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ORIGINAL ARTICLE

Long Non-Coding RNA SPRY4-IT1 Can Predict Unfavorable Prognosis and Lymph Node Metastasis: a Meta-Analysis

Nandi Li[1] · **Qian Tan**[1] · **Wei Jing**[1] · **Ping Luo**[1] · **Jiancheng Tu**[1]

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Abstract Emerging evidences suggested that long non-coding RNAs (lncRNAs) play an interesting role in the tumor development and progression in various types of cancer. The aim of this study was to analyse the potential prognostic value for cancer patients . We systematically searched the reports through PubMed, Web of Science, Medline, CNKI, and the Cochrane Library from inception to March, 2016, and carefully identified according to eligibility criteria. This quantitative meta-analysis collected all relevant articles to investigated the association of SPRY4-IT1 expression status with overall survival (OS) and lymph node metastasis (LNM). A total of 765 patients with cancer from 8 studies were included in the final analysis. The hazard ratio (HR) of OS and the odds ratios (OR) of LNM were calculated to assess the association . The meta-analysis results showed high SPRY4-IT1 expression could predict unfavorable OS in various cancers (pooled HR: 2.18, 95% CI: 1.45–3.27, $p = 0.001$). Moreover, we found high SPRY4-IT1 expression was related to LNM (pooled OR = 3.86, 95%CI:1.31–11.35, $P = 0.01$). LncRNA SPRY4-IT1 can serve as a new molecular marker for cancer metastasis and prognosis.

Keywords LncRNA · SPRY4-IT1 · Lymph node metastasis · Prognosis

✉ Jiancheng Tu
jianchengtu@whu.edu.cn

[1] Department of Clinical Laboratory Medicine and Center for Gene Diagnosis, Zhongnan Hospital of Wuhan University, Wuhan, China

Introduction

Cancer is a major cause of mortality worldwide [1]. Although encouraging progress in treatment for cancer has been achieved, the 5-year survival rate remains low and the majority of patients die due to late diagnosis and metastases [2]. Therefore, it is urgent to found more efficient biomarkers for cancer metastasis and prognosis.

LncRNAs are a RNA molecule longer than 200 nucleotides in length, which are poorly conserved and not capable of being translated into proteins [3]. The role of lncRNAs in cancers has been broadly researched, lncRNAs can act as oncogenes or tumor suppressor genes during tumorigenisis [4]. Recent studies show that lncRNA plays a key role in prognosis and metastasis [5–7]. Moreover, multiple lines of study have demonstrated that the dysregulation of lncRNAs is associated with tumor biological processes including metastasis, cell proliferation and apoptosis [8].

LncRNA SPRY4 intronic transcript 1 (SPRY4-IT1) localized in 5q31.3, was derived from the second intron within SPRY4, which polyadenylated transcript originally identified in melanoma. [9] The expression level of SPRY4-IT1 was upregulated in various carcinomas, such as esophageal squamous cell carcinoma [10], breast cancer [11], renal cell carcinoma [12], melanoma [13], bladder cancer [14] and glioma [15]. Besides, some studies reported aberrant expression of SPRY4-IT1 has association with lymph node metastasis (LNM) and poor prognosis [10, 12, 14–16], but some studies have no significant association [17, 18]. Moreover, this studies exploring the implication of SPRY4-IT1 are limited by small sample size. Thus, we performed the first meta-analysis to investigated the correlation of SPRY4-IT1 with tumor metastasis and prognosis.

Materials and Methods

Search Strategy

PubMed, Web of Science, Medline, CNKI, and the Cochrane Library were systematically searched on March, 2016. The search strategy used both MeSH terms and free-text words to increase sensitivity. The following search terms were used: "sprouty RTK signaling antagonist 4 intronic transcript 1", "SPRY4 intronic transcript 1", "SPRY4- IT1". The citation lists of the retrieved articles were manually screened to ensure the sensitivity of the search strategy. We did not limit our search by country, race and date.

Inclusion and Exclusion Criteria

Studies in this meta-analysis should meet the follow inclusion criteria: (1) studies investigating the correlation bewteen SPRY4-IT1 and cancer patients; (2) Expression of SPRY4-IT1 was measured by RT-PCRor ISH; (3) The relationship between SPRY4-IT1 expression and clinicopathologic characteristics or prognosis were described; (4) Hazard ratios (HR) for overall survival and odds ratios (OR) for lymph node metastasis expression were provided or were extractable from articles. The following criteria were used to exclude studies: (1) duplicate publications; (2) studies of case reports, letters, and reviews; (3) studies without usable data.

Date Extraction

The two investigators extracted the date independently through a same standard. Any disagreements were consulted with the third investigator. The following details were extracted: first author, publication year, country of origin, cancer type, detection method of SPRY4-IT1, total number of patients, number of high SPRY4-IT1 expression group and low expression group, number of patients with LNM, the HR and the corresponding 95% confidence interval (CI) for overall survival (OS).

Quality Assess

Because all included studies were non-randomized studies, we adoped the Newcastle-Ottawa Scale for assessing the quality of these studies. The quality assessment of non-randomized studies is an important component of a thorough meta-analysis of non-randomized studies. Quality assessment was performed independently by two investigators. Any disagreements were resolved by consensus.

Statistical Analysis

We extracted HRs for OS according to the following three methods: (1) The HRs were obtained directly from the publication or by estimation from the O-E statistic and variance; (2) The HRs were calculated through the HRs from the total number of events and the *P*-value in the articles; (3) We estimate the HRs and 95%CIs by extracting several survival rates at specified times from the Kaplan–Meier survival curves using Engauge Digitizer version 4.1 [19]. The first method was accurate, but the second and third method may generate errors by variation .

The ORs for LNM were calculated by the number of high SPRY4-IT1 expression group and low expression group and the number of patients with LNM and patients without LNM.

The meta-analysis was performed through Cochrane Collaboration Review Manager Version 5.2 and State 11. To investigate the heterogeneity among studies, I^2 statistics and chi-square Q test were used. When I^2 value more than 50% and a p value less than 0.05 for Q test, heterogeneity was regraded as significant. First, we used the fixed effects model to estimate the ORs or the HRs and their corresponding 95% CIs. If heterogeneity was significant, we used the random effects model. The funnel plot and the Begg's test were executed for assessing the pulication bias. We also performed sensitivity analyses to test the effect of each study on the overall pooled results. Statistical significance was defined when a p-value less than 0.05.

Results

Characteristics of Studies

A flow diagram of literature search process is presented in Fig. 1. Based on the inclusion criteria, we ultimately included 8 studies in the final analysis [10, 12–15, 17, 18, 20]. These studies included a total of 765 patients. All studies come from China. A total of 6 different types of cancer were included in this analysis, with 2 esophageal squamous cell carcinoma, 2 bladder cancer, 1 melotoma, 1 gastric cancer , 1 clear cell renal cell carcinoma and glioma. Six studies were normalized to glyceralde-hyde-3-phosphate dehydrogenase (GADPH), and one study was normalized to β-actin. The main characteristics were summarized in Table 1. All the diagnoses were based on pathology. No patient received radiotherapy or chemotherapy before surgery. All included studies were assessed to be of high quality by the Newcastle-Ottawa Scale.

All studies divided a high SPRY4-IT1 expression group and a low SPRY4-IT1 expression group by the following methods. (1) The high SPRY4-IT1 group had SPRY4-IT1 expression levels > median value and the low SPRY4-IT1 group had SPRY4-IT1 expression levels < median value; (2)

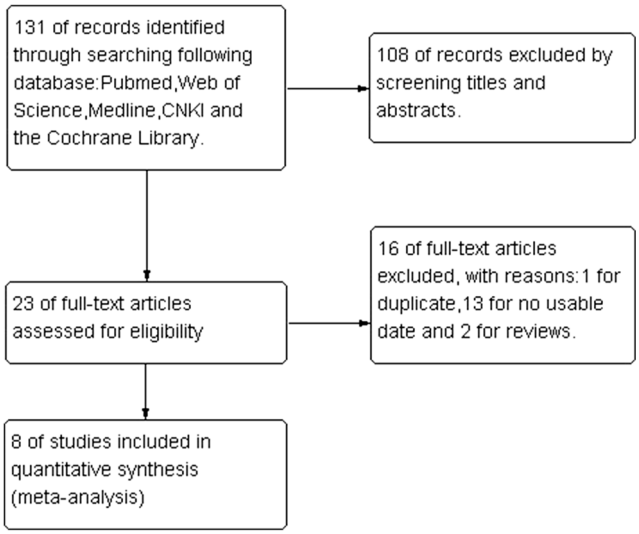

Fig. 1 The flow diagram of this meta-analysis

According to a SPRY4-IT1/ GAPDH ratio of 0.778, high SPRY4-IT1 expression group and a low expression group were divided; (3) Patients were divided into two groups based on the mean value of SPRY4-IT1 expression; (4) Patients were divided into two groups based on the cutoff value.

Relationship Between SPRY4-IT1 and OS

Seven studies investigated the association bewteen SPRY4-IT1 expression and OS in total of 655 patients. Because the heterogeneity test of among the studies was significant ($I^2 = 58\ \%\ , p = 0.03$), the random-effects model was adoped. Meta-analysis of those studies indicated that high SPRY4-IT1 expression was associated with poorer OS in human cancer (pooled HR: 2.18, 95% CI: 1.45–3.27, $p = 0.001$) (Fig.2).

Relationship Between SPRY4-IT1 and LNM

Six studies investigated the association bewteen SPRY4-IT1 expression and LNM in total of 532 patients. There was a significant heterogeneity among the studies (I^2=77%, $p = 0.0007$), and then the random-effects model was used. The result of analysis showed that the pooled OR for LNM was 3.86 (95%CI:1.31–11.35) (Fig.3). Although some studies revealed that high SPRY4-IT1 expression group had a statistic significant elevated LNM rate, this result showed the patients with high SPRY4-IT1 has strong trend to develop LNM.

Sensitivity Analysis and Publication Bias

We used sensitivtiy analysis to explore the source of the heterogeneity. Theheterogeneity decreased from 58% to 0% (pooled HR) and from 77% to 38% (pooled OR) when we excluded Peng et al. ‘s study, but if we remove other studies, The heterogeneity still exist and has no significant change. These results showed that the main heterogeneity derived from Peng et al. ‘s study. After we excluded Peng et al. ‘s study or any other study, we again pooled this studies to analysis, and the pooled HR remained relative stable (Fig.4). Thus, this sensitivity analysis comformed the reliablity of our results.

Table 1 Characteristics of studies in this meta-analysis

Author	Year	Country	Cancer type	Total number	High expression	High with LNM	Low expression	Low with LNM	Method	Cut-off	outcome	Survival analysis	HR
Zhang	2014	China	ccRCC	98	45	13	46	1	RT-PCR	Mean	OS	Multivariate	Reported
Xie	2014	China	ESCC	92	46	29	46	16	RT-PCR	Median	OS	Multivariate	Reported
Zhao	2015	China	UCB	68	56	18	31	1	RT-PCR	Mean	OS	Multivariate	Reported
Liu	2016	China	melanoma	70	-	-	-	-	RT-PCR	2.64 (optimal cutoff)	OS	Multivariate	Reported
Peng	2015	China	GC	175	98	51	77	44	RT-PCR	0.778 (SPRY4-IT1 /GAPDH ra-dio)	OS	Multivariate	Reported
Chen	2016	China	UCB	60	45	4	19	0	RT-PCR	Mean	OS	-	-
Xie	2013	China	ESCC	50	25	14	25	8	RT-PCR	Median	OS	Univariate	Survival curve
Zhou	2016	China	glioma	163					RT-PCR	Median	OS	Multivariate	Reported

ccRCC clear cell renal cell carcinoma, *ESCC* esophageal squamous cell carcinoma, *UCB* urothelial carcinoma of the bladder, *GC* gastric cancer, *LNM* lymph node metastasis, *OS* overall survival, *HR* hazard ratio;

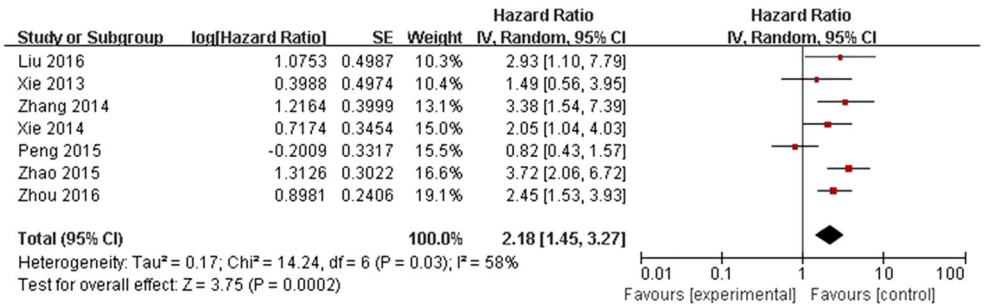

Fig. 2 Forest plot for the asscociation between SPRY4-IT1 expression with OS

Publication bias of this meta-analysis was evaluated by the Begg's test and the Begg's funnel plot, the Begg's test show that no publication bias (p = 0.834 for HR). As shown in the Begg's funnel plot (Fig.5), no significant publication bias was observed.

Discussion

LncRNAs were previously regarded as transcriptional noise or garbage [21]. Recently, with the development of highthroughput sequencing and microarray, more and more studies have revealed that lncRNAs play vital roles in various biological processes [22]. In particular, it has been found that lncRNAs involved in tumor initiation and progression [23]. Dysregulation of lncRNAs exerts impacts on the biological processes of tumors such as cell proliferation, apoptosis, angiogenesis, metastasis, and evasion of tumor suppressors [24]. For instance, in hepatocellular carcinoma, Metastasis associated lung adenocarcinoma transcript 1 (MALAT1) is overexpressed compared with adjacent normal tissue and can serve as an independent prognostic factor for HCC recurrence after liver transplantation. The inhibition of MALAT1 in HepG2 cells reduces cell viability, motility, and invasiveness and sensitivity of the resistance to apoptosis [25]. In addition, lncRNA HOX transcript antisense RNA (HOTAIR) and MALAT1 has been proved to serve as a reliable prognosis marker for human cancers through meta-analysis [26, 27].

SPRY4-IT1, is derived from an intron of the Sprouty 4 (SPRY4) gene and is predicted to contain several long hairpins in its secondary structure [28]. Besides, SPRY4-IT1 is cleaved to release a mature product that localizes to the cytoplasm. It regulates levels of lipin 2, and therefore may be involved in lipid biosynthesis [29]. Emerging evidence revealed that SPRY4-IT1 can mediate cell growth, proliferation, apoptosis and invasion. *Divya* et al. found deletion of SPRY4-IT1 expression attenuates cell growth, invasion, and increases rates of apoptosis in melanoma cells. They predicted that the function of SPRY4-IT1 is likely to be related to the biological pathway of SPRY4 and raised the hypothesis that SPRY4-IT1 may also be involved in the mitogen-activated protein kinase (MAPK) signaling pathway [9]. In gastric cancer, SPRY4-IT1 was upregulated in GC tissues and than noncancerous tissues. Further investigating indicated that lncRNA SPRY4-IT1 contributes to the gastric cancer cell proliferation, migration, and invasion via regulating cyclin D1, matrix metalloproteinase 2 (MMP2) and matrix metalloproteinase 9 (MMP9) expression [17]. Some studies found downregulation of SPRY4-IT1 in significantly increased the expression of E-cadherin and meanwhile remarkably decreased the expression of fibronectin and vimentin. The results suggested that upregulation of SPRY4-IT1 promotes metastasis via induction of epithelial-mesenchymal transition (EMT) [30–32]. The discovery of prognostic factors is critical for the identificationof high-risk patients who are candidates for individual therapy. There were many studies indicated that high SPRY4-IT1 expression has a significant association with prognosis for OS in melanoma,

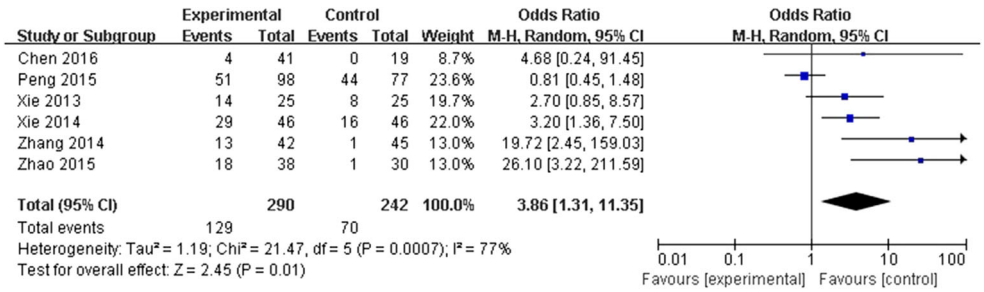

Fig. 3 Forest plot for the association between SPRY4-IT1 expression with LNM

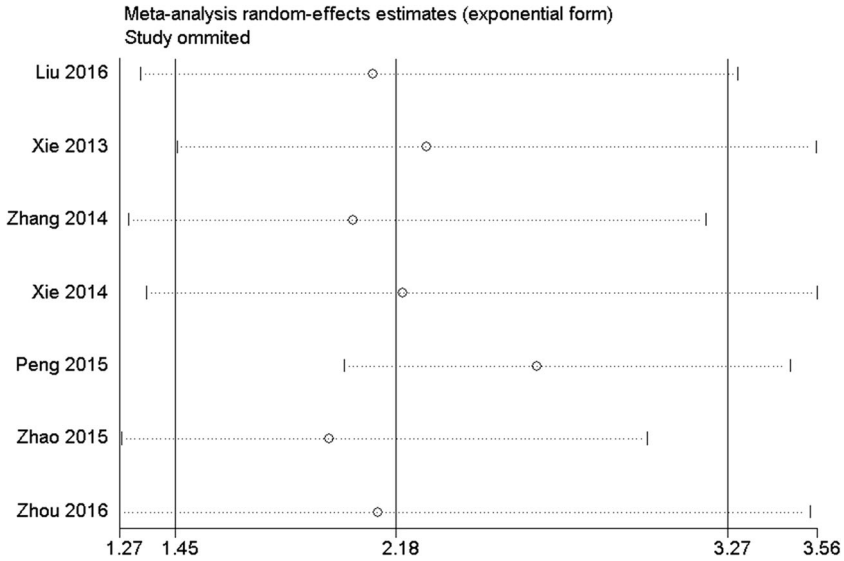

Fig. 4 Sensitivity analysis of the pooled HRs of SPRY4-IT1 expression and OS

esophageal squamous cell carcinoma, renal cell cancer, glioma, and bladder cancer [10, 12–15, 20]. However, in gastric, multivariate analyses show high SPRY4-IT1 expression has no significant correlation with prognosis and LNM [17]. To investigated the correlation of SPRY4-IT1 with prognosis and LNM, we performed this meta-analysis. In this meta-analysis, we found high expression of SPRY4-IT1 was significantly associated with unfavorable OS and LNM in cancers.

Nevertheless, The meta-analysis still has some potential limitations. First, the pooled data were calculated by different types of cancer, which may increase the heterogeneity. Second, the cut-off value of SPRY4-IT1 expression differed in included studies. Third, two studies used risk ratio (RR) as index for OS, and these studies was also included in analysis of the pooled HR for OS. Compared with HR, RR ignored the situation of censoring and loss to follow-up. Fourth, we estimated the HR and 95%CIs from the Kaplan–Meier survival curves in one study, which might influence the accuracy of result.

Conclusion

This meta-analysis investigated the correlation of SPRY4-IT1 expression levels with LNM and OS in various cancers. The result showed that high expression of SPRY4-IT1 was significantly associated with unfavorable OS in cancers (pooled HR: 2.24, 95% CI: 1.24–4.04, $p = 0.007$), and LNM (OR = 3.86, 95%CI:1.31–11.35, $P = 0.01$). These results suggesting that

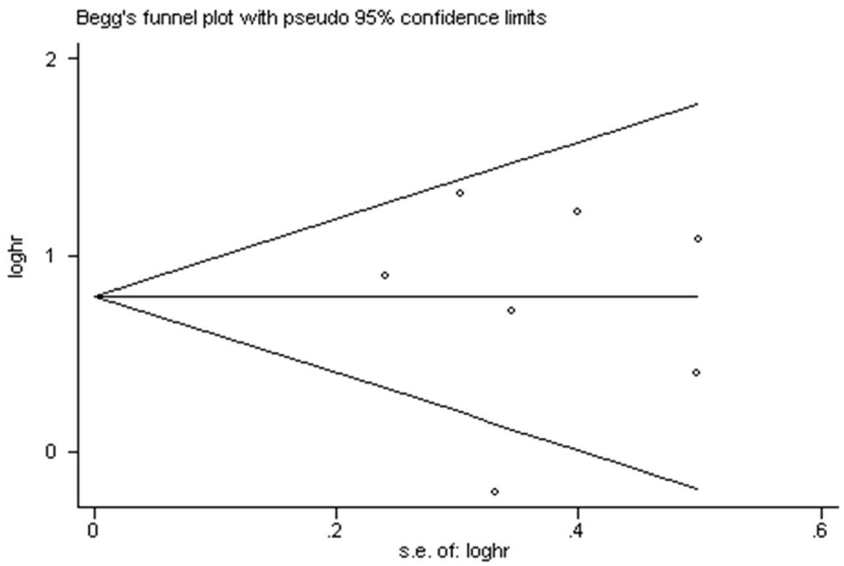

Fig. 5 Funnel plot of the publication bias for the analysis of the pooled HRs of OS

lncRNAs SPRY4-IT1 can serve as a new molecular marker for cancer prognosis and metastasis. In the future, we still need further studies to confirm its precise role in cancers.

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