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RESEARCH ARTICLE

Low expression of let-7 predicts poor prognosis in patients with multiple cancers: a meta-analysis

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Abstract The connection between microRNA expression and cancers has been identified, and microRNAs may be considered as important prognostic biomarkers. However, it is still inconsistent whether expression of let-7 can predict prognosis in patients with multiple cancers. A meta-analysis was performed by searching PubMed, EMBASE, and ISI Web of Science databases. All data were extracted from articles comparing prognosis in patients with multiple cancers having low expression of let-7 with those having high expression. Pooled hazard ratios (HRs) and corresponding 95 % confidence intervals (CIs) were calculated. Subgroup analyses were conducted for cancer type and ethnicity. A total of 1,757 cases of multiple cancers were involved for this meta-analysis. The HR of low let-7 expression in multiple cancers was 1.80 (95 % CI 1.18–2.76), and that in lung cancer was 1.99 (95 % CI 1.17–3.40). A subgroup analysis was performed on ethnicity; combined HR was 1.61 (95 % CI 0.84–3.11) for Asians and 1.94 (95 % CI 1.11–3.39) for non-Asians. Low expression of let-7 might predict poor prognosis in patients with multiple cancers, especially in lung cancer. Furthermore, let-7 might be a biomarker in non-Asian patients with favorable prognosis.

Keywords Multiple cancers · Let-7 · Prognosis · Meta-analysis

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Introduction

MicroRNAs (miRNAs) have been identified as an abundant class of small, non-coding RNAs that play vital roles in the posttranscriptional regulation of different biological processes [1], such as proliferation, apoptosis, and invasion. Recently, expression of miRNAs is found to correlate with the pathogenesis of various cancers, suggesting that miRNAs may act as molecular markers for the prognosis of cancers [2, 3].

Lots of evidence suggest that miRNAs play an important role in pathological processes of various cancers [4]. For instance, a well-known tumor suppressor, let-7, has been detected to modulate the important components of the STAT3 [5]/P53 [6] signal pathway. Increasing numbers of targets for let-7 have been investigated such as EZH2 [7] and LIN28 [8]. The rs7963551 located at the hsa-let-7 binding site may alter expression of RAD52 through modulating miRNA-mRNA interaction and contribute to the development of breast cancer in Chinese women [9]. Overexpression of let-7a can inhibit the growth of lung cancer transplanted subcutaneously in nude mice by suppression of k-Ras and c-Myc [10]. Induction of LIN28 could mediate repression of let-7 family members, promote cell cycle progression, and suppress cell proliferation [11].

In this study, we performed a meta-analysis to evaluate the relationship between let-7 expression and prognosis in patients with multiple cancers. Furthermore, we discussed the effect of let-7 as a prognostic biomarker in these diseases.

Material and methods

Study selection

We selected literatures by searching PubMed, EMBASE, and ISI Web of Science databases from 2003 to February 2013. Studies were chosen by using the following keywords or text words: "cancer," "carcinoma," "tumor," "microRNA," "let-7," "recurrence," "relapse," and "cancer prognosis." For each identified literature, additional studies were selected from its references and citations and from the PubMed option "Related Articles." The following three criteria were used to select published studies: (1) they were English language studies, (2) they had to discuss the patients with cancers, (3) they had to detect the relationship between the expression levels of let-7 and survival outcome with a follow-up time of more than 1 year. The criteria were used to exclude published studies: (1) letters and reviews and (2) lack of data information, including hazard ratio (HR) and 95 % confidence interval (CI). Zhu checked the titles, abstracts, full texts, and reference lists of the identified studies carefully.

Quality assessment

This research was systematically evaluated according to the guidelines of the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines [12, 13]. The following factors were checked: study population, country of origin, study design, method of detecting let-7, outcome, and period of follow-up. We excluded the studies without these points to ensure the quality of this meta-analysis.

Data extraction

According to the selection criteria, the data were extracted from each study. In some studies, survival data were extracted from Kaplan-Meier curves. The first author's name, year of publication, country of origin, total number of cases (*N*), follow-up time, etc. were collected in a form. Additional data were reviewed as follows: age, gender, method of detecting let-7, HRs of let-7 for prognosis, 95 % CIs, and *P* value. An HR of >1 was regarded as associated with a poorer prognosis.

Statistical analysis

In this study, the random effects model or fixed effects model was used for meta-analysis, according to the heterogeneity between studies. Heterogeneity was tested by the *Q* test ($P<0.10$ was considered indicative of statistically significant heterogeneity) and the I^2 statistic (values of 25, 50, and 75 % were considered to represent low, medium, and high heterogeneity, respectively). The fixed effects model was used when there was no significant heterogeneity ($I^2<50$ %); otherwise, the random effects model was used. *P* values were calculated by I^2 tests. All the reported *P* values were analyzed with Student's two-sided test, and *P* values <0.05 were regarded as statistically significant for all included studies.

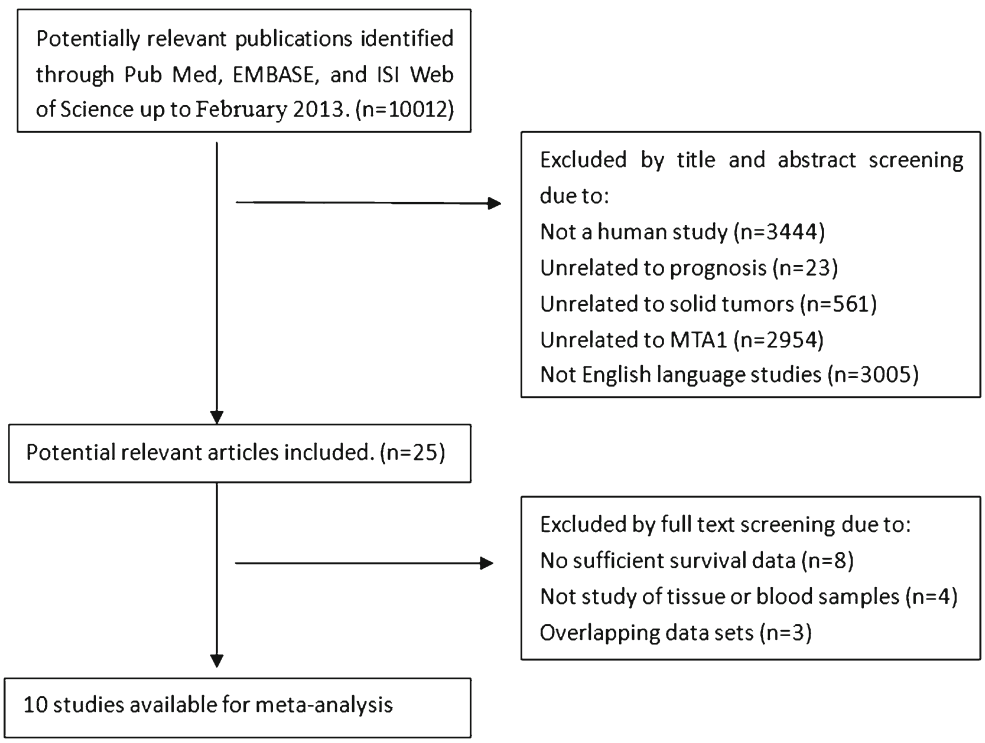

Fig. 1 Flow chart of study selection in this meta-analysis

Table 1 Summary of studies reporting let-7 expression and prognosis in multiple cancers

First author	Year	Country	Cancer	*N*	Follow-up years	Survival analysis	Median age (year)	Gender Male/ female	Stage I–II/III–IV	Histotype SC/AC/other
Takamizawa	2004	Japan	Lung	143	>5	Multivariate Cox analyses	62	53/90	94/49	25/105/13
Yanaihara	2006	USA	Lung	104	>1	Multivariate Cox analyses	67	65/39	82/22	39/65/0
Kenraro	2007	Japan	Bronchioloalveolar	66	>5	Kaplan-Meier analyses	62	33/33	49/17	NR
Yang	2008	USA	Ovarian	72	>5	Kaplan-Meier analyses	60	0/72	0/72	NR
Yu	2008	China	Lung	56	>2	Multivariate Cox analyses	67	45/11	35/21	25/25/6
Voortman	2010	USA	Lung	638	>2	Multivariate Cox analyses	60	518/120	371/267	244/218/76
Landi	2010	USA	Lung	290	>5	Kaplan-Meier analyses	67	211/79	202/88	125/165/0
Xia	2010	China	Lung	31	>3	Kaplan-Meier analyses	61	22/9	16/15	12/19/0
Kahlert	2011	Germany	Colorectal	30	>2	Kaplan-Meier analyses	68	22/8	NR	NR
Jusufovic	2012	Bosnia	Lung	327	>1	Kaplan-Meier analyses	60	NR	NR	NR

qRT-PCR quantitative real-time PCR, *NR* not reported

Calculation of dichotomous variables was carried out using the HR with the 95 % CI as the summary statistic. The Mantel-Haenszel method was used to combine HRs for the outcome parameters. Yate's correction was performed for studies containing a "zero" value in one cell for the number of positive cases in one of the two groups. Begg's test was used to evaluate the publication bias. Analyses were performed using STATA statistical software (Version 12.0).

We defined the "parent" microRNA as the root number assigned to microRNA without any annotation [14]. Here, the

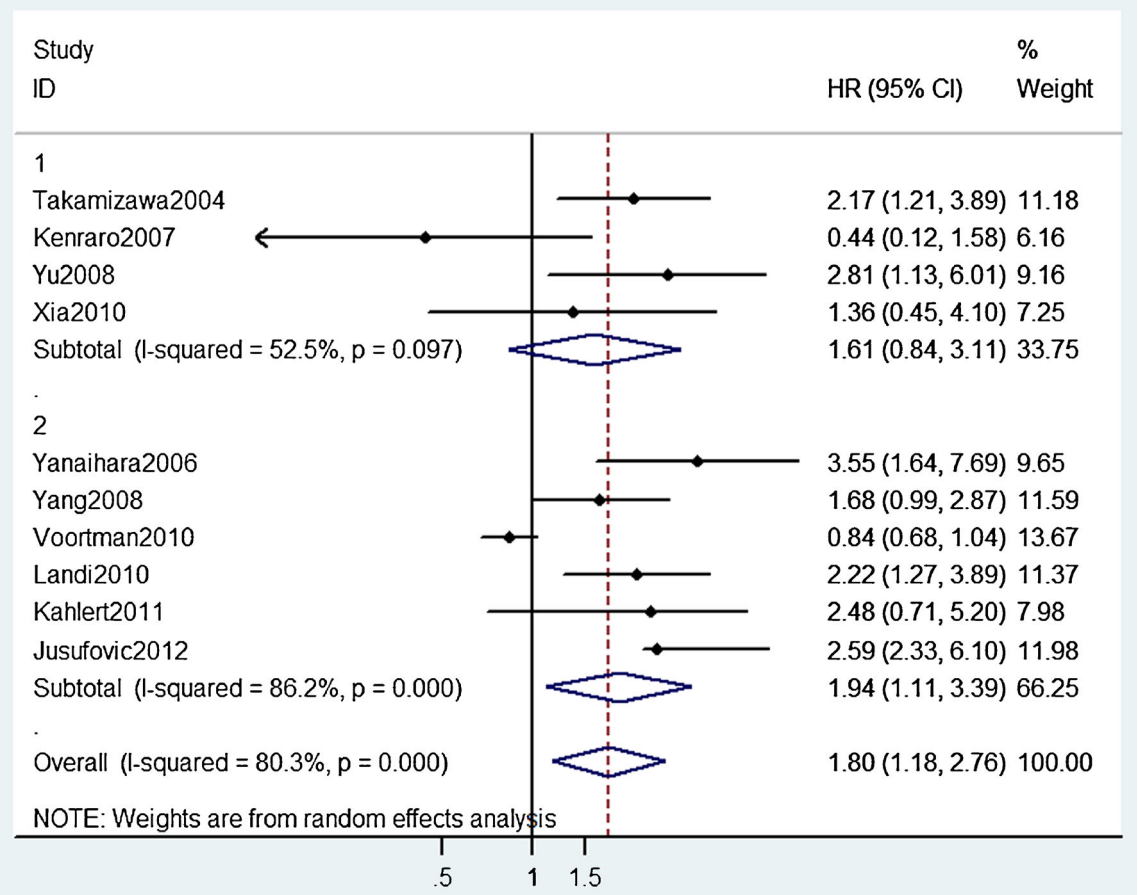

Fig. 2 Meta-analysis (forest plot) of the evaluable studies assessing the association between low expression of let-7 and prognosis of multiple cancers and by subgroup analysis for Asians (*up*)/non-Asians (*down*)

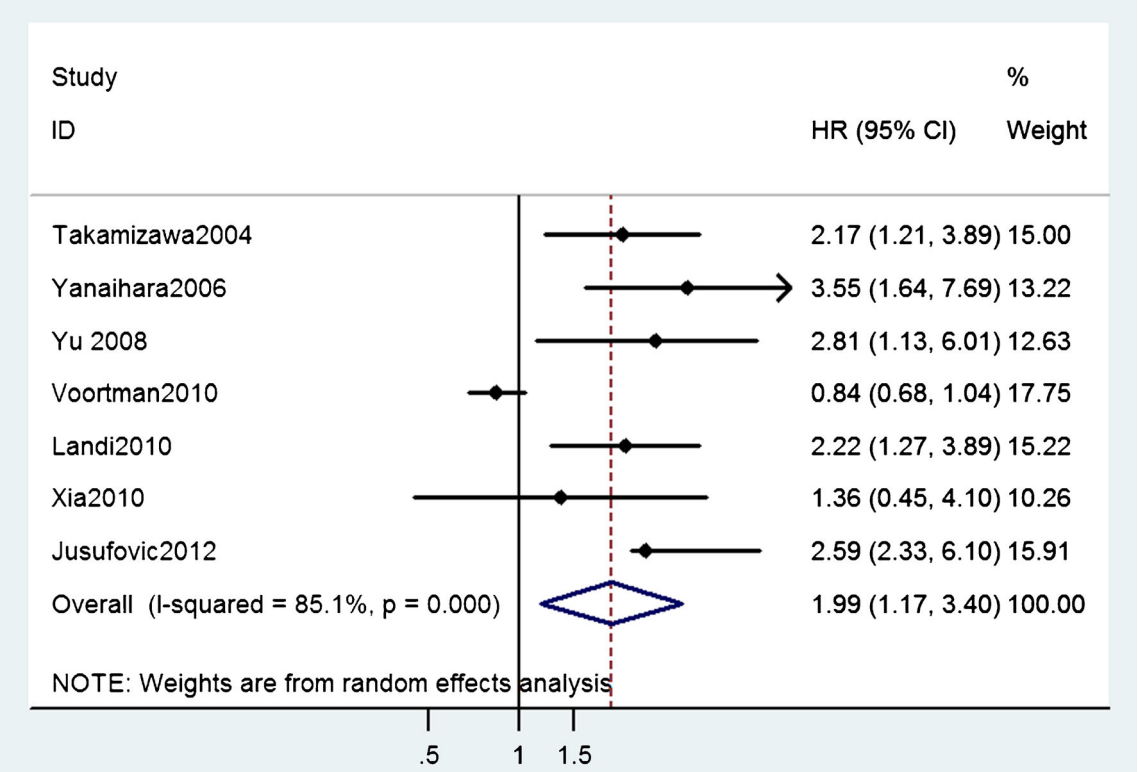

Fig. 3 Forest plot of the studies assessing the association between low expression of let-7 and prognosis of lung cancer

parent microRNA of microRNAs let-7a, let-7a-2, let-7b, let-7c, let-7d, let-7f, let-7g, and let-7i would be defined as let-7.

Results

A total of 1,757 cases of multiple cancers were collected in this meta-analysis

This meta-analysis was performed on the basis of the remaining ten studies (Fig. 1): seven that evaluated lung cancer and one each that evaluated bronchioloalveolar cancer, ovarian cancer, and colorectal cancer. All ten studies reported data that allowed for the calculation of overall survival (OS). The main information of these studies is shown in Table 1.

Low expression of let-7 predicts poor prognosis in patients with multiple cancers

The data of 1,757 patients with multiple cancers from Japan, USA, Bosnia, Germany, and China among the ten studies were summarized. The method for investigating the expression of let-7 was quantitative real-time PCR (qRT-PCR). The combined analysis of the ten studies showed that let-7 low expression was associated with worse overall survival (1.80, 95 % CI 1.18–2.76), and the heterogeneity (I^2 statistic) was 80.3 % (Fig. 2). Analysis by tumor type showed that for the seven lung cancer studies, an obvious association of let-7 low expression and survival was evident (HR=1.99, 95 % CI 1.17–3.40) (Fig. 3). Then a subgroup analysis was performed on ethnicity; no association of let-7 low expression and

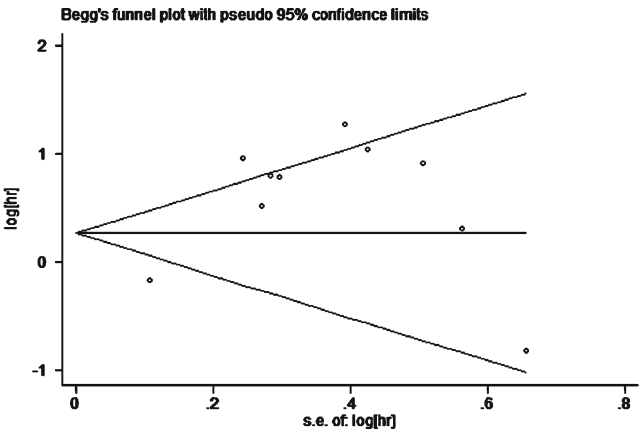

Fig. 4 Begg's funnel plot with 95 % confidence intervals for publication bias testing

survival was evident (HR=1.61, 95 % CI 0.84–3.11) for Asians; however, low expression of let-7 predicts poor prognosis in non-Asian patients (HR=1.94, 95 % CI 1.11–3.39) (Fig. 2).

Funnel plot analysis did not show any evidence of publication bias (Begg's test $z=0.89$, $P=0.371$, continuity-corrected) (Fig. 4).

Discussion

The connection between aberrant expression of microRNAs and prognosis for multiple cancers has been demonstrated in emerging studies [15, 16]. Here, we paid more attention to one microRNA (let-7) in tumors separately, collected complete articles, and pooled the prognostic value. The HR of low let-7 expression in tumors suggested a moderately strong discriminatory ability.

In previous literatures, let-7 was obviously downregulated in lung cancer. In this meta-analysis, we described that low expression of let-7 was significantly associated with poor prognosis in tumors. Empirically, HR of less than 1.5 is regarded as a weak prognostic factor [17]. The HR of low let-7 expression in multiple cancers was 1.80. However, the conclusion is not persuasive enough and needs to be discussed. For instance, the HR value of low expression of let-7 in one study [18] was 0.84 (95 % CI 0.68–1.04, $P=0.11$) and the weight was 13.67 %. This study provided the most samples ($n=638$) and may be more precise. This prompted us to detect more about the relationships between let-7 expression and prognosis in more patients with tumors.

Yet, some questions are unknown and are still poorly defined which limit the transition of microRNA applications from bench to bedside as prognostic biomarkers. For example, how much microRNAs are required to be significant prognostic biomarkers? Some classifiers included a single microRNA, whereas others were a combination of microRNAs and special genes. From our data, in the Voortman et al. 2010 study, on the population evaluable for both let-7a and KRAS ($n=582$), let-7a was a good prognostic indicator (HR=0.79 [0.64; 0.99], $P=0.04$). However, in a total of 638 samples evaluable for let-7a, this prognostic association was no longer significant (HR=0.84 [0.68; 1.04], $P=0.11$).

Furthermore, there are several limitations for this meta-analysis: It excluded non-English articles and studies that lack important survival data (e.g., HR, CI, or survival curve). There was statistical heterogeneity in the papers which may be because of the differences in the characteristics of patients, geographical distribution, technical platforms, normalization controls, and other technical issues. Publication bias in these studies may also affect the prognosis. Several microRNAs chosen without clear justification may result in imprecise prognosis. The number of studies in this paper was only five and may limit the statistical power. Luckily, standardized protocols are expected to improve the quality of this study in the future.

To sum up, this paper is not somewhat perfect due to heterogeneity, biases, and other limitations, but we have to pay attention to the candidate role of microRNAs as prognostic biomarkers. Quantitative synthesis of the articles has demonstrated that aberrant expression of let-7 is related to prognosis in patients with multiple cancers. More large-scale and standard investigations are required to contribute to the role of microRNAs in tumor prognosis and clinical application.

Conclusions

Taken together, low expression of let-7 was significantly related to poor prognosis in patients with tumors. Let-7 may be considered as an important prognostic factor in this disease. The targets of let-7 would drive us to pay more attention to the candidate role of let-7 in cancer therapy and prognosis.

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