Long Noncoding RNA \texttt{HOTAIR} as an Independent Prognostic Marker in Cancer: A Meta-Analysis

Shenghong Zhang\textsuperscript{1,}\textsuperscript{*}, Shuling Chen\textsuperscript{1,}*, Guang Yang\textsuperscript{1}, Fang Gu\textsuperscript{2}, Minrui Li\textsuperscript{3}, Bihui Zhong\textsuperscript{1}, Jifan Hu\textsuperscript{3,4}, Andrew Hoffman\textsuperscript{3,4}, Minhu Chen\textsuperscript{1,}*

\textsuperscript{1}Division of Gastroenterology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, P.R. China, \textsuperscript{2}Division of Reproductive Medicine, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, P.R. China, \textsuperscript{3}VA Palo Alto Health Care System and Stanford University Medical School, Palo Alto, California, United States of America

\textbf{Abstract}

\textbf{Background:} \texttt{HOTAIR}, a newly discovered long intergenic noncoding RNA (lincRNA), has been reported to be aberrantly expressed in many types of cancers. This meta-analysis summarizes its potential role as a biomarker in malignancy.

\textbf{Methods:} A quantitative meta-analysis was performed through a systematic search in Pubmed, Medline and Web of Science for eligible papers on the prognostic impact of \texttt{HOTAIR} in cancer from inception to Feb. 28, 2014. Pooled hazard ratios (HRs) with 95\% confidence interval (95\% CI) were calculated to summarize the effect.

\textbf{Results:} Nineteen studies were included in the study, with a total of 2033 patients. A significant association was observed between high \texttt{HOTAIR} expression and poor overall survival (OS) in patients with cancer (pooled HR 2.22, 95\% CI: 1.68–2.93). Place of residence (Asian or Western countries), type of cancer (digestive or non-digestive disease), sample size (more or less than 100), and paper quality (score more or less than 85\%) did not alter the significant predictive value of \texttt{HOTAIR} expression between high and low groups, while age (young vs. old), sex (male vs. female), smoking status, stage of disease, disease-free survival (DFS), place of residence (Asian or Western countries), type of cancer (digestive or non-digestive disease), sample size (more or less than 100), and paper quality (score more or less than 85\%) did not alter the significant predictive value of \texttt{HOTAIR} expression between high and low groups. Combining HRs from Cox multivariate analyses, we found that \texttt{HOTAIR} expression was an independent prognostic factor for cancer patients (pooled HR 2.26, 95\% CI: 1.62–3.15). Subgroup analysis showed that \texttt{HOTAIR} abundance was an independent prognostic factor for cancer metastasis (HR 3.90, 95\% CI: 2.25–6.74). For esophageal carcinoma, high \texttt{HOTAIR} expression was significantly associated with TNM stage (III/IV vs. I/II: OR 6.90, 95\% CI: 2.81–16.9) without heterogeneity. In gastric cancer, \texttt{HOTAIR} expression was found to be significantly associated with lymph node metastases (present vs. absent: OR 4.47, 95\% CI: 1.88–10.63) and vessel invasion (positive vs. negative: OR 2.88, 95\% CI: 1.38–6.04) without obvious heterogeneity.

\textbf{Conclusions:} \texttt{HOTAIR} abundance may serve as a novel predictive factor for poor prognosis in different types of cancers in both Asian and Western countries.

\textbf{Introduction}

GOBOCAN 2012 reports that an estimated 14.1 million new cancer cases and 8.2 million cancer deaths occurred in 2012, and most of them occurred in less developed countries [1]. Cancer has now become a major cause of morbidity and mortality in most regions worldwide [2]. The 3-year survival rate remains low in many types of cancers, and numerous investigators are searching for biomarkers that may help with diagnosis or prognosis of cancer [3].

Recently, genome-wide transcriptome studies have confirmed that there are a large number of long intergenic noncoding RNAs (lincRNAs), which in the past had been dismissed as simply transcriptional “noise” [4]. lincRNAs are non-protein coding RNA molecules greater than 200 nucleotides in length. Diverse biological functions, including cell differentiation, development and many disease processes, have been attributed to lincRNAs. \texttt{HOTAIR} is a lincRNA that is crucial for cell growth and viability [5,6]. It is transcribed from the antisense strand of the \texttt{HOXC} gene on chromosome 12q13.13 [5]. \texttt{HOTAIR} has been implicated in cancer invasion and metastasis through its role in chromatin remodeling. By targeting polycomb repressive complex 2 (PRC2) and LSD1 complexes to chromatin for coupled histone methylation and demethylation processes, \texttt{HOTAIR} silences various target genes, including the \texttt{HOXD} cluster [5].
**HOTAIR** is aberrantly expressed in a variety of human cancers, including breast cancer, colorectal cancer, laryngeal squamous cell carcinoma, and liver cancer [6–9]. It has been suggested that **HOTAIR** expression may play a useful prognostic role in some tumors. However, most studies examining the implications of **HOTAIR** expression are limited by small sample size. Therefore, we conducted a systematic review and quantitative meta-analysis to clarify the prognostic value of **HOTAIR** expression in human cancers.

**Materials and Methods**

**Study strategy**

The present review was performed in accordance with the standard guidelines for meta-analyses and systematic reviews of tumor marker prognostic studies [10,11]. To obtain relevant articles for this review, two authors (SH Zhang and SL Chen) independently used the following research tools: Medline, Pubmed, and Web of Science to identify all relevant articles about **HOTAIR** as a prognostic factor for survival of patients with any cancer. The literature search ended on Feb 28, 2014. The search strategy used both MeSH terms and free-text words to increase the sensitivity of the search. The following search terms were used: “**HOTAIR**”, “long intergenic noncoding RNA”, “lincRNA”, “lncRNA”, “noncoding RNA”, “cancer”, “carcinoma”, “neoplasm”, “prognosis”, “prognostic”, “outcome”, “mortality”, “survival”, and “recurrence”.

**Study selection**

The same two investigators independently assessed all the eligible studies and extracted the data. Studies were considered eligible if they met the following criteria: any type of human cancer was studied; **HOTAIR** expression was determined in human tissue using quantitative PCR or microarray expression analysis; the relationship between **HOTAIR** expression and survival was examined; sufficient data was provided to estimate hazard ratios (HRs) for survival rates and their 95% confidence intervals. If data subsets were published in more than one article, only the most recent one was included. Citations were limited to those published in the English language. Animal studies [10] and single case reports were excluded [11]. If the data could not be extracted or calculated from the original article, the study was excluded. Disagreements were resolved through discussion with a third investigator (G Yang).

---

**Figure 1. The flow diagram of the meta analysis.**

doi:10.1371/journal.pone.0105538.g001
Table 1. Characteristics of studies included in the meta-analysis.

| Study            | Year  | Region     | Tumor type            | Sample size (n) | Clinical stage of tumor | Cut-off value | Elevated HOTAIR (%) | Preoperative treatment | Outcome measures | Survival analysis | Method* | Quality score (%) |
|------------------|-------|------------|-----------------------|-----------------|-------------------------|---------------|---------------------|-----------------------|-------------------|-------------------|---------|------------------|
| Chen et al. 2013 | China | Esophageal carcinoma | 78                 |                  | 0-IV                     | 26.6          | 34.6                | No                     | OS                | Multivariate analysis | 1       | 85.0             |
| Li et al. 2013   | China | Esophageal carcinoma | 100                |                  | I-IV                    | 125-fold      | 30                  | No                     | OS                | Multivariate analysis | 1       | 84.4             |
| Ge et al. 2013   | China | Esophageal carcinoma | 137                |                  | N/A                     | N/A           | 65.7                | No                     | OS, MFS            | Multivariate analysis | 1       | 78.1             |
| Lv et al. 2013   | China | Esophageal carcinoma | 93                 |                  | I-IV                    | an SI score of 6 | 52.7                | No                     | OS                | Multivariate analysis | 1       | 72.5             |
| Yang et al. 2011 | China | Hepatocellular carcinoma | 60                |                  | Within or beyond Milan criteria | N/A           | 53.3                | NA                     | RFS               | Multivariate analysis | 1       | 85.0             |
| Xu et al. 2013   | China | Gastric cancer     | 83                  |                  | I-IV                    | N/A           | 67.5                | Yes                    | OS                | Univariate and multivariate analysis | 1       | 87.5             |
| Endo et al. 2013 | Japan | Gastric cancer     | 68                  |                  | I-IV                    | HOTAIR/GAPDH of 1.0 | 63.2                | No                     | OS                | No                | 3       | 73.1             |
| Gupta et al. 2010| USA   | Breast cancer      | 132                 |                  | I-IV                    | 125-fold      | 33.3                | NA                     | OS, MFS            | Multivariate analysis | 1       | 92.5             |
| Lu et al. 2012   | USA   | Breast cancer      | 292                 |                  | I-IV                    | 10.01 E1 (median level) | 33.2                | Yes                    | OS, DFS            | Univariate and multivariate analysis | 1       | 87.5             |
| Sørensen et al. 2013 | Denmark | Breast cancer | 164                 |                  | NA                    | Density plot of gene expression of 0.6 | 48.2                | No                     | MFS               | Multivariate analysis | 1       | 90.0             |
| Kogo et al. 2011 | Japan | Colorectal cancer  | 100                 |                  | Dukes' stage A,B,C,D | HOTAIR/GAPDH of 0.273 | 20.0                | No                     | OS                | Univariate and multivariate analysis | 1       | 90.0             |
| Kim et al. 2013  | USA   | Pancreatic cancer  | 102                 |                  | I-IV                    | 15% of HOTAIR expression | 13.7                | NA                     | OS                | Multivariate analysis | 1       | 81.3             |
| Niinuma et al. 2012 | Japan | Gastrointestinal stromal tumor | 39              |                  | Very low, low, intermediate, high | HOTAIR/GAPDH of 0.0002 | 28.2                | NA                     | OS                | Univariate and multivariate analysis | 1       | 85.0             |
| Nakagawa et al. 2013 | Japan | Non-small cell lung cancer | 77               |                  | I-IV                    | 2-fold compared with normal tissue | 22.0                | No                     | DFI               | No                | 1       | 71.9             |
| Liu et al. 2013  | China | Non-small cell lung cancer | 42               |                  | I-IV                    | HOTAIR/GAPDH of 0.57 | 50.0                | No                     | OS                | No                | 3       | 67.5             |
| Li et al. 2013   | China | Laryngeal carcinoma | 72                  |                  | I-IV                    | N/A           | 45.8                | No                     | OS                | Multivariate analysis | 1       | 81.9             |
| Nie et al. 2013  | China | Nasopharyngeal carcinoma | 160              |                  | I-IV                    | an SI score of 6 | 56.9                | No                     | OS, DFS, LRFS, DMFS | Univariate and multivariate analysis | 1,2,2 | 85.0             |
| Zhang et al. 2013 | China | Mesenchymal glioma | 89                  |                  | high grade/low grade | N/A           | 49.4                | NA                     | OS                | Univariate and multivariate analysis | 1       | 87.5             |
Data extraction

The two investigators (SH Zhang and SL Chen) extracted data independently and reached a consensus on all items. For each study, the following characteristics of the individual research articles were collected: author, journal name, year of publication, country of the population enrolled, ethnicity, number of patients, study design, follow-up, overall survival (OS), methods, cut-off values, treatment data, disease-free survival (DFS), metastasis-free survival (MFS), and recurrence-free survival (RFS).

Quality assessment of primary studies

Quality assessment was performed independently by three investigators (SH Zhang, SL Chen, and MH Chen). All eligible studies were scored as previously reported [12,13]. The final scores are expressed as percentages, with a higher percentage denoting better methodological quality.

Statistical analysis

We extracted HRs according to the following three methods [14]. The first and most accurate method was to obtain the reported HRs directly from the publication, or to estimate the HRs from O-E statistic and variance. If that was not possible, we calculated the HRs from the published data including the number of patients at risk in each group, the number of events and the log-rank statistic or its $p$ value. However, there were still some HRs that could not be retrieved using the above methods, as they were presented in the form of Kaplan-Meier Curves. Therefore, with the assumption of a constant rate of the censored patients during follow-up, we reconstructed the HR estimate by extracting several survival rates at specified times from the survival curves. Since the approximation of the survival curves introduces error, we attempted to minimize this error by using the Engauge Digitizer version 2.11 to obtain the necessary points. We inputted the extracted survival rates at specified times into the spreadsheet developed by Tierney JF et al and estimated censoring using the minimum and maximum follow-up [14]. Then an approximated curve was produced; we compared it with published curves to confirm the accuracy of our data extraction and to assist in data adjustment [14]. If needed, we sought original data directly from the authors of the relevant studies.

Pooled hazard ratios or odds ratios (HRs or ORs) and their associated 95% confidence intervals (CI) were estimated using a fixed-effect model (Mantel-Haenszel), while the random effects model was performed when significant heterogeneity was present [15]. For each study, HR was estimated as previously reported [16]. The individual HR estimates were pooled into a summary HR using published methods [17]. Statistical heterogeneity among studies was assessed by using the I² statistic, with significant heterogeneity defined as an I² > 50% [18]. Subgroup analysis and meta regression by factor of region, sample size, type of cancer and paper quality score were both performed to determine if the number of included studies was sufficient. Univariate meta-regression was conducted to explore the potential heterogeneity in the analysis of the association between HOTAIr and survival. Furthermore, factors identified as significant by univariate analysis were further analyzed with multivariate meta-regression if necessary. We also conducted sensitivity analyses to test the effect of each study on the overall pooled results. The presence of publication bias was evaluated by using funnel plots, Begg’s test and Egger’s test [16]. Statistical analysis was performed using Stata software statistical software version 12.0 (Stata, College Station, TX). A $p$ value of less than 0.05 was considered statistically significant.
Results

Included studies and characteristics

As shown in the flow diagram (Figure 1), our search terms revealed 160 articles. After the titles and abstracts were reviewed, 113 irrelevant or duplicate articles were excluded. After a more careful inspection of the abstracts, a total of 21 articles were reviewed in detail [6–9,19–35]. Two papers were excluded because of insufficient data to estimate HR for further analysis [34,35]. As a result, 19 published articles were included in the current meta-analysis [6–9,19–33]. Among these 19 studies, a total of 2033 patients were included, with a maximum sample size of 292 and a minimum sample size of 39 patients (Mean 107.0). Nine studies enrolled more than 100 participants. The accrual period of these studies ranged from 2010 to 2014. The studies were published by groups throughout the world: 11 from China, 4 from Japan, 3 from the United States and 1 from Denmark. A total of 12 different types of cancer were evaluated in studies in this meta-analysis, with the greatest number being digestive system malignancies (4 esophageal carcinoma, 2 gastric cancer, 2 hepatocellular cancer, 1 colorectal cancer, 1 pancreatic cancer and 1 gastrointestinal stromal tumor); other types of cancer were also included (3 breast cancer, 2 non-small cell lung cancer, 1 nasopharyngeal carcinoma, 1 laryngeal carcinoma, 1 mesenchymal glioma and 1 endometrial carcinoma). Treatment information was not available in 4 studies and of the remaining researches, the participants in two received preoperative treatment.

Table 1 summarizes the main characteristics of the included studies. A total of 21 HRs were analyzed. HRs from two studies were calculated by using one of the three methods noted in the Materials and Methods section. HRs could be obtained directly in seventeen studies, and HRs were approximated in two studies by using the total number of events and its p-value. We extrapolated the remaining HRs from two studies using graphical representations of the survival distributions.

Figure 2. Meta analysis of the pooled HRs of OS of different types of cancer with increased HOTAIR expression. (A) Subgroup analysis of HRs of OS by factor of region. (B) Subgroup analysis of HRs of OS by factor of score. (C) Subgroup analysis of HRs of OS by factor of sample size. (D) Subgroup analysis of HRs of OS by factor of type of cancer.

doi:10.1371/journal.pone.0105538.g002
Table 2. Results of subgroup analysis of pooled hazard ratios of overall survival of different types of cancer with increased HOTAIR expression.

| Subgroup analysis                  | No. of studies | No. of patients | Pooled HR(95%CI) | Meta regression (p-value) | Heterogeneity |
|------------------------------------|----------------|-----------------|------------------|---------------------------|---------------|
|                                    |                |                 | Fixed            | Random                    |               |
| **Region**                         |                |                 |                  |                           |               |
| Asian countries                    | 13             | 1206            | 2.15[1.74–2.66]  | 2.15[1.74–2.66]           | 0.98          | 0.00% | 0.938 |
| Western countries                  | 3              | 526             | 1.96[1.36–2.83]  | 2.79[1.09–7.14]           | 81.3%         | 0.000 |
| **Sample size**                    |                |                 |                  |                           |               |
| < 100                              | 8              | 564             | 2.21[1.70–2.88]  | 2.21[1.70–2.88]           | 0.145         | 0.00% | 0.625 |
| ≥100                              | 8              | 1168            | 2.00[1.55–2.59]  | 2.16[1.30–3.58]           | 71.9%         | 0.001 |
| **Type of cancer**                 |                |                 |                  |                           |               |
| Digestive system carcinoma         | 9              | 800             | 2.42[1.89–3.10]  | 2.42[1.89–3.10]           | 0.071         | 0.00% | 0.538 |
| Non-digestive system carcinoma     | 7              | 932             | 1.76[1.34–2.32]  | 1.84[1.07–3.16]           | 70.9%         | 0.002 |
| Preoperative treatment             | 12             | 1611            | 2.01[1.62–2.48]  | 2.13[1.50–3.04]           | 58.4%         | 0.006 |
| No                                 | 10             | 1236            | 2.34[1.86–2.96]  | 2.34[1.86–2.96]           | 0.038         | 0.00% | 0.615 |
| Yes                                | 2              | 375             | 0.927[0.551–1.56] | 0.953[0.199–4.57]         | 89.0%         | 0.003 |
| **Quality score (%)**              |                |                 |                  |                           |               |
| <85.0                              | 8              | 877             | 2.28[1.78–2.92]  | 2.28[1.78–2.92]           | 0.316         | 71.4% | 0.001 |
| ≥85.0                              | 8              | 855             | 1.90[1.45–2.50]  | 2.07[1.19–3.59]           | 0.00%         | 0.657 |
All of the studies were comprised of a high HOTAIR expression arm and a low HOTAIR expression arm. The average percentage of tumors with increased HOTAIR expression was 42.0%, with a maximum of 67.5% in gastric cancer and a minimum of 13.7% in pancreatic cancer. OS, DFS, RFS and MFS were estimated as survival outcome measures in 84.2% (16/19), 5.26% (1/19), 15.8% (3/19) and 21.1% (4/19) of the studies, respectively. Multivariable analyses were performed in 84.2% (16/19) of studies and HOTAIR expression was found to be an independent prognostic factor for OS in 91.7% (11/12) of studies, for recurrence in 1 of 2 of studies, and for metastasis in 2 of 2 of studies.

Association between HOTAIR and survival in twelve types of cancers

Sixteen studies reported the overall survival (OS) of twelve types of cancer based on different HOTAIR expression levels in a total of 1732 patients. A significant association was observed between HOTAIR and OS in cancer patients (pooled HR 2.22, 95% CI: 1.68–2.93) (Figure 2). Significant heterogeneity existed between studies ($\chi^2 = 30.47$, df = 15, $p = 0.010$; $I^2 = 50.8\%$).

Due to the presence of heterogeneity, subgroups were analyzed based on the region, sample size, type of cancer, preoperative treatment and paper quality (Table 2) (Figure 2). We detected a significant association between HOTAIR and OS of cancer patients in both Asian (HR 2.15, 95% CI: 1.74–2.66) and western countries (HR 2.79, 95% CI: 1.09–7.14). HOTAIR was found to be significantly associated with OS of patients with digestive system malignancies (HR 2.42, 95% CI: 1.89–3.10) and with OS of patients with non-digestive system malignancies (HR 1.84, 95% CI: 1.07–3.16). The association between HOTAIR and OS of patients was present in studies with more than 100 or fewer than 100 subjects. After excluding the four studies without available treatment information, HOTAIR was found to be significantly associated with OS in patients without preoperative treatment (HR 2.34, 95% CI: 1.86–2.96) but not in those who received preoperative treatment (HR 0.953, 95% CI: 0.199–4.57). Paper quality did not change the result of the estimated HR (HR 2.07, 95% CI: 1.19–3.59; HR 2.28, 95% CI: 1.78–2.92 respectively), but in those studies with a paper quality score of less than 85.0, there was more heterogeneity across studies than in the subgroup with higher quality. Significant heterogeneity existed across studies in the subgroup of western countries, in the subgroup of patient number more than 100 and in the subgroup of patients with non-digestive system malignancy but there was not significant heterogeneity in the subgroups of Asian countries, patient number fewer than 100 and patients with digestive system malignancy. Thus, the region, type of cancer and sample size did not alter the significant predictive value of HOTAIR in OS of various kinds of cancer.

In order to further explore the sources of heterogeneity, we performed meta-regression by the covariates including region, type of cancer, sample size, preoperative treatment and paper quality to quantify the heterogeneity (Table 2). As was found in the subgroup analysis, only the factor of preoperative treatment accounted for the inter-study heterogeneity which was consistent with the result of subgroup analysis. Moreover, HR did not change significantly after the exclusion of any of the studies in the sensitivity analysis (Figure S1A). For meta-analysis of the association between HOTAIR expression and OS, Begg’s test ($P = 0.022$) showed significant publication bias across studies; the
| Subgroup analysis               | No. of studies | No. of patients | Pooled HR(95%CI)  | Meta regression (p-value) | Heterogeneity |
|--------------------------------|----------------|----------------|-------------------|--------------------------|---------------|
|                                |                |                | Fixed            | Random                   |               |
| Overall survival               | 12             | 1377           | 2.15[1.76–2.63]   | 2.26[1.62–3.15]           | —             | 59.6% 0.004 |
| Region                         |                |                |                  |                          |               |
| Asian countries                | 9              | 883            | 2.42[1.94–3.03]   | 2.42[1.94–3.03]           | 0.185 0.00%   |
| Western countries              | 3              | 494            | 1.32[0.839–2.08]  | 1.66[0.405–6.79]          | 88.2% 0.000   |
| Sample size                    |                |                |                  |                          |               |
| <100                           | 6              | 454            | 2.38[1.79–3.17]   | 2.38[1.79–3.17]           | 0.554 0.00%   |
| ≥100                           | 6              | 923            | 1.95[1.47–2.59]   | 2.08[1.10–3.93]           | 78.8% 0.000   |
| Type of cancer                 |                |                |                  |                          |               |
| Digestive system carcinoma     | 7              | 632            | 2.45[1.87–3.20]   | 2.45[1.87–3.20]           | 0.326 0.00%   |
| Non-digestive system carcinoma | 5              | 745            | 1.82[1.34–2.47]   | 1.85[0.905–3.78]          | 80.6% 0.000   |
| Preoperative treatment         | 8              | 1015           | 1.94[1.54–2.45]   | 2.00[1.29–3.10]           | 68.8% 0.002   |
| No                             | 6              | 640            | 2.34[1.80–3.04]   | 2.34[1.80–3.04]           | 0.069 0.00%   |
| Yes                            | 2              | 375            | 0.927[0.551–1.56] | 0.953[0.199–4.57]         | 89.0% 0.003   |
| Quality score (%)             |                |                |                  |                          |               |
| <85.0                          | 4              | 404            | 2.36[1.71–3.26]   | 2.36[1.71–3.26]           | 0.746 0.00%   |
| ≥85.0                          | 8              | 973            | 2.03[1.57–2.63]   | 2.22[1.31–3.75]           | 72.5% 0.001   |
| Recurrence                     | 2              | 352            | 1.03[0.64–1.65]   | 1.28[0.18–9.29]           | 94.0% 0.000   |
| Metastasis                     | 2              | 269            | 3.90[2.25–6.74]   | 3.90[2.25–6.74]           | —             | 0.00% 0.065 |

doi:10.1371/journal.pone.0105538.t003
funnel plot was slightly asymmetrical although Egger’s test \(p = 0.103\) did not show significance (Figure S2A).

HRs from Cox multivariate analyses were recorded in 12 studies to investigate whether HOTAIR was predictive for OS of cancer. Combining these HRs suggests that HOTAIR expression might be an independent prognostic factor for cancer patients (pooled HR 2.26, 95% CI: 1.62–3.15) (Figure 3), but a significant heterogeneity was detected among studies (\(\chi^2 = 27.25, df = 11, p = 0.004, I^2 = 59.6\%\)). In addition to the independent role of HOTAIR in OS, two other studies respectively found that HOTAIR was an independent factor for cancer metastasis but not for the recurrence of cancer (pooled HR 3.90, 95% CI: 2.25–6.74; pooled HR 1.28, 95% CI: 0.18–9.29) (Table 3) (Figure 3). Heterogeneity was significant in studies examining the association between HOTAIR and recurrence, whereas no heterogeneity was found in studies looking at the independent role of HOTAIR in metastasis.

Subgroup analysis, sensitivity analysis and meta-regression were performed to illustrate the heterogeneity across studies concerning the independent role of HOTAIR in OS, but not in the recurrence or metastasis (Table 3). Subgroup analysis showed that HOTAIR was an independent prognostic factor for digestive system cancer patients without preoperative treatment in Asian countries, and sample size and paper quality did not change the overall result. However, we found that none of the examined factors, including region, sample size, type of cancer, preoperative treatment and manuscript quality, were responsible for heterogeneity across studies in meta-regression. Sensitivity analysis showed no significant change after exclusion any of the included studies (Figure S1B). There was no significant publication bias for studies concerning an independent prognostic role for HOTAIR in different types of cancer (Begg’s test: \(p = 0.064\), Egger’s test: \(p = 0.25\)) (Figure S2B).

The prognostic significance of HOTAIR in recurrence-free survival (RFS) and metastasis-free survival (MFS) was evaluated in 3 studies with 512 patients and in 4 studies with 593 patients, respectively (Table 4). HOTAIR was not significantly associated with RFS (HR 1.40, 95% CI: 0.48–4.05) with obvious heterogeneity (\(\chi^2 = 18.95, df = 2, p = 0.00\), \(I^2 = 89.4\%\)) (Figure 4A), but a significant relation was demonstrated in the subgroup of Asian countries (HR 2.31, 95% CI: 1.13–4.71), the subgroup of sample size less than 100 (HR 3.56, 95% CI: 1.67–7.63) and the subgroup of digestive system carcinoma (HR 3.56, 95% CI: 1.67–7.63). However, it showed that patients with high HOTAIR expression were more likely to have significantly shorter MFS (HR 2.30, 95% CI: 1.50–3.53) with heterogeneity (Figure 4B). Heterogeneity existed across studies in the subgroup of Asian countries (\(\chi^2 = 2.64, df = 1, p = 0.104; I^2 = 62.1\%\)) while there was only one study in the Western subgroup with digestive system carcinoma. The subgroup of digestive system carcinoma (HR 4.47, 95% CI: 1.99–10.05 vs. HR 1.93, 95% CI: 1.36–2.74) and paper quality less than 85.0 (HR 4.47, 95% CI: 1.99–10.05 vs. HR 1.93, 95% CI: 1.36–2.74) reported larger HR than did the other two subgroups without significant heterogeneity. However, patients from Asian and western countries showed similar HR from HOTAIR in MFS with heterogeneity. Meta regression analysis showed that no included stratifying factors contributed to main heterogeneity across studies. Furthermore, we estimated HRs in 4 studies with available multivariate data regarding the independent prognostic role of HOTAIR in recurrence and metastasis (Figure 3). This analysis showed that HOTAIR was an independent prognostic factor for cancer metastasis (HR 3.90, 95% CI: 2.25–6.74) without heterogeneity but not for cancer recurrence (HR 1.28, 95% CI: 0.18–9.29). Sensitivity analysis changed after omitting any of the included studies in this part (Figure S1C and 1D). Meta-regression was not applicable in analysis of the association between HOTAIR and RFS because of the limited number of included studies. There was no significant publication bias across studies in analyzing HOTAIR and RFS (\(p = 1.00\) in Begg’s test and \(P = 0.74\) in Egger’s test) (Figure S2C). Publication bias was significant in studies regarding the association between HOTAIR and MFS, with a \(p\) value less than 0.05 in Egger’s test and asymmetry of the funnel plot, although Begg’s test demonstrated a \(p\) value larger than 0.05 (Figure S2D).

Association between HOTAIR and clinicopathological characteristics of cancer

There were seven studies examining the correlation between HOTAIR and clinicopathological characteristics of cancer, including 3 studies regarding esophageal carcinoma, 2 studies involving hepatocellular cancer and 2 studies of gastric cancer (Table 5). In esophageal carcinoma, high HOTAIR expression was significantly associated with TNM stage (III/IV vs. I/II: OR 6.90, 95% CI: 2.81–16.9) and N status (N2/3 vs. N0/1: OR 3.29, 95% CI:1.18–9.16) whereas no significant correlation was found with T classification (T3/4 vs. T1/2: OR 2.15, 95% CI: 0.24–19.5) or grade of differentiation (G3/4 vs. G1/2: OR 1.14, 95% CI: 0.10–13.0). The analysis between HOTAIR expression and T classification, N status and grade of differentiation in esophageal carcinoma displayed significant heterogeneity across studies except TNM stage. However, we did not observe a significant correlation between HOTAIR and TNM stage (III/IV vs. I/II: OR 0.92,
Table 4. Results of subgroup analysis of pooled hazard ratios of recurrence-free survival and metastasis-free survival of cancer with increased HOTAIR expression.

| Subgroup analysis                      | No. of studies | No. of patients | Pooled HR(95%CI) | Meta regression (p-value) | Heterogeneity |
|----------------------------------------|----------------|-----------------|------------------|---------------------------|---------------|
|                                        |                |                 | Fixed            | Random                    | i²            | p-value |
| Recurrence-free survival               | 3              | 512             | 1.33[0.96–1.85]  | 1.40[0.48–4.05]           | 89.4%         | 0.000   |
| Region                                 |                |                 |                  |                           |               |         |
| Asian countries                        | 2              | 220             | 2.08[1.40–3.09]  | 2.31[1.13–4.71]           | 62.1%         | 0.104   |
| Western countries                      | 1              | 292             | 0.47[0.26–0.86]  | 0.47[0.26–0.86]           | —             | —       |
| Sample size                            |                |                 |                  |                           |               |         |
| ≤100                                   | 1              | 60              | 3.56[1.67–7.63]  | 3.56[1.67–7.63]           | —             | —       |
| ≥100                                   | 2              | 452             | 0.91[0.26–3.21]  | 1.06[0.73–1.53]           | 90.9%         | 0.001   |
| Type of cancer                         |                |                 |                  |                           |               |         |
| Digestive system carcinoma             | 1              | 60              | 3.56[1.67–7.63]  | 3.56[1.67–7.63]           | —             | —       |
| Non-digestive system carcinoma         | 2              | 452             | 0.91[0.26–3.21]  | 1.06[0.73–1.53]           | 90.9%         | 0.001   |
| Metastasis-free survival               | 4              | 593             | 2.03[1.57–2.61]  | 2.30[1.50–3.53]           | —             | —       |
| Region                                 |                |                 |                  |                           |               |         |
| Asian countries                        | 2              | 297             | 1.98[1.41–2.79]  | 2.55[0.98–6.65]           | 0.831         | 78.7%   | 0.030   |
| Western countries                      | 2              | 296             | 2.09[1.43–3.05]  | 2.30[1.19–4.44]           | 58.6%         | 0.120   |
| Type of cancer                         |                |                 |                  |                           |               |         |
| Digestive system carcinoma             | 1              | 137             | 4.47[1.99–10.1]  | 4.47[1.99–10.1]           | 0.777         | —       |
| Non-digestive system carcinoma         | 3              | 456             | 1.86[1.43–2.34]  | 1.93[1.36–2.74]           | 35.5%         | 0.212   |
| Quality score (%)                      |                |                 |                  |                           |               |         |
| <85.0                                  | 1              | 137             | 4.47[1.99–10.1]  | 4.47[1.99–10.1]           | 0.777         | —       |
| ≥85.0                                  | 3              | 456             | 1.86[1.43–2.34]  | 1.93[1.36–2.74]           | 35.5%         | 0.212   |

doi:10.1371/journal.pone.0105538.t004
| Clinicopathological features | No. of studies | No. of patients | Pooled OR  | p-value | Heterogeneity |
|-----------------------------|----------------|----------------|------------|---------|---------------|
|                             |                |                | Fixed      | Random  | i²            | p-value |
| Esophageal carcinoma        |                |                |            |         |               |         |
| TNM stage                   | 2              | 171            | 6.93[2.79–17.2] | 6.90[2.81–16.94] | 0.000 | 0.00% | 0.953 |
| T classification            | 2              | 230            | 1.60[0.79–3.27] | 2.15[0.237–19.5] | 0.497 | 83.0% | 0.015 |
| N status                    | 3              | 215            | 2.84[1.73–4.67] | 3.29[1.18–9.16] | 0.023 | 74.3% | 0.020 |
| Grade of differentiation    | 2              | 230            | 0.75[0.366–1.55] | 1.14[0.101–13.0] | 0.915 | 85.1% | 0.010 |
| Hepatocellular carcinoma    |                |                |            |         |               |         |
| TNM stage                   | 2              | 124            | 0.92[0.398–2.12] | 0.917[0.398–2.12] | 0.840 | 0.00% | 0.967 |
| Invasion of portal vein     | 2              | 124            | 1.23[0.547–2.78] | 1.23[0.545–2.78] | 0.616 | 0.00% | 0.765 |
| Gastric cancer              |                |                |            |         |               |         |
| TNM stage                   | 2              | 151            | 1.92[0.959–3.83] | 1.94[0.646–5.85] | 0.238 | 59.6% | 0.116 |
| Lymph node metastasis       | 2              | 151            | 4.40[1.99–9.71] | 4.47[1.88–10.6] | 0.001 | 13.6% | 0.282 |
| Depth of invasion           | 2              | 151            | 1.65[0.601–4.55] | 1.32[0.625–2.77] | 0.470 | 0.00% | 0.513 |
| Vessel invasion             | 2              | 151            | 1.01[0.335–3.01] | 2.88[1.38–6.04] | 0.005 | 2.10% | 0.312 |

doi:10.1371/journal.pone.0105538.t005

Table 5. Results of meta-analysis of increased HOTAIR expression and clinicopathological features of three types of cancer.
95%CI: 0.40–2.12) and invasion of the portal vein (positive vs. negative: OR 1.23, 95%CI: 0.53–2.76) in hepatocellular cancer. This result was strengthened by the low heterogeneity between studies. In terms of gastric cancer, HOTAIR expression was found to be significantly associated with lymph node metastasis (present vs. absent: OR 4.47, 95%CI: 1.80–10.63) and vessel invasion (positive vs. negative: OR 2.88, 95%CI: 1.30–6.04) while TNM stage (III/IV vs. I/II: OR 1.94, 95%CI:0.65–5.85) and depth of invasion (T3/4 vs. T1/2: OR 1.32, 95%CI:0.63–2.77) tended to have relatively weaker correlations with HOTAIR expression. Except for TNM stage, there was no significant heterogeneity between studies in lymph node metastasis, vessel invasion and depth of invasion. Subgroup analysis, sensitivity analysis, meta-regression analysis and assessment of publication bias was not performed due to the relatively little heterogeneity across studies and limited number of included papers.

Discussion

In recent years, numerous studies have demonstrated that lncRNAs are involved in various biological processes, including cancer progression and metastasis, via chromosome remodeling, transcription and post-transcriptional processing [36]. The lncRNA HOTAIR is aberrantly expressed in different types of cancer. In this meta-analysis, we have examined the prognostic role of HOTAIR in cancer and the relation between HOTAIR and clinicopathological characteristics of cancer. We believe that this meta-analysis is the first to investigate the relationship between a lncRNA and cancer prognosis. The fact that we included studies from both Asian and Western countries may enhance the generalizability to some extent. Subgroup analysis in a fixed or random model, meta regression analysis and sensitivity analysis were all performed in the current study, enhancing the statistical power to detect a role of HOTAIR in different types of cancer.

A total of 19 papers comprising 2033 patients were included into this meta-analysis. We found that HOTAIR expression was associated with a poorer prognosis in patients with different types of cancer. Since significant heterogeneity existed across these studies, subgroup analyses were performed. Factors including region (Asian or western countries), type of cancer (digestive or non-digestive disease), sample size (more or less than 100), and paper quality (score more or less than 85%) did not alter the significant predictive value of HOTAIR expression in OS in different kinds of cancer. By combining HRs from Cox multivariate analyses, we found that HOTAIR was an independent prognostic factor for cancer patients (pooled HR 2.26, 95% CI: 1.62–3.15). However, heterogeneity existed. Subgroup analysis showed that sample size and paper quality did not change the overall result, but that the type of cancer, preoperative treatment and region did. In addition to this, biological types of carcinoma might also be a resource of heterogeneity which was not analyzed in our study due to limited data. For example, a majority of participants in the studies on breast cancer were estrogen-receptor (ER) positive and progesterone receptor (PR) positive, and it is theoretically possible that the prognostic value of HOTAIR expression might be different in ER and PR-negative breast cancer. Of note, the prognostic significance of HOTAIR in OS was observed in patients without preoperative treatment rather than those with preoperative treatment which to some extent showed the true prognostic value of HOTAIR in survival with a controlled population in which a treatment modality modified the predictive effect of HOTAIR. HOTAIR could be used as an independent prognostic factor for digestive system cancer patients in Asian countries without heterogeneity. Both Begg’s test and Egger’s test found no significant publication bias concerning on independent prognostic role of HOTAIR in different types of cancer.

TNM stage is associated with cancer prognosis. In this meta-analysis, only seven studies examined the correlation between HOTAIR and TNM stage. Among these, 3 studies investigated esophageal carcinoma. We found that high HOTAIR expression in esophageal carcinoma was significantly associated with TNM stage (III/IV vs. I/II: OR 6.90, 95%CI: 2.81–16.9) without obvious heterogeneity.

Previous studies have shown that N status, vessel invasion and depth of invasion were associated with an unfavorable outcome in cancer patients [37–39]. In our meta-analysis, we found that in gastric cancer, HOTAIR expression was significantly associated with lymph node metastasis and vessel invasion without heterogeneity. However, no such association was found in liver cancer or esophageal cancer. One potential explanation for these differences might be that the number of included studies was small. Therefore, more studies should be conducted in order to clarify the relationship between HOTAIR and clinicopathological features in other types of cancer.

The prognostic significance of HOTAIR in RFS and MFS was evaluated in 3 studies with 529 patients and in 4 studies with 593 patients, respectively. Subgroup analysis showed that patients with high HOTAIR expression were more likely to have significantly shorter MFS albeit with heterogeneity, but HOTAIR expression was not significantly associated with poorer RFS. Considering the limited number of studies concerning the relationship between HOTAIR and RFS or MFS, we cannot draw a definite conclusion regarding the relationship, as more studies with large sample size are needed.

Since HOTAIR overexpression in breast cancer cells promoted cancer cell invasion, and HOTAIR silencing reduced cancer invasiveness through Matrigel in vitro [6], we estimated HRs in 4 studies with available multivariate data regarding the independent prognostic role of HOTAIR in recurrence and metastasis. We show that HOTAIR is an independent prognostic factor for cancer metastasis (HR 3.90, 95%CI: 2.25–6.74) without heterogeneity.

Through subgroup analysis, we identified for the first time that HOTAIR was a novel predictive factor for poor prognosis in different types of cancers for both Western and Asian populations; however, HOTAIR was an independent prognostic factor for OS of Asian patients rather than Western ones. Secondly, we found that the predictive significance of HOTAIR in OS, RFS and MFS was more significant in patients with digestive system carcinoma than in those with non-digestive system carcinoma. The above two findings suggest that HOTAIR expression might be more meaningful in predicting OS of Asian patients or patients with digestive system carcinoma than that of Western patients or those with non-digestive system cancer. Finally, we showed that the pooled HRs in the studies with poorer quality were larger than those in the studies with better quality, suggesting that the results in some individual studies with poor quality might overestimate the predictive effect of HOTAIR.

It should be emphasized that there are several limitations in our study. First, the cut-off value of high and low HOTAIR expression varied in different studies. It was difficult to reach a consensus value. Second, the treatment protocols after surgery differed in the various studies, and these differences might have a great impact on survival and thus result in some heterogeneity. Third, most of the HRs could not be directly obtained from the primary studies, requiring us to calculate them ourselves or to reconstruct the survival curves to extract the HR estimates. Fourth, we only included English language papers. Fifth, most of the included
studies reported positive results because those with negative results are generally less likely to be published. Thus, our results might overestimate the predictive significance of HOTAIR in prognosis of cancer to some extent. Sixth, differences of paper quality across the studies might have led to bias in the meta-analysis although subgroup analysis and meta-regression did not show the paper quality as the resource of heterogeneity. Seventh, we could not investigate the role of HOTAIR in different biological subtypes of a given cancer as this distinction was not available for most studies.

In conclusion, our study found that HOTAIR might be a novel predictive factor for assessing poor prognosis in different types of cancer both in Asian and western countries. This is the first example of a lincRNA being shown to be a biomarker in predicting cancer prognosis.

Supporting Information

Figure S1 Sensitivity analysis of the association between HOTAIR expression and OS/RRF/MFS of cancer.

(A) Sensitivity analysis of the pooled HRs of OS of different types of cancer with increased HOTAIR expression; (B) Sensitivity analysis of the independent role of HOTAIR in OS/recurrence/metastasis of different types of cancer; (C) Sensitivity analysis of the pooled HRs of RFS of cancer with increased HOTAIR expression; (D) Sensitivity analysis of the pooled HRs of MFS of cancer with increased HOTAIR expression.

References

1. Globocan (2012) IARC. http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx. Accessed July 18, 2014.
2. Bray F, Ren JS, Masuyer E, Ferlay J (2013) Global estimates of cancer prevalence for 27 sites in the adult population in 2008. Int J Cancer 132: 1133–1143.
3. Gonzalez CA, Agudo A (2012) Carcinogenesis, prevention and early detection of gastric cancer: where we are and where we should go. Int J Cancer 130: 745–753.
4. Gutman M, Donahue J, Carey BW, Garber M, Grenier JK, et al. (2011) lincRNAs act in the circuitry controlling pluripotency and differentiation. Nature 477: 295–300.
5. Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, et al. (2007) Functional Demarcation of Active and Silent Chromatin Domains in Human HOX Loci by Noncoding RNAs. Cell 129: 1311–1323.
6. Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, et al. (2010) Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 464: 1071–1076.
7. Kogo R, Shimamura T, Mimori K, Kavahara K, Imoto S, et al. (2011) Long Noncoding RNA HOTAIR Regulates Polycomb-Dependent Chromatin Modification and Is Associated with Poor Prognosis in Colorectal Cancers. Cancer Res 71: 6320–6326.
8. Li D, Feng J, Wu T, Wang Y, Sun Y, et al. (2013) Long Intergenic Noncoding RNA HOTAIR Is Overexpressed and Regulates PTEN Methylation in Laryngeal Squamous Cell Carcinoma. Am J Pathol 182: 64–70.
9. Yang Z, Zhou L, Wu LM, Lai MC, Xie HY, et al. (2011) Overexpression of Long Non-coding RNA HOTAIR Predicts Tumor Recurrence in Hepatocellular Carcinoma Patients Following Liver Transplantation. Ann Surg Oncol 18: 1243–1250.
10. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, et al. (2005) Statistics Subcommittee of the NC-IORTC Working Group on Cancer Diagnostics. Reporting recommendations for tumor marker prognostic studies (REMARK). J Natl Cancer Inst 97: 1180–1184.
11. Altman DG, McShane LM, Sauerbrei W, Taube SE. (2012) Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK): explanation and elaboration. PLoS Med 9: e1001219.
12. Steele E, Paansmans M, Berghmans T, et al. (2001) Role of p53 as a prognostic factor for survival in lung cancer: a systematic review of the literature with a meta-analysis. Eur Respir J 18: 705–719.
13. Xing N, Tang YR, Yuan G, Wang X, Wang J, et al. (2013) The prognostic value of E-cadherin in gastric cancer: a meta-analysis. Int J Cancer 132: 2589–2596.
14. Tierney JF SL, Gherzi D, Burdett S, Sydes MR (2007) Practical methods for incorporating summary time-to-event data into meta-analysis. Trials 8:16.
15. Higgins JP, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta-analyses. BMJ 327: 557–560.
16. Parmar MK, Torri V, Stewart L (1998) Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. Stat Med 17: 2133–2143.
17. Yusuf S, Peto R, Lewis J, Collins R, Sleight P (1995) Beta blockade during and after myocardial infarction: an overview of the randomized trials. Prog Cardiovasc Dis 27: 335–371.
18. Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical test. BMJ 315: 629–634.
19. Chen JJ, Sun M, Li SQ, Wu QQ, Ji L, et al. (2013) Upregulation of the long non-coding RNA HOTAIR promotes esophageal squamous cell carcinoma metastasis and poor prognosis. Mol Carcinog 52: 908–915.
20. Li X, Wu Z, Mei Q, Guo M, Fu X, et al. (2013) Long non-coding RNA HOTAIR, a driver of malignancy, predicts negative prognosis and exhibits oncogenic activity in oesophageal squamous cell carcinoma. Br J Cancer 109: 2266–2276.
21. Le XB, Lian GY, Wang HR, Song E, Yao H, et al. (2013) Long noncoding RNA HOTAIR is a prognostic marker for esophageal squamous cell carcinoma progression and survival. PLoS One 8: e65316.
22. Nie Y, Liu X, Qiu S, Song E, Zou H, et al. (2013) Long non-coding RNA HOTAIR is an independent prognostic marker for nasopharyngeal carcinoma progression and survival. Cancer Sci 104: 458–464.
23. Lu L, Zhu G, Zhang C, Deng Q, Katsaros D, et al. (2012) Association of long noncoding RNA HOTAIR expression and its downstream intergenic CpG island methylation with survival in breast cancer. Breast Cancer Res Treat 136: 875–883.
24. Kim K, Juturu I, Chadalapaka G, Johnson G, Frank J, et al. (2012) HOTAIR is a negative prognostic factor and exhibits pro-oncogenic activity in pancreatic cancer. Oncogene 32: 1616–1625.
25. Nakagawa T, Endo H, Yokoyama M, Abe J, Tamai K, et al. (2013) Long 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.
26. He X, Bao W, Li X, Chen Z, Che Q, et al. (2014) The long non-coding RNA HOTAIR is upregulated in endometrial carcinoma and correlates with poor prognosis. Int J Mol Med 33: 325–332.
27. Sørensen KP, Thomassen M, Tan Q, Bak M, Cold S, et al. (2013) Long 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.
28. Parmar MK, Torri V, Stewart L (1998) Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. Stat Med 17: 2133–2143.
29. Parmar MK, Torri V, Stewart L (1998) Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. Stat Med 17: 2133–2143.
30. Nakagawa T, Endo H, Yokoyama M, Abe J, Tamai K, et al. (2013) Long 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.
31. He X, Bao W, Li X, Chen Z, Che Q, et al. (2014) The long non-coding RNA HOTAIR is upregulated in endometrial carcinoma and correlates with poor prognosis. Int J Mol Med 33: 325–332.
32. Sørensen KP, Thomassen M, Tan Q, Bak M, Cold S, et al. (2013) Long 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.
30. Niinuma T, Suzuki H, Nojima M, Nosho K, Yamamoto H, et al. (2012) Upregulation of miR-196a and HOTAIR Drive Malignant Character in Gastrointestinal Stromal Tumors. Cancer Research 72: 1126–1136.

31. Ge XS, Ma HJ, Zheng XH, Ruan HL, Liao XY, et al. (2013) HOTAIR, a prognostic factor in esophageal squamous cell carcinoma, inhibits Wnt pathway. Cancer Sci 104: 1675–1682.

32. Zhang JX, Han L, Bao ZS, Wang YY, Chen LY, et al. (2013) HOTAIR, a cell cycle-associated long noncoding RNA and a strong predictor of survival, is preferentially expressed in classical and mesenchymal glioma. Neuro Oncol 15: 1595–1603.

33. Xu ZY, Yu QM, Du YA, Yang LT, Dong RZ, et al. (2013) Knockdown of Long Non-coding RNA HOTAIR Suppresses Tumor Invasion and Reverses Epithelial-mesenchymal Transition in Gastric Cancer. Int J Biol Sci 9: 587–597.

34. Ishibashi M, Kogo R, Shihata K, Sawada G, Takahashi Y, et al. (2013) Clinical significance of the expression of long non-coding RNA HOTAIR in primary hepatocellular carcinoma. Oncol Rep 29: 946–950.

35. Geng YJ, Xie SL, Li Q, Ma J, Wang GY (2011) Large Intervening Non-Coding RNA HOTAIR is Associated with Hepatocellular Carcinoma Progression. J Int Med Res 39: 2119–2128.

36. Shi X, Sun M, Liu H, Yao Y, Song Y (2013) Long non-coding RNAs: a new frontier in the study of human diseases. Cancer Lett 339: 159–166.

37. Laerum OD, Ovrebo K, Skarstein A, Christensen JJ, Alpizar-Alpizar W, et al. (2012) Prognosis in adenocarcinomas of lower oesophagus, gastro-oesophageal junction and cardia evaluated by uPAR-immunohistochemistry. Int J Cancer 131: 558–569.

38. Xu J, Zhang C, He Y, Wu H, Wang Z, et al. (2012) Lymphatic endothelial cell-secreted CXCL1 stimulates lymphangiogenesis and metastasis of gastric cancer. Int J Cancer 130: 787–797.

39. Zhang CH, Xu GL, Jia WD, Ge YS, Li JS, et al. (2012) Prognostic significance of osteopontin in hepatocellular carcinoma: a meta-analysis. Int J Cancer 130: 2685–2692.