level ($P < 0.01$) were found to be significantly associated with prognosis (Table 2). Multivariate analyses were further used to determine whether *HOTAIR* expression levels were an independent prognostic factor of clinical outcomes. According to

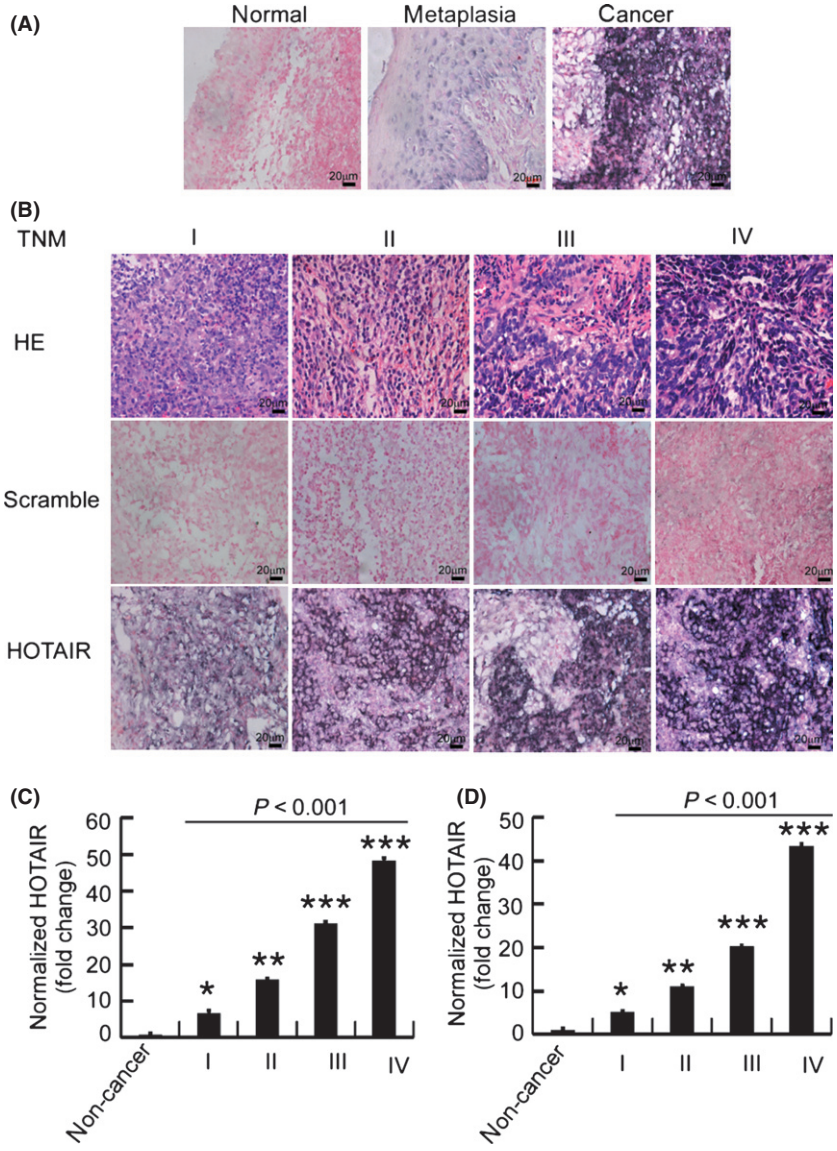

Fig. 1. High levels of *HOTAIR* are correlated with nasopharyngeal carcinoma (NPC) progression. (A) Representative images (×400) of *in situ* hybridization for *HOTAIR* in normal nasopharyngeal tissue, metaplasia with atypical hyperplasia and NPC tissue. (B) Representative images (×400) of H&E and *HOTAIR* staining in paraffin-embedded NPC tissue of different clinical stages. Scramble RNA was used as a negative control. (C,D) Quantification of *HOTAIR* levels was performed using real-time PCR of paraffin-embedded samples (C) and fresh frozen tissue (D). *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared with non-cancerous tissue. Bar, 20 μm.

our results, clinical stage, lymph node (N) classification and *HOTAIR* expression levels showed significant prognostic effects on overall survival, independent of various clinical variables including age, gender and histological classification. Thus, our findings indicate that *HOTAIR* expression levels were significantly correlated with NPC patient prognosis.

***HOTAIR* is an indicator for predicting the prognosis of advanced-stage NPC patients.** Moreover, the prognostic value of *HOTAIR* expression in selective patient subgroups stratified by clinical stage, tumor size and lymph node status was evaluated. In light of our analysis, there was no difference between high and low *HOTAIR*-expression groups in NPC patients with an early stage (Fig. 3A, left) or small tumor (T1–T2; Fig. 3B, left) or without lymph node metastasis (N0 classification; Fig. 3C, left). However, patients with tumors exhibiting high *HOTAIR* expression had significantly shorter overall survival compared with those with low expression of *HOTAIR* in the T3–T4 subgroup ($n = 77$; $P = 0.001$; Fig. 3B, right) or N1–N2 subgroup ($n = 106$; $P = 0.014$; Fig. 3C, middle). There was a trend toward shorter overall survival times of patients with high *HOTAIR* expression in the clinical stage III–IV ($n = 114$; $P = 0.059$; Fig. 3A, right) and N3 subgroups ($n = 54$; $P = 0.144$; Fig. 3C, right), although there were no significant differences between patients with low or high *HOTAIR* expression levels.

Next we constructed ROC curves to evaluate the average sensitivity and specificity of *HOTAIR* for NPC patients. The ROC curve analysis showed that *HOTAIR* performed better in the N2–N3 subgroups (area under the curve [AUC] = 0.814; 95% confidence interval [CI], 0.717–0.911; Fig. 3D, right) than the N0–N1 groups (AUC = 0.688; 95% CI, 0.560–0.816; Fig. 3D, left). Similarly, *HOTAIR* showed slightly better sensitivity and specificity for the III–IV subgroups (AUC = 0.755; 95% CI, 0.668–0.843; Fig. S2B) than the I–II groups (AUC = 0.724; 95% CI, 0.556–0.891; Fig. S2A). Together, these results suggest that *HOTAIR* might better serve as a prognostic marker for predicting the prognosis of advanced-stage NPC patients.

***HOTAIR* mediates the migration and invasion of NPC cells.** To further investigate the functional role of *HOTAIR* in NPC cells, we examined the expression levels of *HOTAIR* in normal, immortalized NPEC and several paired NPC cell lines with different invasive potentials. The corresponding materials and methods, including cell culture and treatment, boyden chamber assay, cell viability and proliferation are described in detail in the Method S1. Compared with NPEC, the NPC cells had up-

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Table 1. Correlation between the clinicopathological features and expression of *HOTAIR*

Characteristics	*HOTAIR in situ* hybridization (%)		*P*
	SI < 6	SI ≥ 6	
Age (years)			
<46	36 (50.7)	35 (49.3)	0.495
≥46	33 (37.1)	56 (62.9)	
Gender			
Male	44 (40.4)	65 (59.6)	0.303
Female	25 (49.0)	26 (51.0)	
Histological classification			
WHO type I	3 (27.3)	8 (72.7)	0.532
WHO type II	11 (42.3)	15 (57.7)	
WHO type III	55 (44.7)	68 (55.3)	
Clinical stage			
I–II	27 (58.7)	19 (41.3)	0.012
III–IV	42 (36.8)	72 (63.2)	
T classification			
T1–T2	43 (51.8)	40 (48.2)	0.021
T3–T4	26 (33.8)	51 (66.2)	
N classification			
N0	11 (42.3)	15 (57.7)	0.005
N1	32 (60.4)	21 (39.6)	
N2–N3	26 (32.1)	55 (67.9)	
Metastasis			
Yes	56 (48.7)	59 (51.3)	0.023
No	13 (28.9)	32 (71.1)	

N, lymph node; T, tumor; WHO, World Health Organization.

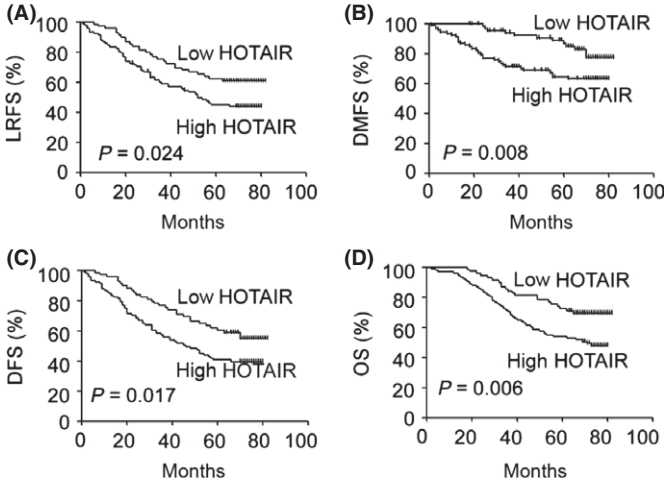

Fig. 2. High *HOTAIR* levels are correlated with poor survival in nasopharyngeal carcinoma (NPC) patients. Kaplan–Meier survival curve of NPC patients with low (staining index [SI] <6) and high HOTAIR (SI ≥ 6) levels, with a median follow-up period of 69 months. (A) Local recurrence-free survival (LRFS). (B) Distant metastasis-free survival (DMFS). (C) Disease-free survival (DFS). (D) Overall survival (OS).

regulated *HOTAIR* levels (Fig. 4A). Consistent with increased capacity for invasion, the NPC cell lines with high invasive potential (5–8F, S18 and CNE2 cells) had increased *HOTAIR* expression levels compared with the paired lines with low invasive potential (6–10B, S26 and CNE1 cells). The *HOTAIR* expression levels in 5–8F, S18 and CNE2 cells increased 2.7-fold ($P < 0.001$), 8.55-fold ($P < 0.001$) and 4.47-fold ($P < 0.001$), respectively (Fig. 4A).

Because *HOTAIR* is upregulated in NPC cells exhibiting high invasion, we further investigated whether *HOTAIR* mediates NPC cell migration and invasion using Boyden chamber assays with or without Matrigel coating on the inserts. Transfecting S18 and 5–8F cells with siRNA against *HOTAIR* reduced the expression levels to levels similar to the paired S26 and 6–10B cells (Fig. S3). Transfection with *HOTAIR* siRNA, but not si-GFP, decreased the number of migrating S18 cells and 5–8F cells by approximately 63% ($P < 0.01$; Fig. 4B,C) and 59.4% ($P < 0.01$; Fig. 4B,D), respectively. In agreement, knocking down *HOTAIR* expression significantly abrogated invasion in S18 cells by 61% ($P < 0.01$; Fig. 4B,C) and 5–8F cells by 58% ($P < 0.01$; Fig. 4B,D). These data suggest that *HOTAIR* plays a critical role in NPC cell migration and invasion.

It has been reported that *HOTAIR* mediates proliferation in breast cancer cells.[17] Therefore, the effect of *HOTAIR* on the proliferation of NPC cells was also investigated using cell viability and clonogenic assays. Cancer cell growth was dramatically suppressed after knocking down *HOTAIR* expression (Fig. S4A,B), indicating that *HOTAIR* might promote NPC cell proliferation. These data suggest that *HOTAIR* contributes to NPC development through involvement in diverse cellular processes.

Discussion

Nasopharyngeal carcinoma is one of the most common cancers in southern China and Southeast Asia.[1] This remarkable racial and geographic distribution of NPC indicates that the development of this cancer might be related to genetic factors. Identification of new NPC biomarkers will be helpful for the diagnosis and treatment of NPC and might provide new insight into its pathogenesis. In the present study, we found that clinically, high expression levels of *HOTAIR*, a cancer-related lncRNA, correlate with NPC progression. *HOTAIR* is an indicator for predicting the prognosis of advanced-stage NPC patients. In addition, *HOTAIR* contributes to the malignant character of NPC cells through involvement in diverse cellular processes, including migration, invasion and proliferation.

Recently, the roles for lncRNA as drivers of tumor suppressive and oncogenic functions have appeared in prevalent cancer types. It has been demonstrated that *HOTAIR*, a trans-regulatory lncRNA, is upregulated in breast cancer, hepatocellular carcinoma and colorectal cancer.[17–19] The *HOTAIR* expression levels assessed were found to be higher in cancerous tissue than in the corresponding non-cancerous tissue in primary breast tumors[17] and colorectal cancers,[19] and upregulated *HOTAIR* has also been detected in hepatocellular carcinoma (HCC) compared with the adjacent non-tumorous tissue in HCC patients.[18,21] Similarly, the present study showed that higher levels of *HOTAIR* staining were observed in NPC tissue than in non-cancerous tissue; furthermore, the expression levels of *HOTAIR* were upregulated in samples with a higher tumor burden, including larger tumor size, advanced clinical staging and increased tumor burden of lymph nodes, as well as the presence of distant metastases. Therefore, *HOTAIR* is crucial for the oncogenesis and development of NPC.

Dysregulated expression of lncRNA in cancer marks the spectrum of disease progression[22] and might serve as an independent predictor for patient outcome.[17] *HOTAIR* is one of the few biologically well-studied lncRNA. Previous studies have demonstrated that high *HOTAIR* expression strongly correlates with the presence of liver metastasis.[19] Similarly, overexpression of *HOTAIR* is linked to earlier recurrence in HCC patients who underwent surgical resection.[18] Tumor recurrence and distant metastases are also responsible for poor survival of NPC patients. Here, the present study demonstrated that high expression levels of *HOTAIR* correlate with a poor LRFS, DMFS, DFS and overall survival in NPC patients.

Table 2. Univariate and multivariate analysis of different prognostic variables in patients with nasopharyngeal carcinoma using Cox regression analysis

	Univariate analysis			Multivariate analysis	
	No. patients	*P*	Regression coefficient (SE)	*P*	Relative risk (95% CI)
Histological classification					
WHO type I	11	0.936	−0.066 (0.199)	0.990	1.003 (0.680–1.478)
WHO type II	26				
WHO type III	123				
Clinical stage					
I–II	46	0.000	1.283 (0.358)	0.004	2.631 (1.278–5.417)
III–IV	114				
N classification					
N0	26	0.000	0.897 (0.240)	0.003	2.042 (1.273–3.274)
N1–N2	115				
N3	19				
HOTAIR					
SI < 6	69	0.008	0.702 (0.263)	0.012	1.902 (1.132–3.194)
SI ≥ 6	91				

CI, confidence interval; N, lymph node; SE, standard error; WHO, World Health Organization.

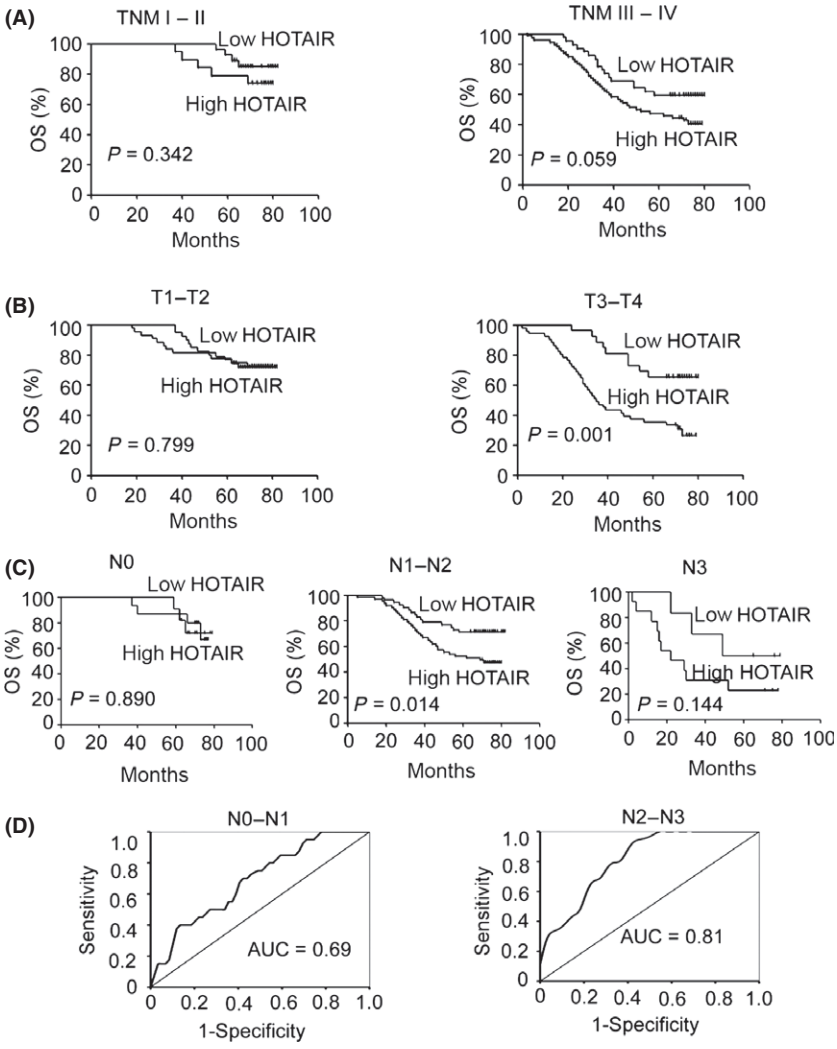

Fig. 3. *HOTAIR* shows better prognostic value in more advanced nasopharyngeal carcinoma (NPC) patients. Kaplan–Meier analysis showing the overall survival (OS) of NPC patients with low (staining index [SI] < 6) and high (SI ≥ 6) *HOTAIR* levels categorized according to TNM stage (A), tumor size (B) and status of lymph nodes (C). The *P*-value was calculated using the log-rank test. (D) The receiver operating characteristic curves were conducted to evaluate the average value of sensitivity and specificity of *HOTAIR* for selected NPC patients stratified by lymph node (N) classification. AUC, area under the curve.

Multivariate analyses revealed that *HOTAIR* expression levels were a powerful independent prognostic factor of clinical outcomes, suggesting that *HOTAIR* could be a candidate biomarker for predicting tumor recurrence in NPC patients.

To date, in addition to tumor size and status of lymph nodes, molecular markers are considered important factors in predicting treatment and prognosis. Heterogeneous ribonucleoprotein K and thymidine phosphorylase are therapeutic markers for

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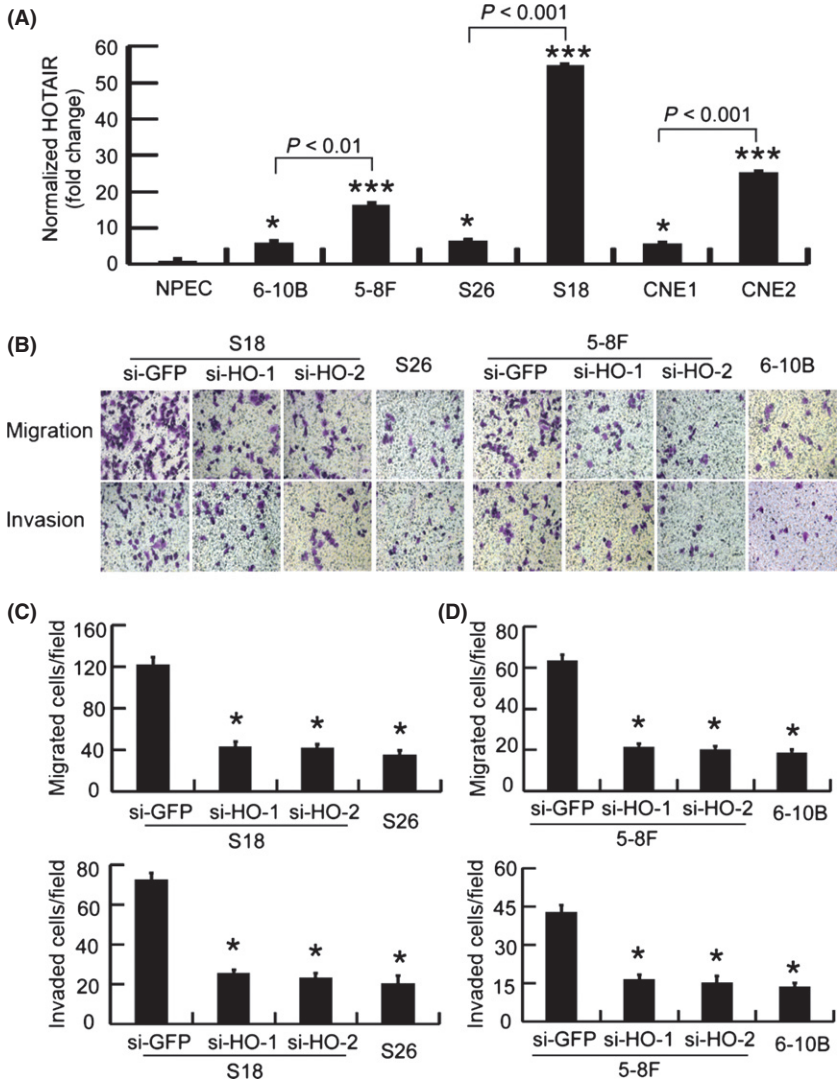

Fig. 4. *HOTAIR* mediates nasopharyngeal carcinoma (NPC) cell migration and invasion. (A) The expression levels of *HOTAIR* were determined using real-time PCR in NPEC and paired NPC cell lines with high (5–8F, S18 and CEN2) and low invasive potential (6–10B, S26 and CNE1). $*P < 0.05$ and $***P < 0.001$ compared with nasopharyngeal epithelial cell line (NPEC). (B) Representative images are shown of a Boyden chamber assay for migrated and invaded NPC cells treated with si-GFP or si-*HOTAIR* (si-HO). (C,D) The number of migrated and invaded NPC cells treated as in (B). $*P < 0.01$ compared with NPC cells treated with si-GFP.

NPC.[23] Epstein-Barr virus (EBV) is widely used for screening and early diagnosis of NPC.[2] Centromere protein H might function as a prognostic marker of NPC at earlier clinical stages.[24] Interestingly, the prognostic value of *HOTAIR* expression was improved for advanced-stage NPC patients. According to our data, NPC patients with tumors exhibiting high *HOTAIR* expression had significantly shorter overall survival compared with patients with low expression of *HOTAIR* in the subgroup with large tumors and high tumor burden in the lymph nodes. The sensitivity and specificity of *HOTAIR* for NPC patients was slightly improved for the III–IV and N2–N3 subgroups. These results suggest that *HOTAIR* might serve as a better prognostic marker for predicting the prognosis of advanced-stage NPC patients, which differs from many biomarkers that are usually used for early stage NPC.

Emerging evidence suggests that lncRNA constitute an important component of tumor biology. Suppression of *HOTAIR* in liver cancer cells reduces cell viability and invasion, sensitizes TNF-α-induced apoptosis and increases chemotherapeutic sensitivity.[18] In breast cancer, overexpression of *HOTAIR* increases lung metastases in mice.[17] Upregulation of *HOTAIR* drives the malignant character of gastrointestinal stromal tumors and promotes cell invasiveness by altering the expression of reported *HOTAIR*-targeted genes.[25] Similarly, our data demonstrate that *HOTAIR* is upregulated in NPC cell lines with high invasive potential and the capacity for migration, invasion and proliferation was suppressed after knocking down *HOTAIR* expression. However, further studies are required to investigate the potential mechanisms of *HOTAIR* involvement in the development of NPC. The turnover of *HOTAIR* is not well understood and one study reported that depletion of *HOTAIR* induced a significant change in the gene expression profile, suggesting that *HOTAIR* might regulate a spectrum of genes by mechanisms other than directly interacting with histone modification complexes.[25]

The above findings not only suggest a useful candidate molecular marker for NPC and an indicator for advanced-stage NPC but also provide new insights into the role of lncRNA in cancer biology. In addition to the prognostic value for NPC, *HOTAIR* combined with other biomarkers might be useful to stratify patients for individual therapies.

Acknowledgments

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Disclosure Statement

The authors have no conflict of interest.

Abbreviations

AJCC	American Joint Committee on Cancer
AUC	Area under the curve
CI	Confidence interval
DFS	Disease-free survival
DMFS	Distant metastasis-free survival
EBV	Epstein-barr virus
HCC	Hepatocellular carcinoma
LNA	Locked nucleic acid
LRFS	Local recurrence-free survival
MOD	Mean optical density
NPC	Nasopharyngeal carcinoma
ROC	Receiver operating characteristic
SI	Staining index
WHO	World health organization

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Expression of *HOTAIR* was quantified using the mean optical density (MOD) in non-cancerous tissues and nasopharyngeal carcinoma (NPC) tissues of different clinical stages.

Fig. S2. Receiver operating characteristic curves were conducted to evaluate the average value of sensitivity and specificity of *HOTAIR* for selected nasopharyngeal carcinoma patients stratified by TNM stage.

Fig. S3. Expression of *HOTAIR* was determined using real-time PCR in paired NPC cells treated with si-GFP or si-*HOTAIR*.

Fig. S4. Effect of *HOTAIR* on nasopharyngeal carcinoma cell proliferation.

Table. S1. Clinicopathological characteristics of patient samples and expression of *HOTAIR* in nasopharyngeal carcinoma.

Methods. S1. Including: cell culture and treatment; Boyden chamber assay; and cell viability and proliferation.

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