Int J Clin Exp Med 2017;10(7):9848-9856
www.ijcem.com /ISSN:1940-5901/IJCEM0051928

Review Article

MicroRNA-21 as prognostic molecular signatures in oral cancer: a meta-analysis

Yufei Xie[1], Jinyang Wu[2]

Departments of [1]Orthodontics, [2]Oral and Cranio-Maxillofacial Surgery, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Key Laboratory of Stomatology, Shanghai 200011, China

Received March 1, 2017; Accepted April 6, 2017; Epub July 15, 2017; Published July 30, 2017

Abstract: Many studies have showed the prognostic value of microRNAs (miRNAs) in patients with oral cancer. However, many of these included a small number of patients. To increase statistical power, we performed a systematic review and meta-analysis to determine a pooled conclusion. The aim of this meta-analysis was to assess the prognostic role of miRNA-21 expression in patients with oral cancer. PubMed, Embase, Web of science and wanfang databases were searched for eligible studies with data assessing the prognostic role of miRNA-21 expression in oral cancer. Pooled hazard ratios (HRs) and their 95% confidence intervals (CI) were calculated. A total of 9 eligible studies comprising 777 patients with oral cancer were included in this meta-analysis. For overall survival, the pooled HR of higher miRNA-21 expression in patients with oral cancer was 1.71 (95% CI 1.20-2.44). After excluding one study causing heterogeneity, the combined HR was 1.93 (95% CI: 1.44-2.59). The pooled HR of higher miRNA-21 expression in patients with oral cancer were 2.65 (95% CI: 1.49-4.73), 2.04 (95% CI: 1.09-3.80) and 2.70 (95% CI: 1.08-6.76) for cancer specific survival and recurrence-free survival, respectively. This is the first rigorous pooled analysis suggested that miRNA-21 is associated with the prognosis of patients with oral cancer, and high expression of miRNA-21 can predict poor prognosis in patients with oral cancer.

Keywords: MiRNA-21, oral cancer, survival, meta-analysis

Introduction

An estimated 300,400 new cases and 145,400 deaths from oral cavity cancer occurred 2012 in worldwide [1]. Some of the major reasons contributing to the high mortality rate are late diagnosis, lack of treatment options and high prevalence of tobacco consumption [2]. In the past decade, there has been an increasing use of molecular markers in the assessment and management of oral cancer [3]. To improve the prognosis of patients, identification of useful prognostic factors is important for clinicians to choose optimal treatment for patients and improve the treatment effect.

In spite of widespread investigation of genetic biomarkers for oral cancer, epigenetic biomarkers including miRNAs received considerable attention because of the clinical and biological utility in oral cancer diagnosis and treatment [4]. MiRNAs are RNA molecules of 19-25 nucleotides in length [5]. Over the last few years, reports have shown that miRNAs could play a key role in not only the whole aspects of carcinogenic process [6]. Prognostic role of miRNAs has been shown in several tumors including colon, breast ovarian and brain cancers [7-10]. The meta-analysis also showed that miRNA-21 could be a significant biomarker in the prognosis of various cancers [10, 11]. Some miRNAs have been associated with distinctive pathologic features in oral cancer. The relationship between the expression of particular miRNAs and survival of patients with oral cancer has also been increasingly reported. However, there was still lack of evidence for the prognostic role of miRNA-21 expression in oral cancer. We thus performed a meta-analysis of studies to assess the prognostic role of miRNA-21 expression in patients with oral cancer.

Materials and methods

Search strategy

This meta-analysis was carried out in keeping with the guidelines of the Meta-analysis of

Table 1. Criteria for the inclusion of prognostic miRNA studies

Study design	Prospective or retrospective cohort design with a well-defined study population
Tumors type	Oral cancer
miRNA measure	Microarray, qRT-PCR or ISH (clear cut-off described)
Outcome Measure	OD, RFS, CSS or DFS
Analysis	Reporting of the resulting HRs including 95% CIs or data/Kaplan Meier survival curves to allow their calculation
Oral cancer stage	Any
Length of follow-up	≥ 60 months
Source	Peer-reviewed journals

qRT-PCR, quantitative real time reverse transcription PCR; ISH, in situ hybridisation; OS, overall survival; DFS, disease free survival; RFS, recurrence free survival; CSS, cancer special survival; HR, hazard ratio; CI, confidence interval.

Observational Studies in Epidemiology group (MOOSE) and Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) criteria [12]. The database of Pubmed, Embase and Web of science were searched for the last time on December 30, 2016. The search strategy included the following keywords: (microRNA-21 OR miRNA-21 OR miR-21) AND (oral cancer) AND (prognosis OR prognostic OR survival OR outcome OR mortality). There was no language limitation in the literature search. Two investigators (Y Xie and J Wu) inspected the title and abstracts of citations to identify publications appearing to report the study of oral cancer samples by miRNA expression and association with survival outcomes and obtained the full texts. Further articles were searched by hand-searching reference lists of all the articles retrieved and relevant reviews.

Eligibility criteria

The main criteria considered in including a study were investigating the prognosis of oral cancer, measuring the expression of specific miRNAs in tissue or serum and studying their association with survival outcome. The details were listed in **Table 1**. Survival outcome was further explored considering the Hazard ratio (HR) with 95% confidence interval (CI), *P* value, Kaplan-Meier curves or obtaining the required data by contacting correspondent author. Articles were excluded if they (1) were case reports, letters, commentaries, meeting records, review articles or laboratory articles; (2) concerned genetic alterations of a miRNA including polymorphisms or methylation patterns; (3) lacked sufficient data for estimating HRs and their 95% CIs, or calculated HRs based on multiple miRNAs. When duplicate studies were retrieved, we included the most informative and recent article.

Quality assessment

The quality of all the studies included was systematically determined according to a critical review checklist of the Dutch Cochrane Centre proposed by MOOSE [12]. The current checklist included the following key points: (1) clear definition outcome assessment by representing it in overall survival (OS), disease free survival (DFS), recurrence free disease (RFS) or cancer special survival (CSS); (2) clear definition of measurement miRNAs; (3) clear definition of study population and origin of country; (4) clear definition of study design; (5) clear definition of cut-off and follow-up. Any study without mentioning these points was excluded from the systematic review.

Data extraction

Data were extracted independently by two investigators (Y Xie and J Wu) Disagreements were resolved by discussion. The following data were extracted: first author's last name, year of publication, the kind of study design, origin of the study population, number of participants, the kind of samples, the method of detecting miRNA-21 expression, and follow-up duration, cut-off value; and HRs of elevated miRNAs for OS, CSS, DFS, RFS, as well as their 95% CIs and *P* values. If available, the HRs with their 95% CIs and *P* values were collected from the original article or the correspondent author. If Kaplan-Meier curves were available, we extracted data from the graphical survival plots and estimated the HRs and their 95% CI. All the calculations mentioned above were based on the methods illustrated by Parmar et al. [13] and Tierney et al. [14]. An observed HR > 1 was considered as a worse survival for the group with elevated miRNA expression. Conversely, an observed HR < 1 was considered as a worse survival for the group with decreased miRNA expression.

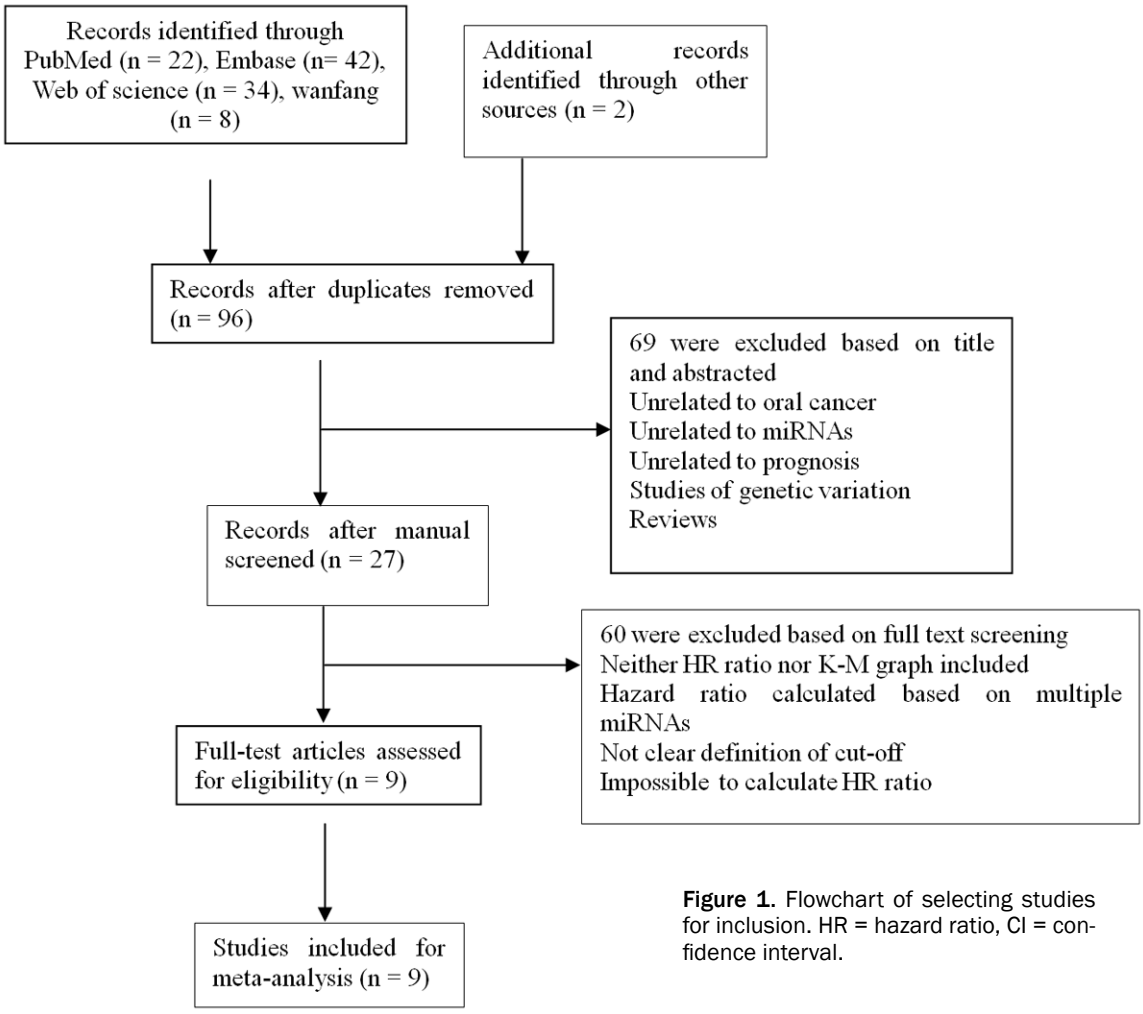

Figure 1. Flowchart of selecting studies for inclusion. HR = hazard ratio, CI = confidence interval.

Statistical analysis

Heterogeneity was conducted using Cochran's Q test and Higgins *I*-squared statistic. A *P* value of less than 0.05 was considered significant. I^2 values of > 50% indicate heterogeneity among studies. A random effect model was applied if heterogeneity was observed ($P < 0.05$). When there was no obvious heterogeneity, the fixed effect model was used ($P > 0.05$). Sensitivity analysis was performed by excluding one study by turns and examining the impact of each single study on the combined of HRs. The publication bias was assessed by funnel plot and Begg's linear regression test. All analyses were performed using Stata 13.0 software (StatCorp, College Station, TX, USA).

Results

Selection of studies

This search strategy returned 108 publications for consideration within the meta-analysis (**Figure 1**). Title and abstract assessment identified 27 manuscripts appropriate for evaluation of prognostic miRNA biomarkers in oral cancer and full text articles were obtained for these. The full text versions of these 27 publications were then retrieved and their reference lists were screened for further relevant publications. The titles and abstracts were retrieved through this search were screened by two of the authors. Finally, 9 articles remained for detailed screening and data extraction [15-23] (**Figure 1** and **Table 2**). Inclusion criteria can be seen in **Table 1**. The full texts of 9 papers (published 2009-2016) fulfilling the criteria for meta-analysis were obtained (**Table 3**).

Characteristics of the included studies

The 9 included studies analyzed 777 patients with oral cancer and correlated individual miRNA levels with survival. They had a retrospective design, and the miRNAs expression were measured in tissue samples. Detailed

Table 2. Main charateristics of the eligible studies

Study (year)	Population	Study design	Number	Assay	Cut-off	Detected sample	Survival analysis	Source of HR	Adjusted	Follow up (months)
Zheng (2016)	China	R	72	ISH	Score ≥ 2	Tissue	OS	SC	Mo	60
Li (2009)	China	R	103	qRT-PCR	Median	Tissue	OS	SC	No	68
Ko (2014)	Korea	R	167	ISH	Score ≥ 2	Tissue	CSS/RFS	Rep	Yes	210
Kawakita (2014)	Japan	R	79	ISH	Score ≥ 1	FFPE	CSS	SC	No	65
Jung (2012)	USA	R	17	qRT-PCR	Median	Tissue	OS	Rep	No	180
Hedback (2014)	Denmark	R	86	ISH	Tertile	FFPE	DFS	Rep	No	60
Ganci (2015)	Italy	R	92	qRT-PCR	Signal score	FFPE	RFS	Rep	Yes	60
Li (2013)	China	R	63	ISH	Score > 3	FFPE	OS	SC	No	150
Chang (2015)	China	R	98	qRT-PCR	Median	Tissue	OS	Rep	No	60

R, retrospective; qRT-PCR, quantitative real time reverse transcription PCR; ISH, in situ hybridisation; OS, overall survival; CSS, cancer special survival; RFS, recurrence free survival; DFS, disease free survival; HR, hazard ratio; CI, confidence interval; FFEP; Formalin-fixed paraffin-embedded; Rep = report; SC = survival curve.

Table 3. Main charateristics of the eligible studies

Study (year)	OS		CSS		DFS		RFS	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Zheng (2016)	1.22 (1.09-1.36)	0.0005						
Li (2009)	2.06 (1.21-3.51)	0.008						
Ko (2014)			2.97 (1.34-6.59)	0.007			1.66 (0.82-3.34)	0.156
Kawakita (2014)			1.16 (0.15-9.02)	0.521				
Jung (2012)	5.31 (1.39-20.38)	0.015						
Hedback (2014)					2.70 (1.1-6.9)	0.032		
Ganci (2015)							4.20 (1.1-15.98)	0.040
Li (2013)	2.13 (1.11-4.10)	-						
Chang (2015)	1.58 (1.02-2.47)	0.034						

OS, overall survival; CSS, cancer special survival; RFS, recurrence free survival; DFS, disease free survival; HR, hazard ratio; CI, confidence interval.

screening revealed that four of 9 studies did not report the HR value. All of them we were able to calculate the HR by one of methods explained in Materials and Methods section. The method of miRNA-21 expression detection was quantitative realtime polymerase chain reaction (qRT-PCR) and ISH.

Meta-analysis

Five studies described high miRNA-21 as predictive of poor OS in oral cancer (n = 353). Significant inter-study heterogeneity was found (I^2 = 64.5%, *P* = 0.024). The random model was applied to calculate a pooled HR with 95% CI. A combined HR of 1.71 (95% CI: 1.20-2.44) was found for those patients (**Figure 2A**). The *P* value of Begg's linear regression test was 0.086. The funnel plot showed that there was no obvious risk of publication bias in the meta-analysis (**Figure 3**). Sensitivity analysis by omitting one study by turns showed that the study of Zheng et al. had obvious influence of individual study on the pooled HR (**Figure 4**). After excluding the study causing heterogeneity, no significant inter-study heterogeneity was found (I^2 = 3.7%, *P* = 0.374). The fix model showed that a higher expression level of miRNA-21 was a predictor of poorer OS, with a combined HR of 1.93 (95% CI: 1.44-2.59) (**Figure 5**).

The pooled HR for CSS was 2.63 (95% CI: 1.25-5.51), for RFS was 2.04 (95% CI: 1.09-3.80) and for DFS was 2.70 (95% CI: 1.08-6.76) (**Figure 2B**). Due to the small size of this study considering CSS, RFS and DFS, no conclusive graph could be generated, and we therefore did not evaluate publication bias.

Discussion

Oral cancer is one of the 10 most common cancers in the world, with a limited screening, delayed clinical detection, poor prognosis, with-

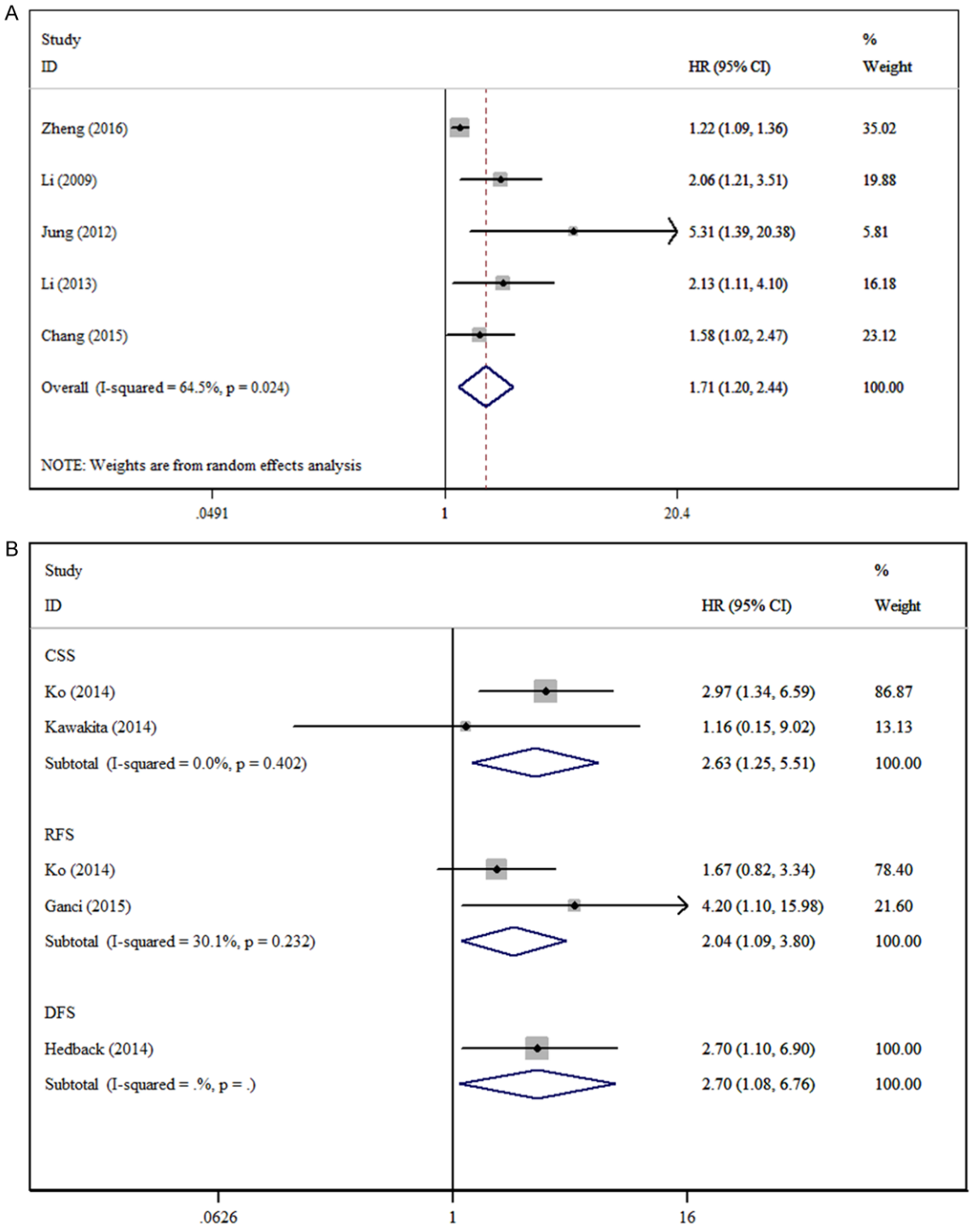

Figure 2. Forrest plots of studies evaluating HRs of aberrant miR-21 expression. A. OS; B. CSS, RFS, DFS. OS = overall survival; CSS = cancer specific survival; RFS = recurrence free survival; DFS = disease free survival.

out specific biomarkers for the disease and expensive therapeutic alternatives [24]. Though there are many new advances in the understanding of the molecular pathogenesis of oral cancer, there are still short of effective treatment and bad prognosis for patients. The prognostic factors predicting survival of oral cancer patients can help us not only get a better

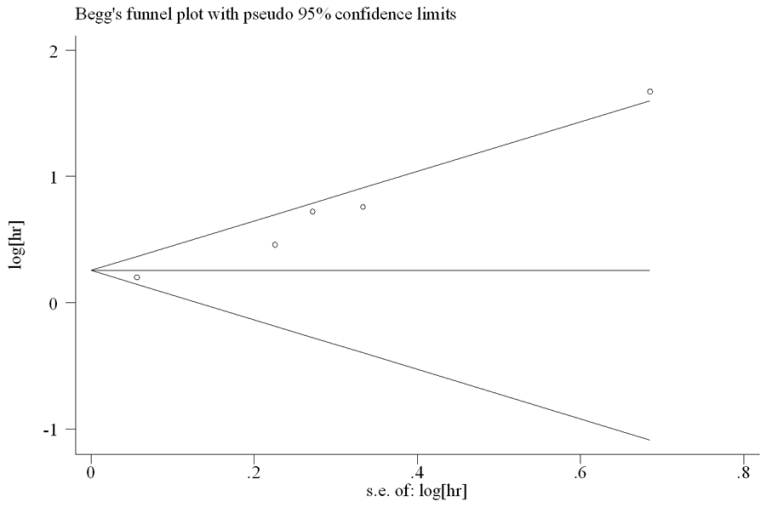

Figure 3. Funnel plot in the meta-analysis of the association between miRNA-21 expression and OS in patients with oral cancer.

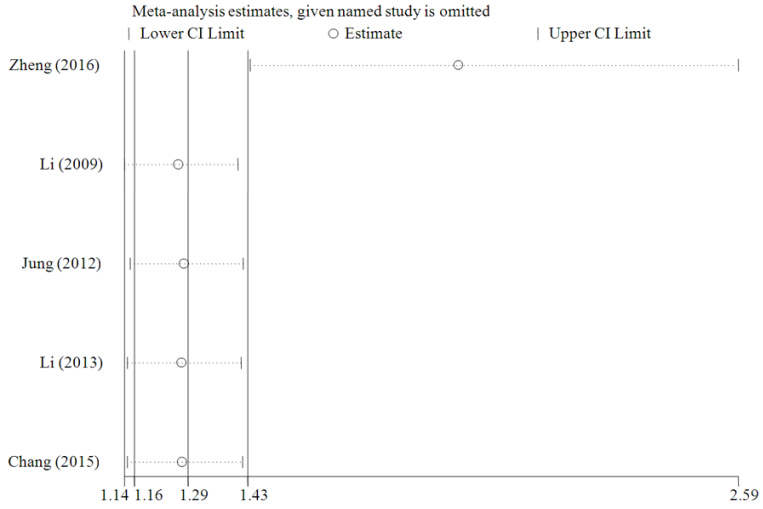

Figure 4. Forest plot in the sensitivity analysis by omitting one study by turns.

understanding of the pathogenesis of oral cancer, but provide much help in choosing optimal treatment for patients.

MiRNAs are a kind of short noncoding RNAs and they are ~22 nucleotides in length [5]. It has been clear that miRNAs can promote mRNA degradation, prevent mRNA from being translated, and thus regulate post-transcriptional expression of target genes by binding to the complementary target mRNA. MiRNAs also are believed to have important roles in the in the carcinogenesis of various cancers [25, 26]. MiRNAs can act as tumor suppressor genes or oncogenes, and thus mediate the growth, development, and progression of cancers.

miRNA-21 is the most studies miRNA in cancer and is repeatedly found to be associated with the prognosis of human cancer, such as digestive system cancer [27], breast cancer [7], or lung cancer [28]. However, there was still lack of evidence for the prognostic role of miRNA-21 expression in oral cancer. Study showed that miRNA-21 could modulate chemosensitivity of tongue squamous cell carcinomas (TSCC) cells to cisplatin by targeting PDCD4, and may serve as a potential target for TSCC therapy [29]. MiRNA-21 is an independent prognostic indicator for TSCC, and may play a role in the development of TSCC by inhibiting cancer cell apoptosis partly via TPM1 silencing [16]. We thus performed this meta-analysis of published studies to assess the prognostic role of miRNA-21 expression in patients with oral cancer.

Nine studies were finally included into the meta-analysis. Those nine studies included a total of 777 patients with oral cancer. For overall survival, the pooled HR of higher miRNA-21 expression in patients with oral tumors was 1.71 (95% CI: 1.20-2.44). After excluding the study causing heterogeneity, the fix model showed that a higher expression level of miRNA-21 was a predictor of poorer OS, with a combined HR of 1.93 (95% CI: 1.44-2.59). High miRNA-21 also predicted reduced DFS, CSS and RFS. Thus, the present meta-analysis suggests that miRNA-21 is associated with the prognosis of patients with oral cancer and high expression of miRNA-21 can predict poor prognosis in patients with oral cancer. The findings from the meta-analysis suggested that miRNA-21 is associated with the prognosis of patients with oral cancer and high expression of miRNA-

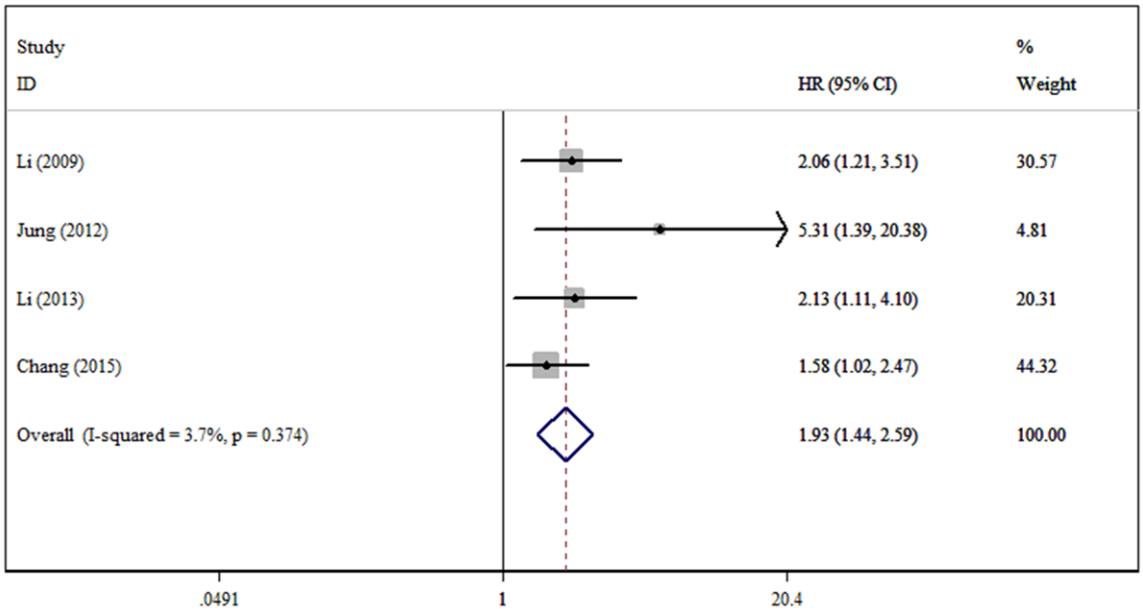

Figure 5. Forest plots of studies evaluating HRs of aberrant miR-21 expression, after omitting one study. HR = hazard ratio, CI = confidence interval.

21 can predict poor prognosis in patients with oral cancer. Thus, the findings of the meta-analysis provided new and strong evidence for the important role of miRNA-21 in the development and progression of oral cancer. It might facilitate individualise management of patients with oral cancer.

The present meta-analysis had some limitations to be considered. First, there still was limited number of studies on the prognostic role of miRNA-21 expression in oral cancer. More studies are needed to further assess the association between miRNA-21 expression and prognosis of oral cancer. Secondly, whilst the method of HR extrapolation from survival curves has been previously validated, there may have been errors due to inaccurate readings, even though two independent reviewers were used to minimise this variation. Therefore, the extrapolated HRs may be less reliable compared to published statistics. Thirdly, several important clinicopathologic factors affecting prognosis have been identified for oral cancer, but only two studies adjusted for these co-variates. Finally, the expression of miRNA-21 expression was detected in the samples of tumor tissues. The prognostic roles of miRNA levels in blood or saliva are need further studies.

In summary, the present meta-analysis suggests that miRNA-21 is associated with the prognosis of patients with oral cancer, and high expression of miRNA-21 can predict poor prognosis in patients with oral cancer. More studies with large number of patients and the miRNA levels in blood or saliva are needed to further identify the prognostic roles of miRNAs expression in oral cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Yufei Xie, Department of Orthodontics, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Key Laboratory of Stomatology, Zhizaoju Road 639#, Shanghai 200011, China. Tel: 8621-23271699-5206; E-mail: YFX2017@yeah.net

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Alnuaimi AD, Wiesenfeld D, O'Brien-Simpson NM, Reynolds EC, McCullough MJ. Oral Candida colonization in oral cancer patients and its relationship with traditional risk factors of oral

cancer: a matched case-control study. Oral Oncol 2015; 51: 139-45.
[3] Zlotogorski-Hurvitz A, Dayan D, Chaushu G, Salo T, Vered M. Morphological and molecular features of oral fluid-derived exosomes: oral cancer patients versus healthy individuals. J Cancer Res Clin Oncol 2016; 142: 101-10.
[4] Wu BH, Xiong XP, Jia J, Zhang WF. MicroRNAs: new actors in the oral cancer scene. Oral Oncol 2011; 47: 314-9.
[5] Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014; 15: 509-24.
[6] Hayes J, Peruzzi PP, Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. Trends Mol Med 2014; 20: 460-9.
[7] Pimentel F, Bonilla P, Ravishankar YG, Contag A, Gopal N, LaCour S, Lee T, Niemz A. Technology in MicroRNA profiling: circulating MicroRNAs as noninvasive cancer biomarkers in breast cancer. J Lab Autom 2015; 20: 574-88.
[8] Hollis M. MicroRNAs potential utility in colon cancer: early detection, prognosis, and chemosensitivity. World J Gastroenterol 2015; 21: 8284.
[9] Fan Y, Fan J, Huang L, Ye M, Huang Z, Wang Y, Li Q, Huang J. Increased expression of microRNA-196a predicts poor prognosis in human ovarian carcinoma. Int J Clin Exp Pathol 2015; 8: 4132-7.
[10] He XY, Liao YD, Guo XQ, Wang R, Xiao ZY, Wang YG. Prognostic role of microRNA-21 expression in brain tumors: a meta-analysis. Mol Neurobiol 2016; 53: 1856-61.
[11] Wang Y, Zhang Y, Pan C, Ma F, Zhang S. Prediction of poor prognosis in breast cancer patients based on microRNA-21 expression: a meta-analysis. PLoS One 2015; 10: e0118647.
[12] Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis of Observational Studies in Epidemiology (MOOSE) Group. JAMA 2000; 283: 2008-12.
[13] Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. Stat Med 1998; 17: 2815-34.
[14] Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials 2007; 8: 16.
[15] Zheng G, Li N, Jia X, Peng C, Luo L, Deng Y, Yin J, Song Y, Liu H, Lu M, Zhang Z, Gu Y, He Z. MYCN-mediated miR-21 overexpression enhances chemo-resistance via targeting CADM1 in tongue cancer. J Mol Med 2016; 94: 1129-41.
[16] Li J, Huang H, Sun L, Yang M, Pan C, Chen W, Wu D, Lin Z, Zeng C, Yao Y, Zhang P, Song E. MiR-21 indicates poor prognosis in tongue squamous cell carcinomas as an apoptosis inhibitor. Clin Cancer Res 2009; 15: 3998-4008.
[17] Ko YH, Won HS, Sun DS, An HJ, Jeon EK, Kim MS, Lee HH, Kang JH, Jung CK. Human papillomavirus-stratified analysis of the prognostic role of miR-21 in oral cavity and oropharyngeal squamous cell carcinoma. Pathol Int 2014; 64: 499-507.
[18] Kawakita A, Yanamoto S, Yamada S, Naruse T, Takahashi H, Kawasaki G, Umeda M. MicroRNA-21 promotes oral cancer invasion via the Wnt/beta-catenin pathway by targeting DKK2. Pathol Oncol Res 2014; 20: 253-61.
[19] Jung HM, Phillips BL, Patel RS, Cohen DM, Jakymiw A, Kong WW, Cheng JQ, Chan EK. Keratinization-associated miR-7 and miR-21 regulate tumor suppressor reversion-inducing cysteine-rich protein with kazal motifs (RECK) in oral cancer. J Biol Chem 2012; 287: 29261-72.
[20] Hedback N, Jensen DH, Specht L, Fiehn AM, Therkildsen MH, Friis-Hansen L, Dabelsteen E, von Buchwald C. MiR-21 expression in the tumor stroma of oral squamous cell carcinoma: an independent biomarker of disease free survival. PLoS One 2014; 9: e95193.
[21] Ganci F, Sacconi A, Manciocco V, Sperduti I, Battaglia P, Covello R, Muti P, Strano S, Spriano G, Fontemaggi G, Blandino G. MicroRNA expression as predictor of local recurrence risk in oral squamous cell carcinoma. Head Neck 2016; 38: E189-E97.
[22] Chang C, Du J, Li E, Yang Y. Expression of MicroRNA-21 in oral squamous cell carcinoma and its correlation with prognosis. J Oral Sci Res 2015; 31: 927-30.
[23] Li S, Zhou X, Zhang Q, Zhang L. Abnormal expression of STAT3 and miRNA-21 in oral squamous cell carcinoma. Chinese Journal of Clinical Oncology 2013; 40: 323-7.
[24] Rivera C. Essentials of oral cancer. Int J Clin Exp Pathol 2015; 8: 11884-94.
[25] Zhang JX, Song W, Chen ZH, Wei JH, Liao YJ, Lei J, Hu M, Chen GZ, Liao B, Lu J, Zhao HW, Chen W, He YL, Wang HY, Xie D, Luo JH. Prognostic and predictive value of a microRNA signature in stage II colon cancer: a microRNA expression analysis. Lancet Oncol 2013; 14: 1295-306.
[26] Ueda T, Volinia S, Okumura H, Shimizu M, Taccioli C, Rossi S, Alder H, Liu CG, Oue N, Yasui W, Yoshida K, Sasaki H, Nomura S, Seto Y, Kaminishi M, Calin GA, Croce CM. Relation between microRNA expression and progression and

prognosis of gastric cancer: a microRNA expression analysis. Lancet Oncol 2010; 11: 136-46.

[27] Ye TT, Yang YL, Liu XY, Ji QQ, Pan YF, Xiang YQ. Prognostic value of circulating microRNA-21 in digestive system cancers: a meta-analysis. Int J Clin Exp Med 2014; 7: 873-8.

[28] Boeri M, Pastorino U, Sozzi G. Role of microRNAs in lung cancer: microRNA signatures in cancer prognosis. Cancer J 2012; 18: 268-74.

[29] Ren W, Wang X, Gao L, Li S, Yan X, Zhang J, Huang C, Zhang Y, Zhi K. MiR-21 modulates chemosensitivity of tongue squamous cell carcinoma cells to cisplatin by targeting PDCD4. Mol Cell Biochem 2014; 390: 253-62.