Review Article

p53 codon 72 polymorphism is associated with human papillomavirus-related esophageal cancer risk: a meta-analysis

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Abstract: Background: p53 codon 72 polymorphism is associated with esophageal cancer (EC). Human papillomavirus (HPV) infection is considered as a risk factor of EC. However, the association of p53 codon 72 polymorphism with the risk of HPV-related EC remains inconsistent. Hence, we aimed to investigate the association between p53 codon 72 polymorphism and HPV-related EC risk through meta-analysis. Methods: All eligible studies published before November 1, 2015 were selected by searching PubMed, Embase, China National Knowledge Infrastructure (CNKI), and WanFang with the following key words: “p53”, “HPV” or “human papillomavirus”, and “esophageal cancer”. Crude odds ratio (OR) with 95% confidence interval (CI) was used to assess the association. Statistical analyses were performed using Review Manager 5.2 and Stata/SE 12.0. Results: Twelve studies including 1682 HPV-related EC cases were included in this meta-analysis. p53 codon 72 genotypes were associated with HPV-related EC in an allelic model (Arg vs. Pro: OR=1.66, 95% CI=1.19-2.32, P=0.003), a homozygous model (ArgArg vs. ProPro: OR=1.73, 95% CI=1.26-2.37, P=0.0007), and a recessive model (ArgArg vs. ArgPro+ProPro: OR=2.39, 95% CI=1.45-3.94, P=0.0006). By contrast, significant association was not seen in a heterozygous model (ArgPro vs. ProPro: OR=0.85, 95% CI=0.61-1.19, P=0.33) and a dominant model (ArgArg+ArgPro vs. ProPro: OR=1.24, 95% CI=0.93-1.65, P=0.14). A greater association was observed in the subgroup of studies performing PCR-RFLP or real-time PCR for p53 genotyping and studies performing PCR to detect HPV status in allelic and recessive models. Conclusion: p53 codon 72 Arg homozygous genotype is a high risk factor of HPV-related EC. Individuals carrying ArgArg genotype exhibit an increased risk of EC with HPV infection.

Keywords: Esophageal cancer, meta-analysis, p53 codon 72 polymorphism, human papillomavirus

Introduction

Esophageal cancer (EC) is the sixth leading cause of cancer-related deaths worldwide [1]. Approximately 455,800 new esophageal cancer cases and 400,200 deaths occurred in 2012 worldwide [2]. EC is considered as a serious malignancy because of its extremely aggressive histopathological features and poor survival rate [3]. Dietary and environmental factors, such as smoking, alcohol consumption, obesity, high soil nitrate levels, and HPV infection, are risk factors that predispose individuals to EC [4-6]. Nevertheless, some individuals without these known risk factors develop EC. This phenomenon suggests that genetic factors also play an important role in esophageal carcinogenesis [7].

P53, a tumor suppressor gene located on chromosome 17p13, contains 11 exons and encodes a 53 kDa nuclear phosphoprotein (TP53; GenBank NM_000546.2). Activated p53 suppresses carcinogenesis mainly by inducing the cell cycle arrest, senescence, and apoptosis of damaged cells [8]. p53 is commonly mutated in all kinds of human tumors. The most common polymorphism of p53 is at the 72nd amino acid residue with an arginine (Arg) to proline (Pro) change because of a G-to-C
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transversion [9]. p53 Arg72Pro polymorphism is associated with some malignancies, such as cervical cancer [10], lung cancer [11], bladder cancer [12], thyroid carcinoma [13], nasopharyngeal carcinoma [14], skin cancer [15], prostate cancer [16], and gastric cancer [17]. The association between p53 codon 72 polymorphism and EC susceptibility has been extensively investigated, but inconclusive results have been obtained [18-29].

HPV infectious agents function as either direct carcinogens or promoters in esophageal carcinogenesis [30]. A meta-analysis has demonstrated that HPV increases the risk of EC by three-fold [31]. The E6 protein of HPV binds to and induces the degradation of p53 tumor suppressor protein via a ubiquitin-mediated process [32]. The role of p53 Arg72Pro polymorphism in the development of HPV-related cancer was first proposed in 1998 [33]. Since then, studies have investigated the combined influences of p53 codon 72 polymorphism and HPV infection on the risk of EC. However, results remain inconsistent. Furthermore, whether p53 Arg72Pro polymorphism can increase the risk of HPV-related EC remains unclear. Thus, this study aimed to assess the relationship of p53 Arg72Pro polymorphisms with the risk of HPV-related EC.

**Materials and methods**

**Search strategy**

We examined the databases of PubMed (1946-), Embase (1945-), China National Knowledge Infrastructure (CNKI) (1979-), and WanFang (1982-) until November 1, 2015 with the following search items: “p53”, “HPV” or “human papillomavirus”, and “esophageal cancer”.

**Inclusion and exclusion criteria**

The following inclusion criteria were used: (1) articles were published in English or Chinese; (2) EC cases were diagnosed pathologically or histologically; (3) the association of p53 Arg72Pro polymorphism with the risk of HPV-related esophageal cancer was evaluated; (4) the number of individual genotypes was provided in HPV-positive and HPV-negative groups or calculated from the original article.

The title and abstract of each study identified in the search was scanned to exclude clearly irrelevant publications. The exclusion criteria were as follows: (1) overlapping of data. When studies were duplicated (identified by author names and institution), the study based on the largest number of patients was selected; (2) reviews, meta-analysis, or case reports; and (3) non-extractable relevant raw data (the number of cases for a given p53 genotype or HPV status). If studies did not report sufficient or clear data, the corresponding authors were contacted by e-mail to request the information. The remaining articles were browsed to determine whether they contained relevant information.

**Statistical analysis**

Odds ratio (OR) and 95% confidence intervals (CI) were used to quantify the strength of the association between p53 Arg72Pro polymorphism and HPV-related EC by using five genetic models: allelic model (Arg vs. Pro), homozygous model (ArgArg vs. ProPro), heterozygous model (ArgPro vs. ProPro), dominant model (ArgArg+ArgPro vs. ProPro), and recessive model (ArgArg vs. ArgPro+ProPro). $I^2$ statistic was employed to quantify the proportion of the total variation because of the calculated heterogeneity. An $I^2$ of > 50% was interpreted as significant heterogeneity among the studies. The fixed-effects model (FEM) was initially used to pool the results of the included studies. If $I^2 > 50\%$, a random-effects model (REM) was used. To evaluate the stability of the results, sensitivity analysis was performed to evaluate the influence of individual study on the estimated summary relative risks. Publication bias was calculated using the Begger’s test and visualized using funnel plot ($P < 0.05$ was considered representative of statistically significant publication bias). All statistical analyses were conducted.
Results

Study characteristics

We initially identified a total of 1696 studies from PubMed, Embase, CNKI, and WanFang databases. After irrelevant studies were duplicated and excluded, 12 studies containing 1682 EC cases were included in this meta-analysis [34-45]. A flow chart illustrating the screening process was shown in Figure 1. The main characteristics of the studies and all of the related distribution patterns of p53 Arg72Pro polymorphism genotype and HPV frequencies in EC cases are summarized in Table 1. Nine studies included pure ESCC cohorts [34, 37-42, 44, 45], two studies included mixed histologic cohorts [35, 36], and one study did not mention histologic clearly [43]. Four studies assessed p53 codon 72 polymorphism by direct sequencing [34, 39, 41, 42], one study detected p53 polymorphism through real-time PCR [40], and the remaining seven studies performed PCR-RFLP [35-38, 43-45]. Six studies assessed HPV status by PCR [35-37, 42, 44, 45].

Figure 1. PRISMA flow chart of study inclusion.
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Table 1. Characteristics of the included studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Ethnicity</th>
<th>Type</th>
<th>Source</th>
<th>ArgArg</th>
<th>ArgPro</th>
<th>ProPro</th>
<th>p53 testing method</th>
<th>HPV testing method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pantelis A, 2007 [34]</td>
<td>Germany</td>
<td>Caucasians</td>
<td>ESCC</td>
<td>Tissue</td>
<td>3</td>
<td>28</td>
<td>5</td>
<td>10</td>
<td>Sequencing</td>
</tr>
<tr>
<td>Peixoto Guimaraes D, 2001 [37]</td>
<td>China</td>
<td>Asians</td>
<td>ESCC</td>
<td>Tissue</td>
<td>40</td>
<td>40</td>
<td>14</td>
<td>10</td>
<td>PCR-RFLP</td>
</tr>
<tr>
<td>Yao E, 2008 [38]</td>
<td>China</td>
<td>Asians</td>
<td>ESCC</td>
<td>Tissue</td>
<td>12</td>
<td>25</td>
<td>2</td>
<td>16</td>
<td>Sequencing</td>
</tr>
<tr>
<td>Yu Q, 2014 [40]</td>
<td>China</td>
<td>Asians</td>
<td>ESCC</td>
<td>Tissue</td>
<td>3</td>
<td>29</td>
<td>14</td>
<td>17</td>
<td>Real-time PCR</td>
</tr>
<tr>
<td>Li T, 2002 [41]</td>
<td>China</td>
<td>Asians</td>
<td>ESCC</td>
<td>Tissue</td>
<td>39</td>
<td>20</td>
<td>8</td>
<td>16</td>
<td>PCR-RFLP</td>
</tr>
<tr>
<td>Yu Q, 2014 [40]</td>
<td>China</td>
<td>Asians</td>
<td>NA</td>
<td>Tissue</td>
<td>3</td>
<td>29</td>
<td>14</td>
<td>17</td>
<td>Sequencing</td>
</tr>
<tr>
<td>Li X, 2012 [42]</td>
<td>China</td>
<td>Asians</td>
<td>NA</td>
<td>Tissue</td>
<td>39</td>
<td>20</td>
<td>8</td>
<td>16</td>
<td>PCR-RFLP</td>
</tr>
<tr>
<td>Lu XM, 2004 [44]</td>
<td>China</td>
<td>Asians</td>
<td>ESCC</td>
<td>Tissue</td>
<td>3</td>
<td>29</td>
<td>14</td>
<td>17</td>
<td>PCR-RFLP</td>
</tr>
<tr>
<td>Zhou X-h, 2012 [45]</td>
<td>China</td>
<td>Asians</td>
<td>ESCC</td>
<td>Tissue</td>
<td>3</td>
<td>29</td>
<td>14</td>
<td>17</td>
<td>PCR-RFLP</td>
</tr>
</tbody>
</table>

ESCC: esophageal squamous cell carcinoma; Mixed: esophageal squamous cell carcinoma and esophageal adenocarcinoma; NA: not available; PCR: polymerase chain reaction; PCR-RFLP: polymerase chain reaction with restriction fragment length polymorphism; ISH: in situ hybridization; ELISA: enzyme-linked immunosorbent assay.
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Figure 2. Forest plot of ORs for the association of p53 codon 72 polymorphism in allelic (A), homozygous (B), recessive (C), heterozygous (D), and dominant (E) models with HPV-related esophageal cancer.

Table 2. Stratified analysis of pooled odds ratios for the association of p53 codon 72 polymorphism in allelic and recessive models with HPV-related esophageal cancer

<table>
<thead>
<tr>
<th>Stratified analysis</th>
<th>No. of studies</th>
<th>Sample size (HPV+/HPV-)</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
<th>Model</th>
<th>I²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allelic model</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>P53 genotyping method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sequencing</td>
<td>4</td>
<td>616/377</td>
<td>1.34</td>
<td>0.77-2.32</td>
<td>0.3</td>
<td>REM</td>
<td>55%</td>
</tr>
<tr>
<td>No sequencing</td>
<td>7</td>
<td>556/440</td>
<td>1.88</td>
<td>1.19-2.97</td>
<td>0.006</td>
<td>REM</td>
<td>72%</td>
</tr>
<tr>
<td>HPV detection method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR only</td>
<td>6</td>
<td>832/430</td>
<td>1.87</td>
<td>1.28-2.74</td>
<td>0.001</td>
<td>REM</td>
<td>51%</td>
</tr>
<tr>
<td>No PCR</td>
<td>5</td>
<td>340/387</td>
<td>1.46</td>
<td>0.81-2.60</td>
<td>0.21</td>
<td>REM</td>
<td>70%</td>
</tr>
<tr>
<td>Recessive model</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P53 genotyping method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sequencing</td>
<td>4</td>
<td>301/167</td>
<td>1.43</td>
<td>0.58-3.54</td>
<td>0.44</td>
<td>REM</td>
<td>67%</td>
</tr>
<tr>
<td>No sequencing</td>
<td>8</td>
<td>285/147</td>
<td>3.07</td>
<td>1.74-5.43</td>
<td>0.0001</td>
<td>REM</td>
<td>70%</td>
</tr>
<tr>
<td>HPV detection method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR only</td>
<td>7</td>
<td>475/191</td>
<td>3.19</td>
<td>1.68-6.06</td>
<td>0.0004</td>
<td>REM</td>
<td>73%</td>
</tr>
<tr>
<td>No PCR</td>
<td>5</td>
<td>111/123</td>
<td>1.54</td>
<td>0.75-3.13</td>
<td>0.24</td>
<td>REM</td>
<td>60%</td>
</tr>
</tbody>
</table>

Overall analyses

All 12 studies were included in the meta-analysis to assess the association of the recessive model (ArgArg vs. ArgPro+ProPro) with HPV-related EC. A total of 11 studies, including 1478 HPV-related EC cases, were evaluated to determine the relationship of allelic (Arg vs. Pro), homozygous (ArgArg vs. ProPro), heterozygous (ArgPro vs. ProPro) and dominant (ArgArg+ArgPro vs. ProPro) models with HPV-related EC. Results showed that p53 codon 72 Arg homozygous genotype was significantly associated with HPV-related EC in three genetic comparison models (OR_{allelic} = 1.66, 95% CI = 1.19-2.32, P = 0.003, I² = 65%; OR_{homozygous} = 1.73, 95% CI = 1.26-2.37, P = 0.0007, I² = 23%; OR_{recessive} = 2.39, 95% CI = 1.45-3.94, P = 0.0006, I² = 72%; Figure 2). By contrast, p53 genotypes were not significantly correlated with HPV-related EC in the heterozygous model (OR = 0.33, 95% CI = 0.61-1.19, P = 0.33, I² = 0%) and the dominant model (OR = 1.24, 95% CI = 0.93-1.65, P = 0.14, I² = 0%).

Subgroup analyses

Due to the significant heterogeneity of included studies in the allelic model and the recessive model (I² = 65% and 72%, respectively; Figure 2), we conducted subgroup analyses according to detection methods of p53 codon 72 polymorphism and HPV status (Table 2). The association between p53 Arg72Pro polymorphisms and the risk of HPV-related EC appeared to be greater among studies performing PCR-RFLP or real-time PCR for p53 genotyping (pooled OR_{allelic} = 1.88, 95% CI = 1.19-2.97, P = 0.006, I² = 72%; OR_{recessive} = 3.07, 95% CI = 1.74-5.43, P = 0.0001, I² = 70%) compared with studies using direct p53 sequencing, and studies performing PCR to detect HPV status (OR_{allelic} = 1.87, 95% CI = 1.28-2.74, P = 0.001, I² = 51%; OR_{recessive} = 3.19, 95% CI = 1.68-6.06, P = 0.0004, I² = 73%) compared with studies using ISH, ELISA or sequencing.

Sensitivity analysis and publication bias

Sensitivity analysis was performed to address the potential bias due to the quality of the included studies. As shown in Figure 3, the results for all studies were stabilized, and thus, no individual study was found to influence the
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Discussion

P53 is a major regulator of cell responses to stress; p53 also functions as a tumor suppressor by inducing cell cycle arrest and apoptosis [46]. A common polymorphism occurs at codon 72 of exon 4 in the transactivation domain of p53 [47]. HPV exhibits a formidable binding affinity to p53 by encoding oncogenic protein E6; as a result, p53 is ubiquitinated and degraded; an uncontrollable cell cycle also occurred [48]. In 1998, Storey and co-workers reported that p53 codon 72 Arg homozygous genotype represents a significant risk factor of the development of HPV-related cancers [33]. Since then, related studies have been published, but results remain inconclusive [49-60]. A meta-analysis has revealed that p53 Arg72Pro polymorphism is not associated with the risk of HPV-related head and neck squamous cell carcinoma [61]. Another meta-analysis has demonstrated that Arg72-Pro is associated with the progression of squamous intraepithelial lesions to cervical cancer in the presence of HPV-positive Arg variant of p53 [62]. However, definitive conclusion between p53 Arg72Pro
p53 codon 72 polymorphism is associated with HPV-related ESCC

We performed this meta-analysis to obtain a more robust estimate of the relationship between p53 polymorphism and the risk of HPV-related EC. A total of 1682 HPV-related EC cases were evaluated in all of the 12 included studies. The results indicated that the p53 codon 72 Arg homozygous genotype is a high-risk factor of HPV-related EC (OR\text{Arg vs. Pro} = 1.66, 95% CI=1.19-2.32; OR\text{ArgArg vs. ProPro} = 1.73, 95% CI=1.26-2.37; OR\text{ArgPro vs. ProPro} = 0.85, 95% CI=0.61-1.19; OR\text{ArgArg+ArgPro vs. ProPro} = 1.24, 95% CI=0.93-1.65; OR\text{ArgArg vs. ArgPro+ProPro} = 2.39, 95% CI=1.45-3.94). Our results are consistent with those of Habbous' study [62], who demonstrated that p53 Arg allele plays an important role in cervical cancer development among HPV-positive patients. Our results also agree with those of Storey's study [33], who stated that the arginine-encoding allele represents a significant risk factor of the development of HPV-associated cancers.

Of the 12 included studies, 2 did not find any association between p53 codon 72 polymorphism and HPV-related esophageal cancer. Pantelis and co-workers analyzed the association of these factors in Germany, a geographic region with a low incidence of esophageal cancer [34]. Peixoto and co-workers extracted DNA from exfoliated cells to analyze p53 codon 72 polymorphism and to detect HPV from 32 cases [37]. These varying results are likely attributed to the regional or DNA extraction source. However, subgroup analysis according to ethnicity or source of samples was not conducted in this meta-analysis because of the limited number of included studies.

Figure 4. Funnel plot for the association of p53 codon 72 polymorphism in allelic (A), homozygous (B), and recessive (C) models with HPV-related esophageal cancer.

polymorphism and HPV-related esophageal cancer has yet to be obtained [34-45].

Heterogeneity between studies is very common in genetic association analysis involved in meta-analysis. Heterogeneity of the allelic model and the recessive model in overall analyses was high. To identify the source of heterogeneity, we removed non-Asian populations [34]. However, we found that heterogeneity was not remarkably decreased (data not shown); thus, ethnicity may not contribute to the observed heterogeneity.

Some possible limitations of our meta-analysis include the following. First, the sample sizes of the studies included in this meta-analysis except those in three studies were small [40, 42, 45]. Second, we included studies published in English and Chinese. Therefore, some relevant reports published in other languages may be missed. Finally, the result of this meta-analysis did not measure potential confounders or adjust for their effects because not all eligible studies have comprehensive information, such as smoking and alcohol consumption.

In conclusion, this meta-analysis suggested that p53 codon 72 Arg homozygous genotype is a high risk factor of HPV-related EC. Individuals carrying ArgArg genotype are at an increased risk of EC with HPV infection. Further well-designed and extensive studies should be conducted to validate this association in different populations.

Acknowledgements

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Disclosure of conflict of interest

None.

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[34] Pantelis A, Pantelis D, Rueemmele P, Hartmann A, Hofstaedter F, Buettner R, Bootz F and


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