RESEARCH COMMUNICATION

Prognostic Role of MicroRNA-21 in Non-small Cell Lung Cancer: a Meta-analysis

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Abstract

    <u>Introduction</u>: Many studies have reported that microRNA-21 (miR-21) mihght predict the survival outcome in non-small cell lung cancers (NSCLCs) but the opposite opinion has also been expressed. The aim of this study was to summarize the evidence for a prognostic role of miR-21. <u>Materials and Methods</u>: All the eligible studies was searched by Medline and EMBASE and patients' clinical characteristics and survival outcome were extracted. Then a meta-analysis was performed to clarify the prognostic role of the miR-21 expression in different subgroups. <u>Results</u>: A total of 8 eligible articles were yielded covering survival outcomes or clinical characteristics. The combined hazard ratio (HR) and 95% confidence interval (95% CI) for overall survival (OS) was 2.19 [0.76, 6.30], while the combined HR (95% CI) of Asian group for OS had a significant result, 5.49 [2.46, 12.27]. The combined HR (95% CI) for recurrence free survival or disease free survival (RFS/DFS) was 2.31 [1.52, 3.49]. Odds ratios (ORs) showed that the miR-21 expression was associated with lymph node status and histological type. <u>Conclusion</u>: miR-21 expression could predict the prognostic outcome of NSCLC in Asians, despite some deficiencies in the study data.

Keywords: MicroRNA-21 - lung cancer - prognosis - survival outcome - meta-analysis

Introduction

    Lung cancer is the most common cause of cancer mortality in the world. Approximately 1.6 million new cases of lung cancer will be diagnosed and 1.4 million deaths will occur from lung cancer during 2008. Among all cases suffered lung cancer, non-small-cell lung cancer (NSCLC) approximately accounts for 80% (Ramalingam et al., 1998; Jemal et al., 2011)

    MicroRNAs (miRNAs) are endogenous, small non-coding and have a length of 18-25 nucleotides RNAs. These miRNAs could identify post transcriptional gene regulators that paired to complementary sequences in the 3' untranslated region (3' UTR) of target mRNAs, leading to mRNA degradation or translational repression (Bartel 2004; Bartel and Chen 2004).

    Many studies have reported that over expression of microRNA-21 (miR-21) play important roles in increasing cell proliferation, migration, invasion and survival (Lu et al., 2008; Yang et al., 2011). Relatively speaking suppression or knock-down of miR-21 could induce apoptosis and repress cell proliferation and invasion (Zhang et al., 2010). The miR-21 was also found to be elevated in many cancers such as lymphoma, prostate cancer, colorectal cancer and breast cancer (Lawrie et al., 2007; Siva et al., 2009; Qian et al., 2009; Nielsen et al., 2011). However, the expression of miR-21 in NSCLC and the prognostic significance remains unclear.

    The aim of this study is to comprehensively and quantitatively summarize the evidence for the use of miR-21 to predict the clinical results of NSCLC patients. And we also want to evaluate the overall risk of elevated miR-21 for survival in patients with NSCLC.

Materials and Methods

    Medline and EMBASE were searched for the last time on Apr 10, 2012. The search strategy included the following keywords variably combined by "microRNA-21", "miR-21", "lung cancer" and "NSCLC".

Study inclusion/exclusion criteria

    Studies were considered eligible if they met all of the following inclusion criteria, (i) discussed patients with NSCLC (ii) measured the miR-21 expression in tumor and serum;(iii) investigated the survival outcome or the correlation between miR-21 expression and the clinical variables. Studies were excluded based on any of the following reasons, (i) were review articles, laboratory articles or letters (ii), described the survival outcome of other tumors or other markers, (iii) lacked key information for calculation with methods developed by Parmar, Williamson , and Tierney (Parmar et al., 1998; Williamson et al., 2002; Tierney et al., 2007), (iv) the articles from one

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author and the studies brought into the repeated samples from the same patients .

Data Extraction

Eligible articles were reviewed independently by two investigators (Ma XL and Liu Lei). Disagreements were resolved by consensus. Multivariate Cox hazard regression analysis reported in the article was included in the our analysis; if these data were not available, we extracted univariate Cox hazard regression analysis or log-rank p value and Kaplan–Meier survival curves of survival outcomes instead. Above primary information had been extracted by two investigators (Ma XL and Liu XX) independently. Additional data were extracted from the studies included first author, publication year, study size, patients age and sexuality, smoker or not, TNM stage, lymph status, histological classification, methods to detect miR-21, positive miR-21 definition, the attitude conclusion and other clinical characteristics.

Statistical Methods

All these HRs and 95% confidence interval(CI) were calculated following Tierney's method and the logHR and SE (logHR) (SE) were used for aggregation of the survival results, but these statistical variables were not given directly in most studies. We calculated the necessary statistics on the basis of a available numerical data with methods developed by Parmar, Williamson, and Tierney. Calculation was accomplished by the software designed by Matthew Sydes and Jayne Tierney with these methods (Medical Research Council Clinical Trials Unit, London, UK) (Tierney et al., 2007).

We also examine the correlation between miR-21 expression and the clinical variables including TNM stage, lymph node status, histological type, sexuality and smoking status. According to clinical characteristics, Stage I and Stage II were combined and Stage III and Stage IV were combined. Odds ratio (OR) was used as the measure index to describe the correlation (CORNFIELD 1951).

Forrest plots were used to estimate the effect of miR-21 expression on survival outcome and the correlation between miR-21 expression and the clinical variables. Heterogeneity was defined as p<0.10 or I2>50%. When homogeneity was fine (p≤0.10, I^2≤50%), a fixed effect model was used for secondary analysis. If not, a random effect model was used (Higgins et al., 2003). An observed HR>1 indicated worse outcome for the positive group relative to the negative group and would be considered statistically significant if the 95% CI did not overlap 1.The Begg's rank correlation also was applied to assess the potential publication bias, p>0.05 was considered that there was no potential publication bias (Begg, 1994). All above calculations were performed using RevMan 5.1 (Cochrane collaboration, Oxford, UK) Publication biases were evaluated using the Begg's funnel plot by STATA 11.0 (STATA Corporation, College Station, TX).

Results

Eligible Studies

The initial search returned 42 studies in PubMed and EMBASE. Following review of these abstracts, 13 potentially relevant studies were identified as eligible for full-text review. 5 studies were excluded, because these studies were short of the necessary data for calculation. One article from Saito M (Saito et al., 2011) gave the CSS for Maryland/Norway cohort, and the recurrence free survival (RFS) for Japan cohort. Because there was only one study for CSS, we just used the RFS for Japan cohort in our meta-analysis. We combined the results disease free survival (DFS) and RFS together as RFS/ DFS. There were 3 articles from Gao W, one article used RFS to describe the survival outcome (Gao et al., 2012), while another two used OS (Gao et al., 2010, 2011). We exclude one article for overall survival (OS) in the meta-analysis, because Gao W referred in the article

Table 1. Clinical Characteristics of Included Studies

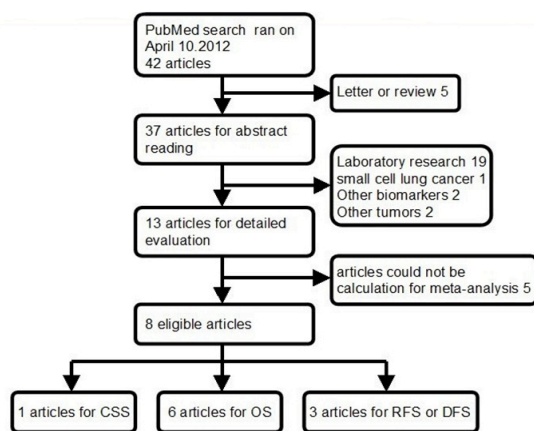| auther	Population	N	Positive	Sampling site	male	smoker	TNM stage	LNM	histological	method	cut-off	Survival analysis	Multivariate analysis	conclusion
Markou A 2008	Greece	48	25	tumor	—	44	I/II32 III/IV16	25	ADC 25 AQC 23	qRT-PCR	2	OS, DFS	No	Positive
Voortman J 2010	France	631	316	tumor	—	—	I/II369 III262	76	ADC 218 AQC 341 others 72	qRT-PCR	median	OS	Yes	Negative
Saito M 2011 Positive	USA and Norwa	126	62	tumor	66	109	I78 II27 III21	—	all ADC	qRT-PCR	USA 2.11 Norway 2.78	CSS	No	No
	Japan	191	95		92	96	I42 II49 III0	—			3.11	RFS	No	Positive
Gao W 2010	China	47	—	tumor	—	31	I 22 II12 III13	22	ADC 21 AQC 26	qRT-PCR	1.4	OS	Yes	Positive
Gao W 2012	China	58	29	tumor serum	32	—	I16 II15 III26	32	ADC 33 AQC 24	qRT-PCR	1.5	DFS	Yes	Positive
Gao W2 2010	China	30	15	tumor	25	24	I 17 II/III 13	11	—	qRT-PCR	1.47	OS	Yes	Positive
Liu XG 2011	China	70	48 42	tumor serum	56	46	I 36 II/III/ IV34	32	ADC 34 AQC 36	qRT-PCR	2	OS	No	Negative
Wang ZX 2011	China	88	49	serum	42	50	I/II 47 III 41	35	ADC 37 AQC 21 others 30	qRT-PCR	5	OS	Yes	Positive

ADC, adenocarcinoma; AQC, squamous cell carcinoma; LNM, lymph node metastasis; CSS, cancer-specific mortality; DFS, disease-free interval; OS, overall survival; RFS, relapse free survival; qRT-PCR, Quantitative reverse transcriptase PCR

Table 2. Meta-analyses of miR-21 Expression to Predict the Survival Outcome

	Survival outcome	Study n.	Patient n.	Model	HR(95% Cl)	P value	Heterogeneity (I², p)	Conclusion
miR-21 total	OS	4	796	Random	2.19 [0.76, 6.30]	0.15	88%, < 0.0001	negative
	RFS/DFS	3	297	Fixed	2.31 [1.52, 3.49]	< 0.0001	0%, 0.56	positive
miR-21 Asian	OS	2	117	Fixed	5.49 [2.46, 12.27]	< 0.0001	0%, 0.59	positive
miR-21 non Asian	OS	2	679	Random	0.89 [0.72, 1.10]	0.27	86%, 0.007	negative
Serum Asian	OS	2	158	Fixed	2.08 [1.55, 2.80]	< 0.00001	13%, 0.28	positive

Table 3. Meta-analyses of miR-21 Expression Classified by Clinical Characteristics

	Analysis	Study n.	Patient n.	Model	OR(95% CI)	p value	Heterogeneity (I², p)	Conclusion
	TNM stage (III/IV vs. I/II)	4	824	Random	1.19 [0.55, 2.58]	0.66	73%, 0.01	negative
	Lymph node (N3/N4 vs.N1/N2)	5	845	Fixed	1.80 [1.24, 2.60]	0.002	0%, 0.98	positive
miR-21	Sexuality (male vs. female)	3	176	Fixed	1.09 [0.59, 2.02]	0.78	0%, 0.51	negative
	Histological differentiation (ADC vs. SQC)	4	722	Fixed	0.60 [0.44, 0.81]	0.0008	0%, 0.72	positive
	Smoking (yes vs.no)	3	166	Fixed	0.79 [0.38, 1.62]	0.08	0%, 0.76	negative

Figure 1. Selection of Studies

Figure 2. Estimated Hazard Ratios (HRs) Summary for (A) overall survival with miR-21 expression in tumor tissue, (B) disease free survival or recurrence free survival with miR-21 expression in tumor tissue, and (C) overall survival with miR-21 expression in serum
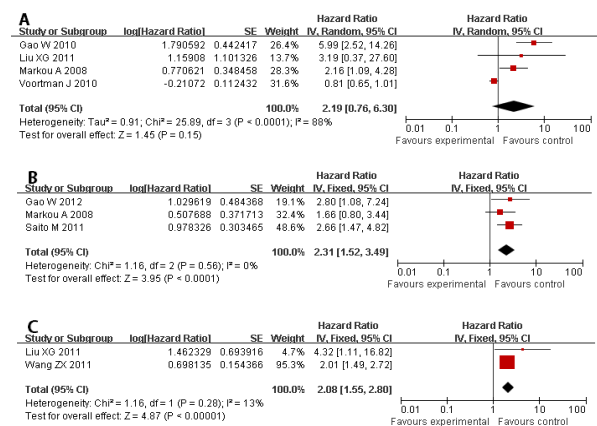
that they used the repeated data have been defined and described previously (Gao et al. 2011). Liu XG's study (Liu et al., 2011) gave the HRs and CI both in the tumor and the serum, we used the correspond data in different meta-analysis. Finally, we enrolled 8 eligible articles containing survival outcomes (Markou et al., 2008; Gao et al., 2010 2011, 2012; Voortman et al., 2010; Liu et al., 2011; Saito et al., 2011; Wang et al., 2011), respectively, 6 articles (Markou et al., 2008; Gao et al., 2010, 2011, 2012; Voortman et al., 2010; Saito et al., 2011) for tumor, and 2 articles (Liu et al., 2011; Wang et al., 2011) for serum in our meta-analysis (Figure 1).

These eligible studies were published from 2008 to 2012. The 8 eligible studies included a total of 1163 patients with a median number of 145 patients per study. The patients' clinical characteristics and other useful information have been extracted in Table 1.

Correlation between miR-21 expression and survival outcome (OS and RFS/DFS)
Overall Analyses

The meta-analysis of all studies for OS showed no significant prognostic effect on miR-21 detected in tumor samples. The combined HR (95% CI) of 4 studies (Markou et al., 2008; Gao et al., 2010; Voortman et al., 2010; Liu et al., 2011) for OS was 2.19 [0.76, 6.30] (n=796, I² = 88%, P<0.0001), and the HR (95% CI) of 3 studies (Markou et al., 2008; Saito et al., 2011; Gao et al., 2012) for RFS/DFS

was 2.31 [1.52,3.49] (n=297, I² = 0%, P=0.56). The pooled HR for serum miR-21 was 2.08 [1.55, 2.80] (n=158, I² = 13%, P=0.28) (Liu et al., 2011; Wang et al., 2011). All the above meta-analysis used the fixed effect model except the HR for OS (Table 2, Figure 2).

Subgroup analysis

When we grouped the meta-analysis by the patients' nation, we found that the combined HR of Asian studies (Gao et al., 2010; Liu et al., 2011) was 5.49 [2.46, 12.27] (n=128, I² = 0%, P=0.59), while the combined HR of other yielded non-Asian studies (Markou et al., 2008; Voortman et al., 2010) was 1.25 [0.48, 3.25] (n=679, I² = 86%, P=0.007) (Table 2). We also tried to use the other grouping term to examine the prognostic role of miR-21, such as histological type, TNM stage, and lymph status. No result could give clinical significance.

Correlation between miR-21 expression and clinical characteristics

The studies which referred the correlation between miR-21 expression and some clinical characteristics (TNM stage, lymph metastasis, sexuality, histological type and smoking status) have been pooled to calculate
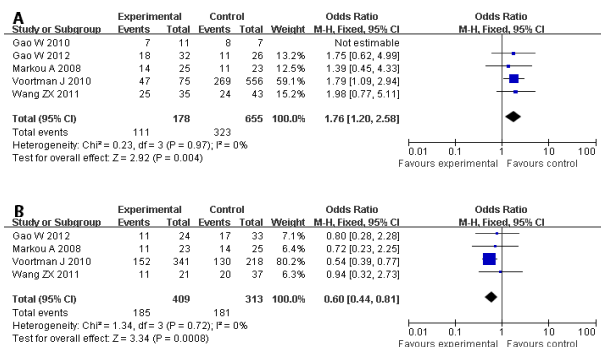
Figure 3. Estimated Odds Ratios (ORs) Summary for Correlation of (A) miR-21 expression and lymph node status, (B) miR-21 expression and histological differentiation

the ORs. In our study we did not find miR-21 expression had significant correlation with the TNM stage (Markou et al., 2008; Voortman et al., 2010; Jemal et al., 2011; Wang et al., 2011; Gao et al., 2012), sexuality (Gao et al., 2011, 2012; Wang et al., 2011) and smoking status (Markou et al., 2008; Gao et al., 2011; Wang et al., 2011) in the eligible studies. The ORs and the corresponding CIs were 1.19 [0.55, 2.58], 1.09 [0.59, 2.02] and 0.79 [0.38, 1.62], respectively. Meanwhile, the correlation results for lymph metastasis (Markou et al., 2008; Voortman et al., 2010; Gao et al., 2011, 2012; Wang et al., 2011) and histological type (Markou et al., 2008; Voortman et al., 2010; Wang et al., 2011; Gao et al., 2012) were evidently significant (Figure 3). The ORs and long-rank p-value of studies for lymph metastasis and histological type were 1.80 [1.24, 2.60], p=0.004 and 0.60 [0.44, 0.81], p=0.0008, respectively. All these results could be reviewed in Table 3.

Assessment of publication bias

Begg's test was used to examine publication bias. No significant publication biases were found in results of meta-analyses of miR-21 prediction value for OS both using tumor and serum samples (P=0.497 and P=0.317 respectively). There was also no publish bias in the studies for RFS/DFS (P=0.602) (Figure 4).

Discussion

As we know, it was the first time that a comprehensive and detailed meta-analysis revealed the prognostic role of tumor and serum miR-21 for NSCLC. The prognostic role of miR-21 expression was still a great puzzle according to the evidence-based medicine in our study.

The prognostic values of miR-21 have been proven in the recent meta-analysis in head and neck squamous cell carcinoma (HNSCC) and carcinomas in digestion system. Our result of HR for OS was 2.19 [0.76, 6.30]. The interval of HR overlapped 1, it showed that the miR-21 expression had no prognostic significance on NSCLC. The subgroup analysis showed that the HR for Asian group was 5.49 [2.46, 12.27], it suggested that the miR-21 expression played significant prognostic role on NSCLC. Meanwhile, the combined HR for non-Asian group was 1.25 [0.48, 3.25],it suggested that the miR-21 was no find the effect to predict survival outcome in non-Asian group.
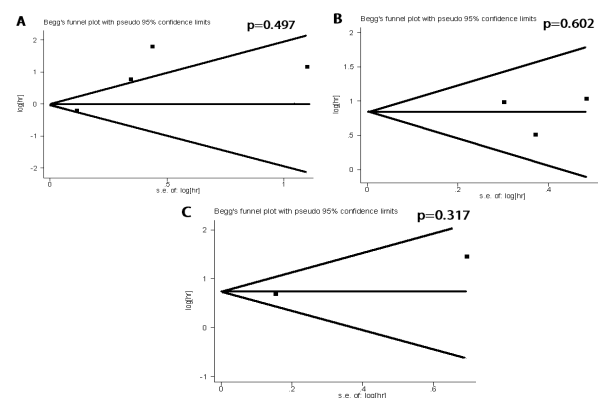

Figure 4. Funnel Plots of Publication Bias Summary for Corresponding Meta-analysis in Figure 2. Orderly, they are Funnel plots of publication bias for meta-analysis of hazard ratios (HRs) for (A) overall survival with miR-21 expression in tumor tissue (B)disease free survival or recurrence free survival with miR-21 expression in tumor tissue, and (C) overall survival with miR-21 expression in serum

The HR for RFS/DFS to predict the survival outcome was statistically significant. As referred in Hayes (Hayes et al. 2001), a prognostic factor with RR>2 is considered as useful practical value. All the meta-analysis except the pooled result for OS had a well result. It may suggest that detected miR-21 expression in NSCLC patients could predict their prognosis practically. However, Voortman J's study (Voortman et al. 2010) indicated that the miR-21 expression patterns examined were neither predictive nor prognostic. And this study have the greatest patients cohort with radically resected NSCLC. This clinical discovery was opposite to the recent laboratory studies, these studies most considered the miR-21 as a risky microRNA for lung cancer (Diederichs and Haber 2006; Wang et al., 2009; Hatley et al., 2010). This puzzle could be solved when much more studies were conducted to confirm clinical value of the miR-21 expression. And the prognostic role of miR-21 for Asian group could be confirmed by adequately multi-center designed prospective studies in Asian country in future. We could also find the serum miR-21 could play prognostic role on NSCLC (HR 2.08[1.55, 2.80]). This result indicated that serum miR-21could be a diagnostic tool to detect the NSCLC. Some clinical studies and review have been proven the application of the microRNA (Liang 2008; de Planell-Saguer M and Rodicio, 2011; Wei et al., 2011).

In the correlation study of miR-21 expression with patients' clinical characteristics, TNM stage, sexuality and smoking status showed complete no correlation with miR-21 expression while ORs for lymph metastasis and histological type were significant.The miR-21 have been proven to regulates the metastatic behavior in both mouse and human by activating many signal ways (Gao et al., 2012). These mechanism indicated that miR-21might be correlated with the metastasis. Much more clinical studies were required to research the correlation between miR-21 and metastatic factors.

Significant heterogeneity was found in the meta-analysis for OS of the prognostic role of miR-21 ($I^2 = 88\%$, P<0.0001). To exclude technique biases, subgroup analyses were performed by the country group, detecting

method, and histological classification. These entire attempts could not eliminate the heterogeneity. The heterogeneity came from the Voortman J's study. This study had a opposite survival outcome. When we removed this study, the adjusted HR was 3.37 [1.60, 7.13] ($I^2 = 39\%$, P=0.19). It suggested that the miR-21 expression could predict prognostic outcome in lung cancer.

In another article, we found that the software we used before, designed by Matthew Sydes and Jayne Tierney retained only percentile when calculated the logHR and SE. To eliminate this bias, we designed a new software following Tierney's method by Lei Deng. The publication biases were additional problem for the meta-analysis. Fortunately, the Begg's test showed no significant publication bias in this study (p>0.05).

In conclusion, the meta-analysis suggested that it was still a puzzle whether the miR-21 expression could predict the poor prognosis. Meanwhile our meta-analysis showed that serum miR-21 expression had a prognostic role on NSCLC. The miR-21 expression might have some correlation with the metastasis behavior in the NSCLC, though the p-value were on borderline. Future adequately multi-center designed prospective with larger sample size were of great value to confirm these findings.

Acknowledgements

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