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Review

Prognostic and clinicopathological significance of cyclin D1 expression in oral squamous cell carcinoma: A systematic review and meta-analysis

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ABSTRACT

Objectives: To evaluate the prognostic significance of cyclin D1 (CD1) overexpression in OSCC.
Material and methods: We searched studies published before August 2017 (Pubmed, Embase, Web of Science, Scopus). We evaluated the quality of the studies included (Quality in Prognosis Studies [QUIPS] tool). The impact of CD1 overexpression on overall survival and disease-free survival, T status, N status, stage, and histological degree was meta-analyzed. We analyzed heterogeneity among studies, conducted sensitivity analyses, analyzed small-study effects, and conducted subgroup analyses.
Results: 31 studies (2942 patients) met inclusion criteria. Qualitative evaluation demonstrated that not all studies were performed with the same rigor, finding the greatest risk of bias in the study confounding domain. Quantitative evaluation showed that CD1 overexpression had a strong statistical association with worse overall survival (HR = 2.00, 95% CI = 1.59–2.51, $p < 0.001$), worse disease-free survival (HR = 1.46, 95% CI = 1.13–1.87, $p = 0.003$), higher T status (OR = 1.51, 95% CI = 1.07–2.13, $p = 0.02$), N+ status (OR = 2.16, 95% CI = 1.60–2.92, $p < 0.001$), advanced stage (OR = 1.44, 95% CI = 1.15–1.81, $p = 0.002$), and high histological grade (OR = 1.60, 95% CI = 1.12–2.29, $p = 0.010$). We observed heterogeneity in all parameters except for disease-free survival and clinical stage. We found effect of small studies on T and N status. The tonguel SCC subgroup showed the strongest association between CD1 overexpression and worse development. In addition, application of a cutoff point ≥10% tumor cells with nuclear CD1 expression maintained most of the significant associations reported.
Conclusions: These findings indicate that immunohistochemical assessment of CD1 overexpression may be useful as a prognostic biomarker for OSCC.

Introduction

Oral cancer has a worldwide incidence of 300,400 cases and is responsible for 145,400 deaths a year (GLOBOCAN, IARC, WHO) [1]. Oral squamous cell carcinoma (OSCC) represents around 90% of malignant oral neoplasms [2] and has a 5-year survival rate of 50–60% [2,3]. Prediction of the prognosis is of major importance and is usually based on the Tumor Node Metastasis (TNM) staging system, with N+ status and the presence of extracapsular spread predicting a worse prognosis [4,5]. The prognostic value of molecular biomarkers has attracted considerable research interest [6,7], and evidence has accumulated on a key role for cyclin D1 (CD1) in oral oncogenesis [8]. CD1 is encoded by the CCND1 gene in chromosomal band 11q13 [9] and promotes G1 cell cycle progression, regulating cell proliferation [10]. CD1 functions that have emerged over the past few years include cell growth regulation, mitochondrial activity modulation, DNA repair, and cell migration control [8,11]. The frequent amplification and overexpression of the CCND1 gene and its CD1 protein [8,9,12] has been strongly implicated in the development of breast, lung, and colon cancers, melanoma, and head and neck squamous cell carcinomas, including OSCC [8,13]. Since its first description [14], numerous publications have explored a possible relationship between CD1 expression and OSCC prognosis [8,9], associating its overexpression with risk factors for a poor prognosis, including N+ and T status, advanced

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clinical stage, undifferentiated tumor, and reduced survival [8]). Out of a panel of candidate biomarkers of oral carcinogenesis, one of the best performances was observed for CD1 [8,15], but its prognostic value in OSCC remains controversial [8] and it is not utilized as a standardized marker in the clinical setting. In this systematic review and meta-analysis, we have carried out qualitative and quantitative analyses of scientific evidence on the prognostic significance of CD1 in OSCC with the objective to establish whether its overexpression can predict the progression of this disease. If this association is confirmed, CD1 expression may be useful in routine clinical practice for the prognosis of patients with OSCC and for therapeutic decision-making, with potential benefits for their survival.

Material and methods

This systematic review and meta-analysis complied with PRISMA guidelines [16] and closely followed the criteria of *Cochrane Prognosis Methods Group* [17], *Cochrane Handbook for Systematic Reviews of Interventions* [18], and *Centre for Reviews and Dissemination (CRD)'s guidance for undertaking reviews in healthcare* [19].

Protocol

In order to minimize the risk of bias and improve the transparency, precision, and integrity of this study, we registered a protocol on its methodology *a priori* in the PROSPERO international prospective register of systematic reviews (www.crd.york.ac.uk/PROSPERO, registration number CRD42018081746) [20]. The protocol adheres to PRISMA-P guidelines to ensure a rigorous approach [21].

Search strategy

We searched Pubmed, Embase, Web of Science, and Scopus databases for studies published at any time before the search date (July 2017). Searches were conducted by combining thesaurus terms used by the databases (e.g., MeSH and EMTREE) with free terms. In order to maximize sensitivity, the search strategy in Pubmed combined the following terms: ("cyclin d1"[MeSH Terms] OR ("cyclin"[All Fields] AND "d1"[All Fields]) OR "cyclin d1"[All Fields] OR "cyclind1"[All Fields] OR "ccnd1"[All Fields] OR "ccnd 1"[All Fields]) AND ("mouth"[MeSH Terms] OR "mouth"[All Fields] OR "oral"[All Fields]) AND ("carcinoma, squamous cell"[MeSH Terms] OR ("carcinoma"[All Fields] AND "squamous"[All Fields] AND "cell"[All Fields]) OR "squamous cell carcinoma"[All Fields]). An equivalent search strategy was adapted to the syntax of each database consulted (see protocol).

We also manually screened the reference lists of retrieved studies for additional relevant studies. All references were managed using software Mendeley v.1.17.10 (Elsevier. Amsterdam, The Netherlands), and duplicate references were eliminated.

Eligibility criteria

Study eligibility criteria were applied independently by two authors (PRG and MAGM). Any discrepancies were resolved by consensus.

Inclusion criteria: (1) Original research studies published in English. (2) Evaluation of CD1 expression using immunohistochemistry (IHC) in human tissues from primary OSCCs. (3) Analysis of the association between CD1 overexpression with at least one of the following clinicopathological and/or prognostic variables: T status, N status, histological grade, clinical stage, overall survival (OS), or disease-free survival (DFS). OS was defined as the time elapsed from date of diagnosis/surgery to date of death by any cause. DFS was defined as the time elapsed from surgery to the detection of locoregional or distant recurrence or to death without recurrence. Given the lack of international consensus standards to define survival endpoints, we included studies that used the direct designation of the aforementioned terms (OS/DFS) or other terms that are defined in the original studies as in the present article (e.g., recurrence-free survival) (4). The names and affiliations of authors and the recruitment period and setting were examined to determine whether studies were conducted in the same study population. In such cases, we included the most recent study or that which published more complete data.

Exclusion criteria were: (1) Reviews, meta-analyses, case reports, editorials, letters, abstracts from scientific meetings, personal opinions or comments, book chapters, and any study in a language other than English. (2) Study with no OSCC cases. (3) *In vitro* or animal studies. (4) Studies using techniques other than IHC or analyzing CCND1 gene alterations alone. (5) Studies with no analysis of relationships with clinicopathological and/or survival variables of interest. (6) Studies with insufficient data to estimate odds ratios (ORs) in analyses of clinicopathological variables, and studies of time-to-event variables alone (OS/DFS) that reported inadequate data for survival analysis, e.g., hazards ratio (HR) with 95% confidence interval (CI).

We selected articles in two phases, first screening the titles and abstracts of retrieved articles in an initial selection, and then reading the complete text of the article selected, excluding articles that did not meet the review eligibility criteria.

Data extraction

Two authors (PRG and MAGM) independently extracted data from the articles selected for reading of the complete texts, completing a data collection form in a standardized manner using Excel v.2015 (Microsoft. Redmond, WA). These data were additionally reviewed by two different authors (LGR and IRA), solving discrepancies by consensus. Data were gathered on the first author, year of publication, study country and continent, sample size, tumor localization, recruitment period, treatment modality, follow-up time, anti-CD1 antibody used, intracellular immunostaining (nuclear/cytoplasmic), cutoff point, CD1 overexpression (high/low), N and T status, histological grade, clinical stage, and survival data (OS and DFS).

Evaluation of quality and risk of bias

Two authors (PRG and MAGM) evaluated the quality of studies and the risk of bias using the Quality in Prognosis Studies (QUIPS) tool of the *Cochrane Prognosis Methods Group* [22], which explores six main potential bias domains: (1) Study participation, (2) Study attrition, (3) Prognostic factor measurement, (4) Outcome measurement, (5) Study confounding, and (6) Statistical analysis and reporting [23]. The risk of bias was evaluated as low, moderate, or high for each domain. Discrepancies were resolved by consensus.

Statistical analysis

CD1 expression was considered as "high" (above cutoff) or "low" (below cutoff). ORs with 95% CIs were calculated to determine the correlation between CD1 expression and clinicopathological variables in patients with OSCC. We used HRs with 95% CIs to estimate the impact of CD1 expression on time-to-event variables (OS and DFS). When reported, HRs and 95% CIs were directly extracted from the original articles. When HRs were determined in univariate and multivariate models, we used data from the multivariate model. When HR data were not reported, these were calculated following the methods of Parmar et al [24] and Tierney et al [25] or, in some studies, relative risk (RR) values and adjusted ORs were extracted as an approximation of the same measure [26]. When only a survival curve was given, data were extracted using Engauge Digitizer 4.1 (open-source digitizing software developed by M. Mitchell).

In the meta-analysis, studies were grouped by association measure. Combined associations were analyzed using both fixed-effect models (Mantel-Haenszel methods and inverse variance) and random-effect