Original Article
Association between long non-coding RNA MALAT-1 expression and cancer progression: a meta-analysis

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Abstract: The aim of this meta-analysis is to investigate the relationship between lncRNA MALAT-1 and cancer progression. We collected all the relevant articles by searching the following database: PubMed, Cochrane Library, OVID, Web of Science and Chinese National Knowledge Infrastructure (CNKI). We calculated the odds ratio (OR) and its corresponding 95% confidence interval (CI) to assess the strength of the association by using RevMan5.0. In total, 751 patients from 7 studies were included in this meta-analysis. The subgroup analysis showed that MALAT-1 expression was significantly associated with tumor distant metastasis (Absent subgroup: OR=0.41, 95% CI 0.19-0.91, P=0.03; Present subgroup: OR=2.42, 95% CI 1.09-5.37, P=0.03), but not significantly associated with gender differences (female subgroup: OR=0.86, 95% CI 0.63-1.17, P=0.33; male subgroup: OR=1.17, 95% CI 0.86-1.59, P=0.33), tumor differentiation (Well moderate subgroup: OR=0.79, 95% CI 0.50-1.25, P=0.31; Poor subgroup: OR=1.27, 95% CI 0.80-2.00, P=0.31) and lymphatic metastasis (Absent subgroup: OR=1.09, 95% CI 0.77-1.55, P=0.63; Present subgroup: OR=0.92, 95% CI 0.65-1.30, P=0.63). The results indicated that MALAT-1 could be a potential biomarker for tumor distant metastasis.

**Keywords:** MALAT-1, lncRNA, cancer, meta-analysis

Introduction

The human RNA acts not only as a supporting role in the intermediation of genetic information, but more importantly as a regulator in a variety of physiological and pathological processes. More than 80% of human RNAs cannot directly encode protein so that they are named as non-coding RNAs (ncRNAs). Long non-coding RNAs (lncRNAs) are ncRNAs with more than 200 nucleotides. Nowadays lncRNA has become a research hotspot after microRNA (miRNA). The structure of lncRNA is similar to mRNA [1, 2]. The vast majority of lncRNAs are transcripted by RNA polymerase II and alternative splicing. They widely participate in almost all physiological and pathological processes in view of genetic and transcription level [3].

Using lncRNA microarray, high-throughput sequencing and qRT-PCR (real-time fluorescent quantitative-PCR), researchers have found a large number of lncRNAs which differentially express in human cancer such as prostate cancer, breast cancer, liver cancer, colorectal cancer, etc [4-6].

Metastasis associated lung adenocarcinoma transcript 1 (MALAT-1), also known as the nuclear-enriched abundant transcript 2 (NEAT2), exist widely in normal human cells. It is a very important gene which can regulate the function of endothelial cells and angiogenesis [7]. In 2003, Ji et al [8] found that MALAT-1 was a predictive indicator for I stage lung adenocarcinoma or squamous cell carcinoma by using subtractive hybridization. Since then, many researchers subsequently confirmed that the expression level of MALAT-1 was up regulated in tumor cells including liver cancer, breast cancer, pancreatic cancer, bladder cancer and prostate cancer [9-11].

Studies in vitro also revealed that up or down regulating the expression of MALAT-1 had a remarkable impact on the proliferation, migration and invasion of tumor cells. Tripathi et al [12] found that MALAT-1 can regulate the proliferation of tumor cells by regulating the expression of carcinogenic transcription factor B-MYB. Some researches indicated that silencing MALAT-1 could reduce the proliferation and invasion of renal cell cancer and promote the apoptosis of tumor cells [13].
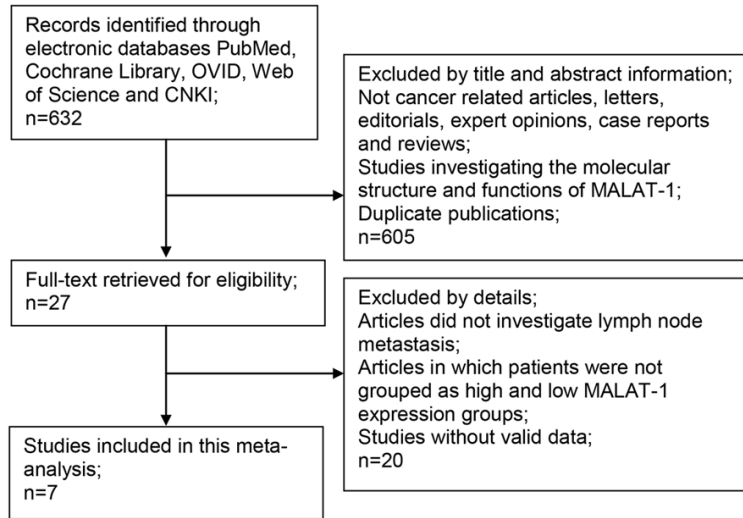
Figure 1. Flowchart presenting the steps of literature search and selection.

Recently, the association between long non-coding RNA MALAT-1 expression and tumor has been investigated by some studies. However, there are still some disputes about the results and the sizes of the samples are small. Besides, there is no meta-analysis reporting the association between long non-coding RNA MALAT-1 expression and cancer progression. Therefore, we conducted this meta-analysis to explore the potential relationship.

Materials and methods

Search strategy

We performed a systematic computer literature search to collect potentially eligible articles. The following electronic databases were used for finding relevant studies: PubMed, Cochrane Library, OVID, Web of Science and Chinese National Knowledge Infrastructure (CNKI). The keywords we used were "MALAT1" OR "MALAT-1" OR "metastasis associated lung adenocarcinoma transcript 1". The literatures were retrieved for further screening.

Study selection

We used the following criteria to evaluate the retrieval literatures which were consistent with our analysis included in the request: (a) studies investigating the roles of MALAT-1 in the genesis and progression of cancer, (b) the expression levels of MALAT-1 in tumor tissues were measured, (c) patients were divided into groups according to the expression levels, (d) related

clinic pathologic parameters were described, (e) there were sufficient data for us to calculate the odds ratios (OR) and corresponding 95% confidence intervals (CI), (f) all the patients were diagnosed by pathological examination. At the same time, we used the following exclusion criteria: (a) letters, editorials, expert opinions, case reports and reviews, (b) studies which focus on the molecular structure and functions of MALAT-1, (c) articles which contain no usable data, (d) duplicate publications. Two of our research members (Xie and Yan) read the full text versions and discussed whether they met the selection criteria and the eligible articles were included in our study.

Data extraction

We extracted the following data from the included articles: first author, publish date, country, tumor type, and total number of patients, number of high MALAT-1 expression groups and low expression groups and detection methods of MALAT-1 expression. Two investigators (Xie and Dong) extracted data independently. If there was any disagreement about the extraction, another investigator (Yan) adjudicated the results.

Study quality

Two authors (Xie and Yan) evaluated the quality of retrieved studies independently according to the Newcastle-Ottawa Scale (NOS) for case-control studies. The NOS ranged from zero to nine stars. The controversial part solved by discussion. And the third investigator (Dong) adjudicated the disagreements.

Statistical analysis

Heterogeneity was analyzed by calculating the Q test statistics with RevMan 5.0 software. Counting data was estimated with Risk Ratio (RR) and 95% confidence intervals (CI); Measurement data was assessed by pooled Standard Mean Difference (SMD). If the data suggests $P > 0.10$ with no significant heterogeneity, we will use the fixed effects model, otherwise, use the random effects model. Considering the incidence rate of PCA is low, HR could be

Table 1. Characteristics of the eligible studies in this meta-analysis

First author	Year	Country	Ethnicity	Cancer type	Total number	MALAT-1 expression		Detection method
						High	Low	
Lai	2012	China	Asian	LC	60	33	27	qRT-PCR
Liu	2014	China	Asian	PC	45	26	19	qRT-PCR
Pang	2014	China	Asian	PC	126	63	63	qRT-PCR
Zhang	2014	China	Asian	RC	106	56	50	qRT-PCR
Zheng	2014	China	Asian	CRC	146	73	73	qRT-PCR
Ma	2014	China	Asian	Glioma	118	59	59	qRT-PCR
Okugawa	2014	Japan	Asian	GC	150	88	62	qRT-PCR

LC: liver cancer, PC: pancreatic cancer, RC: renal cancer, CRC: colorectal cancer, GC: gastric cancer, qRT-PCR: real-time fluorescent quantitative-PCR.
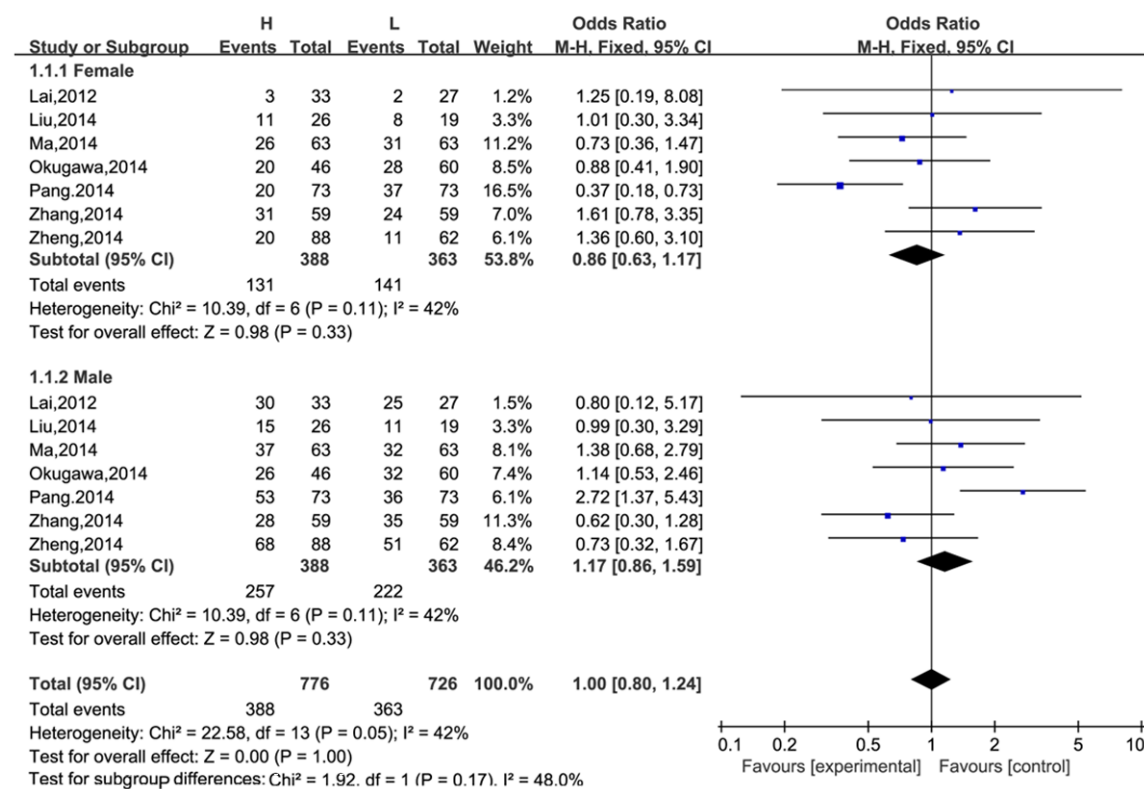
Figure 2. MALAT-1 expression was not significantly associated with tumor differentiation.

approximately equal to OR. Here we used OR instead of HR. Egger's funnel plot was explored to find if there is any evidence of publication bias [14].

Results

Information from the literature

Based on the above search strategy, we achieved 632 relevant studies. 605 articles were excluded by title and abstract information.

Then we further excluded the rest articles by details which we show in **Figure 1**.

Study characteristics

All studies were published from 2012 to 2014. Patients enrolled were all Asian [15-21]. They compared the differences in sex, tumor differentiation, lymphatic metastasis and distant metastasis. The average NOS score was 6 indicating the reliable quality. The characteristics of included studies were showed in **Table 1**.

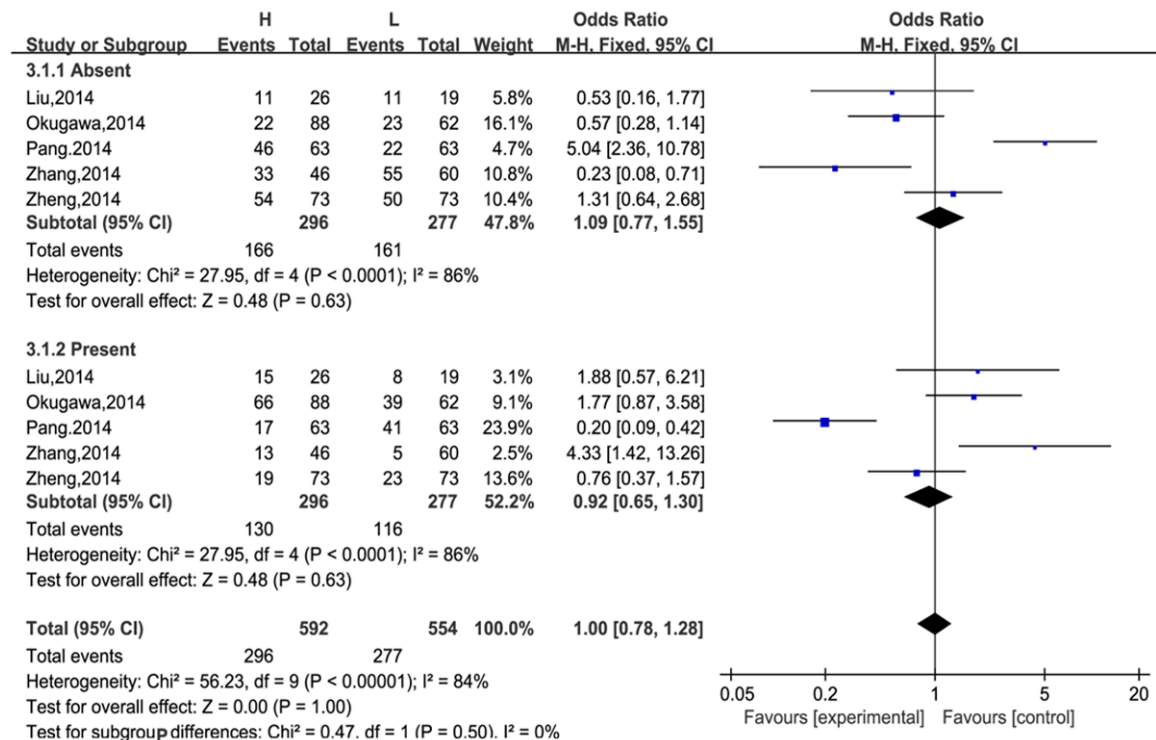| Study or Subgroup	H Events	Total	L Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI	Odds Ratio M-H, Fixed, 95% CI
2.1.1 Well+Moderate							
Lai,2012	23	33	19	27	8.5%	0.97 [0.32, 2.94]	
Liu,2014	19	26	17	19	7.1%	0.32 [0.06, 1.75]	
Pang.2014	37	63	41	63	22.6%	0.76 [0.37, 1.57]	
Zheng,2014	56	73	57	73	17.7%	0.92 [0.43, 2.01]	
Subtotal (95% CI)		195		182	55.9%	0.79 [0.50, 1.25]	
Total events	135		134				

Heterogeneity: Chi² = 1.38, df = 3 (P = 0.71); I² = 0%
Test for overall effect: Z = 1.02 (P = 0.31)

2.1.2 Poor							
Lai,2012	10	33	8	27	8.2%	1.03 [0.34, 3.14]	
Liu,2014	7	26	2	19	2.3%	3.13 [0.57, 17.18]	
Pang.2014	26	63	22	63	17.3%	1.31 [0.64, 2.69]	
Zheng,2014	17	73	16	73	16.4%	1.08 [0.50, 2.35]	
Subtotal (95% CI)		195		182	44.1%	1.27 [0.80, 2.00]	
Total events	60		48				

Heterogeneity: Chi² = 1.38, df = 3 (P = 0.71); I² = 0%
Test for overall effect: Z = 1.02 (P = 0.31)

Total (95% CI)		390		364	100.0%	1.00 [0.73, 1.38]	
Total events	195		182				

Heterogeneity: Chi² = 4.61, df = 7 (P = 0.71); I² = 0%
Test for overall effect: Z = 0.00 (P = 1.00)
Test for subgroup differences: Chi² = 2.07. df = 1 (P = 0.15). I² = 51.6%

Favours [experimental] Favours [control]

Figure 3. MALAT-1 expression was not significantly associated with lymphatic metastasis.

Study or Subgroup	H Events	Total	L Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI	Odds Ratio M-H, Fixed, 95% CI
3.1.1 Absent							
Liu,2014	11	26	11	19	5.8%	0.53 [0.16, 1.77]	
Okugawa,2014	22	88	23	62	16.1%	0.57 [0.28, 1.14]	
Pang.2014	46	63	22	63	4.7%	5.04 [2.36, 10.78]	
Zhang,2014	33	46	55	60	10.8%	0.23 [0.08, 0.71]	
Zheng,2014	54	73	50	73	10.4%	1.31 [0.64, 2.68]	
Subtotal (95% CI)		296		277	47.8%	1.09 [0.77, 1.55]	
Total events	166		161				

Heterogeneity: Chi² = 27.95, df = 4 (P < 0.0001); I² = 86%
Test for overall effect: Z = 0.48 (P = 0.63)

3.1.2 Present							
Liu,2014	15	26	8	19	3.1%	1.88 [0.57, 6.21]	
Okugawa,2014	66	88	39	62	9.1%	1.77 [0.87, 3.58]	
Pang.2014	17	63	41	63	23.9%	0.20 [0.09, 0.42]	
Zhang,2014	13	46	5	60	2.5%	4.33 [1.42, 13.26]	
Zheng,2014	19	73	23	73	13.6%	0.76 [0.37, 1.57]	
Subtotal (95% CI)		296		277	52.2%	0.92 [0.65, 1.30]	
Total events	130		116				

Heterogeneity: Chi² = 27.95, df = 4 (P < 0.0001); I² = 86%
Test for overall effect: Z = 0.48 (P = 0.63)

Total (95% CI)		592		554	100.0%	1.00 [0.78, 1.28]	
Total events	296		277				

Heterogeneity: Chi² = 56.23, df = 9 (P < 0.00001); I² = 84%
Test for overall effect: Z = 0.00 (P = 1.00)
Test for subgroup differences: Chi² = 0.47. df = 1 (P = 0.50). I² = 0%

Favours [experimental] Favours [control]

Figure 4. MALAT-1 expression was significantly associated with distant metastasis.

Tumor differentiation

There were 4 studies with a total of 195 cases and 182 controls comparing the MALAT-1 ex-

pression level in different stage of tumor differentiation with that in case-control groups. The result showed no significant heterogeneity (I²=0%, P=0.71), and the pooled OR was 0.79
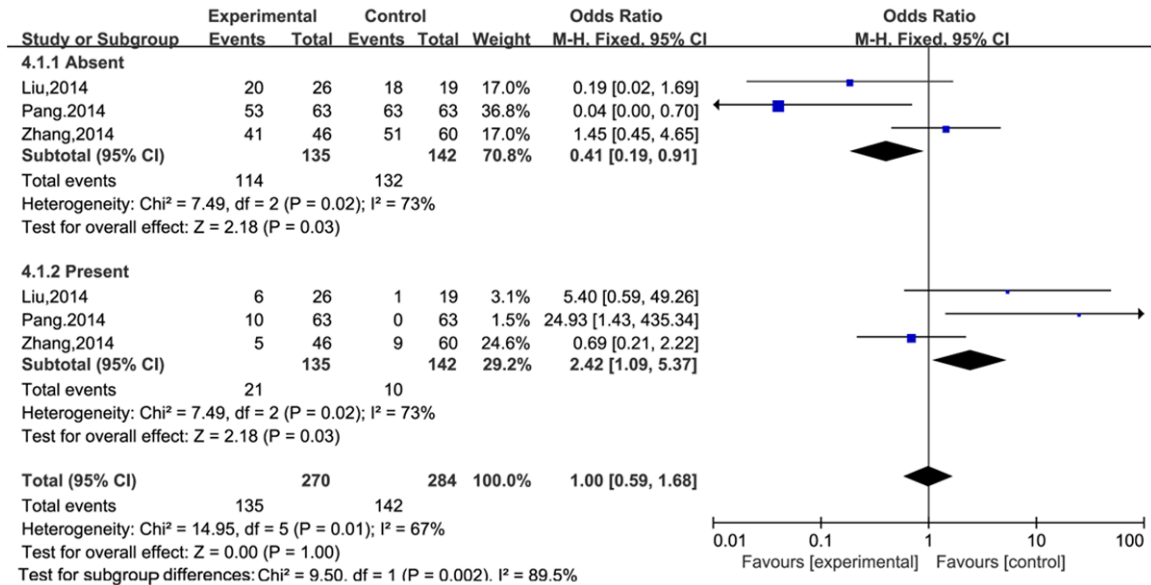
Figure 5. MALAT-1 expression was not significantly different between female and male.

(95% CI: 0.50, 1.25; Z=1.02; P=0.31) in the absent subgroup, 1.27 (95% CI: 0.80, 2.00; Z=1.02; P=0.31) in the present subgroup. It indicated that MALAT-1 expression was not significantly associated with tumor differentiation (**Figure 2**).

Lymphatic metastasis

There were 5 studies with a total of 296 cases and 277 controls comparing the MALAT-1 expression level in cancer with lymphatic metastasis with that in case-control groups. There was significant heterogeneity in both subgroups (I^2=86%, P<0.0001). And the pooled OR was 1.09 (95% CI: 0.77, 1.55; Z=0.48; P=0.63) in the absent subgroup, 0.92 (95% CI: 0.65, 1.30; Z=0.48; P=0.63) in the present subgroup. The results showed that MALAT-1 expression was not significantly associated with lymphatic metastasis (**Figure 3**).

Distant metastasis

There were 3 studies with a total of 135 cases and 142 controls comparing the MALAT-1 expression level in tumor with distant metastasis with that in case-control groups. There was significant heterogeneity in both subgroups (I^2=73%, P=0.02). And the pooled OR was 0.41 (95% CI: 0.19, 0.91; Z=2.18; P=0.03) in the absent subgroup, 2.42 (95% CI: 1.09, 5.37; Z=2.18; P=0.03) in the present subgroup. The

results showed that MALAT-1 expression was significantly associated with distant metastasis (**Figure 4**).

Sex

There were 7 studies with a total of 388 cases and 363 controls comparing the MALAT-1 expression level in different sex with case-control groups. There was significant heterogeneity in both subgroups (I^2=42%, P=0.11). And the pooled OR was 0.86 (95% CI: 0.63, 1.17; Z=0.98; P=0.33) in the female subgroup, 1.17 (95% CI: 0.86, 1.59; Z=0.98; P=0.33) in the male subgroup. The results showed there was no significant difference between female and male groups (**Figure 5**).
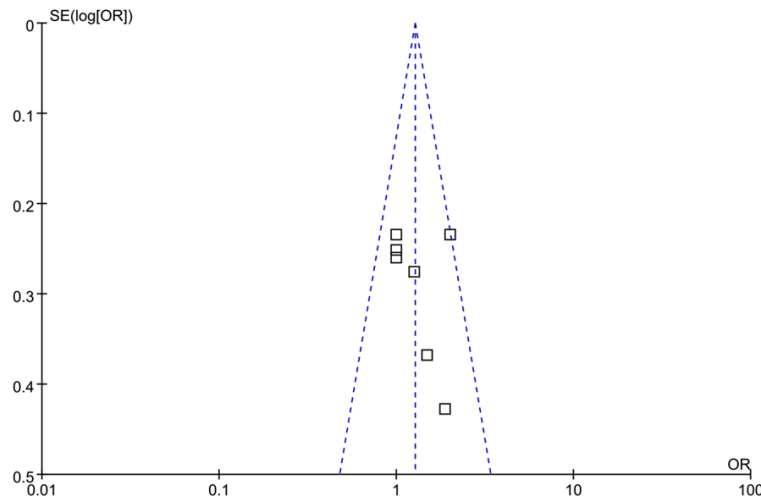
Publication bias and sensitivity analysis

In order to test whether the final result of this meta-analysis was affected by individual study and gauge the stability of the results, a sensitivity analysis was conducted (**Figure 6**). The pooled OR in the meta-analysis was not effect by single study. The result of Egger's regression test showed the asymmetrical distribution in the funnel plot in the expression of MALAT-1 between high and low Gleason score groups.

Discussion

Our results confirm that the expression level of MALAT-1 is significantly related to the tumor

Figure 6. Funnel plot of publication bias on the differences of MALAT-1.

induce MALAT-1 expression and EMT in bladder cancer cells in vitro suggested that MALAT-1 was an important mediator of TGF-beta-induced EMT and participated in the progress of tumor metastasis. Although these studies found significant correlations between high expression level of MALAT-1 and cancer progression, we found they were not significantly associated with each other in every clinic pathological features. There might be several reasons why the results showed that the expression level of MALAT-1 was associated with distant metastasis rather than lymphatic metastasis. First, all the studies we included didn't put hematogenous metastasis into consideration for its difficulty to detect in clinical practice. Second, the heterogeneities of the studies were at least moderate. Third, the number of the patients in the included studies was small.

For clinical practice, if the expression of MALAT-1 is found rising, we should be on high alert for the risk of tumor distant metastasis. Some appropriate adjuvant therapies such as systemic chemotherapies could be considered post operation to reduce the possibility of tumor distant metastasis. This might play an effective role improving the quality of patients' life, improving the prognosis and increasing survival rate.

However, there are still some limitations. Firstly, the literatures we searched may accept their positive results predominantly, leading the results of our meta-analysis expanding. Secondly, samples of this meta-analysis were relatively small which may lead the results unstable. Thirdly, only those studies written in Chinese or English were included in the meta-analysis even though we set no language restriction. Last but not least, most of the included studies were based on the population from Asian countries.

Conclusions

Taken together, our meta-analysis showed that MALAT-1 expression was significantly associat-

distant metastasis, however, it is not significantly associated with sex, tumor differentiation and lymphatic metastasis. It reveals that the expression level of MALAT-1 could be a reference indicator for tumor distant metastasis and provides a clinical guide for further treatment.

The subgroup analysis showed that MALAT-1 expression was significantly associated with tumor distant metastasis (Absent subgroup: OR=0.41, 95% CI 0.19-0.91, P=0.03; Present subgroup: OR=2.42, 95% CI 1.09-5.37, P=0.03), but not significantly associated with gender differences (female subgroup: OR=0.86, 95% CI 0.63-1.17, P=0.33; male subgroup: OR=1.17, 95% CI 0.86-1.59, P=0.33), tumor differentiation (Well moderate subgroup: OR=0.79, 95% CI 0.50-1.25, P=0.31; Poor subgroup: OR=1.27, 95% CI 0.80-2.00, P=0.31) and lymphatic metastasis (Absent subgroup: OR=1.09, 95% CI 0.77-1.55, P=0.63; Present subgroup: OR=0.92, 95% CI 0.65-1.30, P=0.63).

The function of MALAT-1 in promoting cancer progression has been confirmed by many researches. Yang et al [22] found that MALAT-1 was up-regulated in human primary CRC tissues with lymph node metastasis, and MALAT-1 may promote CRC tumor development via its target PRKA kinase anchor protein 9 (AKAP-9). Tumor invasion and metastasis is known associated with epithelial-mesenchymal transition (EMT). Fan et al [23] found that TGF-beta could

ed with tumor distant metastasis. Therefore, MALAT-1 could be a potential indicator for tumor distant metastasis. However, more well-designed researches with large quantities of samples are need to further verify the results of this meta-analysis.

Disclosure of conflict of interest

None.

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