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Prognostic significance of immunohistochemical biomarkers in oral squamous cell carcinoma

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Abstract. Advances in understanding of the molecular mechanisms underlying oral squamous cell carcinoma (OSCC) have resulted in an increasing number of biomarkers that can be used to predict the behaviour of this disease. The authors conducted a literature review of studies examining the role of immunohistochemistry-based protein biomarkers in predicting OSCC outcome. Only articles published in PubMed-indexed journals over the past 5 years were considered. 22 molecular biomarkers were identified and classified into five groups based on their biological functions: cell cycle acceleration and proliferation; tumour suppression and apoptosis; hypoxia; angiogenesis; and cell adhesion and matrix degradation. The cell cycle acceleration and proliferation biomarkers showed the most divergent prognostic findings. Studies on tumour suppression and apoptosis biomarkers were the most prevalent. There were only a few studies examining molecular biomarkers of hypoxia and angiogenesis, and studies examining cell adhesion and matrix degradation biomarkers have shown that this group has the greatest potential for assessing prognostic parameters. Amongst the several proteins analysed, the immunohistochemical expression levels of epithelial growth factor receptor (EGFR), p53, and matrix metalloproteinases (MMPs) have demonstrated the greatest potential for survival prediction in OSCC, but this review demonstrates that their prognostic relevance is debatable and requires further standardisation.

Key words: oral cancer; oral squamous cell carcinoma; biomarkers; clinical outcome; survival.

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Oral squamous cell carcinoma (OSCC) results from the multistep accumulation of heterogeneous genetic changes in squamous cells. These changes progressively increase the ability of transformed cells to proliferate and invade[54]. The heterogeneity of these changes explains why tumours at the same clinical stage and localisation often show significant differences in their clinical outcomes and treatment responses[18,21,25].

OSCC remains one of the most difficult malignancies to control because of its high propensity for local invasion and cervical lymph node dissemination[18]. The biological factors that underlie the locoregional and distant spreading of these neoplasms are not completely understood[56]. The behaviour of OSCC is difficult to predict solely using conventional clinical and histopathological parameters, and due to location of the disease, the multimodal tumour therapy usually prescribed leads to a reduction in quality of life, making the

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psychosocial consequences of OSCC greater than other malignancies[54,57]. For these reasons, despite advances in therapeutic strategies, the survival rate of OSCC patients is still poor.

Investigation of phenotypic changes in OSCC cells may have a strategic prognostic value, and special attention has recently been focussed on the use of potential molecular biomarkers as reliable predictors of tumour aggressiveness[54]. The assessment of prognostic biomarkers can also be useful in the selection of patients who would best benefit from intensive adjuvant therapy[66].

In recent years, the number of molecular-based assays has increased but histopathology remains the gold standard for most diagnostic and therapeutic decisions. Immunohistochemistry (IHC) is a globally available tool that complements histopathological analysis by detecting gene expression at the protein level. Advances in the understanding of cancer at the regulatory protein expression level have resulted in the identification of some prognostic tumour biomarkers associated with the clinical outcome of OSCC, and there are diverse types of cell and tissue molecular biomarkers that can provide information complementary to that which can be obtained from clinical examination and histopathological studies.

Tumour suppressor genes, oncogenes, cell proliferation markers, angiogenic markers, and cell adhesion molecules have been studied as potential tools to predict the prognosis of patients with OSCC[54].

An immunohistochemical panel using multiple prognostic molecular biomarkers can provide useful information for the identification of high-risk OSCC patients. The objective of this study is to identify, through an extensive review of recently published literature, the major IHC biomarkers that have been significantly associated with the prognosis of OSCC.

Material and methods

This review investigated the scientific literature examining the role of the major biomarkers associated with the clinical outcome of OSCC over the past 4 years (June 2005 to June 2009). A web-based search was performed in the PubMed database to retrieve studies on this subject. The search terms were 'oral cancer', 'OSCC', 'prognostic biomarker', 'molecular biomarker', 'prognostic factor', and 'survival, prediction, outcome, mortality'. This search was subsequently refined, and the research manuscripts were then selected based on the abstract text.

The studies selected for this review were retrospective investigations focussing on prognostic and survival parameters in which IHC was performed in the primary OSCC tumour. In the selected manuscripts, all surgical specimens had been taken prior to the initiation of radiotherapy or chemotherapy. The following anatomical localisations were included in this review: C00, C02–C06, C06.1, and C06.2[26]. Reports detailing with *in vitro* investigations were excluded from this review.

Results

The search terms used for this review identified 22 different molecular biomarkers that had been investigated using IHC to assess prognostic parameters in OSCC patients. Table 1 demonstrates the prognostic relevance of all tumour biomarkers divided by group, as well as the variations

Table 1. Prognostic relevance of 22 different biomarkers investigated through IHC in OSCC.

Molecular biomarker	Prognostic significance	Sample size and *P* value variation (min.-max.)[a]	No prognostic significance	Sample size and *P* value variation (min.-max.)[a]	Total
Cell cycle acceleration and proliferation					
EGFR	5[b]	41–140 – 0.0002–0.02	2	47–165 – 0.114	7
Cyclin D1	2[b,c]	140–348 – <0.0001–0.0181	–	–	2
Ki-67	3	41–113 – 0.0001–<0.001	1	74 – 0.091	4
PCNA	–	–	3	60 – 113 – >0.05	3
Akt1	1	84 – 0.013	–	–	1
Tumour suppression and apoptosis					
p53 and p63	9[b]	45–140 – 0.0002–0.049	–	–	9
p21 and p27	2	106–192 – 0.018–0.03	1	192 – 0.43	3
Bcl-2 family members	4	35–110 – <0.005–0.0489	1	110 – 0.4901–0.8287	5
pRb	2	220–348 – 0.004–<0.05	–	–	2
Survivin	1	78 – 0.002	–	–	1
Hypoxia					
HIF-1α	3	57–112 – 0.004–0.048	–	–	3
CA IX	2	60–117 – 0.02–<0.05	1	68 – 0.062	3
GLUT-1	1	40 – 0.035	–	–	1
EPOR	1	43 – 0.030	–	–	1
Angiogenesis					
VEGF	4	59–1002 – <0.001–0.045	1	84 – 0.168	5
CD105	3	26–176 – <0.001–0.02	–	–	3
Ephs	1	59 – 0.037	–	–	1
Cell adhesion and matrix degradation					
MMPs	6	68–138 – 0.008–0.032	1	38 – data not shown	7
CD44	3	36–138 – 0.01–0.03	–	–	3
Cadherins	3	47–67 – <0.0001–<0.05	1	84 – data not shown	4
Catenins	1	135 – 0.0088	–	–	1
Versican	1	139 – 0.048	–	–	1
Total	58		12		70

[a] Variations regarding the number of investigated patients and concerning the *P* statistical value (Cox regression or Kaplan–Meier analysis) according to each specific marker studied amongst the different publications.

[b] Combined expression of EGFR, p53, and cyclin D1 was associated with an unfavourable prognosis in OSCC patients[72].

[c] Tumours with downregulation of p16 and overexpression of cyclin D1 exhibited the worst prognosis[28].

in sample sizes and in *P* statistical values according to each specific marker studied amongst the different publications. The *P* values were obtained from multivariate Cox regression or Kaplan–Meier analysis (preferably overall survival) when the first was lacking. The biomarkers were classified into five groups based on their biological function: cell cycle acceleration and proliferation; tumour suppression and apoptosis; hypoxia; angiogenesis; and cell adhesion and matrix degradation.

Cell cycle acceleration and proliferation molecules

Five biomarkers were identified belonging to this group: epithelial growth factor receptor (EGFR); cyclin D1; Ki-67; proliferating cell nuclear antigen (PCNA); and serine/threonine kinase 1 (Akt1) (Table 1).

EGFR (EGF-R, c-erb1–4, Her-2/neu)

EGFR is a transmembrane cell surface receptor that binds to some ligands such as EGF and TGF-α, thereby activating the protein–tyrosine kinase system, which regulates the signalling involved in cell proliferation and differentiation[72]. It belongs to a family of four similar receptors: HER-1 (ErbB1), HER-2 (neu/ErbB2), HER-3 (ErbB3), and HER-4 (ErbB4). EGFR activation can enhance the malignant potential of epithelial tissues[37]. In some studies, EGFR overexpression had been correlated with poor prognostic in OSCC patients[2,38,73,74], but these results were not confirmed in other investigations[19,75]. A single study had identified a significant association between the combined expression of EGFR, p53, and cyclin D1 and an unfavourable overall survival (OS) in OSCC patients[72].

Cyclin D1

Tumour cell proliferation is constantly associated with genetic or epigenetic modifications in key cell cycle molecules. The D-type cyclins are expressed during the progression from G0/G1 to S phase of the mammalian cell cycle[69]. Cyclin D1 is an oncogene that drives cell cycle progression, and the decision for cell growth or arrest may depend on the concentration of cyclin D1[72]. Cyclin D1 amplification is one of the most frequent molecular alterations in head and neck squamous cell carcinomas (HNSCC)[81]. Cyclin D1 expression alone has not been associated with OSCC progression. Shiraki et al.[72] showed that the combined expression of cyclin D1, EGFR, and p53 was significantly associated with an unfavourable OS in OSCC patients. Similarly, Jayasurya et al.[28] demonstrated the decreased expression of p16, coupled with the overexpression of cyclin D1, in tumours is associated with an unfavourable clinical outcome.

Ki-67

The expression of the human Ki-67 protein is strictly connected with cell proliferation. The fact that the Ki-67 protein is not expressed in G0 resting cells, but is expressed during all active phases of the cell cycle (G1, S, G2, and mitosis), makes it an excellent biomarker for determining the fraction of actively proliferating cells in a given tumour[8]. In an analysis of 113 OSCC patients, Myoung et al.[57] correlated the expression of Ki-67 expression with the cumulative survival rate, confirming that this biomarker provides useful information in predicting a worse prognosis for OSCC patients. This finding has been corroborated by other authors[73,74], whilst Lee et al.[39] have recently observed no independent association between Ki-67 expression and OSCC survival.

PCNA

PCNA is a well-known cell cycle marker protein that plays an important role in nucleic acid metabolism as a component of the replication and repair machinery[58]. In several recent investigations, PCNA expression has not been significantly associated with survival in OSCC patients[32,40,57].

Akt1

Akt plays a pivotal role in cell survival and proliferation through a number of downstream effectors[5]. Akt overexpression was an independent and significant indicator of poor prognosis in a study examining OSCC outcome[40].

Tumour suppression and apoptosis biomarkers

Five subsets of biomarkers were identified in this group (Table 1): p53/p63, p21/p27, Bcl-2 family members, pRb, and Survivin.

p53 and p63

The *p53* gene is one of the most studied biomarkers in OSCC. Functional inactivation of *p53* causes defects in DNA repair and apoptosis, with a subsequent increase in genetic instability that can lead to the accumulation of mutations[11]. The high expression of p53 has been associated with a poor prognosis[60–62], and the combined expression of p53, cyclin D1, and EGFR has been correlated with an unfavourable OS in OSCC patients[72]. The data examining the prognostic value of the expression of p63, a p53 homologue, is controversial. Some authors have found that overexpression of p63 is associated with better prognosis in OSCC[60–62], whilst others have found the opposite[44,47].

p21 and p27

Cell cycle progression is regulated by the cyclins and cyclin-dependent kinases (CDKs). The activity of these enzymes is coordinated by the inhibitory action of the Cip/Kip family, including both $p21^{waf1/cip1}$ and $p27^{Kip1}$ [21]. $P21^{waf1/cip1}$ plays an important function in the regulation of the G1-to-S transition of the cell cycle, because it is an inhibitor of the CDKs[59]. The expression of $p27^{Kip1}$ can result in cell cycle arrest or progression, thereby regulating cell proliferation, cell motility, and apoptosis. Based on its post-translational modifications, $p27^{Kip1}$ can both positively and negatively regulate these processes. The loss of the expression or function of these two G1-checkpoint CDK inhibitors has been implicated in the progression of many human tumours[1]. There are controversial findings concerning the clinical outcome of $p21^{waf1/cip1}$-positivite OSCCs [21,59], and no significant association between $p27^{Kip1}$ expression and OSCC prognostic has been identified[21].

Bcl-2 family members

Bcl-2 family members are apoptosis regulatory proteins. This family includes both anti-apoptotic (e.g. Bcl-2, Bcl-X) and pro-apoptotic proteins (e.g. Bax and Bak), and it is the balance between them that determines the cell fate[9]. Camisasca et al.[9] found that the expression of Bcl-2, Bax, and Bcl-X could be correlated with a favourable outcome in OSCC. de vicente et al.[17] demonstrated that Bcl-2 protein expression was associated with poor prognosis, and they failed to identify any association between Bax expression and disease outcome. Some investigations have shown that the survival rate was significantly higher in OSCC patients with Bcl-2-negative and Bax-positive tumours[30,82].

pRb

The Rb pathway plays a crucial regulatory role in cell cycle progression, and its

function can be inhibited by specific mutations. The phosphorylation of pRb occurs following the activation of CDK4 or CDK6 through cyclin D1 and results in its functional inhibition and liberation of transcription factors required for cell cycle progression[77]. Although JAYASURYA et al.[28] have demonstrated a significant association between pRb overexpression and reduced disease-free survival (DFS), SONI et al.[76] have shown that OSCCs that lost pRb expression were aggressive carcinomas and had a poor prognosis.

Survivin

Survivin is an inhibitor of apoptosis that generally is undetectable in normal mucosa, but is overexpressed in most head and neck cancers[42]. LO MUZIO et al.[45] identified Survivin expression as a potential biomarker of aggressive and invasive OSCCs.

Hypoxia biomarkers

Four IHC hypoxia biomarkers were identified as putative prognostic parameters: hypoxia inducible factor 1α (HIF-1α); carbonic anhydrase IX (CA IX); glucose transporter 1 (GLUT-1); and erythropoietin receptor (EPOR).

HIF-1α

HIF-1 is a heterodimeric transcription factor composed of alpha and beta subunits. The HIF-1α subunit mediates HIF-1 function in response to cellular hypoxia. Under normal oxygen conditions, the HIF-1α subunit is rapidly degraded by the proteasome and has a very short half-life. Under hypoxic conditions, the proteolytic degradation is suppressed, resulting in overexpression of this subunit. In response to decreased oxygen, HIF-1α induces the transactivation of more than 70 genes involved in hypoxia adaptation and/or reversion[20,43]. The diffuse overexpression of HIF-1α has been associated with a good prognosis in OSCC patients[20], but some recent investigations have found the opposite result[41,43].

Carbonic anhydrase IX

Carbonic anhydrases (CAs) form a large family of genes that encode zinc metalloenzymes. They are involved in the reversible hydration of carbon dioxide to carbonic acid, thereby maintaining a stable intracellular pH. The CAs also participate in other biological processes such as cellular respiration and dystrophic calcification[25]. CA IX is a HIF-1-dependent member of the CA family and a transmembrane glycoprotein involved in pH homeostasis[68]. CHOI et al.[13] have shown that CA IX overexpression is significantly associated with disease recurrence and a worse OS in OSCC patients. KIM et al.[32] have demonstrated a statistically significant association between high CA IX expression and a poorer OS in a series of tongue squamous cell carcinomas. In contrast, SAKATA et al.[68] did not find any association between CA IX expression and DFS.

GLUT-1

The glucose transporters (GLUT) are also regulated via the HIF-1 pathway and can mediate cellular glucose uptake, thereby perpetuating anaerobic glycolysis[25]. It has been suggested that, like CA IX and HIF-1α, GLUT-1 might represent an endogenous marker of hypoxia[34]. GLUT has been identified in diverse human tumours, and there is some evidence that GLUT-1 is related to aggressiveness in HNSCC[29]. The increased expression of GLUT-1 was significantly associated with a shorter OS and radiotherapeutic failure in OSCC[34].

EPOR

Erythropoietin (EPO) is a glycoprotein hormone that mediates the production of red blood cells. It is synthesised in the kidney in response to hypoxia, and its biological effects are regulated through its interaction with a specific transmembrane EPOR and signalling mediated through HIF-1α[3]. Head and neck cancer cells are known to express EPOR[3], and ROH et al.[67] have found that high EPOR expression can be associated with a significantly worse prognosis in patients with oral tongue squamous cell carcinoma.

Angiogenesis biomarkers

Three angiogenic biomarkers were identified as possible prognostic parameters (Table 1): vascular endothelial growth factor (VEGF); endoglin (CD105); and Eph receptor tyrosine kinases (Ephs).

VEGF

VEGF acts functions mainly as an angiogenic cytokine that promotes proliferation, differentiation, and migration of vascular endothelial cells. It also induces vessel permeability and promotes endothelial cell survival by preventing apoptosis[35]. One study failed to identify an independent and significant association between VEGF expression and prognostic parameters in patients with OSCC[40], but VEGF overexpression was identified as an adverse DFS prognosticator in another investigation[4]. Two studies confirmed that patients with VEGF-positive tumours have significantly poorer survival[12,71]. In a meta-analysis carried out on HNSCC patients (71% of them diagnosed as OSCC), KYZAS et al.[35] demonstrated that VEGF overexpression was significantly associated with a worse OS.

CD105

Endoglin is a regulatory component of the cellular transforming growth factor-β (TGF-β) receptor complex and can modulate angiogenesis by the regulation of different cellular functions including proliferation, differentiation, and migration. The assessment of neo-vascularisation using CD105 expression has been considered a potential predictor of prognosis in different solid malignancies[52]. It has been shown that the evaluation of micro-vessel density through CD105 expression in primary OSCC may identify patients at risk of recurrence of the disease or poor outcome following treatment[12,53]. In another study performed only on biopsies of early tongue squamous cell carcinomas staged as T1 or T2, CHUANG et al.[14] also demonstrated that CD105 expression was a significant and independent prognostic predictor of a worse prognosis.

Ephs receptors

Ephs receptors (erythropoietin-producing human hepatocellular carcinoma) comprise the largest family of vertebrate receptor tyrosine kinases. Together with their membrane-anchored ephrin ligands (eph family receptor interacting proteins), they form a vital cell–cell communication system capable of bi-directional signalling[10]. This Eph receptor/ephrin system has been shown to play a crucial role in embryonic development, as well as in several human cancers[80]. Investigations suggest that this system can stimulate invasive behaviour in a tumour, thereby promoting a more aggressive and metastatic phenotype[10,80]. Biochemical and genetic evidence has also implicated Ephs receptors as important regulators of angiogenesis[63]. In a recent study, SHAO et al.[71] showed that a high expression of EphA2 was associated with a shorter survival period in tongue squamous cell carcinoma patients.

Cell adhesion and matrix degradation molecules

Five IHC matrix degradation and cell adhesion related molecules were identified as putative prognostic biomarkers associated with OSCC (Table 1): matrix metalloproteinases (MMPs), CD44, cadherins, catenins, and versican.

MMPs

The MMPs are a family of proteases normally expressed by invasive tumours and the adjacent stroma[16]. They are zinc-associated endopeptidases capable of degrading all components of the extracellular matrix (ECM), as well as the basement membrane. MMPs have an essential role in ECM degradation in several situations, including development, inflammation, tissue repair, and tumour invasion and metastasis[48]. There are over 20 known mammalian MMPs[49], and the expression of MMP family members in OSCC tissues has been widely reported. Although a retrospective study with tongue carcinomas found no correlation between MMP-2 and MMP-9 overexpression and survival[31], there are several conflicting studies examining the prognostic role of MMPs in OSCC[2,33,39]. MMP-9 expression was associated with poor prognosis in a subgroup of early stage OSCC patients without neck lymph node metastases[16]. In cases of OSCC presenting with lymph node metastasis, DE VICENTE et al.[18] identified a significant correlation between MMP-7 and MMP-14 (MT1-MMP) expression and poor survival. In another study performed with HNSCC patients (85% of them diagnosed with OSCC), LUUKKAA et al.[48] found that a high MMP-13 (collagenase-3) expression level was significantly associated with a short survival time.

CD44

The CD44 family is a widely expressed transmembrane glycoprotein family that binds hyaluronic acid, growth factors, and ECM proteins[23]. The CD44 family consists of a standard form of CD44 (CD44s) and an alternative splice variant (CD44v). CD44 has been shown to be a major factor in cell–cell and cell–ECM interactions, including cell migration, adhesion, and some lymphocyte functions[33]. The low expression of CD44s on tumour cells has been significantly correlated with poor prognosis in tongue carcinomas[24], and the absence of CD44v expression has been associated with a shorter survival time in lip OSSC[22]. Similarly, KOSUNEN et al.[33] has shown that the irregular expression of CD44 in OSCC was correlated with poor DFS and OS.

Cadherins

Cadherins are a family of transmembrane glycoproteins involved in cell–cell adhesion[56]. In most epithelial cells, this adhesion is established and maintained by the epithelial-cadherin (E-cad) complex, localised mainly in the zonula adherens junctions. The cadherin family also includes more than 20 proteins, such as placental cadherin (P-cad), that have been described in several tissues and organs[56]. E-cad is expressed in the intercellular junctions of keratinocytes, whilst P-cad is predominantly detected on the surface of basal keratinocytes in the normal epidermis. Therefore, cells migrating into the suprabasal compartment tend to down-regulate P-cad expression[46]. The loss of adhesion resulting from the reduced expression of cadherins can play an important role in tumour invasion and dissemination[49]. The lack of P-cad expression has been associated with a low OS rate in OSCC patients[46,56]. In contrast, one study failed to detected an independent and significant association between E-cad expression and prognosis[40]. Some transcriptional repressors have been shown to be important in the loss of E-cad expression by tumour cells. The expression of the transcriptional repressor SIP1, which regulates E-cad expression, has been correlated with a lower disease-specific OS in OSCC[50].

Catenins

The intercellular domain of E-cad binds to proteins known as catenins, forming the cadherin–catenin complex, which is involved in the intracellular transduction of cell-to-cell contact signals[78]. UEDA et al.[78] showed that reduced expression of β- and γ-catenin can be used as a potential marker of poor OSCC prognosis.

Versican

Versican is one of the main components of the ECM. It is a chondroitin sulphate proteoglycan, a member of the aggrecan gene family, and has been associated with diverse interactions in a number of biological and pathological processes[64]. Versican is overexpressed in diverse tumours, and it plays a main role in tumour growth by repressing cell adhesion, stimulating cell proliferation and migration, and regulating angiogenesis[65]. It has been shown in a cohort of OSCC patients that high stromal versican expression is an independent predictor for an unfavourable prognosis[64].

Discussion

In the past few years, significant progress has been made in the identification and understanding of prognostic biomarkers involved in predicting OSCC aggressiveness. The TNM classification system cannot predict the biological features of tumour cells and, therefore, is unable to individualise the prognosis. The evaluation of some important prognostic biomarkers in OSCC at the time of diagnosis might allow for the identification of a subset of patients who require more aggressive management. Therapeutic approaches, such as the use of some molecular inhibitors directed against specific biomarkers along with adjuvant radio- and/or chemotherapy are promising treatments for OSCC patients.

The main advantage of the identification of putative prognostic biomarkers in OSCC by IHC is the establishment of a direct association between the morphology and these biomarkers, which can aid in determining their functional relevance. The use of IHC to evaluate a specific molecular biomarker has the advantage of establishing a pattern of expression in different tumours. IHC can be performed on formalin-fixed paraffin-embedded specimens that can be stored for a long time, thereby allowing retrospective studies of a large population[18].

OSCC carcinogenesis is characterised by a multistep dysregulation of the cell cycle machinery. The prognostic significance of the expression of biomarkers in various HNSCC has been extensively studied, but only a few studies have focussed on OSCC specifically. An extensive array of IHC biomarkers that have been associated with OSCC prognosis is presented in this review, sometimes with contradictory results. This attests the complexity of oral carcinogenesis, but variations in the assessments of some molecular biomarkers, as well as some possible methodological limitations, could also explain these discrepancies.

The utility of a given biomarker as a prognostic tool requires a carefully designed cohort investigation, as well as the defined criteria to designate a tumour as negative or positive for a specific IHC biomarker. Conversely, there is significant heterogeneity amongst experimental procedures, and the literature usually gives no uniform recommendation for a specific

primary antibody or defines a cut-off for positivity. In the absence of uniform biological criteria, scoring categories are usually chosen arbitrarily. It is possible that if a scoring criterion is chosen differently, the correlation with other parameters will change. There is also heterogeneity in the samples amongst the studies, with distinct stages of disease in several of them, which frequently leads to different therapeutic approaches[9]. Therefore, differences observed amongst investigations could be attributed to the use of distinct methods in the evaluation of the biomarker expression, as well the heterogeneity of the samples. To provide adequate measures of predictability and improve the quality of the tumour prognostic biomarkers studies examining OSCC, the recent guidelines proposed by REMARK should be considered to standardise future studies[55]. Table 2 shows the recommendations for reporting studies on tumour markers. Specific items are grouped under the headings: introduction, materials and methods, results, and discussion.

The REMARK reporting guidelines were developed to stimulate complete and reliable information on prognostic studies evaluating tumour biomarkers. Through this literature review the authors found that most research on OSCC is still not following these recommendations, continuing to present heterogeneous results. Besides the non-standardised experimental procedures, some investigations present poor reporting regarding patient/tumour features, event numbers, missing data, end and median follow-up time, and statistical methods[35,36]. The REMARK guidelines make specific reporting recommendations for most of these subjects.

This review highlights some important gaps in the research on OSCC prognostic biomarkers. In several studies, there is a lack of information about their reliability and clinical relevance. Providing inadequate statistical information does not allow confirmatory studies to be made on the predictability and correlation measures, which also makes an adequate meta-analysis difficult. Other systematic reviews on prognostic biomarkers were unable to include data from various investigations because of poor reporting, hampering assessments on their influence in different cancers[51,66]. As shown in Table 1, some studies include a small number of patients, and the *P* value was not described in two studies on cell adhesion and matrix degradation biomarkers.

Despite these problems, the authors think that a carefully selected set of biomarkers obtained through further standardised research may help to predict the prognosis of OSCC patients in future. For this, the individualized molecular biomarker profiles must be analysed in conjunction with other clinicopathological information that could better characterise these tumours and thereby predict the patient response to treatment.

Table 2. REporting recommendations for tumour MARKer prognostic studies (REMARK)[*].

Introduction

1. State the marker examined, the study objectives, and any pre-specified hypotheses.

Materials and methods

Patients

2. Describe the characteristics (e.g., disease stage or co-morbidities) of the study patients, including their source and inclusion and exclusion criteria.
3. Describe treatments received and how chosen (e.g., randomized or rule-based).

Specimen characteristics

4. Describe type of biological material used (including control samples) and methods of preservation and storage.

Assay methods

5. Specify the assay method used and provide (or reference) a detailed protocol, including specific reagents or kits used, quality control procedures, reproducibility assessments, quantitation methods, and scoring and reporting protocols. Specify whether and how assays were performed blinded to the study endpoint.

Study design

6. State the method of case selection, including whether prospective or retrospective and whether stratification or matching (e.g., by stage of disease or age) was used. Specify the time period from which cases were taken, the end of the follow-up period, and the median follow-up time.
7. Precisely define all clinical endpoints examined.
8. List all candidate variables initially examined or considered for inclusion in models.
9. Give rationale for sample size; if the study was designed to detect a specified effect size, give the target power and effect size.

Statistical analysis methods

10. Specify all statistical methods, including details of any variable selection procedures and other model-building issues, how model assumptions were verified, and how missing data were handled.
11. Clarify how marker values were handled in the analyses; if relevant, describe methods used for cutpoint determination.

Results

Data

12. Describe the flow of patients through the study, including the number of patients included in each stage of the analysis (a diagram may be helpful) and reasons for dropout. Specifically, both overall and for each subgroup extensively examined report the numbers of patients and the number of events.
13. Report distributions of basic demographic characteristics (at least age and sex), standard (disease-specific) prognostic variables, and tumour marker, including numbers of missing values.

Analysis and presentation

14. Show the relation of the marker to standard prognostic variables.
15. Present univariate analyses showing the relation between the marker and outcome, with the estimated effect (e.g., hazard ratio and survival probability). Preferably provide similar analyses for all other variables being analyzed. For the effect of a tumour marker on a time-to-event outcome, a Kaplan–Meier plot is recommended.
16. For key multivariable analyses, report estimated effects (e.g., hazard ratio) with confidence intervals for the marker and, at least for the final model, all other variables in the model.
17. Amongst reported results, provide estimated effects with confidence intervals from an analysis in which the marker and standard prognostic variables are included, regardless of their statistical significance.
18. If done, report results of further investigations, such as checking assumptions, sensitivity analyses, and internal validation.

Discussion

19. Interpret the results in the context of the pre-specified hypotheses and other relevant studies; include a discussion of limitations of the study.
20. Discuss implications for future research and clinical value.

[*] Reprinted from Mcshane et al. Eur J Cancer 2005: 41: 1690–1696[55].

The molecular biomarkers affecting cell cycle acceleration and proliferation have been largely explored in premalignant lesions from the oral cavity; this is the most conflicting biomarker group regarding prognostic results. The analysis of EGFR expression in OSCC is the most studied, and EGFR has shown prognostic significance in most investigations.

According to Agra et al.[2], the poor prognosis of OSCC patients associated with EGFR overexpression is related to radiotherapy resistance. No influence on OSCC survival was found in the studies examining PCNA expression. This is an unexpected finding because PCNA is thought to represent the proportion of highly proliferative cells in a tumour[57].

Considerable therapeutic and prognostic interest is focussed on regulators of molecular biomarkers associated with tumour suppression and apoptosis. Amongst these, most studies point to p53 and the Bcl-2 family members. The *p53* and *p63* genes share a similar exon/intron organisation and have been investigated for prognostic associations in OSCC[62]. Despite some contradictory findings implicating high or low p63 expression with a favourable OSCC prognosis[44,60–62], which could be related to the opposing functions of its isoforms, p53 and p63 expression were significantly associated with the prognosis in all selected studies. In regards to Bcl-2, almost all studies were consistent with its biological function and associated high levels of Bcl-2 expression with poor survival in OSCC[17,30,82]. This is most likely because a low level of Bcl-2 usually corresponds to a high level of Bax, which could promote tumour cell apoptosis[30]. The group of biomarkers associated with tumour suppression and apoptosis is the most prevalent in this review, and the high number of significant correlations indicates that there are probably some promising therapeutic targets to be explored in this group.

Recent studies examining potential biomarkers in OSCC have focussed on proteins associated with tumour hypoxia. Hypoxia is a common characteristic of human solid tumours, and it can drive malignant cells to undergo adaptive changes that enable them to survive[25,79]. It has been suggested that tumour hypoxia plays an essential role in promoting chromosome instability, cancer cell invasiveness, and metastasis. It is associated with aggressive tumour growth and treatment failure in several human solid tumours[20,25,27,37]. In agreement with Hoogsteen et al.[25], tumour hypoxia is an important factor that determines the OSCC response to treatment, but it was the least investigated molecular biomarker group in this review. Amongst the main hypoxia biomarkers found with prognostic significance in OSCC was HIF-1α[7]. There are only a few studies examining the expression of this biomarker, and based on the present data, it is difficult to determine its prognostic relevance. The prognostic characterisation of further tumour hypoxia biomarkers in primary OSCC could allow for the assessment of biological aggressiveness in individual tumours, as well as establish a more specific and efficient therapeutic strategy.

Targeted cancer therapy has focussed on angiogenesis inhibitors. The angiogenic process plays a key role in creating a neo-capillary network and is required for cancer growth, progression, and metastasis[53]. In spite of the importance of angiogenesis for tumour development, only a few studies have been performed examining the prognostic value of angiogenesis biomarkers in OSCC. VEGF signalling has been the most investigated angiogenic biomarker in OSCC. VEGF plays a crucial role in the recruitment and maintenance of the tumour vasculature, and is considered the major angiogenic factor during carcinogenesis and tumour dissemination[70,71]. In agreement with a meta analysis carried out by Kyzas et al.[35], despite considerable diversity in the definition of a VEGF-positive tumour documented across studies, VEGF expression can function as a plausible candidate for the prediction of OSCC prognosis, because the prognostic significance of VEGF expression in patients with OSCC seems to be associated with a worse OS[4,12,71]. Further studies should be performed, owing to inconsistent results between tumour angiogenesis and disease progression in OSCC, because this discrepancy may be due to methodological differences including the specificity of different endothelial markers for detecting tumour angiogenesis.

Tumour cell progression, invasion, and migration are dependent on several factors, including the interaction of malignant cells with the ECM[83]. Tumour spread is thought to require diverse matrix degradation enzymes and cell adhesion proteins, and it has been demonstrated that OSCC cells can synthesise and secrete several of them[49,83]. The MMPs were the most investigated molecular biomarker in this group, and even though their predictive value in OSCC remains unclear, it has been hypothesised that their activities are necessary for tumour angiogenesis, invasion, and metastatic dissemination[49]. Owing to this, some inhibitors specific for MMPs are being investigated as potential targeted therapies in the treatment of OSCC[83]. Given the potential functional role of the microenvironment in tumour progression and prognosis, the expression of some molecular biomarkers in the OSCC stroma deserves to be further researched.

The majority of the selected studies in this present review showed statistically significant associations with OSCC survival. The authors findings are in agreement with Kyzas et al.[36] who have demonstrated in an important critical review that almost all investigations examining cancer prognostic biomarkers highlight only the statistically significant results. Even so, the authors identified the subset of candidate IHC-based protein predictors of OSCC, and their findings indicate that the molecular biomarkers EGFR, p53, and MMPs have been shown to be promising prognostic indicators in OSCC, and the current development of new molecular targeted anti-cancer agents through gene therapy or pharmacological intervention is already being applied[6,15,83]. These results need to be further confirmed in additional powered prospective studies, preferably performed with larger number of patients, because the study also revealed important limitations in areas ranging from the choice of assayed proteins to the consistency and quality of selected population and statistical methods used.

The discrepant results presented in this review indicate that there is an increased necessity for identification and standardisation of better molecular biomarkers that can identify OSCC patients with a poor prognosis. The poor quality of reporting found, suggests that adherence to the REMARK recommendations could be used as a reference to facilitate the standardisation of future investigations on this subject. This review demonstrates that unravelling some potential biomarkers significantly associated with OSCC progression across several studies can, together with clinicopathological evaluation, lead to new target opportunities for specific and individualised therapy.

Conflict of interest

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References

1. Abukhdeir AM, Park BH. P21 and p27: roles in carcinogenesis and drug resistance. Expert Rev Mol Med 2008: **10**: e19.
2. Agra IM, Carvalho AL, Pinto CA, Martins EP, Filho JG, Soares FA,

Kowalski LP. Biological markers and prognostic in recurrent oral cancer after salvage surgery. Arch Otolaryngol Head Neck Surg 2008: **134**: 743–749.
3. Arcasoy MO, Amin K, Chou SC, Haroon ZA, Varia M, Raleigh JA. Erythropoietin and erythropoietin receptor expression in head and neck cancer: relationship to tumor hypoxia. Clin Cancer Res 2005: **11**: 20–27.
4. Arora S, Kaur J, Sharma C, Mathur M, Bahadur S, Shukla NK, Deo SV, Ralhan R. Stromelysin 3, Ets-1, and vascular endothelial growth factor expression in oral precancerous and cancerous lesions: correlation with microvessel density, progression, and prognosis. Clin Cancer Res 2005: **11**: 2272–2284.
5. Barnett SF, Bilodeau MT, Lindsley CW. The Akt/PKB family of protein kinases: a review of small molecule inhibitors and progress towards target validation. Curr Top Med Chem 2005: **5**: 109–125.
6. Bouchet BP, de Fromentel CC, Puisieux A, Galmarini CM. p53 as a target for anti-cancer drug development. Crit Rev Oncol Hematol 2006: **58**: 190–207.
7. Brennan PA, Mackenzie N, Quintero M. Hypoxia-inducible factor 1alpha in oral cancer. J Oral Pathol Med 2005: **34**: 385–389.
8. Bullwinkel J, Baron-Lühr B, Lüdemann A, Wohlenberg C, Gerdes J, Scholzen T. Ki-67 protein is associated with ribosomal RNA transcription in quiescent and proliferating cells. J Cell Physiol 2006: **206**: 624–635.
9. Camisasca DR, Honorato J, Bernardo V, da Silva LE, da Fonseca EC, de Faria PA, Dias FL, Lourenço Sde Q. Expression of Bcl-2 family proteins and associated clinicopathologic factors predict survival outcome in patients with oral squamous cell carcinoma. Oral Oncol 2009: **45**: 225–233.
10. Campbell TN, Robbins SM. The Eph receptor/ephrin system: an emerging player in the invasion game. Curr Issues Mol Biol 2008: **10**: 61–66.
11. Chari NS, Pinaire NL, Thorpe L, Medeiros LJ, Routbort MJ, McDonnell TJ. The p53 tumor suppressor network in cancer and the therapeutic modulation of cell death. Apoptosis 2009: **14**: 336–347.
12. Chien CY, Su CY, Hwang CF, Chuang HC, Chen CM, Huang CC. High expressions of CD105 and VEGF in early oral cancer predict potential cervical metastasis. J Surg Oncol 2006: **94**: 413–417.
13. Choi SW, Kim JY, Park JY, Cha IH, Kim J, Lee S. Expression of carbonic anhydrase IX is associated with postoperative recurrence and poor prognostic in surgically treated oral squamous cell carcinoma. Hum Pathol 2008: **39**: 1317–1322.
14. Chuang HC, Su CY, Huang HY, Chien CY, Chen CM, Huang CC. High expression of CD105 as a prognostic predictor of early tongue cancer. Laryngoscope 2006: **116**: 1175–1179.
15. Curran D, Giralt J, Harari PM, Ang KK, Cohen RB, Kies MS, Jassem J, Baselga J, Rowinsky EK, Amellal N, Comte S, Bonner JA. Quality of life in head and neck cancer patients after treatment with high-dose radiotherapy alone or in combination with cetuximab. J Clin Oncol 2007: **25**: 2152–2155.
16. de Vicente JC, Fresno MF, Villalain L, Vega JA, Hernández Vallejo G. Expression and clinical significance of matrix metalloproteinase-2 and matrix metalloproteinase-9 in oral squamous cell carcinoma. Oral Oncol 2005: **41**: 283–293.
17. de Vicente JC, Olay S, Lequerica-Fernandez P, Sánchez-Mayoral J, Junquera LM, Fresno MF. Expression of Bcl-2 but not Bax has a prognostic significance in tongue carcinoma. J Oral Pathol Med 2006: **35**: 140–145.
18. de Vicente JC, Lequerica-Fernández P, Santamaría J, Fresno MF. Expression of MMP-7 and MT1-MMP in oral squamous cell carcinoma as predictive indicator for tumor invasion and prognosis. J Oral Pathol Med 2007: **36**: 415–424.
19. Diniz-Freitas M, García-Caballero T, Antúnez-López J, Gándara-Rey JM, García-García A. Pharmacodiagnostic evaluation of EGFR expression in oral squamous cell carcinoma. Oral Dis 2007: **13**: 285–290.
20. Fillies T, Werkmeister R, van Diest PJ, Brandt B, Joos U, Buerger H. HIF1-alpha overexpression indicates a good prognostic in early stage squamous cell carcinomas of the oral floor. BMC Cancer 2005: **5**: 84.
21. Fillies T, Woltering M, Brandt B, Van Diest JP, Werkmeister R, Joos U, Buerger H. Cell cycle regulating proteins p21 and p27 in prognostic of oral squamous cell carcinomas. Oncol Rep 2007: **17**: 355–359.
22. García-Montesinos-Perea B, Valbernal JF, Saiz-Bustillo R. Epidermoid carcinoma of the lip: an immunohistochemical study. Med Oral Patol Oral Cir Bucal 2005: **10**: 454–461.
23. Georgolios A, Batistatou A, Charalabopoulos A, Manolopoulos L, Charalabopoulos K. The role of CD44 adhesion molecule in oral cavity cancer. Exp Oncol 2006: **28**: 94–98.
24. González-Moles MA, Gil-Montoya JA, Ruiz-Avila I, Esteban F, Delgado-Rodriguez M, Bascones-Martinez A. Prognostic significance of p21WAF1/CIP1, p16INK4a and CD44s in tongue cancer. Oncol Rep 2007: **18**: 389–396.
25. Hoogsteen IJ, Marres HA, Bussink J, van der Kogel AJ, Kaanders JH. Tumor microenvironment in head and neck squamous cell carcinomas: predictive value and clinical relevance of hypoxic markers. A review. Head Neck 2007: **29**: 591–604.
26. World Health Organisation. *ICD-10. International Statistical Classification of Diseases and Related Health Problems*. 10th revision. World Health Organisation: Geneva 1997.
27. Janssen KL, Haustermans KM, Balm AJ, Begg AC. Hypoxia in head and neck cancer: how much, how important? Head Neck 2005: **27**: 622–638.
28. Jayasurya R, Sathyan KM, Lakshminarayanan K, Abraham T, Nalinakumari KR, Abraham EK, Nair MK, Kannan S. Phenotypic alterations in Rb pathway have more prognostic influence than p53 pathway proteins in oral carcinoma. Mod Pathol 2005: **18**: 1056–1066.
29. Jonathan RA, Wijffels KI, Peeters W, de Wilde PC, Marres HA, Merkx MA, Oosterwijk E, van der Kogel AJ, Kaanders JH. The prognostic value of endogenous hypoxia-related markers for head and neck squamous cell carcinomas treated with ARCON. Radiother Oncol 2006: **79**: 288–297.
30. Kato K, Kawashiri S, Yoshizawa K, Kitahara H, Yamamoto E. Apoptosis-associated markers and clinical outcome in human oral squamous cell carcinomas. J Oral Pathol Med 2008: **37**: 364–371.
31. Kim SH, Cho NH, Kim K, Lee JS, Koo BS, Kim JH, Chang JH, Choi EC. Correlations of oral tongue cancer invasion with matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF) expression. J Surg Oncol 2006: **93**: 330–337.
32. Kim SJ, Shin HJ, Jung KY, Baek SK, Shin BK, Choi J, Kim BS, Shin SW, Kim YH, Kim JS, Oosterwijk E. Prognostic value of carbonic anhydrase IX and Ki-67 expression in squamous cell carcinoma of the tongue. Jpn J Clin Oncol 2007: **37**: 812–819.
33. Kosunen A, Pirinen R, Ropponen K, Pukkila M, Kellokoski J, Virtaniemi J, Sironen R, Juhola M, Kumpulainen E, Johansson R, Nuutinen J, Kosma VM. CD44 expression and its relationship with MMP-9, clinicopathological factors and survival in oral squamous cell carcinoma. Oral Oncol 2007: **43**: 51–59.
34. Kunkel M, Moergel M, Stockinger M, Jeong JH, Fritz G, Lehr HA, Whiteside TL. Overexpression of GLUT-1 is associated with resistance to radiotherapy and adverse prognostic in squamous cell carcinoma of the oral cavity. Oral Oncol 2007: **43**: 796–803.
35. Kyzas PA, Cunha IW, Ioannidis JP. Prognostic significance of vascular endothelial growth factor immunohistochemical expression in head and neck squamous cell carcinoma: a meta-analysis. Clin Cancer Res 2005: **11**: 1434–1440.

36. Kyzas PA, Denaxa-Kyza D, Ioannidis JP. Almost all articles on cancer prognostic markers report statistically significant results. Eur J Cancer 2007: **43**: 2559–2579.
37. LaCasse EC, Mahoney DJ, Cheung HH, Plenchette S, Baird S, Korneluk RG. IAP-targeted therapies for cancer. Oncogene 2008: **27**: 6252–6275.
38. Laimer K, Spizzo G, Gastl G, Obrist P, Brunhuber T, Fong D, Barbieri V, Jank S, Doppler W, Rasse M, Norer B. High EGFR expression predicts poor prognostic in patients with squamous cell carcinoma of the oral cavity and oropharynx: a TMA-based immunohistochemical analysis. Oral Oncol 2007: **43**: 193–198.
39. Lee JI, Jin BH, Kim MA, Yoon HJ, Hong SP, Hong SD. Prognostic significance of CXCR-4 expression in oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009: **107**: 678–684.
40. Lim J, Kim JH, Paeng JY, Kim MJ, Hong SD, Lee JI, Hong SP. Prognostic value of activated Akt expression in oral squamous cell carcinoma. J Clin Pathol 2005: **58**: 1199–1205.
41. Lin PY, Yu CH, Wang JT, Chen HH, Cheng SJ, Kuo MY, Chiang CP. Expression of hypoxia-inducible factor-1 alpha is significantly associated with the progression and prognostic of oral squamous cell carcinomas in Taiwan. J Oral Pathol Med 2008: **37**: 18–25.
42. Lippert BM, Knauer SK, Fetz V, Mann W, Stauber RH. Dynamic Survivin in head and neck cancer: molecular mechanism and therapeutic potential. Int J Cancer 2007: **121**: 1169–1174.
43. Liu SY, Chang LC, Pan LF, Hung YJ, Lee CH, Shieh YS. Clinicopathologic significance of tumor cell-lined vessel and microenvironment in oral squamous cell carcinoma. Oral Oncol 2008: **44**: 277–285.
44. Lo Muzio L, Santarelli A, Caltabiano R, Rubini C, Pieramici T, Trevisiol L, Carinci F, Leonardi R, De Lillo A, Lanzafame S, Bufo P, Piattelli A. p63 overexpression associates with poor prognostic in head and neck squamous cell carcinoma. Hum Pathol 2005: **36**: 187–194.
45. Lo Muzio L, Farina A, Rubini C, Pezzetti F, Stabellini G, Laino G, Santarelli A, Pannone G, Bufo P, de Lillo A, Carinci F. Survivin as prognostic factor in squamous cell carcinoma of the oral cavity. Cancer Lett 2005: **225**: 27–33.
46. Lo Muzio L, Campisi G, Farina A, Rubini C, Pannone G, Serpico R, Laino G, De Lillo A, Carinci F. P-cadherin expression and survival rate in oral squamous cell carcinoma: an immunohistochemical study. BMC Cancer 2005: **5**: 63.
47. Lo Muzio L, Campisi G, Farina A, Rubini C, Pastore L, Giannone N, Colella G, Leonardi R, Carinci F. Effect of p63 expression on survival in oral squamous cell carcinoma. Cancer Invest 2007: **25**: 464–469.
48. Luukkaa M, Vihinen P, Kronqvist P, Vahlberg T, Pyrhönen S, Kähäri VM, Grénman R. Association between high collagenase-3 expression levels and poor prognostic in patients with head and neck cancer. Head Neck 2006: **28**: 225–234.
49. Lyons AJ, Jones J. Cell adhesion molecules, the extracellular matrix and oral squamous carcinoma. Int J Oral Maxillofac Surg 2007: **36**: 671–679.
50. Maeda G, Chiba T, Okazaki M, Satoh T, Taya Y, Aoba T, Kato K, Kawashiri S, Imai K. Expression of SIP1 in oral squamous cell carcinomas: implications for E-cadherin expression and tumor progression. Int J Oncol 2005: **27**: 1535–1541.
51. Mallett S, Timmer A, Sauerbrei W, Altman DG. Reporting of prognostic studies of tumour markers: a review of published articles in relation to REMARK guidelines. Br J Cancer 2010: **102**: 173–180.
52. Mărgăritescu C, Simionescu C, Mogoantă L, Badea P, Pirici D, Stepan A, Ciurea R. Endoglin (CD105) and microvessel density in oral squamous cell carcinoma. Rom J Morphol Embryol 2008: **49**: 321–326.
53. Marioni G, Marino F, Giacomelli L, Staffieri C, Mariuzzi ML, Violino E, De Filippis C. Endoglin expression is associated with poor oncologic outcome in oral and oropharyngeal carcinoma. Acta Otolaryngol 2006: **126**: 633–639.
54. Massano J, Regateiro FS, Januário G, Ferreira A. Oral squamous cell carcinoma: review of prognostic and predictive factors. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006: **102**: 67–76.
55. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics.REporting recommendations for tumour MARKer prognostic studies (REMARK). Eur J Cancer 2005: **41**: 1690–1696.
56. Muñoz-Guerra MF, Marazuela EG, Fernández-Contreras ME, Gamallo C. P-cadherin expression reduced in squamous cell carcinoma of the oral cavity: an indicator of poor prognosis. Cancer 2005: **103**: 960–969.
57. Myoung H, Kim MJ, Lee JH, Ok YJ, Paeng JY, Yun PY. Correlation of proliferative markers (Ki-67 and PCNA) with survival and lymph node metastasis in oral squamous cell carcinoma: a clinical and histopathological analysis of 113 patients. Int J Oral Maxillofac Surg 2006: **35**: 1005–1010.
58. Naryzhny SN. Proliferating cell nuclear antigen: a proteomics view. Cell Mol Life Sci 2008: **65**: 3789–3808.
59. Nemes JA, Nemes Z, Márton IJ. p21WAF1/CIP1 expression is a marker of poor prognostic in oral squamous cell carcinoma. J Oral Pathol Med 2005: **34**: 274–279.
60. Oliveira LR, Ribeiro-Silva A, Zucoloto S. Prognostic impact of p53 and p63 immunoexpression in oral squamous cell carcinoma. J Oral Pathol Med 2007: **36**: 191–197.
61. Oliveira LR, Ribeiro-Silva A, Zucoloto S. Prognostic significance of p53 and p63 immunolocalisation in primary and matched lymph node metastasis in oral squamous cell carcinoma. Acta Histochem 2007: **109**: 388–396.
62. Oliveira LR, Ribeiro-Silva A, Costa JP, Simões AL, Matteo MA, Zucoloto S. Prognostic factors and survival analysis in a sample of oral squamous cell carcinoma patients. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008: **106**: 685–695.
63. Pasquale EB. Eph receptor signalling casts a wide net on cell behavior. Nat Rev Mol Cell Biol 2005: **6**: 462–475.
64. Pukkila M, Kosunen A, Ropponen K, Virtaniemi J, Kellokoski J, Kumpulainen E, Pirinen R, Nuutinen J, Johansson R, Kosma VM. High stromal versican expression predicts unfavourable outcome in oral squamous cell carcinoma. J Clin Pathol 2007: **60**: 267–272.
65. Rahmani M, Wong BW, Ang L, Cheung CC, Carthy JM, Walinski H, McManus BM. Versican: signaling to transcriptional control pathways. Can J Physiol Pharmacol 2006: **84**: 77–792.
66. Riley RD, Sauerbrei W, Altman DG. Prognostic markers in cancer: the evolution of evidence from single studies to meta-analysis, and beyond. Br J Cancer 2009: **100**: 1219–1229.
67. Roh JL, Cho KJ, Kwon GY, Ryu CH, Chang HW, Choi SH, Nam SY, Kim SY. The prognostic value of hypoxia markers in T2-staged oral tongue cancer. Oral Oncol 2009: **45**: 63–68.
68. Sakata K, Someya M, Nagakura H, Nakata K, Oouchi A, Takagi M, Hareyama M. Brachytherapy for oral tongue cancer: an analysis of treatment results with various biological markers. Jpn J Clin Oncol 2008: **38**: 402–407.
69. Santamaria D, Ortega S. Cyclins and CDKS in development and cancer: lessons from genetically modified mice. Front Biosci 2006: **11**: 1164–1188.
70. Shang ZJ, Li ZB, Li JR. VEGF is upregulated by hypoxic stimulation and related to tumour angiogenesis and severity of disease in oral squamous cell carcinoma: in vitro and in vivo studies. Int J Oral Maxillofac Surg 2006: **35**: 533–538.
71. Shao Z, Zhang WF, Chen XM, Shang ZJ. Expression of EphA2 and VEGF in squamous cell carcinoma of the tongue: correlation with the angiogenesis and clinical outcome. Oral Oncol 2008: **44**: 1110–1117.

72. Shiraki M, Odajima T, Ikeda T, Sasaki A, Satoh M, Yamaguchi A, Noguchi M, Nagai I, Hiratsuka H. Combined expression of p53, cyclin D1 and epidermal growth factor receptor improves estimation of prognostic in curatively resected oral cancer. Mod Pathol 2005: **18**: 1482–1489.
73. Silva SD, Perez DE, Alves FA, Nishimoto IN, Pinto CA, Kowalski LP, Graner E. ErbB2 and fatty acid synthase (FAS) expression in 102 squamous cell carcinomas of the tongue: correlation with clinical outcomes. Oral Oncol 2008: **44**: 484–490.
74. Silva SD, Cunha IW, Nishimoto IN, Soares FA, Carraro DM, Kowalski LP, Graner E. Clinicopathological significance of ubiquitin-specific protease 2a (USP2a), fatty acid synthase (FASN), and ErbB2 expression in oral squamous cell carcinomas. Oral Oncol 2009: **45**: e134–e139.
75. Smid EJ, Stoter TR, Bloemena E, Lafleur MV, Leemans CR, van der Waal I, Slotman BJ, Langendijk JA. The importance of immunohistochemical expression of EGFr in squamous cell carcinoma of the oral cavity treated with surgery and postoperative radiotherapy. Int J Radiat Oncol Biol Phys 2006: **65**: 1323–1329.
76. Soni S, Kaur J, Kumar A, Chakravarti N, Mathur M, Bahadur S, Shukla NK, Deo SV, Ralhan R. Alterations of rb pathway components are frequent events in patients with oral epithelial dysplasia and predict clinical outcome in patients with squamous cell carcinoma. Oncology 2005: **68**: 314–325.
77. Tashiro E, Tsuchiya A, Imoto M. Functions of cyclin D1 as an oncogene and regulation of cyclin D1 expression. Cancer Sci 2007: **98**: 629–635.
78. Ueda G, Sunakawa H, Nakamori K, Shinya T, Tsuhako W, Tamura Y, Kosugi T, Sato N, Ogi K, Hiratsuka H. Aberrant expression of beta- and gamma-catenin is an independent prognostic marker in oral squamous cell carcinoma. Int J Oral Maxillofac Surg 2006: **35**: 356–361.
79. van de Pol SM, Doornaert PA, de Bree R, Leemans CR, Slotman BJ, Langendijk JA. The significance of anemia in squamous cell head and neck cancer treated with surgery and postoperative radiotherapy. Oral Oncol 2006: **42**: 131–138.
80. Wimmer-Kleikamp SH, Lackmann M. Eph-modulated cell morphology, adhesion and motility in carcinogenesis. IUBMB Life 2005: **57**: 421–431.
81. Yu Z, Weinberger PM, Haffty BG, Sasaki C, Zerillo C, Joe J, Kowalski D, Dziura J, Camp RL, Rimm DL, Psyrri A. Cyclin d1 is a valuable prognostic marker in oropharyngeal squamous cell carcinoma. Clin Cancer Res 2005: **11**: 1160–1166.
82. Zhang M, Zhang P, Zhang C, Sun J, Wang L, Li J, Tian Z, Chen W. Prognostic significance of Bcl-2 and Bax protein expression in the patients with oral squamous cell carcinoma. J Oral Pathol Med 2009: **38**: 307–313.
83. Ziober AF, Falls EM, Ziober BL. The extracellular matrix in oral squamous cell carcinoma: friend or foe? Head Neck 2006: **28**: 740–749.

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