Prognostic Factors of Survival Rate in Oral Squamous Cell Carcinoma: Clinical, Histologic, Genetic and Molecular Concepts

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Abstract

Oral squamous cell carcinoma (OSCC) represents 95% of all forms of head and neck cancers. The five-year survival rate of OSCC patients has been reported approximately 50%, which is not satisfactory despite new treatment modalities. The aim of the current review is to present factors (histologic, clinical, genetic and molecular biomarkers) correlated with survival rate in OSCC patients.

A web-based search for all types of articles published was initiated using MEDLINE/PubMed. The search was restricted to articles focusing on relevant clinical, histologic, genetic and molecular factors of survival rate in OSCC and presenting new concepts in this field.

Mode of invasion, presence of lymph node metastasis, extra-capsular spread, surgical margins and invasive tumor front grade are clinical and histologic parameters, which are strongly associated with survival rate. Focusing on selected proteins, wide range of molecular markers and gene alterations involving in cell cycle regulation, apoptosis, cell migration, cell adhesion and tumor microenvironment have been documented. Among well-known molecular markers, cyclin dependent kinase, survivin, CD44, BUBR1, and heat shock proteins (27,70) can be considered as independent prognostic factors of survival rate.

The identified prognostic factors imply a relatively comprehensive understanding of factors related to survival rate in OSCC patients, and provide an additional tool for selecting patients who need more aggressive treatment design.

Keywords: Oral, prognosis, squamous cell carcinoma, survival rate

Introduction

Squamous cell carcinoma is the most common oral cavity cancer. It is the eight most common cancers in men and fifth most common in women.[1] Tobacco use in various forms (smoking, chewing & snuff dipping) and alcohol consumption both are major risk factors for oral cavity cancer. Frequent use of fresh fruit, vegetables and diet containing micronutrient, β carotene and vitamin E reduce the risk of oral squamous cell carcinoma (OSCC).[2] Evidence shows the human papilloma virus (HPV) has an oncogenic role however, it is likely to be small.[3–4] In spite of significant advances in prevention and treatment, 5-year survival rate after diagnosis remains low due to uncontrolled or recurrent tumors and lack of suitable markers for early detection. Therefore, new approaches for early detection, better understanding of cellular mechanisms leading to malignant transformation and novel treatment based on cellular changes will need to be undertaken.[5] In this review, we draw attention and discuss about prognostic factors related to survival rate in OSCC focusing on histologic and clinical parameters, molecular biomarkers and gene alterations.

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A web-based search for all types of articles published was initiated using MEDLINE/PubMed (since 1999 to 2015), with the key words such as "oral", "squamous cell carcinoma", "survival" and "prognosis". The search was restricted to articles focusing on relevant clinical, histologic, genetic and molecular factors of survival rate in OSCC and presenting new concepts in this field.

Review of the literature
Clinical Parameters

Age and sex: age and sex were reportedly not associated with survival rate in OSCC.[6–9] Although, few cohort studies imply a lower survival rate in men specially in early stage of tongue tumors[10] and patients younger than 50.[11–12] According to Fan, et al. the 5-year survival rate and disease-free survival rate were 61% and 75.5%, respectively, among OSCC patients under the age of 45.[13]

Tobacco and alcohol: Most authors stated higher survival rate in non-smokers and non-drinkers, but there is no difference between ever smokers and current smokers.[12–14] Fang, et al. showed that smoking was associated with an approximately 2-fold increase in the risk of recurrence and 5-fold increase in the risk for disease - related death.[15]

Tumor staging: Staging of OSCC is performed with the use of the "tumor", "node", "metastasis" (TNM) classification system and its variant (PTNM), which are based on clinical and pathologic evaluation of tumor size and lymph node involvement. Most of the authors believe that the most significant factor influencing survival rate is tumor staging "by assessing the primary tumor size and cervical lymph node status". It should be noted that TNM staging alone cannot predict prognosis. Other tumor characteris-

tics particularly histologic parameters must be utilized to identify the prognosis and select favorable treatment.

Loco-regional recurrence (LRR): LRR rate in OSCC has been reported variable according to different researches, but it accounts as one of the important significant factors of survival rate.[6,13,16,18,20,21] Type of treatment, the presence of lymphovascular permeation and observation of malignant cells microscopically in muscles, excluding "the extrinsic muscles of the tongue, pterygoid and master muscles" can affect LRR.[16,21]

Lymph node metastasis: Several studies have indicated that the involvement of cervical lymph node in OSCC patients decreases significantly survival rates than those with disease-free nodes.[18,19,22,23] Regarding the role of lymph node metastasis in prognosis, radical neck dissection is performed when patients present with palpable (N+) cervical lymphadenopathy.

Histologic Parameters

Histologic grading: The histologic grading is used to predict the clinical behavior of OSCC for many decades, but its prognostic value is still controversial. Different histologic grading systems such as broder's, Anneroth's, Bryne's and Jakobsson's are used. Anneroth's classification is the most commonly used comprehensive classification by considering the degree of cell differentiation, and keratinization, as well as pattern and stage of invasion, and lymphoplasmocytic infiltration.[17,20] Among above mentioned parameters, degree of cell differentiation, keratinization and pattern of invasion correlate with survival rate among OSCC patients.[24–28] The pattern of invasion is an independent prognostic factor of survival rate[20,25–28] and lymph node metastasis.[25,29–32] Kademani, et al. showed 44% decrease in survival rate per grade in OSCC.[17]

Perineural invasion: Perineural invasion correlates with larger tumor size, higher depth of tumor invasion, risk of nodal metastasis and lower 5-year survival rates in patient with OSCC.[21,24,28,31]

Surgical margins: Pathologic positive margin has been proven to be an adverse prognostic factor for OSCC patients, which apparently correlates with local recurrence and overall survival (OS).[20,31]

The 5-year OS in early stage OSCC patients with safe margin, positive margin and close margin has been reported 78.2%, 61.4% and 50.8%, respectively.[33] Surgical clear margins > 5mm are recommended to prevent local recurrence.[34]

Extra capsular spread (ECS) and depth of invasion: ECS defined as an extra nodal extension of metastatic deposit outside the lymph node capsule. Most authors have established significant correlation between ECS and lower OS and decreasing survival rate between 29% to 60% when ECS is present.[13–14,16] The same

result has been implicated for depth of invasion.[24,34–36] Liao and coworkers found that tumor thickness < 10 mm is an independent prognostic factor for increasing OS and disease specific survival (DSS).[34]

Genetic alterations and molecular biomarkers of OSCC

Genetic alterations: OSCC, like most other malignancies, arises from the accumulation of a number of discrete genetic events that lead to invasive cancer (Table 1). These changes occur in genes that encode for proteins, which control the cell cycle, cell survival, cell migration and angiogenesis.

Previous cytogenetic analysis has shown a series of alteration in OSCC, most frequently in chromosome 9, chromosome 17 gene as well as 3P, 13q21 and 18q21.[37–41] Significant correlation between hypermethylation of TP73, PIK3R5 and CELSR3,[42] down regulation of MYC,[43] SMAD3/ TGFBR2 genes mutation,[44] amplification of cyclin D1 gene[45] and survival rate in OSCC patients have been reported. Moreover, proteomic analysis of OSCC specimen revealed correlation of thirteen RNAs with their encoded proteins implying transcription control with survival rate. Among these, reduction of DSP, PKP1 and TRIM29 directly related to poorer disease specific survival.[46]

Molecular biomarkers: As previously mentioned, many different proteins, which are genes products, control cell cycle and cell proliferation. Dysregulation and pathological imbalance of these proteins may lead to tumorigenesis. These proteins can be detected by various methods such as immunohistochemistry (IHC) staining, polymerase chain reaction (PCR), reverse transcriptase-polymerase chain reaction (RT-PCR), proteomics analysis and western blot. The novel molecular markers correlated with survival rate in OSCC patients (Table 2) are as below:

Molecular markers related to cell cycle regulation, proliferation and apoptosis

Cyclin dependent kinases (CDKs): Resting cells are in the G0 stage of the cell cycle, need to be recruited to the G1 stage and undergo replication. The orderly progression of cells through the various phases of the cell cycle is programmed by cyclins, CDKs, and their inhibitors. CDKs drive the cell cycle by phosphorylating specialized proteins required for progression of the cells to the next phase of the cell cycle. CDKs are actually inactive and after binding to cyclins become activated.[47]

CDK1 as a key factor for G2- M phase transition as well as cyclin B1 complex pushes cell from G2 phase to M phase and is considered as a maturation promoting factor. Analysis of 77 OSCC tissue sample by Chen, et al. showed expression of CDK1 in 77% of tumor tissue compared with 35% of the control group. Significant reduction in the 5- year accumulative survival rate in CDK1 positive cases was seen compared with CDK1 negative

Table 1. Genetic alterations related to survival rate in OSCC

Gene	Alteration	Relevant references
P16,P14ARF	Mutation	37–41
P53	Mutation	37–41
P73,PIK3R5,CELSR3	Methylation	42
MYC	Amplification	43
SMAD3	Mutation	44
TGFBR2	Mutation	44
Cyclin D1	Amplification	45

Biomarker	Cellular function	Relevant references
CDK1	cell cycle regulation	23
Survivin	apoptosis	49–52
MCM proteins	cell proliferation	54–55
BUBR1	cell proliferation	58–59
HSPs	apoptosis, inflammatory response inhibition	63
UPAR	cell migration, cell adhesion	66–67
CD44	cell adhesion	68–70
CXCR-4	angiogenesis, tumor growth	74–75
CAF	tumor growth	77

CDK: cyclin dependent kinase; MCM: minichromosome maintainace; HPS: heat shock protein; UPAR: urokinase plasminogen activator receptor; CAF: cancer associated fibroblast

cases.[23]

Survivin: Survivin is a member of the inhibitor of apoptosis proteins (IAP) family that inhibits capase 3, 7 and 9. Moreover, it regulates cell proliferation and angiogenesis. Some investigators have suggested that the primary function of survivin is controlling cell division rather than inhibiting apoptosis. Survivin rarely expressed in differentiated tissue, but its up-regulation has been reported in some cancers.[48–49] A retrospective analysis of 96 cases of OSCC and 62 cases of oral epithelial dysplasia showed higher expression of survivin in OSCC samples compared to dysplastic lesions. Indeed, individuals with advanced stage, positive lymph node metastasis and lower survival rate showed high expression (> 25%) of survivin[50] in agreement with YH, et al. study.[51]

Survivin has been shown to play different roles depending on the location within the cell. An examination of 71 pre-therapeutic oral and oropharyngeal SCC tissue by IHC staining showed nuclear expression significantly correlated with favorable relapse free survival.[52]

Minichromosome maintenance (MCM) proteins: MCM proteins are family members involved in the initiation of DNA replication, and functioning for the S phase of cell cycle initiation. The exact role of these proteins in cancers such as OSCC is not well known yet.[53] Few studies in this field implied that MCM 5 LI > 60% and MCM7 LI > 49.5% have been consistent with more aggressive behavior and lower OS in OSCC patients.[54–55] MCM2 expression has been shown to correlate with worse disease specific survival period in OSCC by Szelachowska, et al.[56]

BUBR1: BUBR1, an important protein in the mitotic spindle assembly check-point (SAC), has been associated with some virus-encoded proteins and cancer. The SAC is a surveillance mechanism for proper segregation of chromosome during mitosis.[57–58] It's role in oral carcinogenesis is still controversial and further research is needed. Lira, et al. analyzed a series of OSCC biopsy samples; and found over expression of BUBR1 with less advanced pathologic stage, longer survival period and shorter recurrence-free survival periods.[59] Expression of BUBR1 was also studied in non-malignant oral lesions and OSCC with and without metastasis associated with HPV infection. This is surprising that HPV was more prevalent (76%) in samples with high BUBR1 expression. Besides, higher BUBR1 expression significantly associated with shorter survival rates in malignant lesions.[59]

Heat Shock Proteins: Heat shock proteins (HSPs) are a group of intracellular proteins that play an essential role as molecular chaperones in regulating protein folding, stability, transport and aggregation. HSPs are powerful regulators of apoptosis through the interaction with key components of the apoptotic signaling pathway specially caspase cascade. HSPs are usually up-regulated in cancers. These proteins can inhibit inflammatory response in the tumor stroma and help cancer cells survival.[60–62] HSPs variants are over expressed in some tumors with different results in patient outcome. In OSCC, there have been very few and converse studies on the HSP27. In oral cavity samples, HSP27 over expression has been reported with better OS.[61–62] The compelling study by Kaur, et al. showed negative correlation between HSP70 expression and median disease free survival rate.[63] Although, in another study the correlation between OS and lymph node metastasis with HSP70 over expression was only seen in T2 patients with 20% cut-off point.[64]

Cell migration and adhesion molecules

Invasion of cells into the surrounding tissue and destruction of normal tissue structure are significant hallmarks of malignant tumors. Molecular mechanisms underlying tumor cell migration and invasion have been identified including, dramatic changes in the expression and function of cell adhesion molecules, high expression of urokinase plasminogen activator receptor and extracellular matrix (ECM) remodeling.

Urokinase plasminogen activator receptor: Urokinase plasminogen activator receptor (UPAR) is a part of urokinase plasminogen activator (UPA) system which plays multiple roles in cell migration and tissue remodeling.[65] To investigate UPAR expression, a cohort study of 189 OSCC patients showed patients who positively expressed UPAR, had a lower life expectancy than those who negatively expressed UPAR.[66] This result is similar to those described by Yoshizawa, et al.[67]

CD44: Cell adhesion molecules (CAMs) are necessary for cell-cell or cell- ECM contacts. CD44 is a CAM that binds to hyaluronan, ECM proteins and growth factors.[67] Several studies demonstrated a significant relationship between high expression of CD44 and longer OS in OSCC patients.[68–70] A systematic review and Meta-analysis of CD44 expression in head and neck cancer suggested that CD44 expression predicts tumor stage, tumor

grade and prognosis in pharyngeal and laryngeal cancer, however no clear association was found in oral cancer.[71]

Tumor microenvironment

Malignancy is a state that emerges from a tumor- host microenvironment in which malignant tumor cells recruit vasculature and stroma through the production and secretion of growth factors and chemokines. The locally activated host microenvironment (cellular and extracellular matrix) controls the proliferative and the behavior of the tumor cells. It also creates a permissive field to supply nutrients by angiogenesis and provides a pathway for metastasis through the vascular system.[72–73]

Chemokines: Chemokines are a family of cytokines that affect leukocyte movement. They are classified into four groups according to the arrangement of the conserved cysteine (C) residue in the mature proteins: C-X-C, C-C, C, and CX_3C. Evidence indicates that they have a special role in angiogenesis and tumor growth. The CX-CR and its ligand CXCL12 is one chemokine expressed in OSCC.[74] A retrospective study of 74 OSCC patients indicated that CXCR- 4 expression was an independent prognostic factor for poor survival rate.[74] Albert, et al. study assessed the prognostic value of CXCR-4 in tongue SCC and drew similar conclusion.[75]

Cancer associated fibroblast: Cancer associated fibroblasts (CAFs) were shown to have emerged with SCC cell in vitro and in vivo. Their overall distribution within the tumor microenvironment was determined to be related to disease recurrence.[76] The density of CAFs in a large series of tongue cancer as a parameter of tumor microenvironment was analyzed. CAFs has identified by α- SMA in most of the cases. Furthermore, CAF density was independently and relatively strongly associated with high mortality.[77]

Tumor infiltrating lymphocyte. The tumor microenvironment of head and neck squamous cell carcinoma is highly immune suppressive, mediated by cell- associated inhibitory mediators and host immunosuppressive cells. T cells is considered as the critical immune cells in antitumor immunity via different mechanisms such as antibodies production by CD4 + T cells and cell death by CD8 +T cells products as well as apoptosis induction.[78] High number of natural killer cells (CD57 + cells), infiltrating CD25 + lymphocyte, as well as the density of granzyme B and perforin positive cells correlated with longer OS in OSCC.[79–81] Other studies have shown that CD4 + T regulatory cells (tregs) promote tumor progression by inhibiting the functions of T cells and natural killer cells and their accumulation is associated with worse prognosis.[82] Pretscher, et al. compared the number of CD20 + B-cells and CD8 + T-cells in metastatic and non-metastatic groups of OSCC. In metastatic group high number of CD20 + B-cells, CD8 + T-cells and favorable outcome was seen compare to non-metastatic group.[83] These findings suggest that monitoring the density of infiltrating lymphocytes in lymph nodes and their products in tumor tissue are prognostic factor for prediction of lymph node metastasis and OS in OSCC.

In Summary, despite of the vast amount of studies to clarify the tumorigenesis pathways in OSCC and advances in treatment , the mortality rate is still high. OSCC invasion is a complex process involving multiple proteins in cell proliferation, apoptosis, cell migration, tumor microenvironment and epithelial-mesenchyme transition, which can make clinical and histopathologic variation

in different patients. As reviewed here, clinical and histopathologic characteristics, as well as several genetic alterations and molecular biomarkers have been investigated in OSCC patients. Pattern of invasion, histologic grade, status of surgical margins, disease stage, lymph node metastasis and expression of cyclins, CDK1, survivin, MCMs, CAF, HSPs and CD44 are factors with high influence on survival rate. Host immune defense, especially tumor infiltrating lymphocyte must be noted as critical factors related to survival rate in OSCC patients. Assessment of mentioned parameters and markers might be an additional tool for selecting patients who need more aggressive treatment modalities.

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