MicroRNA-155 Expression has Prognostic Value in Patients with Non-small Cell Lung Cancer and Digestive System Carcinomas

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Abstract

Objective: Published data have shown that microRNAs (miRNAs) could play a potential role as diagnostic and prognostic indicators in cancers. Data for the predictive value of microRNA-155 are inconclusive. The aim of the present analysis was therefore to evaluate the role of miR-155 in prognosis for patients with a variety of carcinomas. Methods: Relevant studies were identified by searching PubMed and EMBASE. Data were extracted from studies comparing overall survival (OS), recurrence-free survival (RFS) or cancer-specific survival (CSS) in patients with carcinoma with higher miR-155 expression and those with lower levels. The pooled hazard ratios (HRs) and 95% confidence intervals (CIs) of miR-155 for clinical outcome were calculated. Results: A total of 15 studies were included. The pooled hazard ratio (HR) for OS of higher miR-155 expression in cancerous tissue was 1.89 (95% CI: 1.20-2.99, P =0.006), which could markedly predict poorer survival in general cancer. For RFS/CSS, elevated miR-155 was also associated with poor prognosis of cancer (HR= 1.50, 95% CI: 1.10-2.05, P = 0.01). On subgroup analysis, the pooled HR for OS in non-small cell lung cancer (NSCLC) was 2.09 (95% CI: 1.48-6.24, P =0.003) and 2.61 (95% CI: 1.98-3.42, P<0.05), respectively. Conclusions: The results indicated that the miR-155 expression level plays a prognostic role in patients with cancer, especially NSCLCs and digestive system carcinomas.

Keywords: Cancer - MiR-155 - prognosis - biomarker - meta-analysis

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Introduction

MicroRNAs are highly conserved, small, single-stranded non-coding RNAs of 19-24 nucleotides in length that were first identified in 1993 (Lee et al., 1993). They can regulate gene expression at a post-transcriptional level and play a significant role in regulation of cell development, metabolism, immunity, proliferation, differentiation, and apoptosis. Published data found that miRNA are involved in carcinogenesis as either oncogenes or tumor suppressors (Ambros et al., 2003; Chen, 2005), and many cancer-related microRNAs have been identified functionally (Calin et al., 2006). Evidences from clinical data indicated that many miRNAs were upregulated or downregulated in diverse carcinomas, and their expression levels associated with the stage of diseases (Ferracin et al., 2010; Nana-Sinkam et al., 2010). Hence, these miRNAs hold great promise as potential biomarkers for cancer prognosis. Evidences from laboratory studies have found that miR-155 is over expressed in a variety of malignant diseases and that it plays a significant role in the process of carcinogenesis (Esquela-Kerscher et al., 2006).

Recent studies have showed that miR-155 gene is one of the miRNAs most consistently over-expressed in both hematopoietic malignancies and solid tumors, such as leukemia (O’Connell et al., 2008; Raponi et al., 2009), thyroid carcinoma (Nikiforova et al., 2008), breast cancer (Iorio et al., 2005; Greither et al., 2010), cervical cancer (Wang et al., 2008), colorectal cancer (Chen et al., 2012), pancreatic ductal adenocarcinoma (PDAC) (Lee et al., 2007; Szafranska et al., 2007), hepatocellular carcinoma (Han et al., 2012; Huang et al., 2012), and lung cancer (Yanaihara et al., 2006; Donnem et al., 2011). Therefore, overexpression miR-155 may be a general characteristic of cancer and be detected as a biomarker for cancer diagnosis and prognosis. However, prognostic role of miR-155 expression in cancers remained inconclusive. In this study, we conducted a systematic review and meta-analysis to pool the overall hazard ratios (HRs) of elevated miR-155 for survival in patients with carcinomas.
Materials and Methods

Literature search strategy

Original articles studying the prognostic value of microRNA-155 in carcinomas were searched with PubMed, EMBASE. Studies were selected by using the following search terms: ‘cancer or carcinoma or neoplasm or tumor’ , ‘microRNA-155 or miR-155’ , ‘prognosis or prognostic or outcome’ . Original and review articles published until February 2013 were sought, considering the latter as an additional source of original works otherwise overlooked. References of the retrieved articles were further screened for earlier original studies. The eligible reports were identified by three reviewers (Xu, Han and Xia), and controversial studies were adjudicated by a fourth reviewer (Shu).

Selection criteria

The inclusion criteria were the following: (1) studied the patients with any types of carcinomas, (2) detected the expression of miR-155 in tissue or serum, (3) analyzed the association between miR-155 expression levels and clinical prognosis, and (4) published in English. Articles were excluded from the analysis if met the following criteria: (1) non-English reports, (2) analysis of a set of miRNAs but not miR-155 alone, (3) no dichotomous miR-155 expression groups and (4) absence of key information such as hazard ratio (HR), 95% CI and P value.

Date extraction

We followed the guidelines of a critical checklist of the Dutch Cochrane Centre proposed by Meta-analysis of Observational Studies in Epidemiology (MOOSE) to guarantee the quality of the meta-analysis (Stroup et al., 2000). The extracted data information included the following: (1) first author’s last name, publication year; (2) characteristics of the studied population: sample size, sample source, disease, stage of disease and histological type; (3) miR-155 assessment and cut-off values; (4) follow-up time; (5) HR of elevated miR-155 for overall survival (OS), recurrence-free survival (RFS) or cancer-specific survival (CSS), as well as their 95% confidence interval (CI) and P value. If the HRs and their 95% confidence interval were not available directly, the total numbers of observed deaths/cancer recurrences and the numbers of samples in each group were extracted to calculate HR (Tierney et al., 2007). If only Kaplan-Meier curves are available, data were extracted from the graphical survival plots and estimation of the HR was then performed using the described method (Tierney et al., 2007).

Quality assessment

We assessed the methodological quality of included studies based on Newcastle-Ottawa scale (NOS) for quality of case-control and cohort studies (Stang et al., 2010). The NOS contains eight items, categorized into three dimensions including selection, comparability, and depending on the study type-outcome (cohort studies) or exposure (case-control studies). A star system of the NOS (range, 0-9 stars) has been developed for the assessment. The highest quality studies are awarded a maximum of one star for each item with the exception of the item related to comparability that allows the assignment of two stars. The highest value for quality assessment was 9 stars (Table 1).

Statistical methods

To identify the prognostic effect of microRNA-155, we evaluated the overall hazard ratios (HRs) and 95% confidence intervals (CIs) of eligible data for elevated miR-155. A HR equal to 1 indicates a lack of association between miR-155 and clinical outcome. A HR greater than 1 corresponds to worse outcome for increased miR-155, while HR less than 1 represents better prognosis. A test of heterogeneity of combined HRs was carried out using Cochran’s Q test and Higgins I-squared statistic. A P value of <0.05 was considered statistically significant. A random effect model was applied if heterogeneity was observed (P<0.05 or I² > 50%), while the fixed effect model was used in the absence of between-study heterogeneity (P>0.05). Sensitivity analyses were performed to check the stability of meta-analysis by comparing the results of
Table 2. Main Characteristics of All Studies Included in the Analysis

<table>
<thead>
<tr>
<th>Studies population</th>
<th>Origin of disease</th>
<th>N</th>
<th>Stage</th>
<th>microRNA assay</th>
<th>Cut-off</th>
<th>Source of samples</th>
<th>Survival analysis</th>
<th>Hazard ratio</th>
<th>Follow up (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huang et al. (2012)</td>
<td>Taiwan HCC</td>
<td>216</td>
<td>I-IV</td>
<td>qRT-PCR</td>
<td>NR</td>
<td>Tissue</td>
<td>RFS</td>
<td>R</td>
<td>High:110 Low:140</td>
</tr>
<tr>
<td>Han et al. (2012)</td>
<td>China HCC</td>
<td>100</td>
<td>I-IV</td>
<td>qRT-PCR</td>
<td>median</td>
<td>FFPE</td>
<td>OS, RFS</td>
<td>R</td>
<td>High:94 Low:100</td>
</tr>
<tr>
<td>Yamaizawa et al. (2006)</td>
<td>USA NSCLC-ADC</td>
<td>64</td>
<td>I-IV</td>
<td>qRT-PCR</td>
<td>mean</td>
<td>tissue</td>
<td>OS</td>
<td>R</td>
<td>High:68 Low:74</td>
</tr>
<tr>
<td>Ishihara et al. (2012)</td>
<td>Japan ATL</td>
<td>35</td>
<td>NR</td>
<td>qRT-PCR</td>
<td>median</td>
<td>plasma</td>
<td>OS, DE</td>
<td>R</td>
<td>High:29.6 Low:18.6</td>
</tr>
<tr>
<td>Papakostas et al. (2012)</td>
<td>Greece PC</td>
<td>88</td>
<td>I-IV</td>
<td>qRT-PCR</td>
<td>mean</td>
<td>FFPE, DE</td>
<td>OS, DE</td>
<td>R</td>
<td>High:40 Low:80</td>
</tr>
<tr>
<td>Shibuya et al. (2010)</td>
<td>Japan CRC</td>
<td>156</td>
<td>I-IV</td>
<td>qRT-PCR</td>
<td>mean</td>
<td>FT</td>
<td>OS</td>
<td>R</td>
<td>60</td>
</tr>
<tr>
<td>Rapioni et al. (2009)</td>
<td>USA SCLC</td>
<td>54</td>
<td>I-IV</td>
<td>qRT-PCR</td>
<td>median</td>
<td>SFT</td>
<td>OS</td>
<td>R</td>
<td>60</td>
</tr>
<tr>
<td>Chen et al. (2012)</td>
<td>China BC</td>
<td>92</td>
<td>I-IV</td>
<td>qRT-PCR</td>
<td>median</td>
<td>FT</td>
<td>OS, RFS</td>
<td>R</td>
<td>66</td>
</tr>
<tr>
<td>Greither (2010)</td>
<td>Germany PDAC</td>
<td>55</td>
<td>I-IV</td>
<td>qRT-PCR</td>
<td>median</td>
<td>SFT</td>
<td>CSS</td>
<td>DE</td>
<td>61</td>
</tr>
<tr>
<td>Shinomi et al. (2012)</td>
<td>Japan RC</td>
<td>137</td>
<td>I-IV</td>
<td>qRT-PCR</td>
<td>median</td>
<td>FFPE, DE</td>
<td>CSS</td>
<td>R</td>
<td>High:188 Low:164</td>
</tr>
<tr>
<td>Monsalve et al. (2012)</td>
<td>Spain B-cell lymphomas</td>
<td>57</td>
<td>I-IV</td>
<td>qRT-PCR</td>
<td>median</td>
<td>FFPE</td>
<td>RFS</td>
<td>R</td>
<td>1-196</td>
</tr>
<tr>
<td>Saito et al. (2011)</td>
<td>USA NSCLC-ADC</td>
<td>89</td>
<td>I-III</td>
<td>qRT-PCR</td>
<td>median</td>
<td>FT, DE</td>
<td>CSS</td>
<td>R</td>
<td>80</td>
</tr>
<tr>
<td>Voortman et al. (2010)</td>
<td>Multicentre NSCLC</td>
<td>639</td>
<td>I-III</td>
<td>qRT-PCR</td>
<td>median</td>
<td>FFPE</td>
<td>OS</td>
<td>R</td>
<td>96</td>
</tr>
<tr>
<td>Donnem et al. (2011)</td>
<td>Norway NSCLC-SCC,</td>
<td>187</td>
<td>I-III</td>
<td>ISH</td>
<td>median</td>
<td>FFPE</td>
<td>CSS, DE</td>
<td>86 (48-216)</td>
<td></td>
</tr>
<tr>
<td>Jung et al. (2009)</td>
<td>USA NSCLC-ADC, DBLCL, ABC-DBLCL</td>
<td>212</td>
<td>I-III</td>
<td>NR</td>
<td>median</td>
<td>FFPE</td>
<td>OS, DE</td>
<td>146 Low:140</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: OS, overall survival; RFS, recurrence-free survival; CSS, cancer-specific survival; HCC, hepatocellular carcinomas; ATL, adult T-cell leukemia; PC, pancreatic cancer; CRC, colorectal cancer; SCLC, small cell lung cancer; BC, breast cancer; PDAC, pancreatic ductal adenocarcinomas; RC, renal cancer; ADC, adenocarcinoma; SCC, squamous cell carcinoma; NSCLC, non-small cell lung cancer; DLBCL, diffuse large B cell lymphoma; ABC, activated B-cell-like; ISH, in situ hybridization; qRT-PCR, quantitative real-time polymerase chain reaction; FFPE, formalin-fixed, paraffin embedded; R, reported; NR, not reported; DE, data-extrapolated

Results

Identification of relevant studies

A total of 82 records for miR-155 and cancer prognosis were identified from a primary literature search in PubMed and EMBASE. After manually screening the titles, abstracts and key words, 55 studies were out of our scope because they were review articles, letters, non-English articles, laboratory studies or studies irrelevant to the current analysis. Of the 27 candidate studies, three studies investigated a set of miRNAs but not miR-155 alone; and nine others lacked the key survival data. A total of 15 studies remained met the inclusion criteria.

Characteristics of studies included in the main analysis

A total of 15 studies were used in the pooled analysis. The main features of identified studies were listed in Table 2. Sample sizes in each ranged from 35 to 639 patients. We collected data from 15 reports including a total of 2463 subjects from the United States, Spain, Greece, Norway, Germany, China, Taiwan and Japan. The patients were of a wide variety of carcinomas, including lung cancer, renal cancer, breast cancer, pancreatic cancer, hepatocellular cancer, colorectal cancer, leukemia and lymphoma. Of all the reports, five studies included a total of 1355 patients assessed lung cancer; and seven studies with a total of 616 subjects evaluated digestion system cancers, including pancreatic ductal adenocarcinoma, colorectal cancer and hepatocellular cancer. The quantitative real-time polymerase chain reaction (qRT-PCR) assay was used in 14 studies, whereas in situ hybridizations (ISH) assay was used in only one study. Frozen or formalin-fixed, paraffin-embedded (FFPE) tumor tissues were used in 14 studies, while one study used plasma as samples. Notably, the cut-off values of miR-155 were different in each study, with median applied in eleven studies and mean used in other studies. All hazard ratios (HRs) were extracted from univariate analysis in each report.

Figure 1. Forrest Plots of Studies Evaluating Hazard Ratios of High miR-155 Expression vs. Low Expression.

Survival data are reported as overall survival (A) and recurrence-free survival or cancer-specific survival (B) in various kinds of carcinomas.
Overall association of miR-155 conditions with the risk of OS and RFS/CSS

For studies evaluating OS, there appeared to have heterogeneity between studies for miR-155 (P < 0.05). Hence, a random model was applied to calculate a pooled HR and its 95% CI. We found that higher expression levels of miR-155 significantly predicted poorer OS, with the pooled HR being 1.89 (95% CI: 1.20-2.99, P = 0.006) (Figure 1A). For studies evaluating RFS/CSS, a random model was also applied because of the heterogeneity between studies. MiR-155 over-expression was also significantly correlated to RFS/CSS, with the combined HR being 1.50 (95% CI: 1.10-2.05, P = 0.01) (Figure 1B). Then subgroup analysis was conducted in two types of common cancer, lung cancer and carcinomas of digestive system. In subgroup of lung cancer, we pooled HR for OS using random effect model, showed statistically insignificant (HR, 2.09; 95% CI, 0.68-6.41, P=0.199; P<0.05 for heterogeneity, I² = 41%) (Figure 2A). While, the combined HR for RFS/CSS indicated that higher expression of miR-155 was correlated to poor survival with the estimated HR being 1.50 (95% CI: 1.10-2.05, P = 0.01) (Figure 2B). In subgroup of patients with digestive system cancer, the pooled HRs for OS and RFS/CSS were 3.04 (95% CI: 1.48-6.24, P=0.003; P=0.009 for heterogeneity, I² = 79%) and 2.61 (95% CI: 1.98-3.42, P=0.05; P=0.17 for heterogeneity, I² = 40%), respectively (Figure 3A, Figure 3B). The result indicated that overexpression of miR-155 was significantly associated with worse clinical outcome in patients with digestive system cancer.

Sensitivity Analysis

In order to compare the differences and to evaluate the sensitivity of the meta-analyses, we also performed the results of the fixed-effect model and are almost consistent with the random-effect model, suggesting stability of the meta-analysis (data not shown).

Publication bias

Publication bias of all included studies for was evaluated by funnel plots and Egger’s tests. The funnel plots were almost symmetric and the P values in OS and RFS/CSS meta-analysis of Egger’s regression intercepts were 0.123 and 0.760, respectively. Hence, there was no evidence for significant publication bias in the meta-analysis, because their P values were > 0.05 (Figure 4A, Figure 4B).

Discussion

Emerging studies have indicated that aberrant expression of microRNAs played a significant role in diagnosis and prognosis for malignant diseases. Most of the protective microRNAs such as let-7 family are down-regulated, while the risky microRNAs including miR-155 are up-regulated in carcinoma (Esquela-Kerscher et al., 2006; Wang et al., 2009). Moreover, overexpression microRNAs such as miR-21 and miR-155 were more common than others in malignant diseases. Recent
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The author(s) declare that they have no competing interests.
References
