Prognostic evaluation of oral tongue cancer: Means, markers and perspectives (I)

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SUMMARY

Oral (mobile) tongue squamous cell carcinoma (OTSCC) is the most common cancer diagnosed within the oral cavity. Due to the inherent disadvantages of the mobile tongue OTSCC behaves aggressively and is generally associated with higher rates of occult metastasis and neck nodal metastasis than any other cancer of the oral cavity. The prognosis remains relatively poor and is still heavily reliant on TNM (tumor, node, metastasis) staging of the tumor despite a vast array of literature on possible prognostic indicators. This is a two-part article which examines the methods by which the behavior and prognosis of OTSCC has been studied, the prognostics markers, and the relevance and future direction of prognostic studies. In this first part, we discuss the relative merits of the methods used in prognostic studies and the clinicopathologic prognostic factors.

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Introduction

Cancer of the head and neck region is the sixth most common malignancy worldwide. Head and neck squamous cell carcinoma (HNSCC) and oral squamous cell carcinoma (OSCC) show variations in clinical progression and prognosis based on the subsite affected.1–3 Oral tongue squamous cell carcinoma (OTSCC) is the most common cancer diagnosed in the oral cavity comprising 25–40% of oral carcinomas, and along with the closely related cancer of the floor of the mouth (15–20%) can account for more than half of all oral carcinomas when excluding those affecting the lips.4 The tongue is disadvantaged because of its unusual histologic makeup (rich lymphatic network and highly muscularized structure) which makes it poorly equipped to protect itself from invasion and metastasis.5 OTSCC is thus more frequently associated with metastasis to draining lymph nodes than any other cancer of the oral cavity.6 The clinical course of OTSCC is also unpredictable because of the relatively high rates of occult metastasis in patients presenting with a very small primary tumor without clinical evidence of metastatic disease.7,8 The presence of nodal metastasis in the neck is the most important prognostic factor. Over the years, the use of neck dissection in the surgical management of clinical stage I–II OTSCC has been a source of debate, mainly because of a relatively high rate of occult metastases.9–11 Metastatic disease (like in other types of cancer) accounts for most cases of mortality and is a result of acquisition of properties such as growth signal self sufficiency, insensitivity to antigrowth signals, evasion of immune surveillance, sustained angiogenesis, cell migration, and invasion.12 Other factors that bear directly on prognosis are local recurrence or relapse, and the development of a second primary tumor. Patients with OTSCC have a significantly worse prognosis than those with similar lesions of the oropharynx, larynx, hypopharynx, and other oral cavity sites.5

This article describes the relative merits of the methods used in assessing the prognosis in solid cancers, such as HNSCC, and discusses the prognostic factors related to OTSCC using subject-specific articles obtained from MEDLINE with emphasis on the inclusion of multivariate survival analyses of known clinical and histological factors that affect prognosis. The factors that were considered to reflect poor prognosis were the presence of neck metastasis, recurrence (relapse), poor disease-specific or overall survival, decreased disease-free survival and development of a second primary tumor. This paper does not discuss premalignant lesions and the prognosis associated with the presence of such lesions.

Methods of investigating prognostic factors in oral cancer

The main goal of cancer research is to discover fundamental reasons for cancer development and to apply the result obtained in patient management in regard to predicting prognosis, formulating specific therapeutics and assisting in adaptation of therapy to the severity of the disease. Cancer is a disease characterized by complex, heterogeneous mechanisms, which means that prognostication and discovery of new treatment strategies is more difficult than for monogenetic diseases.13 The prognostic factors in
cancers of the head and neck are studied using various methods, such as morphological studies, genetic studies, cell and tissue cultures, animal models, and serum or saliva samples.

**Morphological studies**

This is the most widely used method in prognostic studies because it involves direct use of patient material (usually in the form of formalin-fixed, paraffin-embedded (FFPE) tissue blocks of surgical specimens) and is available in most laboratories. It comprises the use of histological or immunohistochemical staining and in situ hybridization on tissue sections. Patient material can also be used for molecular biology techniques such as immunoblotting and polymerase chain reaction. Histological techniques continue to enjoy widespread use because they are easy to perform, cheap and readily available. The limitations of these techniques include subjective quantitative measurements which are associated with variable scoring between observers. Moreover, features of tumor morphology do not always explain the underlying biology, as having the same tumor type and characteristics does not necessarily imply the same prognosis in different patients. In addition to sharing some of the limitations with morphological analysis, molecular markers, studied through immunohistochemistry or other methods, rely on assessment of individual molecules, whereas cancer is a complex (and often heterogeneous) process involving many molecules and pathways. Among the success stories of morphological methods in categorizing cancer patients into prognostic groups is the use of human epidermal growth factor receptor 2 (HER-2) and urokinase plasminogen activator (uPA) and its endogenous inhibitor, plasminogen activator inhibitor 1 (PAI-1) in breast cancer, which is currently supported by a number of expert groups. Presently, no such marker is in routine use for categorization of patients into prognostic groups for HNSCC.

**DNA cytometric methods**

DNA cytometry involves the staining of cell nuclei with stoichiometric DNA binding stain and measuring the amount of staining obtained. Two methods are commonly used: flow cytometry (FCM) or image (static) cytometry (ICM). FCM uses fluorescing probe molecules and fluorescing stains to study cells in fluid. The cells are sucked into a flow cytometer, passed through a laser beam, and the amount of light scatter and fluorescence is measured. In ICM, the cells are placed on a glass slide and stained using modified Fuelgen reaction, and the optical density of the nuclei is measured by image analysis. The relative advantages of using either of the two methods have been discussed by Alanen et al. The essential difference between the two is that while FCM allows the measurement of a large number of cells in a short time, ICM is interactive and much fewer cells can be studied. Since the investigator selects cells interactively in ICM, cellular debris or cells not needed in the measurement, e.g. lymphocytes and other inflammatory cells, can be excluded from the measurement. In addition, internal control can be achieved in image cytometry by selecting benign cells (lymphocytes, fibroblasts) as the standard diploid cells. Aneuploidy has been associated with poor prognosis in OSCC.

**Genetic studies**

Microarray studies (tissue and DNA microarray) have been developed in the last decade to solve the problems encountered in morphological studies, particularly the problem of the latter’s inability to identify multiple molecular markers simultaneously. Microarrays make it possible to study the expression of several thousands of genes simultaneously, and they can identify genes showing different expression (gene signature) in tumors with different outcomes. However, even this method, once heralded as a revolutionary technology that would likely result in development of effective treatments and cures for every human disease by the year 2050, has not significantly outperformed conventionally-derived single prognostic markers in classifying cancer patients into prognostic groups. The problems identified with using this method include non-reproducibility of gene signatures and inability to replicate results in terms of significant genes from different laboratories and from different experimental platforms. The underlying issue is the lack of standard methods for design, data analysis and performance assessment according to clinical aims.

The best examples of the clinical application of this method are found in breast cancer management. In patients with estrogen receptor positive (ER+) tumors, MammaPrint, a 70-gene signature currently available in the market, is reported to predict accurately who will develop metastasis within 5 years and who will not, and also to be able to identify low-risk patients who would not need aggressive chemotherapy. It is used on fresh frozen patient samples. Randomized clinical trials are presently ongoing. Two other gene signatures, Oncotype DX (21 genes) and Mammostrat (5 genes), which can be used on formalin-fixed, paraffin-embedded (FFPE) samples are also commercially available. Genetic studies can also be done on body fluids such as blood and saliva to generate molecular signatures that can aid in the diagnosis and prognosis of cancers along with or independently of tumor tissue.

**Cell culture and organotypic methods**

Cells can be cultured from tissues by disaggregating the tissue either mechanically or enzymatically to produce a suspension of cells capable of attachment to a solid substrate and forming a monolayer. Most cancer cells are capable of proliferation to give rise to cancer cell lines. Much of the present understanding of the cell and molecular biology of cancer is largely a result of cell culture studies using human cancer cell lines. The effects of various chemical compounds, drugs and viruses on human cancers are also initially investigated using human cancer cells lines. Studies using monolayers of human cancer cell lines are, however, limited in their extrapolation to the in vivo situation in cancer because they do not take into account the role of the other cells and the extracellular matrix (ECM) within the tumor microenvironment that play a significant impact in tumor growth, invasion and tumor outcome. This problem was addressed by the development of the organotypic 3D model by Fusenig and colleagues, which made possible the study of cell invasion and its quantitative analysis in 3D collagen gel embedded with fibroblasts. Despite its wide acceptance, this model is still somewhat artificial because only fibroblasts and ECM constitute its “microenvironment”. Our group recently developed a human uterine leiomyoma-based organotypic model and showed that this model mimics the tumor microenvironment better than the existing organotypic models and possibly enhances epithelial-mesenchymal transition in cancer (Fig. 1).

**Animal model experiments**

Animal models of cancer have been widely used to study various mechanisms of the carcinogenic process including invasion and metastasis, investigation of chemopreventive effects of naturally occurring or synthetic agents, and the activity of therapeutic agents on the cancer process. The most commonly used animal model is the mouse. The animal is either subjected to genetic engineering (transgenic or knockout mice) or injected with mouse or human cancer cells (syngeneic or xenograft transplantation, respectively) or administered with carcinogens (chemical carcinogenesis).
The basic mechanism for production of genetically engineered models (using a variety of methods) involves the creation of transgenic mice with random insertion of oncogenes (e.g. using a cell-specific promoter) or genomic deletion of tumor suppressor genes by targeted mutations of endogenous genes or recombination through embryonic stem cell manipulation resulting in replacing of genes with altered ones (knock-out) or altering a gene at its natural location (knock-in).  

Xenograft transplantation is a method carried out by transplanting cultured human cancer cells, cell line or co-cultures of tumor cells and tumor microenvironment cells into experimental animals (in vivo xenografts). It has been used in OTSCC.

Chemical carcinogenesis involves the application of carcinogens such as 7,12-dimethylbenz(a)anthracene (DMBA), 12-O-tetradecanoylphorbol-13-acetate (TPA) or N-methyl-N-nitrosourea (MNU) to induce cancer in normal cells. Subsequent progression and outcome can be observed in the animals. It is probably the most widely used method in OTSCC. The main argument against the use of mouse models is that although they can effectively model human cancer in its early stages, in most cases they do not adequately reflect features of advanced human cancers, mainly because of the short life span of the mouse. Recently, there has been a renewed interest in the use of the zebrafish (Danio rerio), which lives relatively longer than the mouse, for modeling human cancers. It has of late been regarded as a possibly better model because it can develop a wide spectrum of cancers resembling human malignancies, and such cancers share molecular pathways that become dysregulated in similar human cancers. Lee, Chen and colleagues have also shown that this model offers some promise in studying oral carcinogenesis. However, it is not clear if the extracellular matrix or the general tumor microenvironment in the zebrafish is comparable to that of human cancer, or how a model similar to OTSCC can be developed using this animal.

**Serum sample analysis**

Serum tumor markers are useful in all stages of patient management in cancer: diagnosis, prognosis, treatment choice and monitoring during and after treatment. The incentives for using serum include the fact that it can be obtained by a less invasive procedure and the concentration of the sought marker(s) in the serum is usually very high. Easy accessibility, low cost and multiple access for monitoring disease progression are the other obvious advantages. It also lends itself to use in probably all the methods of systems biology (genomics, proteomics, transcriptomics and metabolomics). Success in the use of serum markers in classifying patients into prognostic groups and making treatment decisions has been achieved in patients with nonseminomatous germ cell tumors (NSGCT) by using α-fetoprotein (AFP), human chorionic gonadotrophin (hCG) and lactate dehydrogenase (LDH). The use of these markers is recommended by several expert panels and is now widely applied for prognostication in NSGCT. Although a few other markers have also been found useful in some cancers, they have not achieved the success of the markers in NSGCT because of their much lower sensitivity and specificity for prognostic purposes. They are, however, still found to be useful in patient management: carcinoembryonic antigen (CEA) is recommended for the postoperative follow-up of patients with stage II and III colorectal carcinoma if further surgery or chemotherapy is being contemplated; and prostate specific antigen (PSA) is useful in detecting recurrence and for treatment monitoring in prostate cancer. In OSCC, increased serum p53 antibodies, serum...
C-reactive protein, serum midkine, serum SCC-Ag, serum level of soluble major histocompatibility complex class I-related chain A (MICA), serum vascular endothelial growth factor, serum cyfra 21-1, circulating tumor-associated DNA after surgery, serum sialic acid, and serum hCG-beta have been found to be associated with poor prognosis. In a large prospective study involving 444 patients with HNSCC, Duffy and colleagues reported that increased serum interleukin-6 (IL-6) is predictive of recurrence and poor survival. Our group recently showed that serum tissue inhibitor of matrix metalloproteinase-1 (TIMP) was associated with poor survival in HNSCC.

Saliva analysis

Apart from sharing many of the advantages of serum testing, the use of salivary tumor markers is particularly attractive in OSCC since the tumor is in close proximity to saliva, and also because the procedure is non-invasive. However, studies on saliva have shown that it has greater potential as a tumor diagnostic tool than for prognostication. Saliva and other body fluids are now considered very useful in the fields of genomics, proteomics, transcriptomics and metabolomics for generation of diagnostic and prognostic biomarker signatures. Further validation by multi-institutional studies and randomized clinical trials is still needed before translating the results obtained into clinical practice. Most studies on saliva and head and neck cancer have focused on comparing salivary biomarker concentrations in patients with healthy normal subjects and may not be directly predictive of prognosis. It has also been suggested that saliva biomarkers may be good early predictors of recurrence. Many molecular markers present in cancer tissue and serum may also be present in the saliva, but probably in much lower concentrations.

Clinical prognostic factors of tongue cancer

Sociodemographic factors

Sociodemographic factors are regarded as being of weak prognostic value in oral cancers affecting any subsite. Several studies have looked into age, gender, race and lifestyle as prognostic indices in OTSCC. There is no agreement in literature about the prognostic value of age in patients with mobile tongue cancer. Studies (some of them by matched-pair analysis) show that patients younger than 40 years have an increased frequency of tumor recurrence, distant metastases and cancer-related deaths compared to older patients. Several other studies, however, report that younger age is associated with a better survival. Some investigators have found no difference between age and prognosis. A study by Popovtzer et al. suggested that there are two distinct patterns of tongue cancer in young patients: an indolent form with freedom from disease for over 15 years and an aggressive type associated with up to 40% mortality within 2 years. Most studies are small institutional-based studies. Relatively larger studies such as those where data were extracted from the Surveillance Epidemiology and End Result (SEER) tumor registries have suggested that older age is more likely to be associated with poor survival.

Some studies have shown that relative survival rates in men are lower in than in women with tongue cancers while others have found no such association. Shiboski and co-workers reported a significant mortality in the black (African-American) adult male population compared with whites, mainly because they had a higher proportion of tongue cancer, and presented more often with late-stage disease than whites. It was suggested that whites have a better access to and utilize healthcare facilities more than blacks. In people under 65 years, survival rates fell from 47% to 39% between 1968 and 1987 in Scotland, with the highest increase recorded among subjects from the more socially deprived areas.

Betel quid use, although more important in carcinomas derived from the buccal mucosa and gingiva than the tongue, has also been associated with decreased survival. Smoking and chewing tobacco was found to have a significant adverse effect on survival in a population where alcohol use was assumed to be uncommon. Alcohol usage was also significantly associated with a decreased survival in patients with stage III/IV OTSCC. In summary, sociodemographic factors may be considered in prognostication, but they seem relatively less important when compared with other factors affecting prognosis.

Tumor stage

The tumor, node, metastasis (TNM) staging of tumors has long been used in the treatment planning of OTSCC and still remains the most important tool for the clinician in predicting disease outcome. It seems particularly useful in the prediction of prognosis of later stage cancers. The high propensity for occult locoregional metastases is the single most important disadvantage for the use of clinical staging, particularly in early stage OTSCC. Clinical palpation for neck nodes has a false negative rate approaching 30%. Advanced imaging techniques, such as fine-needle aspiration cytology (FNAC), computed tomography (CT), magnetic resonance imaging (MRI), ultrasonography (US) and positron emission tomography (PET) are used as an aid in detecting cervical lymph node metastases. Despite this, almost a quarter of micrometastases still go undetected. A biopsy of the first lymph node where the tumor emboli is presumed to enter through afferent lymph vessels (sentinel lymph node) is a potential method for the staging of locoregional metastases in tongue cancer. Its effectiveness is yet to be validated by large multicenter studies in the management of patients with head and neck cancers (including OTSCC).

More recently, many workers have used genetically based methods for molecular (gene expression profiling) signatures to predict cervical lymph node metastasis in HNSCC. Some have reported the effectiveness of these methods to be superior to conventional diagnostic methods. These methods have not been widely used because further multicenter validation is needed.

Conflict of interest statement

None declared.

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References