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Review

Metastasis from Oral Cancer: An Overview

JULIANA NOGUTI[1], CAROLINA FOOT GOMES DE MOURA[1], GUSTAVO PROTASIO PACHECO DE JESUS[1], VICTOR HUGO PEREIRA DA SILVA[2], THAIS AYAKO HOSSAKA[1], CELINA TIJUKO FUJIYAMA OSHIMA[1] and DANIEL ARAKI RIBEIRO[1,2]

Departments of [1]Pathology and [2]Biosciences, Federal University of São Paulo, UNIFESP, Sao Paulo, Brazil

Abstract. *Oral cancer is a common neoplasm worldwide. Its incidence and mortality have also increased over the past decades. It is characterized by poor prognosis and a low survival rate despite sophisticated surgical and radiotherapeutic modalities. Metastasis of oral cancer is a complex process involving detachment of cells from tumor tissue, regulation of cell motility and invasion, proliferation and evasion through the lymphatic system or blood vessels. In this review, we will focus on the current knowledge in metastasis from oral cancer regarding facts, such as incidence; stage, histopathology and grade of primary tumor; clinical manifestations; diagnosis; and treatment. Certainly, such information will contribute to the understanding of oral cancer pathogenesis.*

Head and neck cancer is a common neoplasm that encompasses epithelial malignancies of the paranasal sinuses, nasal cavity, pharynx and larynx, representing about 6% of all cases and accounting for an estimated 650,000 new cancer cases and 350,000 cancer-related deaths worldwide every year (1, 2). Oral cancer is the most frequent type of cancer of the head and neck area, with squamous cell carcinoma being the most common single entity. It may appear in any location, although there are certain areas in which it is found more frequently, such as the tongue and floor of the mouth. These areas represent about 90% of all malignancies of the oral cavity (3, 4). However, despite significant advances in surgery and chemotherapy achieved over the past decades (5), oral cancer is still characterized by poor prognosis and a low survival rate (6). In patients diagnosed with tumors at an advanced stage, there is a high occurrence of invasion to surrounding tissues, with lymph node and distant metastasis, and a peculiarly high risk of second malignancy during the patient's lifetime (1). Metastasis has been interpreted in a Darwinistic perspective as being a process whereby genetic instability in the primary tumor fuels cell heterogeneity, allowing for a few metastatic clones to eventually emerge and be positively selected to disseminate cancer at distance (7). It represents the most devastating feared stage of malignancy and the leading cause of death from cancer. Metastasis consists of sequential and selective steps including proliferation, stimulation of angiogenesis, detachment, motility, invasion into bloodstream and cross-talk with components of the new microenvironment, including parenchymal, stromal and inflammatory cells (8, 9). It is clear that only a minority of malignant cells undertake the metastatic route, due to an interplay between host factors and intrinsic characteristics of cancer cells; thus metastasis may represent an escape of these cells from the hostile environment they themselves created, such as shortage of oxygen and nutrients, inflammation and immune system attacks (10-12). This review aims to reveal recent findings, regarding especially oral cancer research thereby raising our level of understanding of this crucial strategy by which cancer cells escape death, and in addition to provide new perspectives on oral cancer diagnosis and treatments.

Correspondence to: Daniel Araki Ribeiro, DDS, Ph.D., Departamento de Biociências, Av. Ana Costa, 95, Vila Mathias, Santos – SP, 11060-001, Brazil. Tel: +55 1332218058, Fax: +55 1332232592, e-mail: daribeiro@unifesp.br

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Incidence

Oral cancer is one of the most common types of tumor in the head and neck (38%) with an incidence of 75% in male patients over age 60 years old, while about 95% of cases are squamous cell carcinomas (13). Oral squamous cell carcinoma is an invasive lesion with the presence of perineural growth. It has a significant recurrence rate and frequently metastasizes to cervical lymph nodes (14). Lymph node metastatic tumors occur in about 40% of patients with oral cancer. Clinically, their manifestations are hidden in rates of 15% to 34% (15, 16).

Metastasis of oral cancer is a complex process involving detachment of cells from the tumor tissue, regulation of cell motility and invasion, proliferation and evasion through the lymphatic system or blood vessels. This process is due to reduced intercellular adhesion of tumor cells as they progress to malignancy because of loss of E-cadherin; they thereby begin to express proteins such as mesenchymal vimentin and N-cadherin, promoting cell elongation and interfering with cell polarity. This morphological transition, called epithelial-mesenchymal transition (EMT) leads to molecular alterations interfering with the behavior of these cells (17).

The major determinant of the prognosis of oral carcinoma is the risk of cervical metastasis. While it is widely accepted that more advanced oral tumors be treated with elective neck dissection, management of stage I disease remains controversial. In the absence of clinical neck disease, stage I oral cancer is often treated with primary tumor resection and clinical follow-up of the neck. However, studies have shown the incidence of occult neck metastases in stage I/II disease to be as high as 42% (18).

At least 50% of patients with locally-advanced head and neck cancer develop locoregional or distant relapses, which are usually detected within the first two years of treatment (19). Investigators suggested that squamous cell carcinoma of the upper aerodisgetive tract with parotid metastasis most often arises from the oral cavity or oropharynx (20, 21). It has been postulated that unlike the parotid gland, the submandibular gland is unlikely to be the host tissue for metastasis because of its lack of lymph nodes and vessels (22). In fact, hematogenous spread of cancer may rise from tissues outside the head and neck region (23). In addition, unlike many other types of cancer, it characteristically metastasizes to the regional lymph nodes through the draining lymphatics in the early stages (24). Lymph node involvement is considered as the first indication for spread and a strong prognostic factor (25). At present, only 25%-40% of patients with lymph node metastasis will achieve 5-year survival, in contrast to approximately 90% of patients without metastasis (26-28). According to published data, the incidence of occult metastases to the neck can range from 15% to 60% depending on different prognostic factors (29). Currently, new and highly sensitive detection methods such as immunohistochemistry and molecular analysis have been developed; these could be an explanation as to why the incidence of metastasis has even increased in the last few years (13, 30).

Stage, Histopathology and Grade of Primary Tumor

Important prognostic indicators that are known to affect regional metastasis and therefore outcome, include size of the primary tumor, site, T stage, grade, depth of invasion, biological tumor markers, perineural invasion and patient compliance (31, 32).

The TNM classification of oral squamous cell carcinoma (33) provides a reliable basis for patient prognosis and therapeutic planning. There are a number of clinically detected or small undetectable primary tumors that display biological aggressiveness, with early regional metastasis and death. Typically, T1-T2 lesions are often associated with a risk of regional metastasis of 10% to 30% respectively, especially to lymph nodes, whereas several studies have shown a clear correlation between increasing tumor thickness and an increased risk of cervical metastasis (34, 35); T3-T4 lesions have a significantly higher risk of regional neck disease (13, 36).

Clinical Manifestations

The basic procedure in checking cervical lymph nodes is physical examination (37). To facilitate treatment planning and to provide a sense of outcome, clinical staging is generally used. The TNM clinical stage system has been adopted worldwide. This system uses the size of the primary tumor (T), the extent of regional lymph node involvement (N), and the presence of distant metastases (M) to predict prognosis (38).

Clinically, lymph nodes are assessed for location, number, size, shape, consistency, and fixation. Nodes are considered to be malignant if their size is greater than 1 cm, and they are hard and fixed (39). Patients without clinical manifestations of the disease are great candidates to be submitted to cervical intervention and/or co-adjuvant therapy (40).

Diagnosis

Auxiliary modern diagnostic modalities such as computed-tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), lymphoscintigraphy (LS), ultrasonography (USG) and USG-guided fine-needle aspiration cytology are recommended to increase the efficacy of the neck evaluation in patients with oral carcinoma, and some have become routine screening procedures in recent years, playing an important role in the detection of metastasis to cervical nodes (37, 41). The main advantage of CT and MRI is less inter-observer variation and these are relatively standardized techniques that can be performed in most institutions and can be interpreted by radiologists without specific knowledge of images of the head and neck (42). Criteria such as size, laterality and rapid increase of diameter of lymph nodes are most important as selection criteria for nodes to be punctured to detect occult metastasis (43).

Sentinel lymph node (SLN) status has been shown to be an excellent predictor of metastatic disease for both melanoma and breast cancer and is now the standard-of-care for cancer. Given this success, SLN biopsy has become of interest in oral squamous cell carcinoma (44, 45). The ability to identify metastases and unpredictable lymphatic drainage patterns is

another advantage of SLN biopsy (45). Multiple validation studies of elective neck dissections revealed SLN detection rates above 95% (46).

Elective neck dissection (END) remains the current gold standard for both staging and treatment of N0 cancer. However, END is associated with morbidity and is unnecessary for approximately three-quarters of all patients with N0 disease. A wait-and-scan policy avoids neck dissection, but raises risks for later presentation of occult metastases. In turn, this leads to more extensive dissection and an increased requirement for post-operative radiotherapy (44).

SLN utilizes lymphatic mapping to locate and harvest the small group of lymph nodes most likely to harbor metastases, minimizing the invasiveness of the procedure (46). The SLN concept states that tumor will spread from the primary site to a single node or group of nodes, termed the sentinel nodes, before progressing to the remainder of the lymph node basin (47).

Histopathological evaluation of SLNs allows for accurate prediction of the disease status of the rest of the basin, with biopsy-positive patients going on to END, while biopsy-negative patients are spared the morbidity of END. More detailed histopathological evaluation, including step-serial sectioning and immunohistochemistry can be carried out on the small number of harvested SLNs, potentially leading to more accurate nodal staging compared with the routine examination of many nodes from a neck dissection specimen (44). SLN navigation surgery is a minimally invasive technique used to help decide whether to perform neck dissection in patients with clinically-early head and neck squamous carcinoma (47).

Efforts have been made to elucidate tumor-related factors that could influence the appearance of metastases in oral squamous cell carcinoma (37). The samples are studied using hematoxylin and eosin (HE) staining and reviewed according to World Health Organization histological criteria (33). Although a number of studies have investigated the potential of these biomarkers in oral squamous cell carcinoma, there is no agreement on a reliable predictor of prognosis. Various hystopathological parameters, keratinization, mode of invasion, and lymphocyte infiltration have been described as being predictors of lymph node metastasis (38). Tumor thickness is an important prognostic factor in carcinomas of the oral cavity. The treatment of tumors smaller than 3 mm might need to be less aggressive than if the tumor is larger than 5 mm (48).

The use of biomarkers could help to avoid the unnecessary surgical treatment of metastasis free patients (40). Although the TMN staging system is used routinely, the technique accurately determines only the size and location of tumor and does not predict their metastatic potential. Further clinical examination can only identify regional metastasis with an accuracy of 70%. Although the use of various forms of imaging can improve this percentage, microscopic disease cannot be detected by these methods (40).

Several proteins and genes are candidates for use as predictors of metastasis due to the heterogeinty of the cells (40, 49). Some studies have tried to relate the expression of proteins in primary tumors with the occurrence of metastasis, for this purpose many key proteins have been searched with intention of establishing more reliable prognostic factors.

The immunohistochemical expression of vascular endothelial growth factor (VEGF)-A and VEGF-C in oral squamous cell carcinoma has been analyzed and suggested to be associated with the prognosis of patients, the association between the expression of VEGF-C in lymph node metastases and prognosis has been suggested, however, the prognostic value of this expression in oral squamous cell carcinoma has not been clarified (50). VEGF-A expression was significantly related to higher tumor stage and invasion grade, while VEGF-C expression was significantly associated with tumor stage, regional lymph node metastasis and invasion grade (49).

E-Cadherins and catenins are important epithelial adhesion molecules. The E-cadherin protein is a transmembrane glycoprotein involved in calcium-dependent cellular adhesion. The cytoplasmic domain of E-cadherin can bind directly to either β- or γ-catenin, whereas α-catenin links E-cadherin (β, γ) to the actin cytoskeleton. Loss or reduction of E-cadherin-mediated adhesion is an important step in the development of invasion and metastasis in many types of epithelial carcinomas, including head and neck carcinomas. In some studies, catenin and E-cadherin were underexpressed in oral tongue carcinoma and nodal metastases (51-52). The EMT is believed to play a crucial role in cancer metastasis (53). EMT type 3 refers to the loss of carcinoma epithelial phenotype and the acquisition of mesenchymal-associated features which play a relevant role in tumor invasion and metastasis. Transformation to tumor spindle cells can be associated with EMT but is not a requisite feature. The syndecans are a family of transmembrane cell surface heparan sulfate proteoglycans, which regulate cell-cell and cell-extracellular matrix adhesion, cell migration and growth factor activity (54).

Focal adhesion kinases (FAKs) are protein tyrosine kinases localized in the focal adhesions, which upon activation, interact with each other, regulating several cellular signaling pathways implicated in malignant transformation and disease progression. FAKs up-regulation has been reported in various tumor types while unopposed FAK signaling appeared to promote tumor growth, progression, metastasis, and angiogenesis (55). FAK signaling has been shown to promote angiogenesis in embryonic development, as well as in various physiological and disease processes in adults, including tumor angiogenesis (56). The detection of occult metastases remains difficult, so that the establishment and validation of prognostic markers in primary tumor specimens have been considered of high priority (55).

Metallothioneins (MTs) are involved in many pathophysiological processes, including metal homeostasis and

detoxification, protection against oxidative damage, maintenance of intracellular redox balance, cell proliferation and apoptosis, drug and radiotherapy resistance, defense against tissue injury and remodeling, and several other aspects of cancer biology. MTs in tumors influence aspects, including proliferation, apoptosis, degree of differentiation, and hormonal dependency of the tumor (55). MT protein expression has been described in several types of head and neck neoplasia such as oral and oesophageal squamous cell carcinoma and is considered to be an informative biomarker for patient management and prognosis. Cellular distribution of MT has been significantly associated with histopathological grade of differentiation and depth of invasion (57, 58).

Tumor invasion is thought to involve the multiple proteolytic enzymes, among which are the matrix metalloproteinases (MMPs). MMPs are a family of proteases commonly expressed in invasive tumors and the adjacent stroma and it are thought to play an important role in tumor invasion and metastasis (59). Tumor cells are capable of utilizing MMPs produced by stromal cells and this indicates an active role for stroma in tumor invasion (59, 60). Some studies suggest that expression of MMP-2 and MMP-9 is related to invasion of oral squamous cell carcinoma on the basis of immunohistological expression. The loss of basement membrane components, such as laminins, type IV collagens, and heparan-sulphate proteoglycans, has been shown to correlate with an increase in invasive and metastatic potential (60, 61).

Treatment

The goal of oral cancer treatment is to eliminate the tumor, to restore the structure and function of the affected area, to minimize sequelae and to prevente subsequent cancer. In cases of incurable disease, the objective changes to improving the quality of patients' life until death (62).

To choose the initial treatment for oral squamous cell carcinoma, there are several factors related to the primary tumor and patient characteristics. The tumor factors include primary site, size, location, proximity to the bone, status of cervical lymph nodes, previous treatment and histological characteristics, such as the type, grade and depth of invasion of the tumor. The patient factors are related to age, general medical condition, tolerance to treatment and lifestyle (62). There are various approaches to squamous cell carcinoma treatment, from combined therapies using radiation and chemotherapy following surgery, commonly used for advanced-stage disease to single-modality therapy, such as radiotherapy, chemotherapy or surgery alone, which are usually applied to early-stage disease (63).

For malignant tumors of the oral cavity, surgical removal has always been the most important procedure used for treatment. For general cancer, it was the first to be accepted as treatment with the intent to excise the tumor with surgical margins comprising healthy tissue (64).

The introduction of radiotherapy and chemotherapy as adjuncts to surgical removal has made surgery-alone no longer the ideal choice as a treatment modality for most types of oral cancer. Currently, surgery-alone is used for accessible early-stage oral cancer (stage 1/2) with no lymph node spread and no clinical or radiographic evidence of metastasis in areas of low risk for metastasis (65). Neck dissections, for detecting neck lymph nodes, have become routine surgical procedures in an attempt to prevent occult metastases (66).

Radiotherapy is not commonly used for oral cancer as the only treatment modality, unless the tumor site is inoperable, or the patient chooses not to have surgery. It can also be given as a palliative treatment option for more advanced and terminal cases. Radiation therapy is usually used with surgery or chemotherapy in order to kill cells in mitosis, by breaking of DNA (67).

For early-stage oral cancer, radiation therapy alone has been found to achieve similar survival rates to surgery (up to 5 years) and can be used for the management of tumors at certain sites (68).

Radiation therapy alone has some advantages over surgery. It has milder complications following treatment and improved quality of life, while surgery of advanced tumours can lead to minimal chance of post-operative death, as well as permanent loss of function of oral structures (69).

Radiotherapy after surgery is a common method for oral cancer. It is not used before surgery because it would lead to fibrosis of the tissues, which makes the surgical removal more difficult. Large primary tumors and signs of adjacent tissue invasion direct the use or not of the radiation therapy, but neck is commonly treated with radiation to prevent potential metastasis and recurrence (70). Many techniques have been developed to improve the effectiveness of this kind of treatment, raising favorable control rates. These include altered fraction ratio by accelerated or hyperfractionated radiotherapy combined with chemotherapy or targeted therapy (70, 71).

Chemotherapy is a systemic treatment which aims to destroy malignant cells, to control the volume of the tumor and to reduce the chance of metastasis. Therefore, it is an important procedure used against advanced oral squamous cell carcinoma. Although it is generally not a curative method when performed alone for oral, head and neck solid tumors, it can be used prior to surgery, concomitantly with post-surgical radiation therapy (chemoradiotherapy), or in both cases (72, 73).

This kind of therapy, like every other, has its advantages and disadvantages. It has optimal drug delivery through undisturbed vasculature, prevents early micrometastases and assessment of tumor response, and allows for decisions to be made regarding the preservation of organs (74). However, chemotherapy

treatment with radiotherapy is usually related to a more frequent toxicity than use of each therapy on its own (75).

The range of drugs used for treating oral and maxillofacial cancer has expanded over the past decades with the introduction of methotrexate, 5-fluorouracil, hydroxyurea, platinum derivatives, anthracyclines, plant alkaloids, and, most recently the taxoids (76).

Targeted therapies have been developed in order to improve oncological treatment. They bear this name because they act on specific cell targets. Regarding head and neck squamous cell carcinoma, there is cetuximab, for example, which fixes the ligand-binding domain of the epidermal growth factor receptor (EGFR), which is abnormally expressed in epithelial malignancies and whose expression is increased in cancerous cells when they are irradiated rendering the tumor more resistant to treatment (13, 77).

Other advances in this field also include research on biological therapies and immunotherapy, virotherapy, gene therapy and cancer vaccines which are all still in developmental stages. Targeted-therapy molecules that can block growth factor receptor-specific enzymes can modify functions of proteins, induce apoptosis, inhibit angiogenesis and stimulate immune cells. Biological therapies or immunotherapies use laboratory-made antibodies and cytokines that help eliminate cancer cells or boost immune function. In virotherapy, viruses are used as vectors to carry genes into malignant cells, in order to disrupt their growth. Gene therapy is the usage of genes to boost immune cell recognition and elimination of cancer cells, or the genes are inserted into cancer cells to increase cytokine production, thus attracting immune cells. Cancer vaccines stimulate immune function by artificially introducing antigens into the body as prevention for future disease (78-80).

Conclusion

In this review, we have highlighted recent advances on the metastasis process in oral cancer. Although many of the underlying mechanisms have been elucidated so far, this is an area that warrants further investigation, since comprehension of these pathways will be added to those already established in the literature as a way to better understand oral cancer pathogenesis.

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