Cyclin D1 Overexpression Is Associated with Poor Clinicopathological Outcome and Survival in Oral Squamous Cell Carcinoma in Asian Populations: Insights from a Meta-Analysis

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Abstract

Background: The clinicopathological significance of cyclin D1 overexpression and prognosis of oral squamous cell carcinoma has not been fully quantified. We performed a comprehensive meta-analysis for evaluation of cyclin D1 overexpression in oral squamous cell carcinoma to determine the strength of this association.

Methods: Using both medical subheadings and free terms, we searched PubMed, Embase and the Institute for Scientific Information Web of Science for all eligible studies published before Nov. 2013. We retrieved 1674 citations, determining that 15 met the selection criteria. We used the odds ratio (OR) and hazard ratio (HR) as the common measures of association to quantitatively determine the correlation between cyclin D1 overexpression and outcomes of oral cancer. We performed a meta-analysis and heterogeneity, sensitivity, and subgroup analyses to clarify and validate the pooled results.

Results: The pooled results provided compelling evidence that cyclin D1 overexpression was significantly correlated with increased tumor size (OR = 1.617, 95% confidence interval [CI] = 1.046–2.498, p = 0.031), lymphoid node metastasis (OR = 2.035, 95% CI = 1.572–2.635, p < 0.001), tumor differentiation (OR = 1.976, 95% CI = 1.363–2.866, p < 0.001), and advancement of clinical stages (OR = 1.516, 95% CI = 1.140–2.015, p = 0.004), and adversely influenced overall survival of OSCC patients (HR = 1.897, 95% CI = 1.577–2.282, p < 0.001). The strength of association varied in different oral cavity subsites.

Conclusion: Our findings indicated that cyclin D1 expression correlates with detrimental clinicopathological outcome and poor prognosis in oral squamous cell carcinoma. Our results may be useful in the management of oral cancer.

Introduction

Oral squamous cell carcinoma (OSCC) is a common malignancy in the head and neck region, with significant incidence and mortality. In 2008, there were an estimated 263,900 newly diagnosed oral cancer patients and 128,000 deaths from oral cancer worldwide [1]. Despite recent advances in diagnosis and treatment, the 5-year overall survival (OS) rate remains at 50–60% [2].

In clinical practice, the conventional prognostic tool for cancers is the tumor-node-metastasis (TNM) system, in which lymph node metastasis is the most relevant [3]. However, there are some disadvantages to this system, namely difficulty in discriminating lymph node status in a timely and accurate manner, when using current physical examinations and imaging techniques. In addition, the biological phenotypes of tumors are often divergent despite identical staging, resulting in different clinical outcomes and response to the selected treatment [4]. Clarifying the correlation between biological characteristics or molecular biomarkers and the aggressiveness of oral cancer may provide be of significant benefit for predicting clinical outcomes and determining the optimal individualized therapy for each patient.

More recently, attention has focused particularly on a panel of molecular markers, which includes cell cycle regulators, as possible predictors of biological behaviors in oral cancer [5]. Cyclin D1 is a vital protein that has a widespread role in cell cycle regulations, providing control over G1 to S phase transition and governing cell proliferation rates [6,7]. In the dynamic regulation over the cell cycle, Cyclin D1 exerts its functions by binding cyclin-dependent kinases (CDKs) subunit 4 and 6 to form a complex, which results in successive phosphorylation of the retinoblastoma (Rb) protein.
On the other hand, this complex activates cyclin E-CDK 2 through sequestering the CDK inhibitors p21 and p27 [8–12]. The phosphorylation of Rb results in its functional inactivation, and further leads to the release of E2F transcription factors, and proceeds to activate genes that are essential to initiate the DNA replication and accelerates cell proliferation [8–11]. These procedures will also in turn lead to the transcriptional activation of E2F-responsive genes that are essential to cyclin E and cyclin A synthesis, therefore further promote the phosphorylation of Rb through activating CDK2 [12]. Aberrant cyclin D1 expression, either by rearrangement, amplification or transcriptional up-regulation, contributes to the loss of normal cell cycle control and is associated with increased risk of tumorigenesis [4,13]. In addition, cyclin D1 overexpression has a direct role in cooperating with other proto-oncogenes in neoplastic transformation in several systems [14]. Previously, it was extensively reported that cyclin D1 overexpression was as an important genetic event in a variety of head and neck cancers [15–17], including OSCC [4,11,18]. In oral cancer patients, immunohistochemical studies indicated a relation between certain prognostic factors and cyclin, including primary tumor size, location, nodal metastasis, tumor differentiation and clinical stage. However, the conclusions of such studies were not always in agreement. It is not known whether the heterogeneity originates from an actual difference or a lack of statistical power due to the relatively small sample size in an individual study.

Therefore, we performed a systematic review and meta-analysis of all eligible studies published to date to gain deeper insight into the clinicopathological and prognostic significance of cyclin D1 in OSCC. We found a significant correlation between cyclin D1 overexpression and clinicopathological outcome and prognosis.

Methods

Search Strategy

We searched PubMed, Embase and the Institute of Scientific Information Web of Science for eligible studies published before November 2013. Searches were carried out using both medical subheadings and free terms. We used a combination of the following search string: ("oral" OR "mouth") AND ("cancer" OR "carcinoma" OR "neoplasm" OR "tumor") AND ("cyclin D1" OR "CCND1") AND ("prognosis" OR "prognostic" OR "marker" OR "survival" OR "clinicopathological"). In addition, we manually screened the reference lists of included studies for further relevant studies. If the identified studies reported on overlapping populations, we selected the study that was published more recently or that contained more information.

Selection Criteria

Two reviewers (Zhao Y. and Yu D.) screened the study selection process independently and in duplicate. Inter-reviewer agreement of the eligibility of the studies between reviewers was good, the kappa value was 0.9. Any disagreement was resolved by arbitration until consensus was achieved. Studies were eligible for inclusion if: (1) they were original articles published in either English or Chinese; (2) they focused on the association of cyclin D1 overexpression with high-risk clinicopathological factors and OSCC prognosis; (3) they used immunohistochemistry (IHC) as the main method to examine the cyclin D1 expression in OSCC specimens. We restricted the included studies to those on Asian populations as previous studies have reported on these populations more recently or that contained more information.

Studies on cutaneous, verrucous and lip carcinoma as well as other types of carcinoma were also excluded.

Data Extraction

All data for selected full-text articles were extracted by two independent reviewers (Zhao Y. and Yu D.) using standardized Excel 2007 worksheets (Microsoft, Redmond, WA). Discrepancies were resolved by discussions and referring to the contents of the articles. We extracted the basic study information (name of first author, year of publication, region or country in which study was conducted, size of study population), participant characteristics (recruitment period, sex and age distributions, treatment modality, duration of follow-up), IHC methodology (staining sites, cyclin D1 cut-off value) and clinicopathological parameters (tumor size, nodal metastasis, histological grade, clinical stage) from each study, and recorded the survival results of each study. From studies that reported hazard ratios (HR) in both univariate and multivariate models, we extracted the latter because these results were more convincing, as there had been adjustment for potential confounders.

Statistical Analysis

We used the odds ratio (OR) as a common measure of association to determine the correlation between cyclin D1 expression and clinicopathological outcomes of oral cancer. The HR was used for quantitative evaluation of the impact of cyclin D1 expression on survival rate. Some studies did not include the point estimates and HR variance, therefore we used the data available in such studies and applied the method reported by Tierney et al. to determine the HR and its 95% confidence interval (CI) [19]. If a study reported only the survival curve, we extracted time-to-event data from the Kaplan–Meier curves of individual studies using Engauge Digitizer 4.1 software (free software downloaded from http://digitizer.sourceforge.net/).

For meta-analysis, the statistical significance of pooled estimates was determined using the Z-test. Heterogeneity across studies was checked by a chi-square based Q test and the Higgins I2 [20]. I2 represented the proportion of inter-study variability attributed to heterogeneity rather than systematic error, which ranges from 0% to 100% [21]. It was suggested that I2 values of 25%, 50% and 75% indicated low, moderate and high bias, respectively [21]. For a Q statistic p-value of >0.1, we used a fixed-effects model (the Mantel-Haenszel method) to calculate the pooled estimates; otherwise a more conservative random-effects model (the DerSimonian–Laird method) was used. However, in the rare events where incidence was <1%, we used the Peto one-step method instead [24]. This method tends to yield the least biased result and strongest statistical power, providing the best CI coverage and no substantial imbalance between case and control sizes [24]. In addition, we performed subgroup analysis to control for potential confounding factors as possible heterogeneity that might have distorted the results. Sensitivity analysis was performed using the leave-one-out method to test the reliability of the overall pooled results [25]. Funnel plot asymmetry was inspected visually to assess the possible effect of publication bias, which was confirmed by Egger’s linear regression [26].

All statistical tests in this meta-analysis were performed using Stata 11.1 software (Stata Corp, College Station, TX) with two-sided p values. A p-value <0.05 was considered statistically significant.
Results

Search Results

Fig. 1 details the selection process. Our search strategy retrieved 1674 unique citations: 254 from PubMed, 1005 from Embase, 413 from the Institute for Scientific Information Web of Science, and two additional studies from the reference lists. After initial screening of the titles and abstracts, we excluded 1633 articles either because of duplication or they did not include the topics cyclin D1 overexpression and oral cancer. An eventual 41 articles underwent full-text evaluation. Upon further review, 26 articles were excluded nine had inadequate clinicopathological parameters for meta-analysis, four did not included IHC testing, four reported cutaneous or verrucous cancer, three were comments or meeting abstracts, two involved Caucasian populations, one contained premalignant data, two included genetic polymorphisms and one involved overlapping populations. Eventually, a total of 15 articles [8,11,13,18,27–37] were included based on the predefined criteria.

Study Characteristics

Table 1 summarizes the characteristics of the included studies. All 15 articles included were of Asian origin; more specifically, six were from China [11,13,18,24,27,37], 5 were from Japan [8,29,31,33,34] and 4 were from India [28,30,32,35]. Altogether,
| Study          | Year | Recruitment period | Country        | Sampling | Tumor site          | Age (years) | Gender (M/F) | Duration of follow-up (months) | Treatment                   | Staining pattern       | Cut-off value | Cyclin D1 Overexpression (%) |
|---------------|------|--------------------|----------------|----------|---------------------|-------------|--------------|-------------------------------|---------------------------|------------------------|--------------|-----------------------------|
| Huang et al.  | 2012 | 1999–2005          | China/Taiwan   | 264      | oral                | 49.3 ± 11.01 | 264/0        | 168                           | surgery                    | nuclei and non-nuclei | 10           | 36.7                        |
| Xing et al.   | 2011 | 2005–2009          | China          | 50       | oral                | 20–76        | NA           | NA                           | NA                        | nuclei and cytoplasm    | 25           | 80                         |
| Das et al.    | 2011 | 2001–2006          | India          | 45       | oral                | 53.2 ± 12.2  | 36/9         | NA                           | surgery                    | nuclei                 | 50           | 66.6                       |
| Yun et al.    | 2010 | 2004–2006          | Japan          | 50       | tongue              | 22–82        | 31/19        | 12–60 (median 40)            | surgery                    | nuclei                 | 10           | 58                         |
| Mishra et al. | 2009 | NA                 | India          | 51       | oral                | NA           | 31/20        | NA                           | NA                        | nuclei                 | NA           | 31.1                       |
| Shah et al.   | 2009 | 2000–2003          | India          | 135      | Buccal and tongue   | 28–75        | 101/34       | > 24                         | surgery and radio-, chemotherapy | nuclei and cytoplasm    | 10           | 43                         |
| Wang et al.   | 2006 | 2000–2003          | China          | 62       | tongue              | 25–86        | 40/22        | NA                           | surgery                    | nuclei                 | 10           | 66                         |
| Shiraki et al.| 2005 | 1986–1998          | Japan          | 140      | oral                | 26–85 (mean 59) | 98/42    | 5–134 (median 66)           | surgery                    | NA                     | 10           | 39                         |
| Miyamoto et al.| 2003 | 1999–2001          | Japan          | 41       | oral                | 21–89 (mean 58.4) | 26/15   | 7.7–39.3 (median 25.4)      | surgery                    | NA                     | 10           | 65.9                       |
| Zhu et al.    | 2003 | 1990–1999          | China          | 50       | oral                | 31–80 (mean 60) | 36/14   | 120                           | surgery                    | nuclei                 | 10           | 52                         |
| Vora et al.   | 2003 | 1986–1990          | India          | 84       | tongue              | NA           | 77/7         | 60                           | surgery (main), radiotherapy | nuclei                 | 10           | 62                         |
| Goto et al.   | 2002 | 1981–1998          | Japan          | 41       | tongue              | 22–82 (mean 59.6) | 24/17   | 2–133 (mean 36.3)          | NA                        | nuclei                 | 33           | 65.9                       |
| Vicente et al.| 2002 | 1990–1999          | Spain          | 35       | oral                | 27–85 (mean 56.6) | 30/5    | 6–107 (mean 68)           | surgery, radiotherapy (37%) | nuclei                 | 50           | 17.1                       |
| Mineta et al. | 2000 | 1977–1995          | Japan          | 94       | tongue              | 16–89 (mean 58) | 68/26   | > 60                         | NA                        | nuclei                 | 50           | 19                         |
| Lam et al.    | 2000 | 1988–1996          | China/Hongkong | 56       | oral                | 37–85 (mean 64) | 45/11   | > 60                         | radiotherapy               | nuclei                 | 5            | 63                         |
| Kuo et al.    | 1999 | 1991–1995          | China/Taiwan   | 88       | oral                | NA           | 76/12        | > 60                         | surgery, radiotherapy      | NA                     | 50           | 44.3                       |
| Bova et al.   | 1999 | NA                 | Australia      | 148      | tongue              | NA           | 104/44       | 1–186 (mean 57)            | surgery, radiotherapy      | nuclei                 | 10           | 68                         |

M/F: Male/Female; NA: not available.

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| Outcomes       | Variables          | Subgroups | Study N. | Samples | Stat. | OR  | 95% CI  | Z      | p   | Z Het x2 | phet   | I² (%) |
|----------------|--------------------|-----------|----------|---------|-------|-----|---------|--------|-----|----------|--------|--------|
| Primary tumor  | Total              | Total     | 11       | 1063    | R     | 1.617| 1.046  | 2.498  | 2.16 | 0.031    | 22.85  | 0.011  | 56.2  |
|                | Country            | China     | 4        | 464     | R     | 1.579| 0.735  | 3.392  | 1.17 | 0.241    | 8.99   | 0.029  | 66.6  |
|                |                    | India     | 3        | 315     | F     | 1.877| 1.148  | 3.068  | 2.51 | 0.012    | 2.93   | 0.403  | 0.0   |
|                |                    | Japan     | 3        | 284     | R     | 1.323| 0.385  | 4.544  | 0.44 | 0.657    | 8.44   | 0.015  | 76.3  |
|                | Cut-off            | 10%       | 6        | 735     | F     | 1.028| 0.751  | 1.407  | 0.17 | 0.861    | 8.74   | 0.120  | 42.8  |
|                |                    | >10%      | 4        | 277     | F     | 2.752| 1.600  | 4.731  | 3.66 | <0.001   | 4.14   | 0.247  | 27.5  |
|                | Tumor              | mixed     | 6        | 638     | R     | 1.431| 0.758  | 2.701  | 1.11 | 0.268    | 13.44  | 0.020  | 62.8  |
|                |                    | tongue    | 4        | 290     | F     | 2.032| 1.200  | 3.441  | 2.64 | 0.008    | 5.36   | 0.147  | 44.1  |
| Nodal metastasis| Total              | total     | 12       | 1099    | P     | 2.035| 1.572  | 2.635  | 5.39 | 0.000    | 33.16  | <0.001 | 66.8  |
|                | Country            | China     | 5        | 514     | P     | 2.325| 1.620  | 3.337  | 4.58 | 0.000    | 24.38  | <0.001 | 83.6  |
|                |                    | India     | 2        | 219     | F     | 1.282| 0.716  | 2.296  | 0.84 | 0.403    | 0.05   | 0.828  | 0.0   |
|                | Cut-off            | Japan     | 5        | 366     | F     | 2.215| 1.361  | 3.604  | 3.20 | 0.001    | 6.12   | 0.190  | 34.7  |
|                |                    | 10%       | 8        | 826     | R     | 1.754| 1.131  | 2.719  | 2.51 | 0.012    | 12.10  | 0.097  | 42.2  |
|                |                    | >10%      | 4        | 273     | P     | 2.954| 1.737  | 5.026  | 4.00 | <0.001   | 19.12  | <0.001 | 84.3  |
|                | Tumor              | mixed     | 6        | 633     | P     | 1.878| 1.343  | 2.627  | 3.68 | <0.001   | 25.26  | <0.001 | 80.2  |
|                |                    | tongue    | 5        | 331     | F     | 2.915| 1.761  | 4.826  | 4.16 | <0.001   | 5.78   | 0.216  | 30.8  |
| Histological grade| Total              | total     | 13       | 1118    | R     | 1.976| 1.363  | 2.866  | 3.59 | <0.001   | 21.48  | 0.044  | 44.1  |
|                | Country            | China     | 6        | 532     | F     | 1.841| 1.279  | 2.649  | 3.29 | 0.001    | 6.61   | 0.251  | 24.4  |
|                |                    | India     | 3        | 270     | R     | 3.841| 1.131  | 13.039 | 2.16 | 0.031    | 8.31   | 0.016  | 75.9  |
|                | Cut-off            | Japan     | 4        | 316     | F     | 1.271| 0.777  | 2.078  | 0.96 | 0.339    | 0.92   | 0.821  | 0.0   |
|                |                    | 10%       | 8        | 826     | R     | 1.781| 1.076  | 2.947  | 2.25 | 0.025    | 17.74  | 0.013  | 60.5  |
|                |                    | >10%      | 3        | 185     | F     | 1.953| 0.974  | 3.916  | 1.89 | 0.059    | 0.71   | 0.702  | 0.0   |
|                | Tumor              | mixed     | 8        | 693     | F     | 1.702| 1.242  | 2.347  | 3.29 | 0.001    | 7.59   | 0.37   | 7.8   |
|                |                    | tongue    | 4        | 290     | R     | 2.660| 0.894  | 7.564  | 1.75 | 0.079    | 11.33  | 0.009  | 74.0  |
| Clinical stage | Total              | Total     | 10       | 949     | F     | 1.516| 1.140  | 2.015  | 2.87 | 0.004    | 8.10   | 0.524  | 0.0   |
|                | Country            | China     | 4        | 458     | F     | 1.363| 0.903  | 2.058  | 1.47 | 0.140    | 2.32   | 0.509  | 0.0   |
|                |                    | Japan     | 5        | 356     | F     | 1.728| 1.091  | 2.737  | 2.33 | 0.020    | 5.25   | 0.262  | 23.9  |
|                | Cut-off            | 10%       | 7        | 764     | F     | 1.530| 1.112  | 2.103  | 2.62 | 0.009    | 4.51   | 0.609  | 0.0   |
|                |                    | >10%      | 2        | 129     | F     | 1.837| 0.840  | 4.019  | 1.52 | 0.128    | 2.64   | 0.104  | 62.1  |
|                | Tumor              | mixed     | 6        | 639     | F     | 1.339| 0.949  | 1.889  | 1.66 | 0.096    | 3.87   | 0.568  | 0.0   |
|                |                    | tongue    | 3        | 175     | F     | 2.482| 1.290  | 4.930  | 2.60 | 0.009    | 1.82   | 0.403  | 0.0   |

N.: Number; Stat.: Statistic models; R: random-effects model; F: fixed-effects model; P: Peto one-step method; OR: odds ratio; 95% CI: 95% confidence intervals; p Z: p value of statistic Z; pHet: p value of heterogeneity chi-squared; p Z, p < 0.05 was regarded as significant; pHet, p < 0.1 was regarded as significant.

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the studies recruited a total of 1251 participants, with sample sizes ranging from 41 to 264 participants. All 15 studies used IHC methods for cyclin D1 staining. The cyclin D1 positive cut-off value varied between studies, ranging 5–50%. The most common selected cut-off was 10%. Regarding the clinicopathological factors, most of the studies reported prognostic factors of cyclin D1 expression referring to tumor size, nodal metastasis, histological grade and clinical stage. For survival analysis, eight studies reported OS [8,11,13,30–33,37], while only two studies investigated disease-free survival (DFS) as a potential outcome of cancer [11,30].

Quantitative Synthesis

Eleven studies investigated the association between cyclin D1 expression and primary tumor size, which involves 1063 participants. Cyclin D1 overexpression was more prevalent in larger tumors (T3, T4) than in smaller tumors (T1, T2), and with OR = 1.617 (95% CI = 1.046–2.498) (Table 2 and Fig. 2). Some subgroup analysis maintained a significant association, more specifically, in the Indian populations (OR = 1.877, 95% CI = 1.148–3.068) and cut-off value >10% (OR = 2.752, 95% CI = 1.600–4.731) (Table 2). When we stratified the pooled estimates according to tumor site, there was a positive association between cyclin D1 expression and increased tumor size in tongue SCC (OR = 2.032, 95% CI = 1.200–3.441) rather than with a mixed tumor site in the oral cavity (OR = 1.431, 95% CI = 0.758–2.701) (Table 2).

We also summarized information associating cyclin D1 overexpression with other clinicopathological parameters as the topic of interest reported in the included studies, including nodal metastasis, histological grade and clinical stage (Table 2 and Fig. 2). The overall estimates indicated that cyclin D1 overexpression was significantly associated with increased risk of nodal metastasis (N1, 2, 3 versus N0) in oral cancer patients (OR = 2.035, 95% CI = 1.572–2.635). Such associations were also found for tumor histological grade (OR = 1.976, 95% CI = 1.363–2.866) and clinical stage (OR = 1.516, 95% CI = 1.140–2.015). When stratified according to tumor site, the pooled estimates changed significantly. The association with tongue SCC was more obvious than that in the oral cavity (nodal metastasis, OR = 2.915 versus 1.878; histological grade, OR = 2.600 versus 1.707; clinical stage, OR = 2.482 versus 1.339, respectively) (Table 2). In addition, we stratified the cut-off value, an important source of heterogeneity at 10%, because most of the studies used this criterion to denote cyclin D1 overexpression. Further, the positive rate of keratinocytes is <10% in normal oral mucosal epithelial [38]. The pooled estimates in the clinicopathological data were altered substantially following stratification of the cut-off value (Table 2). The results associating cyclin D1 overexpression with clinical stage did not have potential heterogeneity ($p_{het}$ >0.1), while there
was some heterogeneity among studies following analysis of other clinicopathological features, with $I^2$ ranging from 44.1 to 66.8% (Table 2).

To investigate whether cyclin D1 overexpression was a prognosis factor in oral cancer patients, we meta-analyzed the HR data extracted from individual studies or derived using the calculations described in the. Most of the studies indicated a stronger link between cyclin D1 overexpression and poor survival. For OS, mortality was higher in cyclin D1-positive groups than in cyclin D1–negative groups ($HR = 1.897$, 95% CI 1.577–2.282, $p = 0.001$), with no potential heterogeneity across studies ($p_{het} = 0.884$, $I^2 = 0.0\%$) (Fig. 3). Significant association was also found for DFS ($HR = 1.421$, 95% CI 1.038–1.947, $p = 0.028$). There was no heterogeneity among studies in the pooled analysis ($p_{het} = 0.403$, $I^2 = 0.0\%$).

**Sensitivity Analysis**

The overall pooled estimates of the relation of cyclin D1 expression to clinicopathological and prognostic outcomes were not substantially altered following the exclusion of any individual study, indicating the reliability of our results.

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**Figure 3. Forest plots of association between cyclin D1 overexpression with poor OS in OSCC.**
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**Figure 4. Begg’s funnel plots for publication bias in cyclin D1 overexpression and clinicopathological outcome in OSCC.** Each point represents a separate study for the indicated estimate; the area of each circle represents the sample size. s.e: standard error; Horizontal line: effect size. (A) Funnel plots of publications for the association between cyclin D1 overexpression and nodal metastasis, random-effects model. (B) Funnel plots of publications for the association between cyclin D1 overexpression and histological grade, Peto one-step model.
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Publication Bias Analysis

Analysis of clinicopathological features and survival data determined no obvious asymmetry in the funnel plots for publication bias (p > 0.05) (Fig. 4). The more sensitive Egger’s regression test confirmed these results, demonstrating that our pooled data contained no potential publication bias.

Discussion

Based on 1251 Asian OSCC patients from 15 studies, we explored the relation of cyclin D1 expression to clinicopathological features and survival in OSCC. Our pooled results are compelling evidence of a significant correlation between cyclin D1 overexpression and increased tumor size, tumor differentiation, lymphoid nodes metastasis, and advancement of clinical stage, which may adversely influence survival in OSCC.

Our findings are in agreement with a recent meta-analysis of esophageal carcinoma that combined the clinicopathological data of 2041 patients [39]. Various studies on head and neck SCC (HNSCC) involving large sample sizes also arrived at similar conclusions: Hanken et al. [16] evaluated the cyclin D1 expression status in 546 HNSCC patients finding a significant association cyclin D1 expression OS in oral subsites (p < 0.001), Rasammy et al. [40], studied 222 samples of upper aerodigestive HNSCC, and reported that strong positive cyclin D1 expression was related to remarkable reduction of overall and disease-specific survival (OS, p = 0.003, disease-specific survival, p = 0.039). Together with these studies, our results suggest that cyclin D1 overexpression is related to local invasiveness and aggressive behavior of SCC, especially in the oral cavity.

In the subgroup meta-analysis, the strength of association of cyclin D1 expression in tongue SCC was stronger than in mixed tumor sites of the oral cavity. There may be two reasons for this difference: First, compared to other subsites in the oral cavity, tongue SCC is characterized by a more aggressive biological phenotype, with a high-degree of cervical lymph node spread that might be reflected at molecular level, such as with cyclin D1 expression. Second, cyclin D1 may be differentially expressed in various anatomical sites in the oral cavity [18, 41, 42]. Regarding ethnicity, two studies previously reported the clinicopathological significance of cyclin D1 overexpression in Caucasian populations. Vicente et al. [4] performed an IHC study in 35 Spanish OSCC patients, finding that cyclin D1 overexpression was related to 2.6 times greater nodal metastasis. Bova et al. [43] investigated 148 Australians with tongue cancer, reporting that cyclin D1 overexpression was associated with higher lymph node stage (approximately 3.43 times higher, p = 0.014), and lower DFS (p = 0.06) and OS (p = 0.01). Compared with our meta-analysis of Asian populations, these disparities may exclude racial difference: First, compared to other subsites in the oral cavity, tongue SCC is characterized by a more aggressive biological phenotype, with a high-degree of cervical lymph node spread that might be reflected at molecular level, such as with cyclin D1 expression. Second, cyclin D1 may be differentially expressed in various anatomical sites in the oral cavity [18, 41, 42].

Conclusion

Our meta-analysis indicates that cyclin D1 overexpression correlates with poor clinicopathological outcome and prognosis in OSCC. The results obtained may aid the management of oral cancer patients.

Supporting Information

Checklist S1  PRISMA Checklist. (DOCX)
Acknowledgments

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