**MDM4 rs4245739 A > C polymorphism correlates with reduced overall cancer risk in a meta-analysis of 69477 subjects**

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**ABSTRACT**

Mouse double minute 4 (MDM4) is a p53-interacting oncoprotein that plays an important role in the p53 tumor suppressor pathway. The common rs4245739 A > C polymorphism creates a miR-191 binding site in the MDM4 gene transcript. Numerous studies have investigated the association between this MDM4 polymorphism and cancer risk, but have failed to reach a definitive conclusion. To address this issue, we conducted a meta-analysis by selecting eligible studies from MEDLINE, EMBASE, and Chinese Biomedical databases. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess the strength of the associations. We also performed genotype-based mRNA expression analysis using data from 270 individuals retrieved from public datasets. A total of 15 studies with 19796 cases and 49681 controls were included in the final meta-analysis. The pooled results revealed that the MDM4 rs4245739C allele is associated with a decreased cancer risk in the heterozygous (AC vs. AA: OR = 0.82, 95% CI = 0.73−0.93), dominant (AC/CC vs. AA: OR = 0.82, 95% CI = 0.72−0.93), and allele contrast models (C vs. A: OR = 0.84, 95% CI = 0.76−0.94). The association was more prominent in Asians and population-based studies. We also found that the rs4245739C allele was associated with decreased MDM4 mRNA expression, especially for Caucasians. Thus the MDM4 rs4245739 A > C polymorphism appears to be associated with decreased cancer risk. These findings would be strengthened by new studies with larger sample sizes and encompassing additional ethnicities.

**INTRODUCTION**

Based on the latest GLOBOCAN estimates, there were approximately 14.1 million new cancer cases and 8.2 million cancer-related deaths worldwide in 2012 [1]. Developing countries accounted for almost 57% of new cancer cases and 65% of cancer-related deaths [1]. According to the trend in cancer incidence, the expected number of new cancer cases will reach 22.2 million worldwide in 2030 [2]. Leading risk factors for cancer development include tobacco use, overweight/obesity, physical inactivity, and infection [1]. Moreover, molecular epidemiological studies have demonstrated that genetic factors including single nucleotide polymorphisms (SNPs), may also play an important role in carcinogenesis [3–9].

As the gatekeeper for cellular growth and division, the tumor suppressor protein p53 maintains genomic stability and regulating cell growth, division, and apoptosis. Dysfunctional p53 protein can lead to the initiation and progression of tumors [10]. Mouse double minute 4 (MDM4) protein is a structural homolog of...
MDM2, which contains a p53 binding domain at the N-terminus and a RING finger domain at the C-terminus. MDM4 has been shown to inhibit p53 transcriptional activity directly by binding to its transcriptional activation domain. Overactive MDM4 reduces p53 tumor suppression function and contributes to tumor formation and progression [11]. The MDM4 protein can also inhibit the degradation of MDM2 by interacting with its RING finger domain [11]. Overexpression of MDM4 is associated with tumor progression and poor prognosis [12–14]. Previous molecular epidemiology studies suggest that genetic variations in MDM4 gene are associated with risk of various types of cancer [15–20].

Among the many MDM4 polymorphisms, a common genetic variant rs4245739 A > C has been widely investigated for its association with cancer susceptibility [21–28]. This polymorphism is located in the 3′ untranslated region (UTR) of the MDM4 gene, and creates a miR-191 target site that can lead to decreased expression of MDM4. However, the studies have generated controversial results regarding the association between this polymorphism and cancer risk. The possible reasons for the inconsistencies include differences in ethnicity and geographic location, as well as the limited sample size. To date, no meta-analysis has been conducted to comprehensively investigate the association of MDM4 rs4245739 A > C with overall cancer risk. To address the controversy regarding this association, we performed the current meta-analysis to precisely define the effect of MDM4 rs4245739 A > C polymorphism on overall cancer risk.

RESULTS

Characteristics of eligible studies

A total of 81 articles were retrieved after an initial literature search in MEDLINE, EMBASE, and Chinese Biomedical (CBM) databases (Figure 1). After full text review, 73 articles were excluded for the following reasons: review articles, duplicate studies, non-case-control study design, genotype distributions were not available, or no evaluation of the association between MDM4 rs4245739 A > C polymorphism and cancer risk. Ultimately, we found that only eight articles [21–28] met the inclusion criteria (Table 1). Among the eight articles, several investigations involving subjects from different areas were divided by area [22, 23, 27] and investigations were also separated by cancer type [26, 28]. As a result, a total of 15 case-control studies with 19796 cases and 49681 controls were included in the final meta-analysis. Of these, sample sizes ranged from 200 to 6512 for cases, and from 400 to 41451 for controls. The genotype distributions of the MDM4 rs4245739 A > C polymorphism were in accordance with Hardy-Weinberg equilibrium (HWE) in the controls in all 15 studies. Studies were performed on various types of cancer. Four studies focused on breast cancer [21, 22, 26], three on lung cancer [26, 27], two on esophageal squamous cell carcinoma [23], and one each on non-Hodgkin lymphoma [24], gastric cancer [25], colon cancer [26], prostate cancer [26], ovarian cancer [28] and endometrial cancer [28]. Seven studies were conducted among Caucasians [21, 26, 28], and eight among Asians [22–25, 27]. All 15 studies were considered high quality; one was scored as 10, eleven as 12 and three as 13.

Meta-analysis results

The overall analysis results are shown in Figure 2 and Table 2. We found the presence of significant heterogeneity under all genetic models (P heterogeneity < 0.10); thus, we chose the random-effects model because it can generate wider confidence intervals (CIs). We found that the MDM4 rs4245739C carriers had a significantly decreased overall cancer risk under the heterozygous [AC vs. AA: odds ratio (OR) = 0.82, 95% CI = 0.73–0.93], dominant (AC + CC vs. AA: OR = 0.82, 95% CI = 0.72–0.93), and allele contrast models (C vs. A: OR = 0.84, 95% CI = 0.76–0.94). In the subgroup analysis by ethnicity, similar results were found among Asians (AC vs. AA: OR = 0.55, 95% CI = 0.43–0.70; AC + CC vs. AA: OR = 0.54, 95% CI = 0.43–0.69; and C vs. A: OR = 0.56, 95% CI = 0.44–0.72), but not among Caucasians. When analyses were stratified by the source of controls, significant association with decreased cancer risk was found among population-based studies (AC vs. AA: OR = 0.70, 95% CI = 0.58–0.83; AC/CC vs. AA: OR = 0.70, 95% CI = 0.59–0.83; and C vs. A: OR = 0.73, 95% CI = 0.63–0.85). In the stratified analysis by quality score, significant associations were found among studies with scores ≥12 (AC vs. AA: OR = 0.81, 95% CI = 0.71–0.93; AC + CC vs. AA: OR = 0.81, 95% CI = 0.71–0.92; and C vs. A: OR = 0.84, 95% CI = 0.75–0.93).

The MDM4 mRNA expression by genotypes

In the genotype-based mRNA expression analysis (Table 3 and Figure 3) using public datasets, we found the rs4245739C allele carriers had trends toward decreased mRNA expression level among Caucasians, Asians, Africans, and all subjects. The decrease in the MDM4 mRNA expression reached a statistical significance among the Caucasians (AC vs. AA: P = 0.002; CC vs. AA: P = 0.004, and AC + CC vs. AA: P = 0.0002), but not among other populations.

Sensitivity analysis and publication bias

We conducted sensitivity analysis to assess the influence of each individual study on the pooled ORs and 95% CIs by omitting one study each time. No individual study could alter the pooled ORs significantly, which demonstrated that the studies were relatively statistically
robust. Additionally, we found that no single study could alter the publication bias in an obvious manner (data not shown).

**DISCUSSION**

In the present meta-analysis comprising 69477 subjects from 15 studies, we completed the first comprehensive evaluation of the association between MDM4 gene rs4245739 A > C polymorphism and overall cancer risk. The pooled results indicate that the MDM4 rs4245739 A > C polymorphism was significantly associated with decreased overall cancer risk, which was consistent with the results of our genotype-based mRNA expression analysis.

SNPs are the most common type of genetic variations. The majority of SNPs are silent or have limited influence on the function and expression of genes. Only a small fraction of SNPs have been reported to be potentially functional and associated with cancer susceptibility [29–32], in accordance with the theory of the driver and passenger somatic mutations in human cancer genome [33]. The influence of genetic variations, particularly SNPs, on an individual’s cancer susceptibility under similar environmental exposures, has been widely investigated and has become a hot research topic worldwide [34]. The association between SNPs and cancer risk may be strongly cancer-specific [35]. Numerous previous studies have investigated the association between MDM4 gene polymorphisms and cancer susceptibility [21–28].

The MDM4 gene (also known as HDMX or MDMX) is located at chromosome 1q32, a region that is frequently found to be amplified in cancer [36]. This gene contains 11 exons and encodes a protein of 490 amino acids [37], in which at least 2709 SNPs have been identified (http://www.ncbi.nlm.nih.gov/projects/SNP). Among these SNPs, a potentially functional polymorphism (rs4245739 A > C) has received a great deal of recent attention. The rs4245739 A > C polymorphism was first identified in 40 German patients with familial breast cancer through sequencing the whole coding and flanking untranslated regions of MDM4 gene [38]. This polymorphism, located in the 3’ UTR of MDM4 gene, generates a miR-191 target binding site. As a result, miR-191 selectively binds to mRNA harboring the MDM4 rs4245739C allele to decrease the expression of MDM4 gene at both mRNA and protein levels, but not mRNA with MDM4 rs4245739A allele (wild-type). The decreased MDM4 expression caused by miR-191 binding might increase the activity of p53 and consequently modify an MDM4 rs4245739A allele carriers’ susceptibility to ovarian cancer and retinoblastoma [39, 40]. Additionally, the MDM4 rs4245739 AC genotype may be associated with increased overall survival in non-small cell lung cancer, when compared to the AA genotype [41]. Despite the biological plausibility, studies investigating the association between this polymorphism and cancer risk have yielded inconclusive results [21–28]. For instance, some studies found that the rs4245739 A > C polymorphism was significantly associated with decreased cancer risk [22–24, 27]; in contrast, Garcia-

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**Figure 1: Flow diagram of included studies for the association between MDM4 rs4245739 A > C polymorphism and overall cancer risk.**
Closas et al. [21] reported that this polymorphism was associated with increased breast cancer risk. Moreover, others found that this polymorphism may have weak or no effect on cancer risk [25, 26, 28]. It is widely recognized that different cancer types have unique characteristics and involve differing signal pathways. Even among the same cancer type, cancers from different patients display significant heterogeneity. The possible reasons for discrepancies regarding cancer susceptibility may be ascribed to tumor specificity, differences in ethnicity, and variations in sample sizes included in each investigation.

When we combined all available investigations, we found that the rs4245739C allele carriers had decreased cancer risk, especially among Asians. Moreover, we also found that the rs4245739C allele was associated with decreased mRNA expression of \( MDM4 \) by genotype-based mRNA expression analysis, which could provide further biological evidence of the possible mechanism of this polymorphism.

Although this is the first meta-analysis investigating the association between \( MDM4 \) gene rs4245739 A > C polymorphism and overall cancer risk, some limitations...
Table 2: Meta-analysis of the association between MDM4 rs4245739 A > C polymorphism and cancer risk

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. of studies</th>
<th>Sample size</th>
<th>Homozygous</th>
<th>Heterozygous</th>
<th>Reciprocal</th>
<th>Dominant</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC vs. AA</td>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td>p-value (%)</td>
<td>OR (95% CI)</td>
<td>p-value (%)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>C vs. A</td>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td>p-value (%)</td>
<td>OR (95% CI)</td>
<td>p-value (%)</td>
<td>OR (95% CI)</td>
</tr>
</tbody>
</table>

Table 3: MDM4 mRNA expression by the genotypes of rs4245739 A > C, using data from the HapMap

<table>
<thead>
<tr>
<th>Population</th>
<th>genotypes</th>
<th>No.</th>
<th>Mean ± SD</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
<th>P&lt;sub&gt;trend&lt;/sub&gt; &lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEU</td>
<td>AA</td>
<td>52</td>
<td>7.28 ± 0.31</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AC</td>
<td>29</td>
<td>7.06 ± 0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>9</td>
<td>6.96 ± 0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dominant</td>
<td>38</td>
<td>7.03 ± 0.26</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>YRI</td>
<td>AA</td>
<td>59</td>
<td>6.75 ± 0.23</td>
<td>0.724</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AC</td>
<td>25</td>
<td>6.72 ± 0.22</td>
<td></td>
<td>0.578</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>6</td>
<td>6.68 ± 0.11</td>
<td></td>
<td>0.271</td>
</tr>
<tr>
<td></td>
<td>Dominant</td>
<td>31</td>
<td>6.71 ± 0.20</td>
<td></td>
<td>0.459</td>
</tr>
<tr>
<td>Asian</td>
<td>AA</td>
<td>80</td>
<td>6.86 ± 0.31</td>
<td>0.530</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AC</td>
<td>10</td>
<td>6.79 ± 0.23</td>
<td></td>
<td>0.530</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>0</td>
<td>/</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dominant</td>
<td>10</td>
<td>6.79 ± 0.23</td>
<td></td>
<td>0.530</td>
</tr>
<tr>
<td>All</td>
<td>AA</td>
<td>191</td>
<td>6.94 ± 0.36</td>
<td>0.377</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AC</td>
<td>64</td>
<td>6.88 ± 0.29</td>
<td></td>
<td>0.271</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>15</td>
<td>6.85 ± 0.22</td>
<td></td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>Dominant</td>
<td>79</td>
<td>6.88 ± 0.28</td>
<td></td>
<td>0.135</td>
</tr>
</tbody>
</table>

CEU, 90 Utah residents with ancestry from northern and western Europe; YRI, 90 Yoruba in Ibadan, Nigeria.
<sup>a</sup>Genotype data and mRNA expression levels for MDM4 by genotypes were obtained from the HapMap phase II release 23 data from EBV-transformed lymphoblastoid cell lines from 270 individuals.
<sup>b</sup>Two-sided Student’s t test within the stratum.
<sup>c</sup>P values for the trend test of MDM4 mRNA expression among 3 genotypes for each SNP from a general linear model.

should be discussed. First, although all of the eligible studies were pooled together, the total number of studies and the sample sizes for most types of cancer were still relatively small. As a result, statistical power might be limited while evaluating the association of interest, especially in the subgroup analysis. For instance, there was only one study available for several types of cancer including non-Hodgkin lymphoma, gastric cancer, colon cancer, and prostate cancer. No pooled study could be performed for these cancers. Second, there was heterogeneity among the included studies, which might stem from the inconsistent results derived from different cancers and ethnicities. Third, nearly all of the studies included in this meta-analysis were conducted...
among Asians and Caucasians. In light of genetic and geographical differences, more investigations from different areas and ethnicities are required to verify our findings. Fourth, the lack of original data limited the further evaluation of potential gene-gene and gene-environment interactions that may modulate cancer risk. Fifth, we may have missed some publications, especially studies without genotype data and those with negative results that were not published. For example, the genotype data for the control subjects were not available in the investigation carried out by Wynendaele et al. [39]. As a result, this study was not included in the current meta-analysis. Finally, publication bias may exist since only published studies were included in our meta-analysis. So, the conclusions drawn from the current study should be interpreted with caution.

In conclusion, our meta-analysis revealed that MDM4 gene rs4245739 A > C polymorphism was associated with a reduction in overall cancer susceptibility. Due to the limitations of the current meta-analysis, future studies with larger sample size and different ethnicities and cancer types are needed to confirm these findings.

MATERIALS AND METHODS

Publication search

We conducted a comprehensive literature search for all relevant publications concerning the association between MDM4 rs4245739 A > C polymorphism and cancer risk from MEDLINE, EMBASE, and CBM database (prior to 5 September, 2016). The following search terms were used: “MDM4 or HDMX or MDMX or MRP1 or rs4245739”, “cancer or carcinoma or tumor or neoplasm”, and “polymorphism or variant or variation”. We also searched for additional relevant studies from the references of retrieved publications.

Inclusion and exclusion criteria

Studies meeting the following inclusion criteria were included: (1) evaluated the association between MDM4 rs4245739 A > C polymorphism and cancer risk; (2) case-control study or cohort study; (3) genotype distributions were available for both cases and controls; (4) published in English or Chinese.

Figure 3: mRNA expression level of the MDM4 gene in Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines. (A) mRNA expression in 90 cell lines from unrelated CEU (Utah residents with ancestry from northern and western Europe) individuals. (B) mRNA expression in 90 cell lines of unrelated YRI (Yoruba in Ibadan, Nigeria) individuals. (C) mRNA expression in 90 cell lines of unrelated Asian individuals. (D) mRNA expression in 270 cell lines of all individuals.
Exclusion criteria included: (1) not case-control study design; (2) did not evaluate the association between MDM4 rs4245739 A > C polymorphism and cancer risk; (3) studies with overlapping participants; (4) conference abstracts, review articles, comments, meta-analyses, or editorial articles. In the case of duplicate or overlapping studies, only the most complete one was included.

Data extraction

Two authors (Chaoyi Xu and Jinhong Zhu) independently extracted the following information from each investigation: the first author’s surname, publication year, country, ethnicity, control source, genotyping method, as well as number of case and control with AA, AC and CC genotypes. All disagreements were resolved through discussion between these two investigators until a consensus was reached.

Genotype-based mRNA expression analysis

We performed genotype-based mRNA expression analysis as we described previously [3, 42–45]. Genotypes data of MDM4 rs4245739 A > C polymorphism for 270 individuals with three ethnicities were obtained from HapMap (http://www.hapmap.org). The MDM4 gene mRNA expression data for the same 270 individuals were downloaded from SNPexp (http://app3.titan.uio.no/biotools/tool.php?app=snpxp).

Statistical analysis

The strength of the association between MDM4 rs4245739 A > C polymorphism and overall cancer risk were assessed using crude OR and 95% CI under the homozygous (CC vs. AA), heterozygous (AC vs. AA), recessive (CC vs. AC + AA), dominant (AC + CC vs. AA), and allele contrast models (C vs. A). Goodness-of-fit $\chi^2$ test was adopted to test deviation from HWE for the genotypes of control subjects. Heterogeneity was assessed using $\chi^2$-based Q test, and was considered as significant when $P < 0.10$. We also qualified the heterogeneity using $I^2$ statistics, a value with a range from 0% to 100%. A higher $I^2$ value indicates a greater degree of heterogeneity [46]. When significant heterogeneity was found, random-effects model [47] would be adopted; otherwise, fixed-effects model (the Mantel-Haenszel method) [48] would be used. The quality of each investigation was evaluated by quality assessment criteria (Supplementary Table S1) as we described previously [43]. Subgroup analysis was conducted by cancer type (investigations with only one study would be merged into the “others” group), ethnicity, source of control and quality score of investigations. Sensitivity analysis was conducted to evaluate the stability of the results. The pooled ORs and 95% CIs were estimated by excluding one study at a time to evaluate the influence of single investigation. The potential publication bias was estimated using Begg’s funnel plot [49] and Egger’s linear regression test [50]. In terms of genotype-based mRNA expression, two-sided Student’s $t$ test was used for the comparison of two groups, and one-way ANOVA was adopted for comparison among three different genotypes. The statistical analysis was performed with STATA software (version 11.0; Stata Corporation, College Station, TX). All the statistics were two-sided, with $P < 0.05$ indicating statistical significance.

CONFLICTS OF INTEREST

The authors declare no competing financial interests.

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