Quantitative assessment of TIM-3 polymorphisms and cancer risk in Chinese Han population

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ABSTRACT

Previous studies have investigated the associations of TIM-3 polymorphisms (-1516G/T, -574G/T, and +4259T/G) with cancer risk in Chinese Han population, but the results remain conflicting. Therefore, we conducted a meta-analysis to derive a more precise estimation of the associations. The pooled data showed that TIM-3 polymorphisms (-1516G/T, -574G/T, and +4259T/G) were significantly associated with an increased risk of overall cancer in Chinese Han population. Subgroup analyses based on cancer system showed that TIM-3 -1516G/T polymorphism was only associated with an increased risk of digestive system cancer in Chinese Han population. TIM-3 -574G/T polymorphism was associated with an increased risk of digestive system cancer and other cancer in Chinese Han population. TIM-3 +4259T/G polymorphism was only associated with an increased risk of other cancer in Chinese Han population. In summary, our results indicated that TIM-3 polymorphisms (-1516G/T, -574G/T, and +4259T/G) were associated with the increased risk of cancer in Chinese Han population.

INTRODUCTION

T-cell immunoglobulin and mucin domain-containing molecule 3 (TIM-3) is a type I cell surface glycoprotein and can inhibit the activation of innate immune cells, such as dendritic cells (DCs), macrophages, and natural killer (NK) cells. For instance, increased TIM-3 expression in tumor-infiltrating DCs could inhibit an innate response to nucleic acids [1]. The in vivo administration of anti-TIM-3 antibody could increase the number and activation of macrophages, suggesting that TIM-3 might inhibit macrophage activation and function [2]. In addition, TIM-3 acted as an inhibitor of macrophage activation, and blockade of the TIM-3 pathway could lead to decreased CD80 costimulatory molecule expression on macrophages and an enhanced inflammatory response [3]. NK cells protect the host against viral infection and cancer. Several lines of studies have shown that the activity of NK cells can be inhibited by TIM-3 [4,5]. For instance, in chronic hepatitis B infection, TIM-3 expression was upregulated on NK cells, which suppressed NK cells function. However, this process was reversed by blockade of the TIM-3 pathway, which supported a negative regulatory role of TIM-3 in the activity of NK cells [5]. In addition to innate immunity, TIM-3 has also been reported to involve adaptive immunity. In HCV-infected HBV vaccine non-responders, TIM-3 blockade improved IL-12p35 and inhibited IL-23p19 productions by CD14+ monocytes, leading to reduction of Th17 cells [6].

Human TIM-3 gene is located in chromosome 5q33.3 and contains a large number of single nucleotide polymorphisms (SNPs). Among them, the following three SNPs are common and widely studied: -1516G/T and -574G/T polymorphisms in the promoter region and +4259T/G polymorphism in the encoding region (amino acid substitution: arginine to leucine). In 2010, the associations between TIM-3 -1516G/T, -574G/T, and +4259T/G polymorphisms and cancer risk were firstly reported in Chinese Han population [7]. Since then, more and more epidemiologic studies from Chinese Han population investigated the role of the three SNPs in the risk of cancer, including non-Hodgkin lymphomas (NHL), hepatocellular carcinoma, non-small-cell lung cancer (NSCLC), pancreatic cancer, and renal cell carcinoma [8-13]. However, the results are inconsistent. Furthermore, a single-center study may have an inadequate sample size
and lack statistical power to obtain reliable conclusions. Thus, we performed a meta-analysis of all eligible studies to obtain a more precise estimation of the associations.

RESULTS

Study selection and characteristics

The study selection process is shown in Figure 1. A total of 36 articles were initially retrieved from electronic databases including PubMed, EMBASE and Chinese National Knowledge Infrastructure (CNKI). After reviewing the titles, abstracts and full text, we excluded 29 irrelevant studies. Finally, 7 articles published between 2010 and 2013 were included in the current meta-analysis. The main characteristics of all eligible studies are shown in Table 1 and S1. All the included studies were conducted in Chinese Han population. Furthermore, all of these studies assessed the association between TIM-3 -1516G/T polymorphism and cancer risk. However, Li Z’s studies in 2012 and 2013 contained overlapping data. According

Table 1: Main characteristics of all studies included in the meta-analysis

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Country</th>
<th>Genotyping method</th>
<th>Cancer type</th>
<th>Source of controls</th>
<th>Case</th>
<th>Control</th>
<th>Polymorphisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Song, H.</td>
<td>2013</td>
<td>China</td>
<td>PCR-RFLP</td>
<td>Non-Hodgkin lymphomas</td>
<td>Hospital</td>
<td>496</td>
<td>512</td>
<td>−1516G/T, −574G/T, +4259T/G</td>
</tr>
<tr>
<td>Li, Z.</td>
<td>2013</td>
<td>China</td>
<td>PCR-RFLP</td>
<td>Hepatocellular carcinoma</td>
<td>NA</td>
<td>271</td>
<td>318</td>
<td>−1516G/T, −574G/T, +4259T/G</td>
</tr>
<tr>
<td>Bai, J.</td>
<td>2013</td>
<td>China</td>
<td>PCR-RFLP</td>
<td>Non-small-cell lung cancer</td>
<td>Population</td>
<td>432</td>
<td>466</td>
<td>−1516G/T, −574G/T, +4259T/G</td>
</tr>
<tr>
<td>Tong, D.</td>
<td>2012</td>
<td>China</td>
<td>PCR-RFLP</td>
<td>Pancreatic cancer</td>
<td>Hospital</td>
<td>306</td>
<td>422</td>
<td>−1516G/T, −574G/T, +4259T/G</td>
</tr>
<tr>
<td>Li, Z.</td>
<td>2012</td>
<td>China</td>
<td>PCR-RFLP</td>
<td>Hepatocellular carcinoma</td>
<td>NA</td>
<td>144</td>
<td>182</td>
<td>−574G/T, +4259T/G</td>
</tr>
<tr>
<td>Cai, C.</td>
<td>2012</td>
<td>China</td>
<td>PCR-RFLP</td>
<td>Renal Carcinoma</td>
<td>Hospital</td>
<td>322</td>
<td>402</td>
<td>−1516G/T, −574G/T</td>
</tr>
<tr>
<td>Cao, B.</td>
<td>2010</td>
<td>China</td>
<td>PCR-RFLP</td>
<td>Gastric cancer</td>
<td>Hospital</td>
<td>212</td>
<td>252</td>
<td>−1516G/T, −574G/T</td>
</tr>
</tbody>
</table>

PCR-RFLP: PCR-restriction fragment length polymorphism; NA: not available.

Figure 1: Flow chart of study selection in the meta-analysis.
to inclusion and exclusion criteria, Li Z’s study in 2013, which contained the latest and most complete data, was adopted. Finally, six articles including 2039 cases and 2372 controls were used to estimate cancer risk associated with TIM-3 -1516G/T polymorphism. For TIM-3 -574G/T and +4259T/G polymorphisms, six articles with 1912 cases and 2236 controls were included.

Quantitative data synthesis

The results of this meta-analysis are shown in Table 2. The pooled risk estimates indicated that TIM-3 -1516G/T polymorphism was associated with an increased risk of overall cancer (GT vs. GG: OR = 1.38, 95%CI: 1.08-1.77; TT+GT vs. GG: OR = 1.40, 95%CI: 1.08-1.83; T vs. G: OR = 1.39, 95%CI: 1.07-1.79, Pz = 0.01) (Figure S1). The similar associations

Table 2: Meta-analysis of the association between TIM-3 polymorphisms and cancer risk in Chinese Han population

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Comparison</th>
<th>Subgroup</th>
<th>Heterogeneity test</th>
<th>Model</th>
<th>Pz</th>
<th>Pe</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1516G/T</td>
<td>GT vs. GG</td>
<td>Overall</td>
<td>53.6</td>
<td>0.06</td>
<td>R</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Digestive system cancer</td>
<td>70.2</td>
<td>0.04</td>
<td>R</td>
<td>0.03</td>
<td>1.75 (1.04-2.92)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other cancer</td>
<td>0</td>
<td>0.89</td>
<td>F</td>
<td>0.14</td>
<td>1.17 (0.95-1.44)</td>
</tr>
<tr>
<td>-1516G/T</td>
<td>TT+GT vs. GG</td>
<td>Overall</td>
<td>58.5</td>
<td>0.03</td>
<td>R</td>
<td>0.01</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Digestive system cancer</td>
<td>72.8</td>
<td>0.03</td>
<td>R</td>
<td>0.03</td>
<td>1.79 (1.05-3.05)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other cancer</td>
<td>0</td>
<td>0.89</td>
<td>F</td>
<td>0.14</td>
<td>1.17 (0.95-1.44)</td>
</tr>
<tr>
<td>T vs. G</td>
<td>Overall</td>
<td>60.6</td>
<td>0.03</td>
<td>R</td>
<td>0.01</td>
<td>0.06</td>
<td>1.39 (1.07-1.79)</td>
</tr>
<tr>
<td></td>
<td>Digestive system cancer</td>
<td>73.7</td>
<td>0.02</td>
<td>R</td>
<td>0.03</td>
<td>1.77 (1.05-2.96)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other cancer</td>
<td>0</td>
<td>0.90</td>
<td>F</td>
<td>0.16</td>
<td>1.15 (0.94-1.41)</td>
<td></td>
</tr>
<tr>
<td>-574G/T</td>
<td>GT vs. GG</td>
<td>Overall</td>
<td>32.7</td>
<td>0.19</td>
<td>F</td>
<td>&lt;0.01</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>Digestive system cancer</td>
<td>28.7</td>
<td>0.25</td>
<td>F</td>
<td>0.02</td>
<td>1.77 (1.08-2.91)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other cancer</td>
<td>54.1</td>
<td>0.11</td>
<td>R</td>
<td>0.01</td>
<td>2.11 (1.26-3.56)</td>
<td></td>
</tr>
<tr>
<td>-574G/T</td>
<td>T vs. G</td>
<td>Overall</td>
<td>30.4</td>
<td>0.21</td>
<td>F</td>
<td>&lt;0.01</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>Digestive system cancer</td>
<td>25.7</td>
<td>0.26</td>
<td>F</td>
<td>0.02</td>
<td>1.75 (1.08-2.85)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other cancer</td>
<td>52.7</td>
<td>0.12</td>
<td>R</td>
<td>0.01</td>
<td>2.07 (1.25-3.44)</td>
<td></td>
</tr>
<tr>
<td>+4259T/G</td>
<td>TG vs. TT</td>
<td>Overall</td>
<td>59.1</td>
<td>0.03</td>
<td>R</td>
<td>&lt;0.01</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>Digestive system cancer</td>
<td>68.1</td>
<td>0.04</td>
<td>R</td>
<td>0.39</td>
<td>1.45 (0.62-3.41)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other cancer</td>
<td>0</td>
<td>0.85</td>
<td>F</td>
<td>&lt;0.01</td>
<td>2.87 (2.04-4.02)</td>
<td></td>
</tr>
<tr>
<td>+4259T/G</td>
<td>G vs. T</td>
<td>Overall</td>
<td>59.8</td>
<td>0.03</td>
<td>R</td>
<td>&lt;0.01</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Digestive system cancer</td>
<td>68.0</td>
<td>0.04</td>
<td>R</td>
<td>0.40</td>
<td>1.43 (0.62-3.29)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other cancer</td>
<td>0</td>
<td>0.85</td>
<td>F</td>
<td>&lt;0.01</td>
<td>2.77 (1.98-3.87)</td>
<td></td>
</tr>
</tbody>
</table>

were also found between \textit{TIM-3} -574G/T and +4259T/G polymorphisms and overall cancer risk. For \textit{TIM-3} -574G/T polymorphism, subjects carrying GT genotype or T allele had a significantly increased risk of overall cancer compared with those carrying the GG genotype or G allele, respectively (GT vs. GG: OR = 1.99, 95\%CI: 1.50-2.64, \textit{P}< 0.01; T vs. G: OR = 1.95, 95\%CI: 1.48-2.58, \textit{P}< 0.01) (Figure S2). For \textit{TIM-3} +4259T/G polymorphism, subjects carrying TG genotype or G allele had a significantly increased risk of overall cancer compared with those carrying the TT genotype or T allele, respectively (TG vs. TT: OR = 2.21, 95\%CI: 1.44-3.38, \textit{P}< 0.01; G vs. T: OR = 2.14, 95\%CI: 1.41-3.26, \textit{P}< 0.01) (Figure 2). In subgroup analyses based on cancer system, we found that \textit{TIM-3} -1516G/T polymorphism was only associated with an increased risk of digestive cancer.

Figure 2: Forest plot of effect estimates for \textit{TIM-3} +4259T/G polymorphism and overall cancer risk.
system cancer (GT vs. GG: OR = 1.75, 95%CI: 1.04-2.92, 
$P_z = 0.03$; TT+GT vs. GG: OR = 1.79, 95%CI: 1.05-3.05, 
$P_z = 0.03$; T vs. G: OR = 1.77, 95%CI: 1.05-2.96, 
$P_z = 0.03$). TIM-3 -574G/T polymorphism was associated with 
an increased risk of digestive system cancer (GT vs. GG: 
OR = 1.77, 95%CI: 1.08-2.91, $P_z = 0.02$; T vs. G: OR = 
1.75, 95%CI: 1.08-2.85, $P_z = 0.02$) and other cancer 
(GT vs. GG: OR = 2.11, 95%CI: 1.26-3.56, $P_z = 0.01$; 
T vs. G: OR = 2.07, 95%CI: 1.25-3.44, $P_z = 0.01$). TIM-3 
+4259T/G polymorphism was only associated with an 
increased risk of other cancer (TG vs. TT: OR = 2.87, 
95%CI: 2.04-4.02, $P_z < 0.01$; G vs. T: OR = 2.77, 95%CI: 
1.98-3.87, $P_z < 0.01$).

Sensitivity analysis and publication bias

The sensitivity analysis showed that no single study 
alted the pooled ORs qualitatively, which provided the 
evidence of the stability of the meta-analysis (Figure 3, 
S3 and S4). Publication bias was assessed by Begg’s test 
and Egger’s test. As shown in Figure S5-S7, the shape 
of Begg’s funnel plot did not reveal obvious asymmetry. 
However, Results of Egger’s tests showed a borderline 
publication bias under the GT vs. GG model for TIM-3 
-1516G/T polymorphism ($P_z = 0.05$), suggesting that the 
number of relevant studies may be insufficient (Table 2).

DISCUSSION

A number of epidemiological studies have assessed 
the associations between TIM-3 genetic polymorphisms 
(-1516G/T, -574G/T, and +4259T/G) and the risk of 
different types of cancer. For instance, Song H, et al. 
found that the prevalence of TIM-3 -574GT genotype and 
+4259TG genotype were significantly increased in the 
NHL cases than in controls [8]. Bai J, et al. confirmed that 
frequencies of TIM-3 +4259TG genotype were 
significantly different between the NSCLC cases and 
controls. Subjects carrying the +4259TG genotype had 
a 2.81-fold increased risk of NSCLC compared to those 
with the TT genotype [10]. There was also a report that 
showed a significant association between TIM-3 -1516G/ 
T polymorphism and the risk and distant metastasis 
of gastric cancer [7]. Compared to the carriers of TIM-3 
-1516GG genotype, the carriers of TIM-3 -1516GT 
genotype had a 2.03-fold increased risk of gastric 
cancer [7]. These data suggest that TIM-3 -1516G/T, 
-574G/T, and +4259TG/G polymorphisms are implicated 
in the development of cancer. However, there were also 
inconsistent results reported in the previous studies. For 
example, the TIM-3 -1516G/T polymorphism did not 
reveal significant difference between NHL patients and 
healthy controls [8]. The TIM-3 -1516G/T and -574G/T 
genotypes did not show any correlation with NSCLC 
risk [10]. No association was observed between TIM-3 
+4259T/G polymorphism and gastric cancer [7]. In order 
to resolve this conflict, we conducted a meta-analysis 
on the association between three TIM-3 polymorphisms 
(-1516G/T, -574G/T, and +4259T/G) and cancer risk. Our 
results showed that TIM-3 polymorphisms (-1516G/T, 
-574G/T, and +4259T/G) were significantly associated 
with an increased risk of overall cancer in Chinese Han 
population. Subgroup analyses based on cancer system 
showed that TIM-3 -1516G/T polymorphism was only 
associated with an increased risk of digestive system 
cancer in Chinese Han population. TIM-3 -574G/T polymorphism was associated with an increased risk 
of digestive system cancer and other cancer in Chinese Han 
population. TIM-3 +4259T/G polymorphism was only 
associated with an increased risk of other cancer in 
Chinese Han population.

A large number of studies have confirmed that 
SNPs in cancer-related genes can contribute to individual 
susceptibility to cancer by affecting gene expression and 
function [14-15]. For instance, the -249T/C polymorphism 
in the promoter region of DEC1 gene reduced risk 
of squamous cell carcinoma of the head and neck by 
enhancing transcriptional activity of the DEC1 promoter 
and the DNA-protein-binding activity [14]. ERBB2 +2246A/G polymorphism (amino acid substitution: 
isoLeucine to valine) is associated with an increased 
familial breast cancer risk. In addition, computational 
analyses showed that a substitution of isoleucine by a 
valine residue would stabilize the formation of active 
HER-2/NEU dimers [15]. Therefore, considering that 
TIM-3 can reduce the antigen-specific T cell responses 
and down-regulate the anti-tumor immunity in vivo by 
inhibiting the Th1 responses [16], we speculated that TIM-3 
polymorphisms (-1516G/T, -574G/T, and +4259T/G) 
conferring individual risk for cancer by increasing TIM-3 
expression or enhancing TIM-3 activity.

Meta-analysis is a very powerful tool for analyzing 
cumulative data of studies where the individual sample 
sizes are small and the statistical power is low. To the 
best of our knowledge, no previous meta-analysis has 
comprehensively assessed the associations between the 
three SNPs and cancer risk. However, there are some 
limitations in the current meta-analysis. First of all, the 
number of published studies was not sufficiently large for 
a comprehensive analysis. Therefore, our analysis should 
be interpreted with caution, and more eligible studies 
on different types of cancer are needed. In addition, our 
results were based on unadjusted estimates because of lack 
of raw data including age, lifestyle, and environmental 
factors, which may cause a confounding bias.

In conclusion, our meta-analysis suggests that TIM- 
3 polymorphisms (-1516G/T, -574G/T, and +4259T/G) 
may increase an individual’s susceptibility to cancer in 
Chinese Han population. However, more large-scale 
studies are warranted to confirm our finding in different 
cancer types.
Figure 3: Sensitivity analysis of the pooled ORs and 95% CIs for TIM-3 +4259T/G polymorphism.
MATERIALS AND METHODS

Search strategy

To identify eligible studies, we systematically searched PubMed, EMBASE and CNKI databases. The keywords used for search were as follows: “T-cell immunoglobulin- and mucin-domain-containing molecule 3 OR TIM-3”, “polymorphism OR variant” and “cancer OR carcinoma OR neoplasm”. There were no limitations on language and publication year. The last search was updated on December 12, 2015. Furthermore, references of all relevant articles were retrieved to identify additional eligible studies.

Inclusion and exclusion criteria

Eligible studies must meet the following inclusion criteria: (a) case-control studies; (b) evaluating the association between TIM-3 polymorphisms (-1516G/T, -574G/T, and +4259T/G) and cancer risk; (c) available genotype frequencies; (d) the genotype distribution in control groups was in the Hardy-Weinberg equilibrium (HWE). Exclusion criteria were as follows: (a) letters, reviews, and case reports; (b) lack of genotype frequency data; (c) duplicate publication. In addition, if multiple studies had overlapping data, only those with complete data were included.

Data extraction

Two authors independently selected the relevant articles and extracted the following data: first author’s name, publication year, country, cancer type, genotyping methods, source of controls, number of cases and controls, genotype and allele frequency, and evidence of HWE in controls. Any disagreement was resolved by discussion between the authors.

Statistical analysis

HWE in the control group of each study was examined by goodness-of-fit chi-square test, and \( P_{\text{HWE}} < 0.05 \) was considered as a deviation from HWE. The association between TIM-3 polymorphisms (-1516G/T, -574G/T, and +4259T/G) and cancer risk was evaluated by pooled OR and 95% CI. The significance of the pooled OR was assessed by the Z test, and \( P_{\text{Z}} < 0.05 \) was considered significant. The chi-square-based Q-test and I\(^2\) tests were used to investigate the heterogeneity between studies. If the \( P_{\text{H}} < 0.05 \) or I\(^2\) >50%, indicating the existence of between-study heterogeneity, the random-effects model was used to calculate the pooled ORs; otherwise, the fixed-effects model was applied to the analysis. Sensitivity analysis was carried out by sequentially omitting one study at a time to estimate the stability of the result. Publication bias among studies was determined using Begg’s test and Egger’s test, and \( P_{\text{E}} < 0.05 \) was considered significant. All statistical tests were performed with the STATA software (version 12.0; StataCorp, College Station, TX, USA).

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CONFLICTS OF INTEREST

The authors declare no competing financial interests.

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