Original Article

Association of rs6505162 polymorphism in pre-miR-423 with cancer risk: a meta-analysis based on 5,891 cases and 7,622 controls

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Received October 8, 2016; Accepted April 8, 2017; Epub June 15, 2017; Published June 30, 2017

Abstract: MicroRNAs (miRNAs) function as negative gene regulators by inhibiting translation or cleaving target mRNAs, for which they are recognized as oncogenes or tumor suppressors. Single nucleotide polymorphisms (SNPs) in miRNAs are closely related with cancer risks. Several studies have evaluated the association of rs6505162 polymorphism in pre-miR-423 with cancer susceptibility. However, the results remain conflicting rather than conclusive. We conducted a meta-analysis of 9 studies that included 5,891 cases and 7,622 controls to identify this association. The meta-analysis showed that the pre-miR-423 rs6505162 polymorphism was not statistically associated with cancer risks in all genetic models. In the stratified analysis of cancer types, the variant AA (CC vs. AA: OR=0.651, 95% CI: 0.482-0.878, P=0.005) and CA/AA genotypes (CC vs. CA+AA: OR=0.644, 95% CI: 0.446-0.931, P=0.019) were associated with a decreased risk of breast cancer compared with wild-type CC genotype. The same association in the allelic contrast (C vs. A: OR=0.808, 95% CI: 0.699-0.934, P=0.004) was also observed. However, an increased risk of lung cancer was found in the co-dominant (CC vs. AA: OR=1.850, 95% CI: 1.049-3.263, P=0.034) and recessive (CC vs. CA+AA: OR=1.364, 95% CI: 1.074-1.732, P=0.011) models. Furthermore, according to the stratified analysis of ethnicity, we found a highly significant association in the Caucasian population (CC vs. AA: OR=0.651, 95% CI: 0.482-0.878, P=0.005; CC vs. CA+AA: OR=0.644, 95% CI: 0.446-0.931, P=0.019; C vs. A: OR=0.808, 95% CI: 0.699-0.934, P=0.004), but no significant association in Asians and other ethnicities. In summary, this meta-analysis suggests a significant association between pre-miR-423 rs6505162 polymorphism and risk of breast cancer and lung cancer. To some extent, this polymorphism is closely related to cancer susceptibility in Caucasians. However, further large-scale case-control studies between this polymorphism and cancer risks are needed in the future.

Keywords: Meta-analysis, miR-423, polymorphism, cancer risk

Introduction

MicroRNAs (miRNAs) are a class of endogenous, small and non-coding RNA molecules (approximately 21-25 nucleotides in length), which negatively regulate gene expression at the post-transcriptional level [1, 2]. Mature miRNAs primarily target the 3’ untranslated region (3’UTR) of their target mRNAs, leading to the translation suppression or the target mRNAs degradation [3, 4]. Numerous miRNAs have been discovered in humans [5, 6]. A single miRNA could bind to the mRNAs with about 200 genes [7]. It has been predicted that half of all protein-coding genes in mammals could be regulated by miRNAs [8]. Meanwhile, massive miRNAs have been considered as key gene regulators in diverse biological pathways, including cell differentiation, proliferation, apoptosis and tumorigenesis [2, 9, 10].

Recent studies have shown that both aberrant expression and genetic variation in miRNAs are closely associated with cancer risk, diagnosis, prognosis, and drug response [11-14]. Single nucleotide polymorphism (SNP) is one of the most common types of genetic variation among the miRNA genes [15]. Previous studies have demonstrated that various SNPs in miRNAs, such as miRNA-146a, miRNA-499 and miRNA-
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Intriguingly, an important polymorphism in the pre-miR-423 with a change of C to A (rs6505162) has been identified [19]. As yet, a number of case-control studies have been conducted to investigate the association between this polymorphism and various cancer risks in diverse populations. However, these results remain controversial and ambiguous. For example, Smith found that the CC genotype of the rs6505162 SNP in pre-miR-423 offered a reduced risk of breast cancer development [20], while Ryan demonstrated that there was no obvious association between rs6505162 polymorphism and esophageal squamous cell carcinoma [21]. Hence, we performed this meta-analysis to combine all the eligible studies to identify whether rs6505162 polymorphism in pre-miR-423 contributes to overall cancer risk, and further evaluate the influence of cancer type and ethnicity.

Materials and methods

Identification of eligible studies

We searched the PubMed and EMBASE databases (last updated on July 14, 2016) for all articles on the association between pre-miR-423 polymorphism and cancer risk. The keywords used for search included “miR-423, pre-miR-423 or rs6505162”, “polymorphism or mutation” and “cancer or tumor”. This literature retrieval work was performed by two independent investigators Wei Liu and Jinjin Ran. There was no limitation on publication years while the searching work went on. References of related studies and reviews were manually retrieved for additional studies.

Inclusion and exclusion criteria

All the studies must meet all the following criteria: (1) case-control study; (2) the association between pre-miR-423 polymorphism and cancer risks; (3) available genotype frequency; and (4) cancers diagnosed by histopathology. The major exclusion criteria were: (1) duplication of the previous publications; (2) the subjects in control group were high-risk individuals with some gene mutations; and (3) abstracts, letters, reviews or editorial articles. When a study reported the results on different ethnicities, we treated them as separate studies.

Data extraction

The two investigators independently extracted all data of eligible studies with selection criteria. The following items were collected: name of the first author; year of publication; country of origin; ethnicity; cancer types; source of control (population- or hospital-based); number of cases and controls; genotype frequency in cases and controls; Hardy-Weinberg equilibrium (HWE) of control subjects. Discrepancies and differences were resolved by discussion and consensus.

Statistical analysis

To begin with, HWE was assessed by the goodness-of-fit chi-square test in controls of each study. Odds ratio (OR) with 95% confidence intervals (95% CI) was used to assess the strength of association between miR-423 rs6505162 polymorphism and cancer risks. The significance of the pooled OR was determined by the Z-test, and P<0.05 was consid-
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Table 1. Characteristics of studies in the meta-analysis

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Ethnicity</th>
<th>Cancer type</th>
<th>Genotyping</th>
<th>Source of controls</th>
<th>Cases (n)</th>
<th>Controls (n)</th>
<th>$P$ value For HWE$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smith</td>
<td>2012</td>
<td>Australia</td>
<td>Caucasian</td>
<td>Breast cancer</td>
<td>Taqman</td>
<td>HB$^a$</td>
<td>24</td>
<td>95</td>
<td>60</td>
</tr>
<tr>
<td>Wang*</td>
<td>2013</td>
<td>South Africa</td>
<td>Black</td>
<td>Esophageal cancer</td>
<td>Taqman</td>
<td>PB$^d$</td>
<td>16</td>
<td>128</td>
<td>207</td>
</tr>
<tr>
<td>Wang</td>
<td>2013</td>
<td>South Africa</td>
<td>Mixed$^b$</td>
<td>Esophageal cancer</td>
<td>Taqman</td>
<td>PB$^d$</td>
<td>14</td>
<td>84</td>
<td>89</td>
</tr>
<tr>
<td>Yin</td>
<td>2013</td>
<td>China</td>
<td>Asian</td>
<td>Esophageal cancer</td>
<td>RT-PCR</td>
<td>HB$^b$</td>
<td>425</td>
<td>207</td>
<td>19</td>
</tr>
<tr>
<td>Ma</td>
<td>2014</td>
<td>China</td>
<td>Asian</td>
<td>Hepatocellular cancer</td>
<td>MassARRAY</td>
<td>HB$^b$</td>
<td>643</td>
<td>313</td>
<td>30</td>
</tr>
<tr>
<td>Zhu</td>
<td>2015</td>
<td>Kazakh Turks</td>
<td>Esophageal cancer</td>
<td>MassARRAY</td>
<td>PB$^d$</td>
<td>99</td>
<td>122</td>
<td>21</td>
<td>109</td>
</tr>
<tr>
<td>Shen</td>
<td>2015</td>
<td>China</td>
<td>Asian</td>
<td>Esophageal cancer</td>
<td>SNaPshot</td>
<td>PB$^d$</td>
<td>920</td>
<td>421</td>
<td>59</td>
</tr>
<tr>
<td>Yin</td>
<td>2016</td>
<td>China</td>
<td>Asian</td>
<td>Lung cancer</td>
<td>Taqman</td>
<td>HB$^b$</td>
<td>389</td>
<td>166</td>
<td>20</td>
</tr>
<tr>
<td>Jiang</td>
<td>2016</td>
<td>China</td>
<td>Asian</td>
<td>Gastric cancer</td>
<td>MassARRAY</td>
<td>HB$^b$</td>
<td>593</td>
<td>255</td>
<td>32</td>
</tr>
</tbody>
</table>

$^a$The article reported by Wang was regarded as two independent studies according to ethnicity; $^b$Mixed: major ancestral components from the indigenous Khoisan, Bantu-speaking Africans, Europeans and Asians; $^c$HB: hospital-based; $^d$PB: population-based; $^e$HWE: Hardy-Weinberg equilibrium.

The pooled ORs were calculated for dominant model (CC vs. AC+AA), recessive model (CA+CC vs. AA), co-dominant model (CC vs. AA), co-dominant model (CA vs. AA) and allele model (C vs. A), respectively. Subgroup analyses were performed by ethnicity and cancer types. Heterogeneity among studies was tested by Chi square-based Q test and $I^2$ [22]. When heterogeneity exists (based on $P>0.05$), a random effect model was used for the meta-analysis. Otherwise, a fixed-effects model was employed [23]. In addition, we used Begg’s funnel plot and Egger’s test to evaluate the publication bias ($P<0.05$ was considered a significant publication bias) [24]. Sensitivity analysis was conducted by deleting one study at a time to examine the influence of individual data set on the pooled ORs. All statistical analyses were performed with STATA software version 11.0 (STATA Corporation, College Station, TX, USA).

Result

Characteristics of studies

According to the inclusion and exclusion criteria above mentioned, a total of 71 articles were identified from the PubMed and EMBASE databases using different combinations of keywords. After preliminarily screening the title and abstract, 47 studies uncorrelated with cancer risk and SNPs were excluded and 25 articles were evaluated in detail. Finally, a total of 9 case-control studies met our inclusion criteria [20, 21, 25-32], including 5,891 cases and 7,622 controls for assessing the association between rs6505162 polymorphism in pre-miR-423 and cancer risk. The detailed selection process was shown in Figure 1. One article of these studies reported by Wang contained two case-control studies, respectively in Black and Mixed Ancestry population. This article was considered as two independent studies according to ethnicity. Cancer cases were diagnosed histologically or pathologically in all studies. Controls in 6 studies were hospital-based and those in the other studies were population-based. A variety of genotyping methods were applied including Taqman, RT-PCR, MassARRAY and SNaPshot. Genotype distribution of controls in all studies was consistent with HWE (Table 1).

Meta-analysis results

The association between rs6505162 polymorphism and cancer risk was analyzed in the 9 eligible studies. As shown in Table 2, no significant association was found in all genetic models. We further performed stratification analysis based on different cancer types and ethnicities.

In the stratification analysis of cancer types (Figure 2), the results showed that the variant AA (OR=0.651, 95% CI: 0.482-0.878, $P=0.005$) and CA/AA (OR=0.644, 95% CI: 0.446-0.931, $P=0.019$) genotypes were associated with a decreased risk of breast cancer compared with the wild-type CC genotype. We observed the same association in the allelic contrast (C vs. A: OR=0.808, 95% CI=0.699-0.934, $P=0.004$). However, a significantly increased risk of lung cancer was found to be associated with the variant AA (OR=1.850, 95% CI: 1.049-3.263, $P=0.034$) and CA/AA (OR=1.364, 95% CI: 1.074-1.732, $P=0.011$) genotypes compared
<table>
<thead>
<tr>
<th>Comparisons</th>
<th>No.</th>
<th>CA vs. AA</th>
<th>CC vs. AA</th>
<th>CC+CA vs. AA</th>
<th>CC vs. CA+AA</th>
<th>C vs. A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td>p^2 (%)</td>
<td>OR (95% CI)</td>
<td>p^2 (%)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>1.119 (0.983-1.274)</td>
<td>0.088b</td>
<td>0.0</td>
<td>1.133 (0.864-1.484)</td>
<td>0.367^</td>
</tr>
<tr>
<td>Cancer type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esophageal cancer</td>
<td>5</td>
<td>1.122 (0.944-1.333)</td>
<td>0.191^</td>
<td>9.0</td>
<td>1.282 (0.899-1.828)</td>
<td>0.170^</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>2</td>
<td>0.977 (0.751-1.270)</td>
<td>0.860^</td>
<td>0.0</td>
<td><strong>0.651 (0.482-0.878)</strong></td>
<td>0.005^</td>
</tr>
<tr>
<td>Digestive tract cancer</td>
<td>2</td>
<td>1.293 (0.913-1.833)</td>
<td>0.148^</td>
<td>0.0</td>
<td>1.263 (0.901-1.772)</td>
<td>0.176^</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>1</td>
<td>1.417 (0.788-2.547)</td>
<td>0.244^</td>
<td>/</td>
<td><strong>1.850 (1.049-3.263)</strong></td>
<td><strong>0.034</strong></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>5</td>
<td>1.172 (0.946-1.451)</td>
<td>0.147^</td>
<td>15.8</td>
<td>1.284 (0.982-1.678)</td>
<td>0.067^</td>
</tr>
<tr>
<td>Caucasian</td>
<td>2</td>
<td>0.977 (0.751-1.270)</td>
<td>0.860^</td>
<td>0.0</td>
<td><strong>0.651 (0.482-0.878)</strong></td>
<td>0.005^</td>
</tr>
<tr>
<td>Others</td>
<td>3</td>
<td>1.168 (0.948-1.438)</td>
<td>0.145^</td>
<td>0.0</td>
<td>1.398 (0.830-2.354)</td>
<td>0.208^</td>
</tr>
</tbody>
</table>

OR: odds ratio; CI: confidence interval. The results in bold indicated the 95% CI excluded 1 or P<0.05. *The statistical significance of the pooled OR was determined by the Z test; No statistical significance was found by the heterogeneity test and the fixed-effects model was adopted here. "Confirmatory analysis with a random-effect model was used when significant heterogeneity existed. "The article reported by Wang was regarded as two independent studies according to ethnicity.

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Table 2. Pooled ORs and 95% CIs of the overall and stratified meta-analyses
Figure 2. Subgroup analysis of the relationship between miR-608 rs4919510 polymorphism and cancer risk by cancer. A: Co-dominant model (CC vs. AA); B: Dominant model (CC vs. AA+AC); C: Allele model (C vs. A).
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Figure 3. Subgroup analysis of the relationship between miR-608 rs4919510 polymorphism and cancer risk by ethnicity. A: Co-dominant model (CC vs. AA); B: Dominant model (CC vs. AA+AC); C: Allele model (C vs. A).
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with the wild-type CC genotype. No significant association was found in esophageal cancer and digestive tract cancer. Furthermore, in the stratification analysis of ethnicity (Figure 3), we found a highly significant association in the Caucasian population with co-dominant model (CC vs. AA: OR=0.651, 95% CI: 0.482-0.878, \(P=0.005\)), dominant model (CC vs. CA+AA: OR=0.644, 95% CI: 0.446-0.931, \(P=0.019\)) and allele model (C vs. A: OR=0.808, 95% CI: 0.699-0.934, \(P=0.004\)). No significant association was found in Asians and other ethnicities.

**Publication bias**

Begg’s funnel plot and Egger’s test were performed to assess the publication bias of the included studies. There was no evidence of publication bias in Begg’s funnel plot for all genetic models (Figure 4). In addition, the results of Egger’s test did not indicate any evidence of publication bias in our meta-analysis (\(P>0.05\)).

**Heterogeneity and sensitivity analysis**

Heterogeneity in all comparisons of the included studies was shown in Table 2. A few of comparisons showed slight or moderate heterogeneities among studies. Sensitivity analysis was performed to explore the influence of individual study on the pooled OR by removing one study at a time from eligible analysis. The results suggested that the omission of any study made no significant difference.

**Discussion**

Polymorphisms in miRNA genes could affect the transcription of miRNA primary transcripts, pre-miRNA maturation or miRNA-mRNA interactions. These mechanisms are involved in the aberrant expression of miRNAs, which have a significant influence on carcinogenesis [32]. MiR-423 is located on chromosome 17 and lies in the first intron of nuclear speckle splicing regulatory protein (NSRP1), which has been confirmed to play an important role in pre-miRNA splicing [33]. Several studies have found different expression patterns of miR-423 in various cancers, such as head and neck cancer [34], breast cancer [35] and hepatocellular cancer [36]. The rs6505162 polymorphism is located in the precurser of pre-miR-423 [20]. Recently, rs6505162 polymorphism in pre-miR-423 has been reported to offer a reduced cancer risk in difference cancer types. For example, Zhao found that the high mutation frequency of the SNP rs6505162 in pre-miR-423 had strong association with the expression of both proliferating cell nuclear antigen (PCNA) and mutant p53, which play an oncogenic role in carcinogenesis [37]. All these studies provoked us to focus on the association of rs6505162 polymorphism and cancer susceptibility.

In the meta-analysis, C-A variation at pre-miR-423 rs6505162 polymorphism sit did not exert significant genetic effect on cancer risk. Subgroup analysis revealed that the variant AA and CA/AA genotypes were associated with a decreased risk of breast cancer compared with wild-type CC genotype. However, an increased risk of lung cancer was found in co-dominant genetic model (CC vs. AA) and the recessive genetic model (CC vs. CA+AA). MiR-423 is highly expressed in multiple cancer types, including breast cancer. Both the mature miR-423-3p and miR-423-5p have been reported to be
involved in tumorigenesis. For example, Lin reported that only miR-423-3p could promote cell growth and regulate G1/S transition by targeting p21Cip1/Waf1 in hepatocellular carcinoma [36]. In gastric cancer cells, miR-423-5p could regulate cell proliferation and invasion by targeting trefoil factor 1 [38]. Another example was that Zhao found miR-423 promoted cell proliferation in breast cancer cell lines through its miR-423-3p strand, but not miR-423-5p. Lower expression level of mature miR-423-3p has the relative lower proliferation ability of pre-miR-423-12C in the stable breast cancer cell population [37]. The different expression levels of mature miR-423-3p and miR-423-5p may result in the different effect of the pre-miR-423 polymorphism on the risk of breast cancer and lung cancer. In the stratification analysis of ethnicity, the variant AA and CA/AA genotypes were associated with a decreased risk of cancer in the Caucasian population, which is consistent with the subgroup analysis of breast cancer. This could be attributed to the fact that 2 eligible studies in the Caucasian population all focused on breast cancer. However, no significant association was found in Asians and other ethnicities in the meta-analysis.

In interpreting the current results, several limitations of this study should also be considered. Firstly, eligible studies are still so inadequate that they may have an influence on our results of subgroup analysis. Secondly, a certain degree of heterogeneity was obvious in some comparisons, which could interfere with our results. Thirdly, lack of original data of the reviewed studies, consisting of age, gender, family history and environment factors, limited our further meta-analysis.

In summary, the results of our meta-analysis indicate a significant association between pre-miR-423 rs6505162 polymorphism and risk of breast cancer and lung cancer. To some extent, this polymorphism is closely related to cancer susceptibility in Caucasians. However, these results should be treated with some caution due to the limitations above. Additional studies from different ethnic groups and different types of cancers are necessary for further identification.

Disclosure of conflict of interest

None.

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References


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