Original Article

P53 status as a biomarker to predict the response to neoadjuvant therapy in esophageal cancer: a systematic review and meta-analysis

Hai-Yuan Xu1*, Zhao-Liang Su2*, Xing Li3, Min-Bin Chen1, Zhang-Yi Ji1, Xiang-Rong Lu4

Departments of 1Medical Oncology, 3Chest Surgery, 4Orthopaedics, Kunshan First People’s Hospital Affiliated to Jiangsu University, 91 Qianjin Road, Kunshan 215300, Jiangsu Province, People’s Republic of China; 2Institute of Immunology, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, Jiangsu Province, People’s Republic of China. * Equal contributors.

Received July 23, 2016; Accepted August 2, 2016; Epub February 1, 2017; Published February 15, 2017

Abstract: Background: Numerous studies published previously have reached inconsistent conclusions regarding the relationship between p53 status and the response to neoadjuvant therapy in patients with esophageal cancer. To acquire a more precise evaluation of such association, we performed this meta-analysis. Methods, findings: 25 eligible studies encompassing 1234 patients were identified and included in this meta-analysis. Significant association was found between wild-type form of p53 status (low expression of p53 protein and/or wild p53 gene) and improved response in esophageal cancer patients who received neoadjuvant therapy (good response: risk ratio [RR] = 1.306; 95% confidence intervals [CI] = 1.131-1.507; P<0.001). In further stratified analysis, wild-type form of p53 status was associated with improved response to neoadjuvant chemoradiotherapy (RR = 1.308, 95% CI = 1.104-1.551, P = 0.002) and neoadjuvant chemotherapy (RR = 1.436, 95% CI = 1.052-1.960, P = 0.023). Patients with wild-type form of p53 status had high complete response rate to neoadjuvant therapy (RR = 1.844, 95% CI = 1.262-2.694, P = 0.002). Additionally, association with good response remained in the Asian population (RR = 1.268; 95% CI = 1.074-1.499; P = 0.005), while in the European subgroup, patients with wild-type form of p53 status tended to have a good response to neoadjuvant therapy, although this did not reach statistical significance (RR = 1.005, 95% CI = 0.667-1.515, P =0.981). Conclusion: The results of this meta-analysis suggested that p53 status may be a useful predictive biomarker for response to neoadjuvant therapy in esophageal cancer, especially in Asian population.

Keywords: P53, biomarker, neoadjuvant therapy, esophageal cancer

Introduction

It is estimated that esophageal cancer is the sixth most common cause of cancer deaths all over the world [1]. In 2015, an estimated 16980 new esophageal cancer cases will occur and 15590 cases will eventually die of their disease in the United States [2]. However, it is more common in the developing nations and is the fourth cause of cancer deaths [1]. Despite advances in surgical treatment and chemotherapy, its prognosis remains poor, mainly because most tumours are diagnosed late either locally advanced or metastatic stages which lost the opportunities of radical surgery for the early stage. CROSS study and MAGIC trial have shown that neoadjuvant therapy significantly enhanced local control, increased resectability rate, and improved disease-free survival in patients with resectable esophageal and esophagogastric cancer [3, 4]. However, in these studies, only those patients who responded to neoadjuvant therapy with tolerable toxicity would potentially benefit from this approach, while a part of patients failed to respond to neoadjuvant therapy, or even progressed during therapy. It is therefore imperative to investigate the predictive markers to identify those individuals who would benefit from neoadjuvant therapy.

As the most studied gene, p53 may be the most suitable biomarker for predicting the response to neoadjuvant therapy [5]. P53, a tumor-sup-
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ergic gene, has central cellular functions in regulating cell cycle, repairing cellular DNA and triggering apoptosis after cellular DNA is injured [6,7]. P53 is the most frequently mutated gene in human cancer, with mutations occurring in at least 50% of human cancers, which plays a critical role in the process of development of human cancer [8]. Experimental evidence indicates that p53 plays a vital role in tumor apoptosis in response to genotoxic agents [9-11].

However, it is inconsistent that the research results for the use of p53 status as a biological marker to predict the response of esophageal cancer to neoadjuvant therapy. Some studies found that patients with wild-type form of p53 status often had a higher response rate to chemotherapy than those with mutant-form of p53 status [12-18]. Other studies, however, drew different conclusions [19-24]. Zhang conducted a meta-analysis and found that wild-type form of p53 status was associated with high response to chemotherapy-based treatment in esophageal cancer [25]. However, the correlation of p53 status with the response to neoadjuvant therapy was not analyzed in detail. Therefore, we proceeded with this meta-analysis with larger sample size specifically to assess p53 status as Biomarker to predict the response of esophageal cancer to neoadjuvant therapy.

Materials and methods

Publication search

Using the following search terms: 'TP53', 'p53', 'p53 protein', 'p53 mutation', '17p13 gene', 'chemoradiotherapy', 'chemotherapy', 'neoadjuvant', 'preoperative' and 'esophageal cancer', studies were identified by a computerized search of the PubMed, Embase, and Web of Science databases (last search up to January 2016). All potentially eligible studies were retrieved and their references were carefully reviewed to identify other eligible studies. If multiple studies of the same patient population were identified, we included the published report with the largest sample size.

Inclusion and exclusion criteria

Studies included in this meta-analysis should suit all of the following features: (a) evaluation of p53 status for predicting the response to neoadjuvant therapy in esophageal cancer, (b) description clinical or pathological therapeutic response, (c) retrospective or prospective cohort study, (d) inclusion of adequate data to allow the estimation of a risk ratio (RR) with 95% confidence intervals (95% CI), and (e) only studies published in English language. Reviews, letters to the editor, and articles published in books were excluded.

Data extraction and definitions

The following information was extracted from each study: the first author’s surname, publication year, country of origin, cases of patients analyzed, treatment, chemotherapy regimen, methods of detection of p53, p53 positive (overexpression or mutation) rate, type of therapeutic response, response criteria, and the response rate. Data was entered in tables showing the clinical or pathological response to neoadjuvant therapy with respect to p53 status. Data was carefully and independently extracted from all eligible publications by two reviewers (Haiyuan Xu and Xiangrong Lu). Any disagreement between the reviewers was resolved by discussions until a consensus was reached. A third reviewer (Zhaoliang Su) was employed to resolve the discrepancies when they failed to reach an agreement.

The definitions and standardizations for ‘p53’ and ‘response to neoadjuvant therapy’ we used were in the light of the study reported by Pakos et al. [26]. For consistency, we used ‘p53 status’ to refer to both the gene and protein markers. Mutation-type form of p53 status means patients with high expression of p53 protein and/or mutant p53 gene. Wild-type form of p53 status means patients with low expression of p53 protein and/or normal p53 gene. Response was defined as grade 3 or complete response (CR), grade 1b+2 or grade 2 or partial response (PR), or major response (MR) (MR = grade 3+ grade 1b+2 or MR = CR+PR), according to the guidelines for the clinical and pathologic studies on carcinoma of the gastric by JSED (the Japanese Society for Esophageal Disease), WHO (World Health Organization) or RECIST (Response Evaluation Criteria in Solid Tumors) criteria [27-30]. For consistency, we defined the response classification in Table 1 [31].

Statistical analysis

STATA version 12 (StataCorp, College Station, TX) was applied to perform the data analysis.
## Table 1. Criteria for response evaluation and standard definition

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Minor response</th>
<th>Major response</th>
<th>Complete response</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO [29]</td>
<td>NC+PD, &lt;50% decrease in tumor load</td>
<td>PR+CR, &gt;50% decrease in tumor load</td>
<td>CR, disappearance of all known disease</td>
</tr>
<tr>
<td>RECIST [30]</td>
<td>PD+SD, &gt;30% regression of the disease</td>
<td>PR+CR, &gt;30% regression of the disease</td>
<td>CR, 100% regression of the disease</td>
</tr>
<tr>
<td>JSED [27, 28]</td>
<td>PD+SD, Grade 0+1, viable cancer cells account for more than 1/3</td>
<td>PR, Grade 2+3, viable cancer cells account for less than 1/3</td>
<td>CR, Grade 3, no residual viable tumor cells</td>
</tr>
<tr>
<td>Nasierowska et al. [32]</td>
<td>PR2+SD</td>
<td>PR1+CR, single cells or small nests of cancer cells or no cancer cells</td>
<td>CR, no microscopic evidence of cancer cells</td>
</tr>
<tr>
<td>Miyata et al. [13]</td>
<td>RR, Residual viable cancer cells</td>
<td>SR, no residual cancer cells</td>
<td>SR, no residual cancer cells</td>
</tr>
<tr>
<td>Sarbia et al. [33]</td>
<td>PD+NC, PD: increasing tumor diameter assessed by CT; NC; &lt;50% regression of tumor extension, and no progression</td>
<td>CR+PR, &gt;50% reduction assessed by CT</td>
<td>Normal barium esophagogram, no visible tumor by esophagoscopy, biopsies free of tumor tissue, and normal CT</td>
</tr>
<tr>
<td>Yang et al. [34]</td>
<td>ORT, gross residual tumor and/or residual tumor in 2 or more tissue blocks</td>
<td>NRT+MRT, absence of tumor either grossly or microscopically</td>
<td>NRT, absence of tumor both grossly and microscopically</td>
</tr>
</tbody>
</table>

The statistical heterogeneity for each pooled estimate was assessed and quantified by the Q test and the $I^2$ statistic. According to the heterogeneity, the pooled RR was calculated using a random-effects model (the DerSimonian and Laird method) or a fixed-effects model (the Mantel-Haenszel method). Pooled analysis was performed using the Mantel-Haenszel model and reported as risk ratio (RR) with 95% CIs. The significance of the pooled RR was determined by the z test. $P<0.05$ was considered to be statistically significant. The potential publication bias was estimated by the Begg’s funnel plot and Egger’s test. We also performed sensitivity analysis by omitting each study or specific studies to find potential outliers.

Results

Eligible studies

On the basis of different combinations of key terms, 408 studies were identified by literature search. As the search flow diagram (Figure 1) demonstrated, 25 studies and a total of 1238 patients were finally included in our analysis.

Subgroup analysis

Among the 25 studies, 18 studies used NCRT and 7 studies used NCT, we also have these data for statistical analysis respectively, and found that wild-type form of the p53 status was associated with improved response to NCRT...
## Table 2. Characteristics of studies included in the meta-analysis

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Cases</th>
<th>Pathology</th>
<th>Treatment</th>
<th>Detection</th>
<th>p53 (%)</th>
<th>Response</th>
<th>Response rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kandioler et al.</td>
<td>2014</td>
<td>Australia</td>
<td>36</td>
<td>SCC+AC</td>
<td>NCT</td>
<td>Gene</td>
<td>50</td>
<td>pathologic</td>
<td>PD+SD</td>
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<tr>
<td>Arsenijevic et al.</td>
<td>2012</td>
<td>Serbia</td>
<td>41</td>
<td>SCC</td>
<td>NCRT</td>
<td>IHC</td>
<td>85</td>
<td>clinical</td>
<td>PD+SD</td>
</tr>
<tr>
<td>Yamamoto et al.</td>
<td>2012</td>
<td>Japan</td>
<td>37</td>
<td>SCC</td>
<td>NCT</td>
<td>IHC</td>
<td>49</td>
<td>pathologic</td>
<td>JSED</td>
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<tr>
<td>Makino et al.</td>
<td>2010</td>
<td>Japan</td>
<td>64</td>
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<td>NCRT</td>
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<td>78</td>
<td>clinical</td>
<td>WHO</td>
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<td>78</td>
<td>clinical</td>
<td>WHO</td>
</tr>
</tbody>
</table>

P53 status as a biomarker


Figure 2. Forest plots of RR were estimated for association between p53 status and good response among esophageal cancer patients treated with neoadjuvant therapy.

Figure 3. Forest plots of RR were estimated for association between p53 status and good response among esophageal cancer patients treated with NCRT.

(RR = 1.308, 95% CI = 1.104-1.551, P = 0.002, Figure 3) and NCT (RR = 1.436, 95% CI = 1.052-1.960, P = 0.023, Figure 4). Ten of the studies supplied complete response data. We found that patients with wild-type form of p53 status had high complete response rate to neoadjuvant therapy (RR = 1.844, 95% CI = 1.262-2.694, P = 0.002, Figure 5). In the studies that histopathology revealed squamous cell carcinoma, we found that wild-type form of p53 status had improved response rate to neoadjuvant therapy (RR = 1.194, 95% CI = 1.109-1.398, P = 0.028). 17 studies were conducted in Asian populations (902 patients), whereas 5 studies were conducted in European populations (179 patients). The results of the Asian subgroup and the European subgroup were therefore calculated separately (Table 3). Wild-type forms of p53 status was associated with improved response in esophageal cancer patients who received neoadjuvant therapy in Asian subgroup (RR = 1.268; 95% CI = 1.074-1.499; P = 0.005, Figure 6). In European subgroup, however, patients with wild-type form of p53 status trended to have a high response rate to neoadjuvant therapy, but did not reach statistical significance (RR = 1.005, 95% CI = 0.667-1.515, P = 0.981).

Publication bias and sensitivity analysis

Both Begg’s funnel plot and Egger’s test were performed to assess the potential publication bias of the included literatures. The shapes of the funnel plots indicated no evidence of obvious asymmetry (Figure 7), and Egger’s test showed the absence of publication bias (P>0.05). Furthermore, sensitivity analysis was conducted to evaluate the influence of each study on the summary effect. No individual study dominated this meta-analysis, and the removal of any single study had no significant effect on the overall results (data not shown).
p53 status and the response to neoadjuvant therapy in a large population with esophageal cancer.

The present meta-analysis of 25 studies systematically estimated the relationship between p53 status and response to neoadjuvant therapy in a large population. The results showed that wild-type form of p53 status may predict the good response rate to neoadjuvant therapy in patients with esophageal cancer. The wild-type form of p53 status was associated with improved total OR. Stratification according to different population indicated that wild-type form of p53 status was significantly associated with increased OR in Asian population. And with respect to the NCRT and NCT respectively, results showed there were good response rate in patients with the wild-type form of p53 status. Significant association was also found in patients with the wild-type form of p53 status and high complete response rate to neoadjuvant therapy.

However, despite our attempts to perform a comprehensive analysis, there were still some limitations of this meta-analysis. First, selection bias might occur because only studies published in English language were included in our meta-analysis, and we did not search conference proceedings and abstract books. Second, the evaluation criterion of response to treatment among the studies was of a great difference and variety. Standardization is therefore of great importance for obtaining an accurate assessment of the clinical significance of p53 status. Despite of our considerable efforts to standardize definitions, some variability among studies was inevitable. Third, different regimens of treatment were employed among these studies and the dose of chemotherapy or

Studies have shown that p53 status play a key role in the response to a large panel of anticancer drugs and radiation-based therapy. However, the conclusion is inconsistent that p53 mutation might be either sensitive or resistant to neoadjuvant therapy in patients with esophageal cancer, because most of the available clinical reports involved small sample sizes, and the results were therefore unable to determine the value of p53 status for predicting the response to neoadjuvant therapy. Thus, we supposed that a meta-analysis might be the best way to evaluate the association between

Figure 4. Forest plots of RR were estimated for association between p53 status and good response among esophageal cancer patients treated with NCT.

Figure 5. Forest plots of RR were estimated for association between p53 status and complete response among esophageal cancer patients treated with neoadjuvant therapy.

Discussion

Studies have shown that p53 status play a key role in the response to a large panel of anticancer drugs and radiation-based therapy. However, the conclusion is inconsistent that p53 mutation might be either sensitive or resistant to neoadjuvant therapy in patients with esophageal cancer, because most of the available clinical reports involved small sample sizes, and the results were therefore unable to determine the value of p53 status for predicting the response to neoadjuvant therapy. Thus, we supposed that a meta-analysis might be the best way to evaluate the association between
radiation and courses of treatment were variable. Forth, the sample size is relatively small. Especially, the stratified studies regarding Asians and Europeans might have insufficient statistical power to expound the real association.

Despite the limitations above, compared to the previously published meta-analysis, our study has the following highlight: (1) this meta-analysis is the first meta-analysis specialized in evaluating the usefulness of p53 status for predicting the response of esophageal cancer patients to neoadjuvant therapy, (2) the sample size is relatively larger and provides more potent statistical power in comparison to other relevant meta-analysis, (3) has included the latest studies to increase coverage and minimize selection bias, (4) our data indicated that p53 status might be a useful predictive biomarker for evaluating response to neoadjuvant therapy in esophageal cancer patients, especially in Asian population. However, future prospective studies with larger sample sizes and better study designs are required to evaluate the predictive role of p53 status in clinical practice.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 81472786). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
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Figure 7. The funnel plot showed that there was no obvious indication of publication bias for the outcome of good response setting.

design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosure of conflict of interest

None.

Authors’ contribution

HY-X, ZL-S, XR-L contributed to the conception and design of the study, the analysis and interpretation of data, the revision of the article as well as final approval of the version to be submitted. MB-C, XL, ZY-J participated in the design of the study, performed the statistical analysis, searched and selected the trials, drafted and revised the article. All authors read and approved the final version of the manuscript.

Abbreviations

CI, Confidence interval; IHC, Immunohistochemistry; RR, Relative risk; WHO, World Health Organization; RECIST, Response Evaluation Criteria in Solid Tumors; JSED, Japanese Society for Esophageal Disease; CR, complete response; PR, partial response; PD, progressive disease; SD, stable disease; NR, no record; NC, no change; SCC, squamous cell carcinoma; AC, adenocarcinoma; NCT, neoadjuvant chemotherapy; NCRT, neoadjuvant chemoradiotherapy.

Address correspondence to: Zhao-Liang Su, Institute of Immunology, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, Jiangsu Province, People’s Republic of China. E-mail: szl30@yeah.net; Xiang-Rong Lu, Department of Orthopaedics, Kunshan First People’s Hospital Affiliated to Jiangsu University, 91 Qianjin Road, Kunshan 215300, Jiangsu Province, People’s Republic of China. E-mail: luxiangr@126.com

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