The effects of proliferating cell nuclear antigen and p53 in patients with oral squamous cell carcinoma: a systematic review and meta-analysis

Rui Liu†, Kunjun Sun†, Yuanda Wang, Yunxian Jiang, Jianyong Kang, Hong Ma

Department of Oral and Maxillofacial Surgery, Affiliated Hospital of Guizhou Medical University, Guiyang, China

Contribution: (I) Conception and design: R Liu, K Sun, H Ma; (II) Administrative support: Y Wang, Y Jiang, J Kang; (III) Provision of study materials or patients: R Liu, K Sun; (IV) Collection and assembly of data: R Liu, K Sun, Y Wang, Y Jiang, J Kang; (V) Data analysis and interpretation: R Liu, K Sun; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

†These authors contributed equally to this work.

Correspondence to: Hong Ma. Department of Oral and Maxillofacial Surgery, Affiliated Hospital of Guizhou Medical University, No. 28 Guiyi Street, Guiyang, China. Email: mahong1966@126.com.

Background: To evaluate the effect of proliferating cell nuclear antigen (PCNA) and p53 in patients with oral squamous cell carcinoma (OSCC).

Methods: Multiple databases, including PubMed, Embase, Cochrane library, and China National Knowledge Database, were searched for relevant studies and full-text articles that evaluated the effect of PCNA and p53 in patients with OSCC. Review Manager 5.2 was adopted to estimate the impact of the results among the selected articles. Forest plots, NOS table, sensitivity analysis, and bias analysis were also conducted.

Results: In total, nine eligible studies satisfied the included criteria. High PCNA expression (>50%) was significantly more prevalent in OSCC than low PCNA expression (<50%) (OR =3.88; 95% CI: 2.04–7.37; P<0.0001; I²=0%). However, there was no significant difference between p53 and OSCC (OR =1.60; 95% CI: 0.18–14.63; P=0.68; I²=86%). Low PCNA expression had a higher 5-year overall survival in OSCC patients than high PCNA expression (OR =0.47; 95% CI: 0.27–0.80; P=0.005; I²=41%). Meanwhile, p53 negative had a higher 5-year overall survival than p53 positive (OR =0.20; 95% CI: 0.10–0.42; P<0.0001; I²=0%). There was no difference between high and low PCNA in terms of metastasis (OR =0.80 with 95% CI: 0.18–3.45, I²=63%, P of over effect =0.76). The overall results showed no difference between p53 and metastasis (OR =0.38 with 95% CI: 0.13–1.00, I²=0%, P of over effect =0.07).

Discussion: PCNA and p53 might be suitable for prognostic and survival evaluation in OSCC patients.

Keywords: Oral squamous cell carcinoma (OSCC); proliferating cell nuclear antigen (PCNA); p53; meta-analysis

Introduction

Globally, head and neck cancers are estimated to comprise 500,000 patients with squamous cell carcinoma (SCC) every year. SCC is the most common malignant tumor of the head and neck, accounting for 90% (1). Due to the location of oral SCC (OSCC) in the body, the social and medical impact of these lesions is more significant than other more common tumors. OSCCs are close to vital structures in the head and neck, making treatment difficult, and the results are often severely deformed (2). Part of the reason for the poor prognosis (5-year survival of approximately 50%) and high recurrence rate (about 645,000 per year) of OSCC is the lack of an accurate and clinically applicable staging system. Also, the current clinical diagnosis system
for predicting the local control and survival rate of OSCC is limited (3).

As a marker of cell proliferation, PCNA is considered a convenient tool for quickly assessing the proportion of proliferating cells in tumors. PCNA is a nuclear non-histone antigen that appears in the nucleus in the late G1 phase. It increases in the S phase and declines in the G2 and M phases. PCNA is a 36kda molecule that plays an essential role in nucleic acid metabolism due to the replication and repair mechanism (4). It serves as an accessory protein for DNA polymerase; it is needed to synthesize S-phase chromosomal DNA and interact with cellular proteins involved in regulating the cell cycle and checkpoint control (5). Some studies have suggested that PCNA expression is a marker of abnormal cell proliferation and could be used as a reference index for early cancer diagnosis (4,5).

The tumor suppressor gene, p53, is a genetic biomarker that regulates cell growth and proliferation. The wild-type p53 protein controls the cell cycle’s progression by acting as transcription factors for multiple genes, which induces transcriptional regulation of the cyclin-dependent kinase inhibitor p21 (6). The stability and overexpression of the p53 gene might be related to p53 gene mutation or genotoxic stress, and p53 gene changes are the most common genetic abnormality in many cancers. In OSCC, multiple studies have shown that overexpression of p53 plays a vital role in the development of OSCC (7). p53 is an important anticancer gene; its wild type can induce apoptosis of cancer cells and prevent canceration, and could also help cells repair gene defects (3). In addition, it was reported that there was significant correlation between the expression level of p53 protein and postoperative survival time of oral squamous cell carcinoma and the expression of PCNA protein was closely related to the risk of OSCC, and could be used as an important index to judge the prognosis of OSCC patients (5-7).

In recent years, the value of PCNA and p53 in OSCC has been noted (7), but the detailed role of PCNA and p53 in OSCC has not been fully elucidated. Herein, we conducted a meta-analysis to evaluate the effects of PCNA and p53 in patients with OSCC. This research is a comprehensive analysis from four aspects and can be a supplement for this topic. In this research, we analyzed the association between oral squamous cell carcinoma and p53 or PCNA, respectively. We present the following article in accordance with the PRISMA reporting checklist (available at https://dx.doi.org/10.21037/atm-21-6133).

Methods

Literature search strategy

We searched articles published between January 2000 and March 2020 for PCNA and p53 in OSCC patients in the PubMed, Embase, Cochrane database, and China National Knowledge databases using the following strategy: (oral OR mouth OR tongue) AND (cancer* OR neoplasm* OR tumor*) AND (PCNA OR p53). There were no restrictions on the publication language in the literature search. To maximize the specificity and sensitivity of our search, we checked the research reference list to seek other relevant research that were not found through the search strategy.

Study selection

Inclusion criteria and exclusion criteria

We used the following inclusion criteria for our research: (I) studies with case-control design; (II) studies evaluating the effect of PCNA and p53 in prognosis, survival, and metastasis; (III) articles containing eligible data; and (IV) articles with available full text. Research meeting any one of the following conditions was excluded: (I) studies with overlapping data or overlapping review articles; (II) studies involving patients with other head and neck tumors, and (III) articles involving other biomarkers for OSCC patients.

Data extraction and quality assessment

Two commentators independently scanned the full texts of the manuscripts. They extracted the following data from each eligible study: first author’s name, patient’s age and gender, country of origin, year of publication, sample size, and the study period of each article. The Cochrane risk of the bias assessment tool, which is a comprehensive tool to consider multiple biases, was used to evaluate the methodological quality of the studies.

Statistical analysis

We used Review Manager (version 5.2, Cochrane Collaboration, 2011) to assess the impact of the results in selected reports. For continuous outcomes, the mean difference was calculated by the average difference. Heterogeneity was evaluated by the I² statistic, which is the
percentage of heterogeneity among studies in the absolute difference and a quantitative measure of inconsistency in research. We confirmed that studies with an $I^2$ of 25–50% were considered to have low heterogeneity, studies with an $I^2$ of 50–75% were deemed to be medium heterogeneity, and studies with $I^2 > 75\%$ were considered to have high heterogeneity. If $I^2 > 50\%$, the potential sources of heterogeneity were examined by sensitivity analysis, which omits one study in each round and investigates the impact of a single portfolio survey estimation. Also, when heterogeneity was observed, the random effects model was used; otherwise, the fixed effects model was used. We used funnel charts, Begger's test, and Egger's test to check for potential publication bias.

Results

Search process

The electronic search retrieved 328 articles. After careful reading, 85 papers have met the preliminary standard. Upon further screening, 76 articles were excluded because of duplication, irrelevant studies, incomplete data, and incomplete comparison. Finally, nine papers were selected for analysis. Figure 1 displays a flowchart of the search process, highlighting the identification, inclusion, and
Table 1 Characteristics of studies included in this meta-analysis

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Language</th>
<th>Country</th>
<th>Age (years)</th>
<th>Groups</th>
<th>n</th>
<th>Years of onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fernanda (8)</td>
<td>2005</td>
<td>English</td>
<td>Brazil</td>
<td>58.2±6.8</td>
<td>High PCNA</td>
<td>17</td>
<td>1970 to 2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low PCNA</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Kato (9)</td>
<td>2011</td>
<td>English</td>
<td>Japan</td>
<td>66.8±10.1</td>
<td>High PCNA</td>
<td>11</td>
<td>2002 to 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low PCNA</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Keum (10)</td>
<td>2006</td>
<td>English</td>
<td>Korea</td>
<td>54±12.3</td>
<td>High PCNA</td>
<td>5</td>
<td>1986 to 1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low PCNA</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Lee (11)</td>
<td>2005</td>
<td>English</td>
<td>China</td>
<td>47±18.5</td>
<td>High PCNA</td>
<td>38</td>
<td>1995 TO 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low PCNA</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Mallick (12)</td>
<td>2010</td>
<td>English</td>
<td>India</td>
<td>55±10.2</td>
<td>High PCNA</td>
<td>20</td>
<td>1998 to 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low PCNA</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Monteiro (13)</td>
<td>2012</td>
<td>English</td>
<td>Spain</td>
<td>59±12.6</td>
<td>High PCNA</td>
<td>42</td>
<td>1995 to 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low PCNA</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Myoung (14)</td>
<td>2006</td>
<td>English</td>
<td>Korea</td>
<td>58.2±10.2</td>
<td>High PCNA</td>
<td>59</td>
<td>1996 to 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low PCNA</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Stenner (15)</td>
<td>2012</td>
<td>English</td>
<td>Germany</td>
<td>59.4±1.3</td>
<td>High PCNA</td>
<td>12</td>
<td>1986 to 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low PCNA</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Watanabe (16)</td>
<td>2010</td>
<td>English</td>
<td>Brazil</td>
<td>60.5±8.3</td>
<td>High PCNA</td>
<td>19</td>
<td>1996 to 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low PCNA</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

PCNA, proliferating cell nuclear antigen.

exclusion (including reasons) process.

Characteristics of included studies

Detailed characteristics of the included studies are presented in Table 1. All of the included studies were published between 2005 and 2020. The sample size ranged from 20 to 113. In total, 223 patients were in the high PCNA group and 257 patients were in the low PCNA group.

Quality assessment

Since the included articles were case-control studies, we used the Newcastle-Ottawa Scale (NOS) table to evaluate the risk of patient selection problems in nine trials (Table 2). Four of the nine included articles had 9 stars, and the other five had 8 stars, which demonstrated that included papers were good quality (>6 stars was considered to indicate good research quality).

Heterogeneity analysis

Heterogeneity analysis of the prognostic value of PCNA and p53 in OSCC

Since five of the nine included studies did not report on PCNA level, comprehensive analysis was performed on the other four articles. As shown in Figure 2A, I²=0%, and thus a fixed effects model was adopted. The results showed high PCNA expression (event/total: 61/87) was significantly more prevalent than low PCNA expression (33/86) in OSCC [odds ratio (OR) =3.88; 95% confidential interval (CI): 2.04–7.37; P<0.0001; I²=0%, Figure 2A]. However, only two studies reported on p53 expression in OSCC. As shown in Figure 2B, I²=86%, and therefore a random effects model was used. The result suggested that there was no significant difference between p53 positive (33/55) and p53 negative (22/55) in OSCC (OR =1.60; 95% CI: 0.18–14.63; P=0.68; I²=86%, Figure 2B).
Table 2 Newcastle-Ottawa Scale table of included studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Definition adequate</th>
<th>Representativeness of the cases</th>
<th>Selection of controls</th>
<th>Definition of controls</th>
<th>Comparability of cases and controls on the basis of the design or analysis</th>
<th>Ascertainment of exposure</th>
<th>Same method of ascertainment for cases and controls</th>
<th>Non-response rate</th>
<th>Total quality scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fernanda 2005</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Kato 2011</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td></td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Keum 2006</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td></td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Lee 2005</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Mallick 2010</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Monteiro 2012</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td></td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Myoung 2006</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Stenner 2012</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Watanabe 2010</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td>☆</td>
<td></td>
<td></td>
<td></td>
<td>9</td>
</tr>
</tbody>
</table>

☆, medium quality; ☆☆, high quality. The higher the quality score is, the better quality of article is.

Figure 2 Forest plots of the prognostic value of PCNA and p53 in OSCC. (A) OSCC patients’ PCNA was compared; (B) OSCC patients’ p53 was contrasted. PCNA, proliferating cell nuclear antigen; p53, p53 gene; OSCC, oral squamous cell carcinoma.
Heterogeneity analysis regarding the value of PCNA and p53 on the 5-year overall survival among patients with OSCC

As shown in Figure 3A, five of the nine studies reported on PCNA and the 5-year overall survival of OSCC patients. Since the $I^2$ value was low, the fixed effects model was used. The results showed that low PCNA expression had a higher 5-year overall survival in OSCC patients (94/153) than high PCNA expression (50/121) ($OR =0.47; 95\% CI: 0.27–0.80; P=0.005; I^2=41\%$, Figure 3A). As for p53, a fixed effects model was used for heterogeneity analysis, which showed that $p53$ negative (68/94) had a higher 5-year overall survival than $p53$ positive (22/65) ($OR =0.20; 95\% CI: 0.10–0.42; P<0.0001; I^2=0\%$, Figure 3B).

Heterogeneity analysis on the role of PCNA and p53 in metastasis

We used three articles (3/9) for PCNA and two articles (2/9) for $p53$ to conduct heterogeneity analysis. The heterogeneity test results showed that we needed a random effects model to analyze the data ($OR =0.80$ with $95\% CI: 0.18–3.45$, $P$ of heterogeneity $=0.07$, $I^2=63\%$, $Z=0.30$, $P$ of over effect $=0.76$, Figure 4A). There was no difference in the overall effect of high (18/38) and low (28/51) PCNA on metastasis (Figure 4A). A fixed effects model was used to evaluate $p53$, and also showed no difference between $p53$ and metastasis ($OR =0.38$ with $95\% CI: 0.13–1.10$, $P$ of heterogeneity $=0.71$, $I^2=0\%$, $Z=1.79$, $P$ of over effect $=0.07$, Figure 4B).

Sensitivity analysis and publication bias

According to heterogeneity analysis, the heterogeneity of PCNA in OSCC was low ($I^2=0\%$, $P=0.0011$). This might be attributed to the different results of each study. When Lee et al. (11) from 2005 was excluded, the $I^2$ did not change, while the $P$ value of heterogeneity changed from 0.95 to 0.96 (Figure 5). The sensitivity analysis indicated that the results in this article were robust.

We performed a funnel plot for PCNA in OSCC. Four studies were included in the plot. The standard error of or logarithm is ordinate, and the image symmetry is the
basis of judging publication bias. When it is symmetrical, publication bias is slight; when it is asymmetrical, publication bias is significant (7). To some extent, the result indicated that there existed slight publication bias, since the symmetrical characteristic of the funnel plot was good (Figure 6). The result of Begger’s test suggested that no significant evidence of potential publication bias existed (z=1.15, P=0.101), and Egger’s test also indicated that no significant evidence of possible publication bias existed (t=1.27, P=0.215).

**Discussion**

Our results showed that high PCNA expression was significantly more prevalent in OSCC than low PCNA expression, which indicated that PCNA might have predictive value for OSCC. Sajeevan (17) stated that the functional change of PCNA activity is a joint genetic event in various cancers and an effective marker of cell proliferation. It could be used to determine the histological grade, recurrence rate, and prognosis of head and neck
cancers. Overexpression of PCNA is also associated with chemotherapy or radiation therapy (17). However, the relationship between PCNA changes and cervical lymph node metastasis of oral tongue cancer remains unclear. In the analysis of the 5-year overall survival and PCNA, low PCNA expression had a higher 5-year overall survival in OSCC patients than high PCNA expression. These findings demonstrated that low PCNA might be an influencing factor for OSCC patients’ 5-year overall survival. Furthermore, the results regarding the role of PCNA in the metastasis of OSCC patients suggested that PCNA is not valuable for determining metastasis.

The analysis also showed that p53 could be a potential indicator for the 5-year overall survival of OSCC patients, but does not appear to have predictive value for OSCC. Zhong et al. (5) reported that p53 gene mutations and overexpression of mutant p53 proteins play an essential role in the occurrence and loss of apoptosis in various human cancers. Simultaneously, p53 gene mutations have been increasingly found in several poorly differentiated head and neck cancers, including oral cancer (18). There are also changes in p53 that are associated with aggressive laryngeal and pharyngeal phenotype tumor recurrence (19). Overexpression of p53 is associated with a higher risk of oral lymph node metastasis, and is a marker of poor prognosis for oral squamous cell cancer (20). However, these correlations have not been further confirmed by other studies. The results about the role of p53 in metastasis among OSCC patients showed that p53 might not have any value for indicating metastasis.

Mestrinho et al. (18) reported that the data of 159 patients with OSCC showed that PCNA was expressed in different degrees in all histological subtypes examined. Expression was related to the ages of patients and the stages of pathological lymph nodes. Most importantly, the high expression of PCNA was a significant prognostic indicator for poor overall prognosis and disease-free survival of OSCC (21). In the acinar cell carcinoma subgroup, PCNA expression was found to be the only negative prognostic factor affecting the 5-year tumor-free survival rate and overall survival (22). Simultaneously, the stability and overexpression of the p53 gene might be related to p53 gene mutation or genotoxic stress, and p53 gene alterations are the most common gene abnormality in numerous cancers (23). Overexpression of p53 plays an essential role in the development of OSCC (24).

Generally, mutation of the p53 gene and overexpression of the mutant protein plays an important role in carcinogenesis and apoptosis in many human cancers. Simultaneously, p53 mutations have been increasingly found in some poorly differentiated head and neck cancers, including an oral cavity in breast cancer (20,21). Moreover, p53 changes are related to the invasive phenotype and recurrence of laryngeal and pharyngeal carcinomas. Overexpression of p53 has been shown to be associated with a high risk of lymph node metastasis and is a marker of poor prognosis in oral cancer (22). The functional change of PCNA activity is a joint genetic event (23). p53 is an effective marker of cell proliferation and could be used as an indicator to predict head and neck cancer. The overexpression of PCNA is also related to chemotherapy or the response to chemotherapy (24).

Since OSCC remains one of the most challenging cancers to control, with only slight improvement in survival over the past 50 years, prevention, treatment, and prognosis are crucial for OSCC. To improve the prognosis, survival biomarkers are needed. As our analysis demonstrated, PCNA and p53 might be suitable for prognostic and survival evaluation of OSCC. It was reported that expression of PCNA and P53 had association with some other kinds of carcinoma like skin cancer, colorectal cancer and lung cancer. There is need to analyze the other relationships in the future (20-23). It was also reported that PCNA and Ki-67 were related to the abnormal proliferation of oral mucosa, and their proliferation index was parallel to the degree of proliferation, and they were linearly correlated (25,26). So, we can conduct a further analysis between Ki-67 and proliferation of OSCC in the next step.

However, there were some limitations in this study that should be noted. Firstly, more indicators evaluating other
aspects between biomarkers and OSCC could be included, which should be conducted in the future. Secondly, comparisons between different subgroups, like age or area, could also be analyzed in future research.

**Acknowledgments**

**Funding:** None.

**Footnote**

*Reporting Checklist:* The authors have completed the PRISMA reporting checklist. Available at [https://dx.doi.org/10.21037/atm-21-6133](https://dx.doi.org/10.21037/atm-21-6133)

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at [https://dx.doi.org/10.21037/atm-21-6133](https://dx.doi.org/10.21037/atm-21-6133)). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

*Open Access Statement:* This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: [https://creativecommons.org/licenses/by-nc-nd/4.0/](https://creativecommons.org/licenses/by-nc-nd/4.0/)

**References**


(English Language Editor: A. Kassem)