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·生物信息学·

High Expression of Long Non-coding RNA HOTTIP Indicates Poor Prognosis in Cancers: Evidence from Six Studies*

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ABSTRACT Objective: The significant role of long non-coding RNAs (lncRNAs) in early diagnosis and predicting prognosis has been recognized in various cancers recently. However, the prognostic value of HOXA transcript at the distal tip (HOTTIP), a vital lncRNA in tumorigenesis, remains unclear. In this study, we evaluated its prognostic value by analyzing the correlation of HOTTIP expression with overall survival (OS), lymph node metastasis (LNM) and distant metastasis (DM) in different cancer types by meta-analysis. **Methods:** We performed a systematic search in PUBMED, MEDLINE, Web of Science and Cochrane Library update to November of 2016. A total of 604 patients from 6 studies were included in final analysis and went through a quantitative meta-analysis by Review manager 5.3. **Results:** We demonstrated that high expression of HOTTIP had a significant correlation with poor OS (hazard ratio [HR] =2.37, 95% confidence interval [CI] =1.81-3.10, p<0.001), high LNM rate (odds ratio [OR]=2.29, 95%CI=1.54-3.40, p<0.001) as well as more DM occurrence (OR=3.30, 95%CI=1.78-6.12, p<0.001). **Conclusion:** Our results indicated that long non-coding RNA HOTTIP may serve as a potential prognostic biomarker in cancer progression.

Key words: Meta-analysis; Long non-coding RNA; HOTTIP; Cancer; Prognosis

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Introduction

Cancer is a major public health problem world-widely with admitted high mortality [1]. Even with the unremitting efforts of countless clinicians and researchers, there remains no efficient therapy for cancer treatment. This urged the searching for potential biomarkers for not only early diagnosis but also prognosis which concerned with two major factors, lymph node metastasis and distant metastasis[2]. Molecular biomarkers especially long non-coding RNAs (lncRNAs) as a group of newly found potential candidates have attracted tremendous attention and changed the conventional view of cancer development during the past few years[3, 4]. However, the potential value of lncRNA in predicting prognosis of cancer patients remains inconclusive and requires more circumstantial clarification.

LncRNA, an enormous group of non-coding RNA which is about more than 200nt at length, has been highlighted recently as its newly discovered significant role in various diseases, especially in cancer development [5, 6]. HOX transcript antisense intergenic RNA (HOTAIR) is one of the most studied lncRNAs in the HOX-associated lncRNA group. Its oncogenic role has been demonstrated in many types of cancers[7-9]. Moreover, several studies have pointed out its prognostic significance in evaluation of the cancer patients' outcome which has been further proved by systematic reviews and meta-analyses[10-14].

With the fruitful results in HOTAIR research, another HOX-associated lncRNA named as HOXA transcript at the distal tip (HOTTIP) attracted great interests most recently. HOTTIP is a lncRNA transcribed from the 5' tip of the HOXA13 locus with a length of around 3kb [15]. Although the function of HOTTIP in tumorigenesis is still largely unknown, its intriguing role as a prognostic biomarker has already been firstly demonstrated in hepatocellular carcinoma patients by Quagliata's group [16]. The sequestered studies verified its prognostic value in some other cancer types [17, 18]. However, there remains no consensus on the relationship between HOTTIP and cancer prognosis. Thus we conducted this meta-analysis to clarify its prognostic value in predicting outcome of cancer patients and to provide more evidence for further clinical application.

1 Material and Methods

1.1 Search strategy

A systematic search was performed in PUBMED, MEDLINE, Web of Science and Cochrane Library by two authors indepen-

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dently update to November of 2016. The key words included "long non-coding RNA", "lncRNA", "HOXA transcript at the distal tip", "HOTTIP", "cancer", "carcinoma", "tumor", "neoplasm", "outcome", "survival", "prognosis" and "prognostic". There was no other limitation such as country, race during the search process. All search results were evaluated independently by two authors and the disputed studies were rescreened by the third one who made the final decision.

1.2 Inclusion and exclusion criteria

The included articles have to meet the following criteria: 1) articles indicated the correlation between HOTTIP expression and overall survival of cancer patients; 2) clinicopathological data of cancer patients was provided sufficiently; 3) expression levels of HOTTIP in obtained tissues were measured by quantitative RT-PCR (qRT-PCR) or other methods; 4) patients were divided into two groups according to the expression levels of HOTTIP; 5) published in English. Exclusion criteria included: 1) any article that did not meet the inclusion criteria; 2) case reports, reviews, letters and other non-original articles; 3) duplicated studies; 4) short of key information for analysis.

1.3 Data extraction and quality assessment

Two authors extracted the major information from eligible studies and recorded them in a previously designed table independently. The major information included last name of first author, publication year, cancer type, number of all included patients, the number of patients in high and low HOTTIP expression group respectively, HRs and corresponding 95% CIs for OS (extracted from multivariable analysis firstly if available). Quality score was assessed by two authors independently according to the quality scale established by the European Lung Cancer Working Party[19]. The consensus on final score for each article was obtained after discussion. The score was expressed as the percentage and the higher score indicated the better quality.

1.4 Statistical analysis

HRs with their corresponding 95%CIs was used to estimate the correlation between HOTTIP expression and clinical prognosis. Moreover, odds ratios of LNM and DM were assessed to provide metastasis outcome. All pooled data was analyzed by Review Manager, version 5.3.5. The heterogeneity of pooled results was calculated by Higgins I-squared statistic. The fixed-effects model was used in meta-analysis unless a significant heterogeneity (I2>50%) showed among studies. Funnel plot was performed to evaluate the potential publication bias. Differences were considered statistically significant when p value less than 0.05.

2 Results

2.1 Characteristics of included studies

The electronic search throughout the data bases displayed a total of 135 articles. Among them, 97 articles were excluded after screening titles due to the irrelevance or duplicate. The remaining 38 articles were included for further evaluation based on abstract. Only 18 articles left to be carefully screened by viewing the full texts for final eligibility. After excluding 12 articles, 6 studies came to the final stage for meta-analysis (shown as Fig. 1) including 6 different cancer types, which are colorectal cancer (CRC)[20], osteosarcoma (OSC)[18], tongue squamous cell carcinoma [21], pancreatic cancer (PC)[22], hepatocellular cancer (HCC)[16] and gastric cancer (GC)[23] respectively. All major information of those studies was showed in Table 1. Tissue was the only specimen type and well preserved before RNA extraction in all 5 studies. At the meantime, qRT-PCR was the only way for measuring the expression level of HOTTIP.

However, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the internal normalization in three of six studies while the remained three used β-actin. Furthermore, the cut-off value to define high- and low- expression group was largely different. Four studies adopted the median value of HOTTIP expression as the cutoff line [18-20, 23]. One of the other two studies selected cut-off score by evaluating the receiver-operating characteristic (ROC) curve [16]. The remaining one defined the fold change >1.5 fold as high expression after comparing the HOTTIP expression between PC tissues and adjacent non-tumor tissue[22].

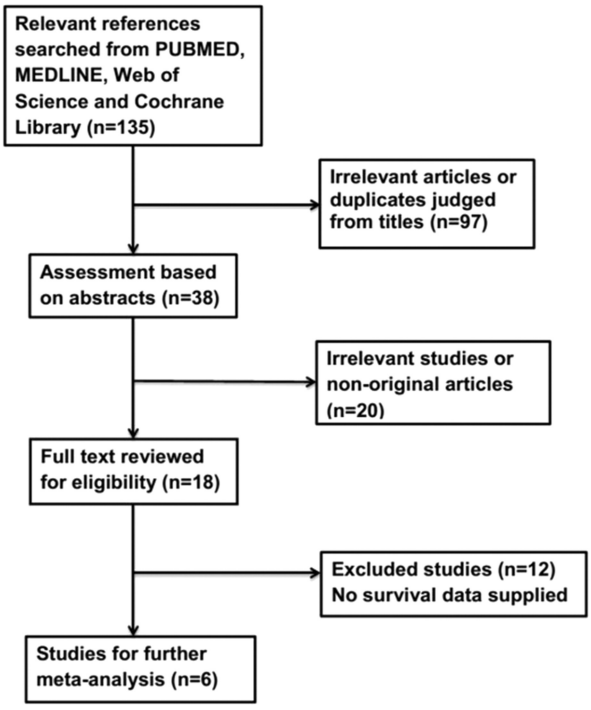

Fig. 1 The flow diagram showing the process of choosing eligible studies.

2.2 Correlation between HOTTIP and OS

We analyzed the pooled HRs of 6 studies with 604 patients. HRs data in our meta-analysis was extracted from the multivariate analysis directly. Fixed-effects model was used in our analysis since no significant heterogeneity showed (P=0.99, I2=0.0%). Analysis displayed a pooled HR of 2.37 (95%CI=1.81-3.10, P<0.001) (Fig. 2A). High HOTTIP expression group had a significant

Table 1 Characteristics of the eligible studies

Author	Country	Year	Ethnicity	Cancer type	Sample size	Distant metastasis		Lymphatic metastasis		Overall survival (HR[95%CI])	Quality Score (%)
						Absent (H/L)	Present (H/L)	Absent (H/L)	Present (H/L)		
Ren	China	2015	Asian	Colorectal cancer	156	50/65	27/14	30/43	47/36	2.151 [1.306-3.415]	77.5
Li	China	2015	Asian	Osteosarcoma	68	23/31	11/3	-	-	2.887 [1.367-7.061]	72.5
Zhang	China	2015	Asian	Tongue squamous cell carcinoma	86	37/41	7/1	16/24	28/18	2.113 [1.062-3.115]	77.5
Wang	China	2015	Asian	Pancreatic cancer	144	-	-	43/16	75/10	2.58 [1.385-4.839]	82.5
Quagliata	Switzer-land	2014	Caucasian	Hepatocellular carcinoma	52	-	-	-	-	2.244 [0.986-5.106]	87.5
Ye	China	2016	Asian	Gastric cancer	98	-	-	10/20	39/29	2.54 [1.209-5.312]	89.5

Note：H, high HOTTIP expression; L, low HOTTIP expression; HR, hazard ratio; CI, confidence interval.

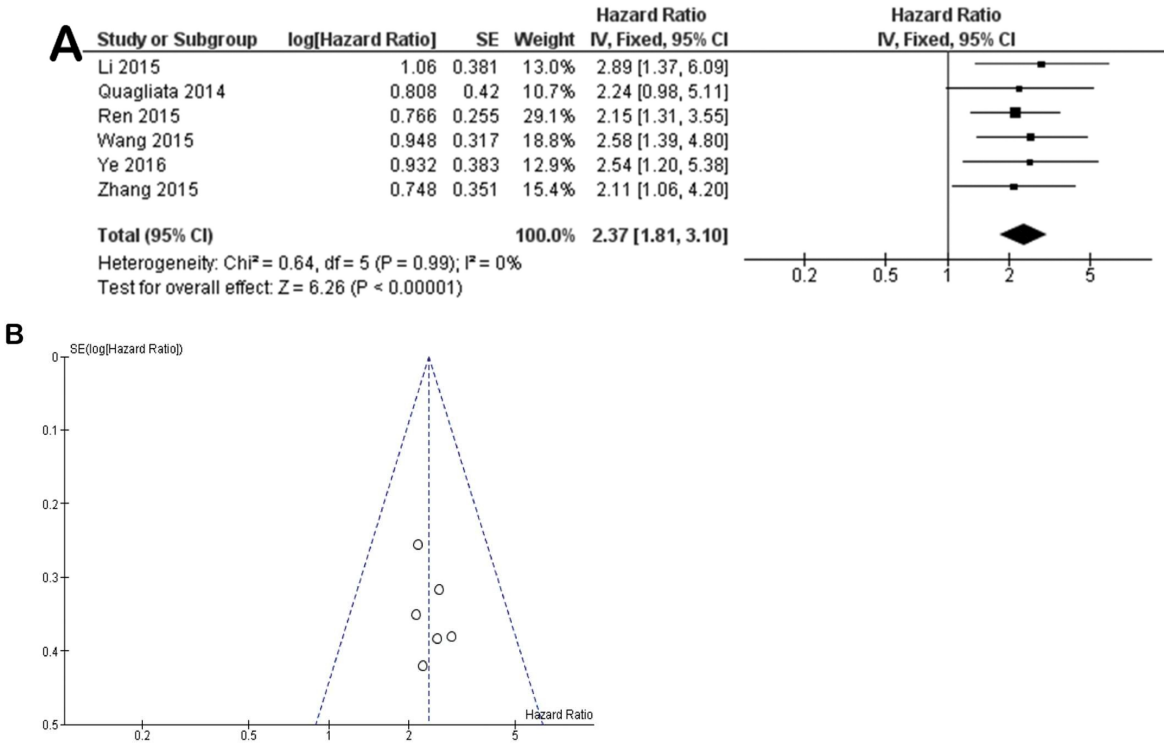

Fig. 2 Meta-analysis of the pooled hazard ratios (HRs) for identifying the correlation between the overall survival (OS) of patients and HOTTIP expression. A. Forrest plots of the meta-analysis of the HRs and their correspondent 95% confidence intervals (CIs). The diamond represents the pooled HR and the correspondent 95% CI. B. Funnel plot to evaluate the publication bias.

reduced OS compared with low expression group. Thus our meta-analysis indicated that high HOTTIP expression is an unfavorable prognostic factor of cancer patients overall survival. Moreover, funnel plot was executed to evaluate the publication bias of study. The funnel plot shape seemed symmetrical which suggested no significant publication bias among those five studies (Fig. 2B).

2.3 Correlation between HOTTIP and LNM

Four of six studies reported the number of patients with LNM in high HOTTIP expression and low HOTTIP expression respectively. We obtained the pooled ORs of four studies with a total of 484 patients. Since no significant h eterogeneity showed (P=0.87, I2=0%), the fixed-effects model was used. The result showed a pooled OR of 2.29 (95%CI=1.54-3.40, p<0.001) (Fig. 3). In this case, the meta-analysis result demonstrated that patients with high HOTTIP expression were more intended to develop LNM.

2.4 Correlation between HOTTIP and DM

Only three of six studies reported the number of patient with DM in two subgroups which defined by different HOTTIP expression. We analyzed the pooled ORs of those 3 studies with 310 patients in all. The fixed-effects model was used since no significant heterogeneity showed (P=0.48, I2=0.0%). The result demonstrated a pooled OR of 3.30, (95%CI=1.78-6.12, p<0.001) (Fig. 4). Compared with low HOTTIP expression group, high HOTTIP expression group showed a significant higher DM rate statistically. Our meta-analysis result showed that patients with high HOTTIP expression had an elevated risk to develop DM.

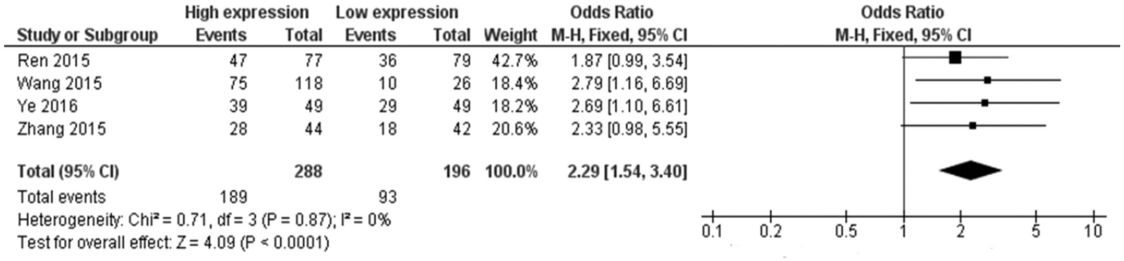

Fig. 3 Forrest plots of the pooled odds ratios (ORs) of the correlation between lymph node metastasis (LNM) of patients and HOTTIP expression. The diamond represents the pooled OR and the correspondent 95% CI.

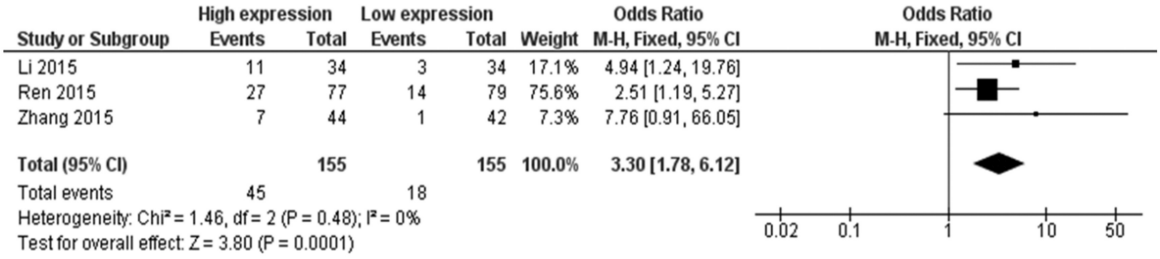

Fig. 4 Forrest plots of the pooled ORs of the correlation between distant metastasis (DM) of patients and HOTTIP expression. The diamond represents the pooled OR and the correspondent 95% CI.

3 Discussion

Emerging evidence has suggested the enormous potential of long non-coding RNA as both diagnostic and prognostic biomarker in cancer management. The most highly concentrated lncRNAs includes HOTAIR, MALAT-1, long intergenic non-coding RNA p21 and GAS5. Since HOTAIR's key role in carcinogenesis and metastasis has been acknowledged, HOTTIP, as another HOX-associated lncRNA, attracts more and more attention as well. HOTTIP's critical role in HOXA locus control was firstly identified by Wang's work [15]. They demonstrated that HOTTIP can drive histone H3 lysine 4 trimethylation and gene transcription by targeting WD repeat containing protein 5 (WDR5) /mixed lineage leukemia 1 (MLL) through the direct binding to WDR5. More importantly, when HOTTIP gets close to its target gene, all the following activation cascade may get started [15]. In this way, HOTTIP can translate location information into chromatin modifications therefore contributing to the tumorigenesis. This thrilling finding attracted attentions from oncologists. They verified the correlation between HOTTIP expression and cancer progression in multiple cancer types, including colorectal cancer [24], hepatocellular carcinoma[25], pancreatic cancer [26] and even tongue squamous cell carcinoma[21]. For example, Lian's work has revealed that HOTTIP's overexpression was associated with advanced tumor stage and larger tumor size in colorectal cancer and this contribution to tumor progression might achieve by suppressing the p21 function [24]. Tsang's group also suggested that HOTTIP may fulfill its pro-oncogenic function in hepatocellular carcinoma by regulating HOXA genes expression especially HOXA13 and has identified miR-125b as a negative post-transcriptional regulator of HOTTIP expression [25]. Meanwhile, Cheng's work showed its similar oncogenic role in pancreatic cancer. However, instead of regulating HOXA13 which was verified in liver cancer cells, HOTTIP turns out to regulate other HOX genes such as HOXA9, HOXA10 and HOXA11 in pancreatic cancer cells[26]. Even with all above findings with HOTTIP functions, further studies are still required to understand its underlying role in different pathways leading to cancer. Our meta-analysis just concentrates on HOTTIP's value as a prognostic predictor in cancer management. This analysis contained 5 independent articles with 506 patients and indicated a remarkable association between high HOTTIP expression and poor OS in patients with included cancer types (pooled HR=2.37, 95%CI=1.81-3.10, P<0.001). No significant heterogeneity showed suggests the clinical use of HOTTIP as potential prognostic biomarker. Our further analysis of the association between HOTTIP and LNM, DM occurrence demonstrated that high HOTTIP expression was significant related with higher LNM rate (odds ratio [OR]=2.29, 95%CI=1.54-3.40, p<0.001) as well as more DM occurrence (OR=3.30, 95% CI=1.

78-6.12, p<0.001).

Nevertheless, there are still several limitations in our analysis that we have to acknowledge. First, there is only one study for each specific cancer type which may cause the increased heterogeneity. Therefore, more studies for each particular cancer type are required for further analysis. Second, all recruited articles were retrospective studies with small sample size which may result in an insufficient statistical power to reveal the real significance in analysis. Moreover, the cut off value to set different group varied in those four studies, thus no agreeable value we can provide for a further clinical use in our analysis. In addition, patients from eligible studies are mostly from China, which suggests the result may not prevail to various ethnicities. Most importantly, the treatment for each cancer patient is variable. However, the impact of non-standardized management on survival of cancer patients was not taking into consideration due to the unavailable information which may bring some bias to assess the relationship between HOTTIP expression and patients overall survival.

Our meta-analysis suggested a significant correlation of high HOTTIP expression with poor OS and higher DM rate which indicates HOTTIP's potential role as a molecular biomarker for prognosis and distant metastasis.

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肿瘤中高表达的长链非编码 RNA HOTTIP 提示较差预后：六项研究的结果汇总 *

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摘要 目的：近些年来长链非编码 RNA（long non-coding RNA, lncRNA）在各类肿瘤的早期诊断及预测预后中的重要作用受到越来越多的重视。然而长链非编码 RNA HOXA 转录本末端 RNA（HOXA transcript at the distal tip, HOTTIP）作为肿瘤发展中的重要长链非编码 RNA，其在肿瘤中的预后价值仍有待研究。本研究通过分析 HOTTIP 表达和不同肿瘤类型的总生存期（overall survival, OS），淋巴结转移（lymph node metastasis, LNM）及远处转移（distant metastasis, DM）来评价其作为肿瘤预后指标的价值。**方法：**通过在 PUBMED，MEDLINE，Web of Science 以及 Cochrane Library 系统性查找截止于 2016 年 11 月所有相关文献，最终纳入 6 项研究共 604 例患者。通过 Review Manager 5.3 对这 6 项研究进行 meta 分析。**结果：**我们发现长链非编码 RNA HOTTIP 高表达的患者总生存较差（风险比 [hazard ratio, HR] =2.37, 95% 置信区间[confidence interval, CI] =1.81-3.10, p<0.001），并具有较高的淋巴结转移率（比值比 [odds ratio , OR]=2.29, 95%CI=1.54-3.40, p<0.001）和较高的远处转移发生率（OR=3.30, 95%CI=1.78-6.12, p<0.001）显著相关。**结论：**我们的分析结果提示长链非编码 RNA HOTTIP 可是作为肿瘤中预测预后的重要生物标志物。

关键词：meta 分析；长链非编码 RNA；HOTTIP；肿瘤；预后

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