Prognostic value of the immunohistochemical detection of epithelial-mesenchymal transition biomarkers in oral epithelial dysplasia: A systematic review

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Abstract

Background: Oral potentially malignant disorders (OPMDs) comprise a range of clinical-pathological alterations that are frequently characterized as architectural and cytological derangements upon histological analysis. Epithelial-mesenchymal transition (EMT) has been proposed as a critical mechanism for the acquisition of the malignant phenotype in neoplastic epithelial processes. This study aims to systematically review the current findings on the immunohistochemical expression of epithelial-mesenchymal transition markers in oral potentially malignant disorders and to evaluate their possible application as biomarkers associated with the progression of oral epithelial dysplasias.
Material and Methods: A systematic search was performed in the following databases: PubMed, EMBASE, Chinese BioMedical Literature Database, and Cochrane Library. Articles that evaluated the relationship between the expression of EMT markers and the degree of oral epithelial dysplasia were selected for the systematic review. The quality of each eligible study was evaluated by independent reviewers that used operationalized prognostic biomarker reporting guidelines (REMARK).
Results: Seventeen articles m*et al*l inclusion criteria and were selected. The EMT markers analyzed exhibited an important association with the prognosis of the cases evaluated. The results showed a progressive increase in the expression of nuclear transcription factors and markers of mesenchymal differentiation, as well as negative regulation of epithelial and cell adhesion markers, according to the stage of oral epithelial dysplasia.

Conclusions: The dysregulation of expression of important EMT components in oral dysplastic epithelium is a potential prognostic marker in OPMDs.

Key words: *Oral potentially malignant disorder, oral epithelial dysplasia, epithelial-mesenchymal transition, biomarker, prognosis.*

Introduction

Oral potentially malignant disorders (OPMDs) comprise a range of clinical-pathological alterations that are frequently characterized as architectural and cytological derangements upon histological analysis (1-3). OPMDs exhibit an increased risk of malignant transformation. A recent meta-analysis estimated an overall risk of malignant transformation of 10.5% for oral epithelial dysplasia (4).

The potential of malignant transformation is believed to be related to the degree of epithelial dysplasia observed (2-4). Several studies have been conducted to identify possible markers that can trigger the development of OPMDs as well as predict their progression (2,5,6). The epithelial-mesenchymal transition (EMT) is a mechanism frequently dysregulated in cancer (5-10,12). However, studies evaluating the role of EMT in the development/progression of dysplastic processes in oral epithelium are scarce.

The EMT, which is an essential event during embryogenesis, is a biological process in which epithelial cells lose their characteristics and shift to a mesenchymal cell-like phenotype. This process has been proposed as a critical mechanism for the acquisition of the malignant phenotype in neoplastic epithelial processes (5,8,9,12). EMT is mediated by nuclear transcription factors and can orchestrate intracellular alterations such as the negative regulation of epithelial markers, as well as the positive regulation of some mesenchymal markers (7,8,10).

A variety of biomarkers are known to be associated with EMT. These biomarkers can be divided into five groups according to their characteristics/functions: 1. cell surface markers; 2. cytoskeletal markers; 3. extracellular proteins; 4. transcription factors; 5. epigenetic markers and microRNAs.8 Recent studies indicate that important EMT markers may be involved in the malignant transformation of cases diagnosed as OPMD (5,11,12). In this study, a systematic review was conducted to clarify the prognostic value of EMT biomarkers in OPMDs. The review includes publications that evaluated the immunodetection of EMT markers and their possible use as prognostic factors/predictors in oral epithelial dysplasia.

Material and Methods

We performed a systematic review to conduct this investigation. The dependent variables were biomarkers of EMT available by immunohistochemistry and the independent variables were clinicopathological parameters and oral epithelial dysplasia grading. The Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed (13).

- Search strategy

The research question was "Are epithelial-mesenchymal transition biomarkers analyzed by immunohistochemistry potential predictors/prognostic factors for oral epithelial dysplasia?" and a keyword search was performed.

To identify all primary research articles that evaluated EMT biomarkers in oral epithelial dysplasia, we searched the MEDLINE/PubMed (1966 to January 2019), EMBASE (1980 to January 2019), Cochrane Collaboration Library (2009 to January 2019), and Chinese BioMedical Literature Databases (1978 to January 2019). The search strategy was based on combinations of the following keywords: ("Oral epithelial dysplasia" [MeSH] AND "Immunohistochemistry" [MeSH] AND "biomarkers of epithelial-mesenchymal transition" [MeSH] AND "clinicopathological parameters" [MeSH] AND "outcome" [MeSH]) AND (risk ratio [Title/Abstract] OR relative risk [Title/Abstract] OR odds ratio [Title/Abstract] OR risk [Title/ Abstract]) AND ("humans"[MeSH Terms]). A manual search of articles was also performed using the references within studies with inclusion potential in the systematic review.

- Selection criteria

Articles were included based on a previously published protocol (14,15). Studies that assessed the relationship between the immunohistochemical expression of EMT markers and histopathological grading of cases diagnosed with oral epithelial dysplasia were selected. The search was carried out without time and language restrictions. The PICOS (population, intervention, comparison, outcome, study design) format was used to construct the research question using the following inclusion criteria: (I) Population: patients diagnosed with oral epithelial dysplasia; (II) Intervention: immunohistochemical analysis of EMT markers; (III) Outcome: risk of progression from oral epithelial dysplasia; (IV) Study Design: observational studies in humans.

We limited selection to human studies on oral epithelial dysplasia defined based on standardized histological assessment as outlined by the WHO (16). We included all studies that reported data for progressing and non-progressing oral epithelial dysplasias. Both prospective and retrospective studies were included. Progressing lesions

were defined as those dysplasias that developed cancer at the same site as the initial biopsy when followed over time. Biomarkers of EMT were defined according to the study by Zeisberg & Neilson (8).

In the screening of titles or abstracts, citations were retained if they were original studies, except for reviews or meeting reports, that explored associations between the immunoexpression of the EMT markers, clinico-pathological characteristics, and outcome/progression of the oral epithelial dysplasia cases analyzed. The articles were selected independently by two reviewers (EFM and JCP). Any disagreement was resolved by consensus.

In the final full-text screening, studies were included if they met the following criteria: (I) designed as a prospective or retrospective cohort study; (II) oral epithelial dysplasia was diagnosed and histopathological grading was performed by pathological examination; (III) data about the association between the EMT biomarker evaluated and histopathological grading were reported. When studies were based on overlapping data, the more comprehensive set was selected.

- Data extraction and analysis

Quality assessment was performed in duplicate for each eligible study by three independent reviewers using the Newcastle operationalized prognostic biomarker reporting guidelines (REMARK) (15). Any disagreement was resolved by consensus. Studies receiving a score of less than 6 were not included in the systematic review. Three reviewers (EFM, JASL, RPM) independently extracted the data from the selected articles.

- Risk of bias in individual studies Methodologically, the authors appraised all of the included studies according to a checklist based in Meta -Analysis of Statistics Assessment and Review Instrument (MAStARI) (17). Two reviewers (EFM and RPM) answered 9 questions for descriptive studies as Y for "yes", N for "no", U for "unclear" and NA for "not applicable". After that, the risk of bias was categorized as high when the study reached up to 49% of a "yes" score, moderate when the study reached 50% to 69% of a "yes" score, and low when the study reached more than 70% of a "yes" score. Disagreements were solved by discussion between the four authors (EFM, RPM, JCP, JASL).

Results

- Study selection and characteristics

The database searches and manual search developed in this systematic review retrieved 378 studies. After reading the titles and abstracts, 54 studies were considered potentially eligible and their full text was read by three reviewers. Seventeen articles met all inclusion criteria and were selected for this systematic review (5,18-33). Fig. 1 illustrates the flow diagram of the screening and selection process of the articles.
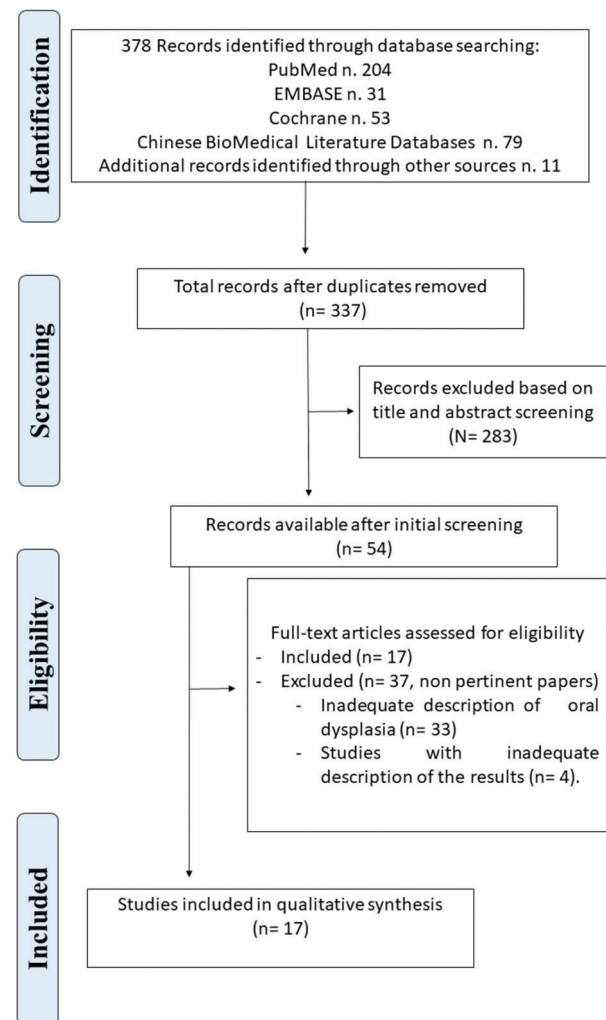

Fig. 1: PRISMA flow diagram of screened studies.

The selected articles were observational studies published between 2007 and 2018, all of them in English. Regarding methodological characteristics, all articles included in this systematic review involved 1,178 patients, with a mean of 69.2 participants per study. Our analysis revealed that 743 (63%) of the cases evaluated were lesions diagnosed in different stages of epithelial dysplasia. Among these cases, 48.7% were graded as mild epithelial dysplasia and 51.3% as moderate or severe epithelial dysplasia. The main characteristics and findings of the studies are shown in Table 1.

Most of the epithelial dysplasia cases were clinically diagnosed as leukoplakia and/or erythroplakia. Anura et al. (25) and Sharada et al. (33) also evaluated tissue samples of oral submucosal fibrosis. Twelve studies included samples of oral squamous cell carcinoma (OSCC) for biomarker analysis, totaling 426 cases analyzed (5,18,21,23,24,26-28,31-33).

Table 1: Summary of the descriptive characteristics and results of the included studies (n=17).

Author (year)	Samples	OPMD lesion subtype (Diagnosis)	Oral Subsite	Marker	Specimen type	Summary of the results
Ishida *et al.* (2007)	Group I (n = 9) - mild ED Group III (n = 3) - severe ED Group IV (n = 6) - NOM Group V (n = 15) - OSCC	Leukoplakia	NE	β-catenin	Tissue (F)	Nuclear β-catenin expression was significantly different between dysplasia and normal oral epithelium ($P < 0.01$), as well as between oral leukoplakia with and without dysplasia ($P < 0.01$).
Lo Muzio *et al.* (2009)	Group I (n = 21) - mild ED Group II (n = 15) - moderate ED Group III (n = 13) - severe ED	NE	NE	β-catenin γ-catenin	Tissue (F)	β- and γ-catenin were differently expressed according to dysplasia grade. As the degree of dysplasia became more severe, it was observed a reduction of catenins expression, loss of the exclusive membranar expression and cytoplasmic delocalization.
Carvalho *et al.* (2010)	Group I (n = 19) - mild ED Group II (n = 26) - moderate ED Group III (n = 3) - severe ED	NE	NE	Claudin-1	Tissue (F)	Alterations in the immunoexpression of claudin-1 between different grades of EDs suggest the involvement of this protein in the progression of oral EDs.
Chaw *et al.* (2012)	Group I (n = 27) - mild ED Group II (n = 8) - moderate/severe ED Group III (n = 18) - NOM Group IV (n = 47) - OSCC	NE	NE	E-cadherin β-catenin Vimentin	Tissue (F)	Aberrant expression of β-catenin and vimentin may be related to malignant transformation.
Inoue *et al.* (2012)	Group I (n = 49) - mild ED Group II (n = 19) - moderate ED Group III (n = 31) - severe ED/CIS Group IV (n = 4) – NOM	Leukoplakia Erythroplakia	Tongue Gingiva Others	Podoplanin	Tissue (F)	Immunoreactivity for podoplanin was detected in 89 (86.4%) of the potentially malignant lesions and staining intensity was correlated with the degree of epithelial dysplasia ($P = 0.016$). Podoplanin may be associated with tumor development via the oral dysplasia–carcinoma.
Silva *et al.* (2012)	Group I (n = 10) - mild ED Group II (n = 10) - moderate ED Group III (n = 10) - severe ED Group IV (n = 10) –NOM Group V (n = 20) - OSCC	Leukoplakia	Floor of the mouth Tongue Palate Gingiva	Twist p-Akt	Tissue (F)	A significant difference in Twist and p-Akt immunoexpression among NOM, EDs and OSCC was observed. In addition, a significant positive correlation was found between Twist and p-Akt expressions. These results suggest that Twist and p-Akt participate of the multistep process of oral carcinogenesis since its early stages.
Rani *et al.* (2013)	Group I (n = 27) - mild ED Group II (n = 8) - moderate ED Group III (n = 29) - severe ED Group IV (n = 24) - EH Group V (n = 48) - OSCC	NE	NE	Laminin-5	Tissue (F)	Laminin-5 expression was significantly increased in SCC, confirming its role as a marker of malignant transformation. The results of this study indicate that overexpression of laminin-5 is found only in SCC and not dysplastic lesions.

Table 1 cont.: Summary of the descriptive characteristics and results of the included studies (n=17).

Anura *et al.* (2014)	Group I (n = 20) - mild ED Group II (n = 10) - moderate ED Group III (n = 10) - severe ED Group IV (n = 10) - NOM Group V (n = 18) - OSFWT	OSFWT	NE	E-cadherin	Tissue (F)	The positive correlation between deregulated expression of epithelial cell–cell adhesion molecule in oral submucous fibrosis with increase in dysplastic grades suggested elucidatory potential of molecular expression features in assessment of malignant potentiality in oral submucous fibrosis.
de Freitas Silva *et al.* (2014)	Group I (n = 10) - mild ED Group II (n = 10) - moderate ED Group III (n = 10) - severe ED Group IV (n = 10) - NOM Group V (n = 20) - OSCC	Leukoplakia	NE	E-cadherin Twist	Tissue (F)	Significant differences in Twist and E-cadherin immunoexpression were observed between NOM and EDs, with an inverse relation since the earliest stages of oral dysplasia (r = 0,512; P < 0.001). The results showed a possible value of Twist and E-cadherin in the prediction of risk of malignant transformation of oral epithelium.
Kyrodimou *et al.* (2014)	Group I (n = 12) - Hyperkeratosis/mild ED Group II (n = 7) - moderate ED Group III (n = 6) - severe ED Group IV (n = 25) - NOM Group V (n = 25) - OSCC	Leukoplakia	Buccal Mucosa Gingiva Tongue Lip	Desmoglein-3 γ-catenin E-cadherin β-catenin	Tissue (F)	The altered expression of Desmogleing3/γ-catenin and E-cadherin/β-catenin, in ODs/OSCC imply their involvement in growth regulation and phenotype of dysplastic/malignant oral epithelial cells, contributing to the better understanding of epithelial dysplasia and OSCCs.
Von Zeidler *et al.* (2014)	Group I (n = 23) - WD / mild ED Group II (n = 8) - moderate/severe ED Group III (n = 12) - OSCC	Leukoplakia	Buccal Mucosa Oral Tongue	E-cadherin	Tissue (F)	Reduced E-cadherin expression was an early phenomenon observed in moderate-severe dysplasia, showing that the loss of epithelial cohesion may be an indicator of progression to oral cavity squamous cell carcinoma.
Reyes *et al.* (2015)	Group I (n = 21) - mild ED Group II (n = 12) - moderate ED Group III (n = 3) - severe ED Group IV (n = 3) –NOM Group V (n = 19) - OSCC	Leukoplakia Erythroplakia	Tongue Palate Floor of mouth Gums Mandible Cheek Others	β-catenin	Tissue (F)	Nuclear expression of β-catenin was observed in all samples with severe and moderate dysplasia, with a median of 267.5, in comparison to mild ED whose median was 103.75. This finding suggests a role for β-catenin in the progression of ED and early malignant transformation to OSCC.
Silva *et al.* (2015)	Group I (n = 19) - mild ED Group II (n = 13) - moderate ED Group III (n = 7) - severe ED Group IV (n = 10) - NOM	Leukoplakia	Buccal Mucosa Oral Tongue Floor of mouth Palate Gingiva	β-catenin	Tissue (F)	Cytoplasmic expression of β-catenin may represent the initial stage of modifications in the E-cadherin-catenin complex, along with changes in cell morphology.
Yagyuu *et al.* (2015)	Group I (n = 58) - WD / mild ED Group II (n = 36) - moderate/severe ED or CIS		Tongue Others	Cytokeratin-13 Cytokeratin-17	Tissue (F)	Cytokeratin-13 loss was found to be an independent diagnostic marker for high-grade dysplasia. Cytokeratin-13 loss may be useful for distinguishing EDs from reactive atypical epithelium.

Table 1 cont.: Summary of the descriptive characteristics and results of the included studies (n=17).

Chandolia *et al.* (2017)	Group I (n = 20) - mild ED Group II (n = 20) - moderate ED Group III (n = 20) - severe ED Group IV (n = 10) - NOM Group V (n = 30) – OSCC	NE	NE	N-Cadhe-rin	Tissue (F)	N-cadherin expression was more evident in OSCC than in oral ED.
Gupta *et al.* (2018)	Group I (n = 7) - mild ED Group II (n = 7) - moderate ED Group III (n = 7) - severe ED/ CIS Group IV (n = 21) - OSCC	NE	NE	E-cadhe-rin	Tissue (F)	Loss of membranar E-cadherin expression and decrease in staining intensity were noted from ED to OSCC.
Sharada *et al.* (2018)	Group I (n = 10) - mild ED Group II (n = 10) - moderate ED Group III (n = 10) - severe ED Group IV (n = 10) - OSFWT Group V (n = 10) - NOM Group VI (n = 10) - OSCC	OSFWT	NE	E-cadhe-rin	Tissue (F)	E-cadherin expression was membranar and continuous in all epithelial layers of NOM and reduced with progressing grades of oral ED to OSCC.

Abbreviators: ♀, female; ♂, male; NOM, normal oral mucosa; OSFWT, oral submucous fibrosis without dysplasia; ED, Epithelial dysplasia; NE, Not specified; EH, Epithelial Hyperplasia; F, Formalin-fixed paraffin-embedded blocks; WD, without dysplasia; CIS, Carcinoma In Situ; OSCC, Oral Squamous Cell Carcinoma.

The following EMT markers were analyzed according to the criteria proposed by Zeisberg & Neilson[8]: β-catenin (18,19,26,28,29), γ-catenin (19,26), claudin-1 (20), E-cadherin (5,21,26,27,32,33), Twist (5,23), p-Akt (23), podoplanin (22), laminin-5 (24), desmoglein-3 (26), cytokeratin 13 and cytokeratin 17 (30), and N-cadherin (31). The methods used for immunohistochemical analysis in the different studies selected were identified in this systematic review (Table 2).

Table 2: Immunohistochemical analysis used in the selected studies.

Author (year)	Marker	Immunohistochemical analysis
Ishida *et al.* (2007)	β-catenin	For counting cells with nuclear and/or cytoplasmic staining, three microscopic fields (x200) were randomly chosen and the mean above 10% of the total cell numbers was deduced to be positive. Evaluation of staining was carried out based on whether nuclear or cytoplasmic staining was detectable. Under microscopic fields (x200), tissues were considered positive for β-catenin if more than 10% of cells showed cytoplasmic or nuclear staining.
Lo Muzio *et al.* (2009)	β-catenin γ-catenin	β-catenin and γ-catenin immunoexpression was quantitatively assessed as a percentage of the final number of 300 cells for each case. Cellular localization of immunolabelling was also classified as membranar or cytoplasmic (including also concurrent nuclear staining) according to the staining distribution pattern.
Carvalho *et al.* (2010)	Claudin-1	Staining distribution was classified into focal (up to 30% of the epithelium) and diffuse (>30% of the epithelium). Epithelial localization, i.e., the predominant site of immunoexpression in epithelium, was divided into upper, middle, and lower third. Cellular localization, corresponding to the site of expression in the cell, was classified as membranar or membranar/cytoplasmic staining. Staining intensity was evaluated subjectively on a qualitative scale and was defined as weak, moderate or strong.
Chaw *et al.* (2012)	E-cadherin β-catenin APC Vimentin	Immunoreactivity for E-cadherin, β-catenin, and APC was assessed using a semiquantitative method based on the proportion of positive cells and staining intensity: Immunoreactivity score = proportion positive score x intensity score. The proportion of positive staining was scored as follows: 0 (0–20% of epithelial cells stained positive), 1 (21–40% of positive cells), 2 (41–60% of positive cells), 3 (61–80% of positive cells), or 4 (>80% of positive cells). The intensity was scored as: 0 (negative staining), 1 (weak intensity), 2 (moderate intensity), or 3 (strong intensity). Immunoreactivity for vimentin was assessed using the Label Index (Li) score, which presents the proportion of positively-stained cells as a percentage.
Inoue *et al.* (2012)	Podoplanin	The intensity of immunoexpression was scored as follows: 0 (no expression in any part of the epithelium); 1 (intensity weaker than that in lymphatic vessels as a positive control, and expression observed in the basal layer; 2 (intensity weaker than that in lymphatic vessels, and expression was observed in more than two layers; 3 (intensity equal to that in lymphatic vessels, and expression observed in basal layer); and 4 (intensity equal to that in lymphatic vessels, and expression observed in more than 2 layers. The score was based on examination of the whole section.
Silva *et al.* (2012)	Twist p-Akt	Five histological fields were randomly chosen, and 1000 cells were counted in each slide. The analysis was performed by counting positive cells in cytoplasmic and/or nuclear compartments.

Table 2 cont.: Immunohistochemical analysis used in the selected studies.

Rani *et al.* (2013)	Laminin-5	The extent of positive immunostaining was calculated by subtracting the negative control strongly positive cytoplasmic or nuclear pixel count from the matching area with a strongly positive cytoplasmic or nuclear pixel count on the study slides. This resultant normalized positive immunohistochemistry pixel count was divided by the area (square millimeters) to provide a comparable indicator of immunopositivity among individual tissue samples.
Anura *et al.* (2014)	E-cadherin	The epithelium was demarcated into proliferative and differentiative layers to evaluate the differential expression pattern of the molecules using intensity scores. To evaluate E-cadherin expression, random points corresponding to each layer were selected from randomly selected microphotographs of the studied groups. Expression intensity scores were given to each point in the range of '0–10', where: '10-8' (intensely deep color), '7-6' (deep color), '5-3' (faint) and '2-0' (very faint color). The intensity of E-cadherin expression in cell membrane and cytoplasm was scored separately.
de Freitas Silva *et al.* (2014)	E-cadherin Twist	The analysis of Twist immunoexpression was performed taking in count the percentage of positive staining cells in the whole examined area. For Twist evaluation, the percentage of positive cells was classified into four categories: 1 (<25%); 2 (25%-50%); 3 (51%-75%); 4 (>75%). For E-cadherin, immunohistochemical staining was evaluated as: 1 (less than 25% positive cells); 2 (heterogeneous staining in 25%-50% of cells); 3 (homogenous staining in 50%–75% of cells); 4 (strong staining in more than 75% of cells).
Kyrodimou *et al.* (2014)	Desmoglein-3 γ-catenin E-cadherin β-catenin	Immunoreactivity of the examined biomarkers was semiquantitatively assessed and positive staining was characterized as membranar, cytoplasmic, or nuclear. The scoring grading was based on the percentage of positive cells. Immunostaining was considered positive if greater than or equal to 5% of tumour cells were stained and the scoring was graded in a I-III scale according to the percentage of positive cells: I (6-35%), II (36–70%), and III (71–100%).
Von Zeidler *et al.* (2014)	E-cadherin	The analysis of E-cadherin immunoexpression was performed by calculating the percentage of positive staining cells in relation to the whole examined area. A semiquantitative scoring system was used, based on the staining pattern on a four-point scale: 0 (negative, with absent or discontinued membranar staining); 1 (weak; 1-50% of cells stained); 2 (moderate; 51-75% of cells stained); and 3 (strong; >75% of cells stained. A total index score was obtained by summing the results of all layers (basal, parabasal and keratinized) in the groups with normal oral mucosa and epithelial dysplasia; tumor front was analyzed at oral squamous cell carcinoma.
Reyes *et al.* (2015)	β-catenin	The cellular localization of β-catenin was classified as membranar, cytoplasmic or nuclear, depending on the immunolocalization pattern. Intensity of membranar staining was assessed as mild, moderate or intense. Five randomly selected fields with presence of dysplastic and/or neoplastic epithelium were assessed. In these photomicrographs, 1000 epithelial cells were counted per case and the number of positive cells was determined with the Image J software.
Silva *et al.* (2015)	β-catenin	A qualitative β-catenin analysis was performed based on the percentage of positive cells, considering as positive only specimens with 25% or more cells presenting β-catenin expression. The cellular localization (membranar, cytoplasmic and nuclear) and the epithelial layer (basal, parabasal, and spinous cell layers) were also considered. β-catenin immunoexpression was analyzed by two independent blind and calibrated examiners under light microscopy at 200-fold magnification.
Yagyuu *et al.* (2015)	Cytokeratin 13 Cytokeratin 17	CK13 was scored as follows: 0, strong and diffuse expression; 1, weak and/or patchy expression; or 2, no expression. CK17 was scored as follows: 0, no expression; 1, weak and/or patchy expression; or 2, strong and diffuse expression. Discrepant slides were re-evaluated using a dual vision microscope to achieve a consensus.
Chandolia *et al.* (2017)	N-Cadherin	Brownish staining in cell membrane and cytoplasm was considered as positive staining. The expression was classified based on the intensity of staining and the percentage of cells exhibiting membranar staining. The sections were observed at low magnification to identify positively stained areas (hot spots). In the control and dysplasia groups, the positivity of N-cadherin expression was analyzed in epithelium in basal and spinous layer. Based on the total score, the final expression was graded as negligible (0-2), mild (3-5), moderate (6-8), or intense (9-12).
Gupta *et al.* (2018)	E-cadherin	Intensity of staining: 0 = absent/loss of expression; 1 = weak/light staining; 2 = moderate staining; 3 = strong/intense staining. Membranar staining expression: 0 = 0 to 10% of cells showing a complete membranar staining; 1 = 11 to 50% of cells showing a complete membranar staining; 2 = 51 to 75% of cells showing a complete membranar staining; 3 = 76 to 100% of cells showing a complete membranar staining.
Sharada *et al.* (2018)	E-cadherin	Cells showing membranar staining were considered positive for E-cadherin. The intensity of immunoexpression was assessed and scores were interpreted as follows: 0 (no positive cells); 1+ (mild intensity); 2+ (moderate intensity); 3+ (strong intensity). The obtained score was tabulated as quick score (QS). The QS was calculated for basal cells, parabasal cells, superficial cells (including intermediate cells) and corneal cells in all six groups and for epithelial cells in the connective tissue stroma of oral squamous cell carcinoma.

- Quality assessment and Risk of bias

We used the criteria established by the REMARK guidelines to evaluate the studies included in the systematic review (Table 3). The selected studies provided details about the objective/hypothesis of the study, characteristics of the patients included in the sample, analysis method used, and relationship of the EMT markers with the degree of epithelial dysplasia in the cases analyzed. In addition, the studies discussed implications for future studies and clinical value.

Based on the MAStARI assessment, 2 articles (18,32) were classified as carrying a high risk of bias, mainly because the answers for questions 3 and 4 (related to co-founding factors, description of the groups and follow-up, respectively) were "No". Fifteen studies were classified as with low risk for bias (5,19-22,25,26-31,33) (Table 4).

Table 3: Evaluation criteria used to assess the quality of studies evaluated (adapted from REMARK guidelines).

Checklist	Criteria
Introduction	The hypotheses and objectives of the study were clearly explained.
Cohort description	Retrospective or prospective cohort with a well-defined study population. Medical treatment of the cases was explained.
Patient data	The basic data such as age, gender, clinical stage and histopathologic grade was provided.
Evaluation method	Well-described method including the microscopic field/s and the cutoff point. Inter-observer variability was evaluated.
Prognostic analysis	The survival end point was defined and/or the relationship between the tumour budding and lymph node metastasis was studied.
Statistical analysis	Estimated effect (e.g., hazard ratio, relative risk with their confidence interval), which reveal the relationship between tumour budding and the survival end point/s. The independence of prognostic value was reported by multivariate analysis
Classical prognostic factors	The prognostic value of the classical prognostic factors (e.g., stage and grade) were reported. The relationship between tumour budding and classical prognostic factors was reported.
Interpretation of the prognostic value and discussion	Comparison of the current findings with other studies. Strengths and limitations of the current data. Recommendation for further research.

Table 4: Analysis of the risk of bias of the articles included in the review was performed with the MAStARI (Meta-Analysis of Statistics Assessment and Review Instrument) critical appraisal tool.

Author (year)	Questions*									% Yes#	Risk of Bias
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9		
Ishida et al. (2007)	NA	Y	N	Y	N	N	NA	Y	Y	57.1	H
Lo Muzio et al. (2009)	NA	Y	N	Y	Y	N	NA	Y	Y	71.4	L
Carvalho et al. (2010)	NA	Y	Y	Y	Y	N	NA	Y	Y	85.7	L
Chaw et al. (2012)	NA	Y	Y	Y	Y	N	NA	Y	Y	85.7	L
Inoue et al. (2012)	NA	Y	Y	Y	N	N	NA	Y	Y	71.4	L
Silva et al. (2012)	NA	Y	Y	Y	Y	N	NA	Y	Y	85.7	L
Rani et al. (2013)	NA	Y	Y	Y	N	N	NA	Y	Y	71.4	L
Anura et al. (2014)	NA	Y	N	Y	Y	N	NA	Y	Y	71.4	L
de Freitas Silva et al. (2014)	NA	Y	Y	Y	Y	N	NA	Y	Y	85.7	L
Kyrodimou et al. (2014)	NA	Y	Y	Y	N	N	NA	Y	Y	71.4	L
Von Zeidler et al. (2014)	NA	Y	Y	Y	N	N	NA	Y	Y	71.4	L
Reyes et al. (2015)	NA	Y	N	Y	Y	N	NA	Y	Y	71.4	L
Silva et al. (2015)	NA	Y	Y	Y	Y	N	NA	Y	Y	85.7	L
Yagyuu et al. (2015)	NA	Y	Y	Y	Y	N	NA	Y	Y	85.7	L
Chandolia et al. (2017)	NA	Y	Y	Y	Y	N	NA	Y	Y	85.7	L
Gupta et al. (2018)	NA	Y	N	Y	N	N	NA	Y	Y	57.1	H
Sharada et al. (2018)	NA	Y	Y	Y	N	N	NA	Y	Y	71.4	L

Q1. Is the study based on a random or pseudorandom sample?
Q2. Are the criteria for inclusion in the sample clearly defined?
Q3. Are confounding factors identified and strategies to deal with them stated?
Q4. Are outcomes assessed using objective criteria?
Q5. If comparisons are being made, was there sufficient description of the groups?
Q6. Is follow up carried out over a sufficient time period?
Q7. Are the outcomes of people who withdrew described and included in the analysis?
Q8. Are outcomes measured in a reliable way?
Q9. Is appropriate statistical analysis used?

*Y=Yes, N=No, NA=Not applicable (which was not considered on the percentage calculation)
Risk of bias was categorized as high (H) when the study reaches up to 49% score "yes", moderate (M) when the study reached 50% to 69% score "yes", and low (L) when the study reached more than 70% score "yes".

- Cell-surface and cytoskeletal markers of the epithelial-mesenchymal transition

Among the 17 selected studies, 15 evaluated cell surface and/or cytoskeletal markers (5,18-22,25-33). These studies demonstrated significant dysregulation of the expression of the proteins analyzed according to the degree of dysplasia.

In our systematic review, β-catenin was a biomarker strongly associated with the progression of oral epithelial dysplasia. Ishida *et al.* (18) detected strong immunoexpression of β-catenin in the cell nucleus of cases diagnosed as oral epithelial dysplasia, with a significant difference between dysplasia samples and normal oral epithelium (*P* < 0.01) and between oral leukoplakia with dysplasia and the group without dysplastic alterations (*P* < 0.01). Lo Muzio *et al.* (19) observed the loss of membrane expression of β- and γ-catenin with increasing degree of oral epithelial dysplasia and a shift of expression to the cytoplasm. The same finding was reported in the other studies included (21,29).

The loss of membrane expression of cell adhesion markers and the translocation of immunopositivity to the cytoplasm was a common finding and was strongly associated with an increase in the degree of oral epithelial dysplasia. Carvalho *et al.* (20) demonstrated that all cases of mild epithelial dysplasia and 73.1% of cases of moderate epithelial dysplasia exhibited only membrane immunopositivity, while membrane/cytoplasmic staining was observed in cases of severe epithelial dysplasia. These findings show the dysregulation of protein expression with increasing degree of dysplasia.

Chaw *et al.* (21) observed a significant correlation between the loss of E-cadherin expression and increased vimentin expression in the cytoplasm in moderate/severe epithelial dysplasias. The expression of vimentin was also positively correlated with the cytoplasmic and nuclear expression of β-catenin (r = 0.467, P < 0.05). De Freitas Silva *et al.* (5) demonstrated a gradual loss of E-cadherin expression with increasing degree of dysplasia of the oral epithelium.

- Extracellular proteins and transcription factors

Although recognized as important markers associated with the EMT process, studies analyzing the role of transcription factors and extracellular proteins in oral epithelial dysplasia by immunohistochemistry are scarce. Silva *et al.* (23) reported a significant variation in the Twist transcription factor among five groups analyzed. The authors observed higher immunopositivity in cases of severe epithelial dysplasia compared to moderate dysplasia (*P* = 0.047) and a progressive increase of expression in cases of OSCC compared to severe epithelial dysplasia (*P* = 0.007).

In the study of De Freitas Silva *et al.* (5) immunostaining for the Twist protein was mainly observed in the parabasal and basal layers of the normal oral epithelium and in the groups with mild and moderate dysplasia, exhibiting a predominant cytoplasmic localization. However, in cases of severe epithelial dysplasia, immunoexpression was also detected in the superficial layers of the epithelium, indicating dysregulation of protein expression in advanced stages of epithelial dysplasia. The authors also showed a statistically significant correlation in the immunoexpression of Twist and E-cadherin, with the observation of an inverse relationship between the immunoexpression of these proteins (r = 0.512; P < 0.001).

Rani *et al.* (24) observed low expression of laminin-5 in cases of epithelial dysplasia, with predominantly weak and cytoplasmic immunostaining. In that study, the expression of laminin-5 did not differ significantly according to the degree of dysplasia. A significant increase in laminin-5 immunopositivity was only found in cases of OSCC compared to the epithelial dysplasia groups, suggesting a role of this protein in the invasion of the already established neoplastic process.

Discussion

The present study analyzed the role of EMT in cases diagnosed with oral epithelial dysplasia. The malignant transformation of OPMDs is an important process in oral carcinogenesis, which is still poorly understood. Thus, the identification of markers that are associated with the development and progression of oral epithelial dysplasia is of paramount importance for establishing a possible relationship with the prognosis of these lesions. The histopathological classification of oral epithelial dysplasia can assist in the monitoring and definition of the most appropriate treatment; however, other factors may be determinant in the process of malignant transformation (34,35).

Several biomarkers are being analyzed by immunohistochemistry in order to evaluate their participation in oral epithelial dysplasia (3,6). In clinicopathological practice, immunohistochemistry is relatively easy to apply and more accessible than other techniques.

The EMT has been indicated as an important process in carcinogenesis that is directly associated with the aggressiveness of OSCC. During this process, epithelial cells lose their capacity of cell-cell adhesion. In addition, reorganization of the cytoskeleton and significant changes in signaling occur that define the shape and structure of the neoplastic cell. EMT is believed to be the result of reprogramming of gene expression mediated by transcription factors such as Twist, Snail, and Slug (10,36-38). This transition increases cell motility and enables the development of an invasive phenotype (37). Despite advances in the understanding of the function of EMT in already established OSCC, it is necessary to identify the role of EMT markers in the development and progression of oral epithelial dysplasia.

The loss of cell adhesion is likely to play a key role in EMT. This phenomenon can be observed in our systematic review by the loss of membrane expression of cell adhesion markers such as claudin-1 and, particularly, E-cadherin in cases diagnosed as epithelial dysplasia (5,10,21,26,27,32,33,36,38). Furthermore, E-cadherin was immunoexpressed in the cytoplasm of epithelial dysplasia cases. In this respect, studies have shown that the cytoplasmic expression of this protein is frequently associated with tumors in advanced stages (39,40). It is possible that the loss of membrane expression and the onset of E-cadherin translocation to the cytoplasm, and consequently the loss of basic functions associated with cell adhesion, occur during the early stages of oral carcinogenesis and are already present in cases of oral epithelial dysplasia, progressing with increasing severity of the latter.

Twist is an important nuclear transcription factor for EMT. De Freitas Silva *et al.* (5) indicated a repressive effect of Twist on the expression of E-cadherin in epithelial dysplasia and oral cancer. Furthermore, previous studies have elucidated the role of Twist in the already established neoplastic process, which negatively regulates the expression of E-cadherin and the overexpression of mesenchymal markers (5,10,37). Other transcription factors with a potential role in the development and progression of oral epithelial dysplasia need to be better investigated. Zheng *et al.* (41) reported the overexpression of the transcription factors Snail and Slug to be associated with a poor prognosis of the neoplastic process. The Twist, Snail and Slug proteins seem to a play a similar role in the repression of E-cadherin and, apparently, the expression of these proteins is also related to the regulation of β-catenin (42).

The binding of E-cadherin to β-catenin in the cytoplasm/membrane represses tumor progression, maintaining cell-cell adhesion and inhibiting EMT, cell motility and tumor metastasis (42). The negative regulation or loss of E-cadherin and β-catenin expression, as well as the immunoexpression of β-catenin in the nucleus, is frequently observed in several type of cancer, including head and neck cancer. In our systematic review, the nuclear expression of β-catenin was a frequent finding in the samples of cases diagnosed as advanced oral epithelial dysplasia and might be an important marker associated with the progression of this condition (18,21,28).

Prgomet, Andersson, & Lindberg44 evaluated the expression of WNT5A, β-catenin and E-cadherin by immunohistochemistry in 21 tissue samples. Each sample contained areas of mucosa with normal appearance, oral epithelial dysplasia, and OSCC. In that study, membrane expression of β-catenin was lower in OSCCs than in dysplasia or normal-appearing mucosa regions, while cytoplasmic expression of β-catenin increased with the severity of dysplasia and was detected in half of the

OSCCs. Similar findings of reduced membrane expression of β-catenin and progression of oral carcinogenesis were reported in the studies included in our systematic review. The cytoplasmic accumulation and subsequent nuclear translocation of β-catenin might be the result of activation of the canonical Wnt signaling pathway or impairment of this pathway due to mutations in some of its components. The cytoplasmic/nuclear overexpression of β-catenin is known to be associated with malignant transformation in different types of cancer (28,45). Recent in vitro studies confirm the role of EMT markers in the progression of oral epithelial dysplasia. Dmello *et al.* (46) evaluated the expression of vimentin in epithelial cells derived from OPMDs. In that study, the exogenous expression of vimentin contributed to the occurrence of EMT and subsequent malignant transformation. Epigenetic markers may regulate EMT. Members of the miR-200 family have been shown to be negatively regulated in human cancer cell lines and play a critical role in the suppression of EMT, tumor cell adhesion, migration, invasion, and metastasis (47). Arunkumar *et al.* (48) demonstrated expressive negative regulation of miR-200 in poorly differentiated OSCC by RT-qPCR analysis ($P = 0.0067$). However, there are no studies investigating epigenetic markers associated with EMT in oral epithelial dysplasia.

Conclusions

In conclusion, the dysregulation of expression of EMT-associated proteins in dysplastic oral epithelium is a potential prognostic marker. The results showed a progressive increase in the expression of nuclear transcription factors and markers of mesenchymal differentiation, as well as negative regulation of epithelial and cell adhesion markers, according to the severity of oral epithelial dysplasia. It should be noted that the studies included in this systematic review examined OPMDs that were not continuously followed up from the time of diagnosis to malignant transformation. Longitudinal studies are necessary to identify the possible association of EMT with the risk of malignant transformation of OPMDs.

References
1. Izumo T. Oral premalignant lesions: from the pathological viewpoint. Int J Clin Oncol. 2011;16:15-26.
2. Sieviläinen M, Passador-Santos F, Almahmoudi R, Christopher S, Siponen M, Toppila-Salmi S, *et al*. Immune checkpoints indoleamine 2,3-dioxygenase 1 and programmed death-ligand 1 in oral mucosal dysplasia. J Oral Pathol Med. 2018;47:773-80.
3. Speight PM, Khurram SA, Kujan O. Oral potentially malignant disorders: risk of progression to malignancy. Oral Surg Oral Med Oral Pathol Oral Radiol. 2018;125:612-27.
4. Shariff JA, Zavras AI. Malignant transformation rate in patients presenting oral epithelial dysplasia: systematic review and meta-analysis. J Oral Dis. 2015;2015:854636.
5. de Freitas Silva BS, Yamamoto-Silva FP, Pontes HA, Pinto Júnior Ddos S. E-cadherin downregulation and Twist overexpression since early stages of oral carcinogenesis. J Oral Pathol Med. 2014;43:125-31.

6. Coletta RD, Salo T. Myofibroblasts in oral potentially malignant disorders: Is it related to malignant transformation? Oral Dis. 2018;24:84-8.

7. Fuchs BC, Fujii T, Dorfman JD, Goodwin JM, Zhu AX, Lanuti M, et al. Epithelial-to-mesenchymal transition and integrin-linked kinase mediate sensitivity to epidermal growth factor receptor inhibition in human hepatoma cells. Cancer Res. 2008;68:2391-99.

8. Zeisberg M, Neilson EG. Biomarkers for epithelial–mesenchymal transitions. J Clin Invest. 2009;119:1429-37.

9. Zhu Y, Zhang W, Wang P. Smoking and gender modify the effect of TWIST on patient survival in head and neck squamous carcinoma. Oncotarget. 2017;8:85816-27.

10. de Morais EF, Santos HBP, Cavalcante IL, Rabenhorst SHB, Dos Santos JN, Galvão HC, et al. Twist and E-cadherin deregulation might predict poor prognosis in lower lip squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol. 2019;127:318-29.

11. Abdalla Z, Walsh T, Thakker N, Ward CM. Loss of epithelial markers is an early event in oral dysplasia and is observed within the safety margin of dysplastic and T1 OSCC biopsies. PLoS One. 2017;12:e0187449.

12. Lopes NM, Xavier FCA, Ortiz RC, Amôr NG, Garlet GP, Lara VS, et al. Subcellular localization and expression of E-cadherin and SNAIL are relevant since early stages of oral carcinogenesis. Pathol Res Pract. 2018;214:1185-91.

13. Moher D, Liberati A, Tetzlaff J, Altman DG. PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6:e1000097.

14. Mallett S, Timmer A, Sauerbrei W, Altman DG. Reporting of prognostic studies of tumor markers: a review of published articles in relation to REMARK guidelines. Br J Cancer. 2010;102:173-80.

15. Altman DG, McShane LM, Sauerbrei W, Taube SE. Reporting recommendations for tumor marker prognostic studies (REMARK): explanation and elaboration. PLoS Med. 2012;9:e1001216.

16. El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ. The fourth edition of the head and neck World Health Organization blue book: editors' perspectives. Hum Pathol. 2017;66:10-12.

17. The JBI. MAStARI: Joanna Briggs Institute Reviewer's Manual 2014, Joanna Briggs Institute: University of Adelaide. 2014.

18. Ishida K, Ito S, Wada N, Deguchi H, Hata T, Hosoda M, et al. Nuclear localization of beta-catenin involved in precancerous change in oral leukoplakia. Mol cancer. 2007;6:62.

19. Lo Muzio L, Lo Russo L, Falaschini S, Ciavarella D, Pentenero M, Arduino P, et al. β- and γ-catenin expression in oral dysplasia. Oral Oncol. 2009;45:501-4.

20. Carvalho MV, Pereira JS, Costa AL, de Souza LB, Freitas Rde A, Miguel MC. Alterations in the immunoexpression of claudin-1 between different grades of oral epithelial dysplasias. Arch Oral Biol. 2010;55:261-7.

21. Chaw SY, Abdul Majeed A, Dalley AJ, Chan A, Stein S, Farah CS. Epithelial to mesenchymal transition (EMT) biomarkers--E-cadherin, beta-catenin, APC and Vimentin--in oral squamous cell carcinogenesis and transformation. Oral Oncol. 2012;48:997-1006.

22. Inoue H, Miyazaki Y, Kikuchi K, Yoshida N, Ide F, Ohmori Y, et al. Podoplanin expression during dysplasia-carcinoma sequence in the oral cavity. Tumour Biol. 2012;33:183-94.

23. Silva BS, Yamamoto FP, Pontes FS, Cury SE, Fonseca FP, Pontes HÁ, et al. TWIST and p-Akt immunoexpression in normal oral epithelium, oral dysplasia and in oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal. 2012;17:e29-34.

24. Rani V, McCullough M, Chandu A. Assessment of laminin-5 in oral dysplasia and squamous cell carcinoma. J Oral Maxillofac Surg. 2013;71:1873-9.

25. Anura A, Das RK, Pal M, Paul RR, Ray AK, Chatterjee J. Correlated analysis of semi-quantitative immunohistochemical features of E-cadherin, VEGF and CD105 in assessing malignant potentiality of oral submucous fibrosis. Pathol Res Pract. 2014;210:1054-63.

26. Kyrodimou M, Andreadis D, Drougou A, Amanatiadou EP, Angelis L, Barbatis C, et al. Desmoglein-3/γ-catenin and E-cadherin/ß-catenin differential expression in oral leukoplakia and squamous cell

carcinoma. Clin Oral Investig. 2014; 18:199-210.

27. Von Zeidler SV, de Souza Botelho T, Mendonça EF, Batista AC. E-cadherin as a potential biomarker of malignant transformation in oral leukoplakia: a retrospective cohort study. BMC Cancer. 2014;14:972.

28. Reyes M, Rojas-Alcayaga G, Maturana A, Aitken JP, Rojas C, Ortega AV. Increased nuclear β-catenin expression in oral potentially malignant lesions: A marker of epithelial dysplasia. Med Oral Patol Oral Cir Bucal. 2015;20:e540-6.

29. Silva BS, Castro CA, Von Zeidler SL, Sousa SC, Batista AC, Yamamoto-Silva FP. Altered β-catenin expression in oral mucosal dysplasia: a comparative study. J Appl Oral Sci. 2015;23:472-8.

30. Yagyuu T, Obayashi C, Ueyama Y, Takano M, Tanaka Y, Kawaguchi M, et al. Multivariate analyses of Ki-67, cytokeratin 13 and cytokeratin 17 in diagnosis and prognosis of oral precancerous lesions. J Oral Pathol Med. 2015;44:523-31.

31. Chandolia B, Rajliwal JP, Bajpai M, Arora M. Prognostic Potential of N-Cadherin in Oral Squamous Cell Carcinoma via Immunohistochemical Methods. J Coll Physicians Surg Pak. 2017;27:475-8.

32. Gupta A, Sharma S, Batra M, Abidullah M, Bhuvinder S, Katragadda P. Role of E-cadherin in Progression of Oral Squamous Cell Carcinoma: A Retrospective Immunohistochemical Study. J Contemp Dent Pract. 2018;19:1105-10.

33. Sharada P, Swaminathan U, Nagamalini BR, Kumar KV, Ashwini BK, Lavanya V. Coalition of E-cadherin and vascular endothelial growth factor expression in predicting malignant transformation in common oral potentially malignant disorders. J Oral Maxillofac Pathol. 2018;22:40-7.

34. Barfi Qasrdashti A, Habashi MS, Arasteh P, Torabi Ardakani M, Abdoli Z, Eghbali SS. Malignant transformation in leukoplakia and its associated factors in southern Iran: A hospital based experience. Iran J Public Health. 2017;46:1110-7.

35. Luiz ST, Modolo F, Mozzer I, Dos Santos EC, Nagashima S, Camargo Martins AP, et al. Immunoexpression of SOX-2 in oral leukoplakia. Oral Dis. 2018;24:1449-57.

36. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol. 2014;15:178-96.

37. Zhou Y, Zhang H, Zhuo X, Liu Y, Zhang G, Tan Y. Over-expression of TWIST, an epithelial-mesenchymal transition inducer, predicts poor survival in patients with oral carcinoma. Int J Clin Exp Med. 2015;8:9239-47.

38. Zang C, Liu X, Li B, He Y, Jing S, He Y, et al. IL-6/STAT3/TWIST inhibition reverses ionizing radiation-induced EMT and radioresistance in esophageal squamous carcinoma. Oncotarget. 2017;8:11228-38.

39. Zhao Z, Ge J, Sun Y, Tian L, Lu J, Liu M, et al. Is E-cadherin immunoexpression a prognostic factor for head and neck squamous cell carcinoma (HNSCC)? A systematic review and meta-analysis. Oral Oncol. 2012;48:761-7.

40. Pereira CH, Morais MO, Martins AF, Soares MQ, Alencar Rde C, Batista AC, et al. Expression of adhesion proteins (E-cadherin and β-catenin) and cell proliferation (Ki-67) at the invasive tumor front in conventional oral squamous cell and basaloid squamous cell carcinomas. Arch Oral Biol. 2016;61:8-15.

41. Zheng M, Jiang YP, Chen W, Li KD, Liu X, Gao SY, et al. Snail and Slug collaborate on EMT and tumor metastasis through miR-101-mediated EZH2 axis in oral tongue squamous cell carcinoma. Oncotarget. 2015;6:6797-810.

42. Wu J, Liao Q, Ele H, Zhong D, Yin K. TWIST interacts with β-catenin signaling on osteosarcoma cell survival against cisplatin. Mol Carcinog. 2014;53:440-6.

43. Zhu GJ, Song PP, Zhou H, Xiao-Hui Shen, Jun-Guo Wang, Xiao-Feng Ma, et al. Role of epithelial-mesenchymal transition markers E-cadherin, N-cadherin, β-catenin and ZEB2 in laryngeal squamous cell carcinoma. Oncol Lett. 2018;15:3472-81.

44. Prgomet Z, Andersson T, Lindberg P. Higher expression of WN-T5A protein in oral squamous cell carcinoma compared with dysplasia and oral mucosa with a normal appearance. Eur J Oral Sci. 2017;125:237-46.

45. Shiah SG, Shieh YS, Chang JY. The Role of Wnt Signaling in Squamous Cell Carcinoma. J Dent Res. 2016;95:129-34.

46. Dmello C, Sawant S, Chaudhari PR, Dongre H, Ahire C, D'Souza ZC, *et al*. Aberrant expression of vimentin predisposes oral premalignant lesion derived cells towards transformation. Exp Mol Pathol. 2018;105:243-51.

47. Mongroo PS, Rustgi AK. The role of the miR-200 family in epithelial-mesenchymal transition. Cancer Biol Ther. 2010;10:219-22.

48. Arunkumar G, Deva Magendhra Rao AK, Manikandan M, Prasanna Srinivasa Rao H, Subbiah S, Ilangovan R, *et al*. Dysregulation of miR-200 family microRNAs and epithelial-mesenchymal transition markers in oral squamous cell carcinoma. Oncol Lett. 2017;15:649-57.