Cancer Metastasis Rev (2007) 26:645–662
DOI 10.1007/s10555-007-9082-y

Metastasis of squamous cell carcinoma of the oral tongue

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Published online: 3 September 2007

Abstract Squamous cell carcinoma of the oral tongue (SCCOT) is one of the most prevalent tumors of the head and neck region. Despite advances in treatment, the survival of patients with SCCOT has not significantly improved over the past several decades. Most frequently, treatment failure takes the form of local and regional recurrences, but as disease control in these areas improves, SCCOT treatment failures are occurring more often as distant metastasis. The presence of cervical lymph node metastasis is the most reliable adverse prognostic factor in patients with SCCOT, and extracapsular spread (ECS) of cervical lymph nodes metastasis is a particularly reliable predictor of regional and distant recurrence and death from disease. Decisions regarding the elective and therapeutic management of cervical lymph node metastases are made mainly on clinical grounds as we cannot always predict cervical lymph node metastasis from the size and extent of invasion of the primary tumors. Therefore, the treatment of these metastases in the management of SCCOT remains controversial. The promise of basing treatment decisions on biomarkers has yet to be fully realized because of our poor understanding of the mechanisms of regional and distant metastases of SCCOT. Here we summarize the current status of investigations of SCCOT metastases and the potential of these studies to have a positive impact on the clinical management of SCCOT in the future.

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Keywords Squamous cell carcinoma of the oral tongue (SCCOT) · Lymphogenic metastasis · Extracapsular spread (ECS) · Predictive marker for lymphatic disease Wnt/β-catenin signaling pathway

1 Introduction

Oral cavity cancer consistently ranks as one of the ten most frequently diagnosed cancers in the world [1], with 363,000 new oral and pharyngeal cancer cases and almost 200,000 deaths annually worldwide [2]. It is also the seventh most common cancer diagnosed in men in the United States [3]. As the incidence of oral cancer continues to increase, the disease becomes an increasingly important public health issue. The World Health Organization predicts a continuing worldwide increase in the number of cases of oral cancer for the next several decades [4]. In 2006, there were 31,000 new oral and pharyngeal cancers diagnosed in the United States, representing approximately 3% of all cancers, and squamous cell carcinoma of the oral tongue (SCCOT) accounted for 9,040 new carcinoma cases, with 1,780 deaths [5].

Despite advances in surgery and radiation therapy, the 5-year survival rate for oral cancer has not improved significantly over the past several decades and remains at 50–55% [6, 7]. This is primarily because patients continue to die from metastatic disease at regional and distant sites, though local recurrence and second primary tumors are also causes of death in these patients. The most reliable prognostic indicator of regional and distant treatment failure for patients with SCCOT is the presence of metastasis in cervical lymph nodes [8–11]. The finding of extracapsular spread (ECS) of cervical lymph node metastasis of SCCOT is associated with even higher rates of

regional and distant failure [12–20]. Therefore, the finding of pathologically involved lymph nodes after neck dissection, especially when there is ECS, often leads clinicians to intensify postoperative adjuvant therapy.

Several studies, however, have shown a high rate of occult nodal metastasis (20–40%) in SCCOT patients with no evidence of regional spread on clinical or radiographic evaluation [21–27]. Therefore, elective management of the neck is an important consideration in patients undergoing treatment for primary SCCOT. While clinico-pathologic characteristics most often guide the clinician's choice of elective and therapeutic neck management strategies, biomarkers of metastasis in SCCOT are currently being investigated to see whether they can reliably characterize SCCOT tumor behavior and thereby guide clinical decision-making. However, as the cellular and molecular mechanisms of metastases in SCCOT remain incompletely understood, the full potential of predictive markers has not yet been realized. This article will review our current understanding of the mechanisms of metastasis of SCCOT and will relate these to the clinical behavior of SCCOT and treatment selection.

2 Lymphatic metastasis of SCCOT

Lymphatic metastasis in the neck is a significant problem in patients with SCCOT. As many as 30% of patients with oral cavity cancer are found to have lymph node metastasis on their initial evaluation, with an even higher rate of nodal metastasis seen in patients with oral tongue cancer [24, 28]. In fact, metastasis to cervical lymph nodes occurs more frequently from the tongue than from any other primary tumor site in the oral cavity. SCCOT grows locally in an invasive manner and has a proclivity to metastasize to regional lymph nodes rather than to spread hematogenously. Primary SCCOT spreads through lymphatic channels to the lymph nodes of the cervical region. Involved nodes usually are enlarged, firm, and nontender to palpation.

2.1 Pathways of spread

For convenience, the location of lymph nodes in the cervical region has been described by levels that correspond to their anatomic location in the neck. The submental and submandibular triangles make up level I, and the upper, middle, and lower jugular nodal groups are considered levels II, III, and IV, respectively. The posterior triangle of the neck is designated as level V, while the pre-laryngeal (Delphian), pre-tracheal, and para-tracheal nodes comprise level VI. Level VII includes the lymph node groups found in the upper mediastum (Fig. 1).

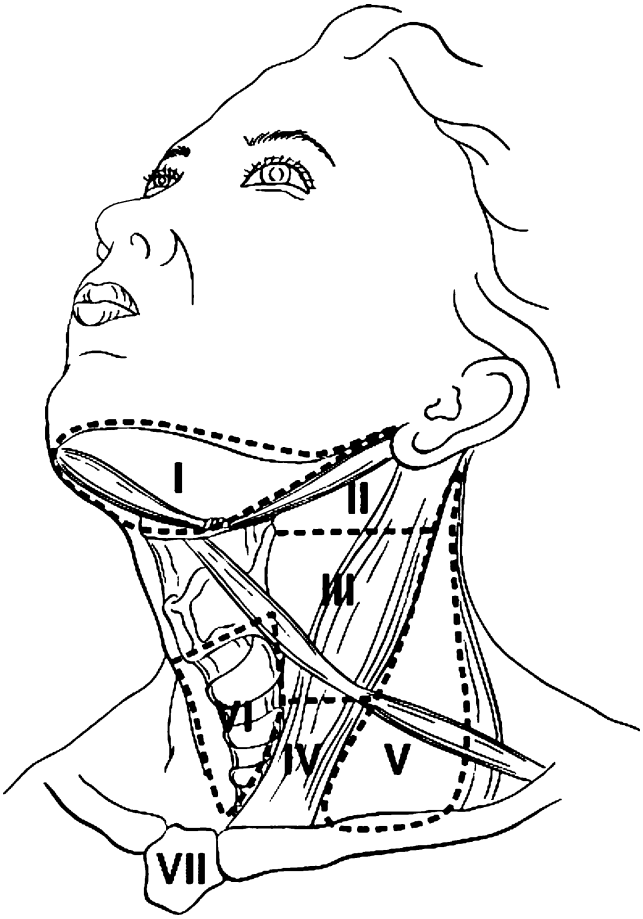

Fig. 1 Levels of cervical lymph nodes. Used with permission of the American Joint Committee on Cancer (AJCC), Chicago, Illinois. The original source for this material is the AJCC Cancer Staging Manual, Sixth edition (2002), published by Springer—New York, www.springer.com

The tongue has a dense lymphatic network, with three main deep muscular lymphatic drainage pathways [29]. The anterior pathway drains the tip of the oral tongue, primarily to level II or level I. The lateral pathway drains the lateral one-third of the dorsum of the tongue from the tip to the circumvallate papillae. These lymphatic channels drain to levels I, II, or III. The central pathway drains the central two-thirds of the tongue. These vessels drain to the level I nodes or course through a sublingual node and terminate in the level III nodes. Most of the lymph from the oral tongue drains to levels I and II. The levels most frequently involved with single pathologic lymph node metastasis in SCCOT are levels I, II, and III (Fig. 2). The incidence varies from 18 to 64% for level I; 43 to 73% for level II; 0 to 26% for level III; 0 to 10% for level IV; and 0 to 2% for level V [30–32]. Therefore cervical metastatic spread of SCCOT usually progresses from higher to lower node levels with only rare involvement of the posterior triangle and/or supraclavicular nodes. Although it is rare, it has been reported that metastasis can occur at level IV without the

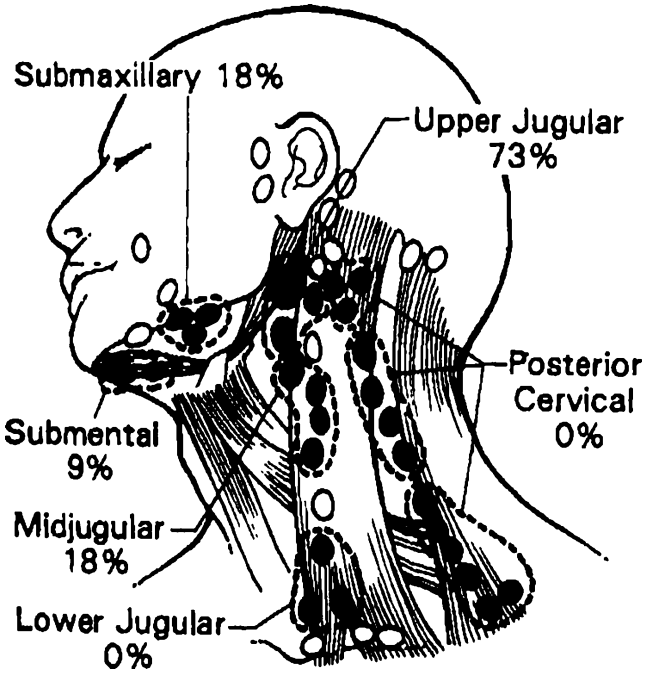

Fig. 2 The percentages of nodes involved with pathologic lymph node metastasis in SCCOT [30]

involvement of levels I–III, also known as skip metastasis [30]. The rich lymphatic network of the tongue also provides extensive connections across the midline, thereby placing both sides of the neck at risk for nodal metastasis, particularly from primary tumors close to or involving the midline of the tongue [29]. The risk of regional metastasis is generally related to tumor size and the depth of infiltration of the primary tumor. In addition, regional lymph nodes should also be described according to the level of the neck that is involved, since metastatic spread to lower cervical nodes, nodal chains and/or multiple nodal groups carries a worse prognosis.

2.2 Impact of lymphatic metastases and extracapsular spread on treatment outcomes

The major type of treatment failure in patients with SCCOT is regional failure. Although patients with early-stage SCCOT have a 2-year survival rate of more than 85% [33], the survival rate decreases by approximately 50% with the finding of cervical nodal metastasis. Grandi et al. [11] found that the presence of neck lymph node metastases in patients with SCCOT reduced the 5-year survival rate from 65 to 29%. Furthermore, pathologic evidence of regional nodal metastasis (pN+) has been associated with an increase in distant metastasis [34] and with marked decreases in overall and disease-specific survival [11]. Recently, in a study of 266 patients treated at our institution from 1980 to 1995 with resection of the primary SCCOT tumor and neck dissection with or without adjuvant radiotherapy, we found that overall and disease-specific survival rates were significantly worse in SCCOT patients who were node-positive. The 5-year overall survival rate was 73% for the pathologically node-negative pN0 group and 43% for the pN+ group. The 5-year disease-specific survival rate was 88% for the pN0 patients and 59% for pN+patients (Fig. 3) [19].

If the tumor has perforated the capsule of the involved node and invaded into the surrounding connective tissue, it is called extracapsular spread (ECS). The prognostic significance of ECS in cervical lymph nodes has been well documented [14]. The impact of ECS on survival in head and neck squamous cell carcinoma (HNSCC) patients has been reported in many articles [12–20, 35]. The 5-year survival rate in these patients ranges from 50 to 70% with the tumor limited to the node, but the presence of ECS reduces it to 25 to 30% [12, 14]. The presence of ECS also has been reported to be another poor prognostic factor for SCCOT [19, 20, 36], and it is a significant predictor of the development of distant metastasis [19]. The critical importance of microscopic ECS, which is only evident on

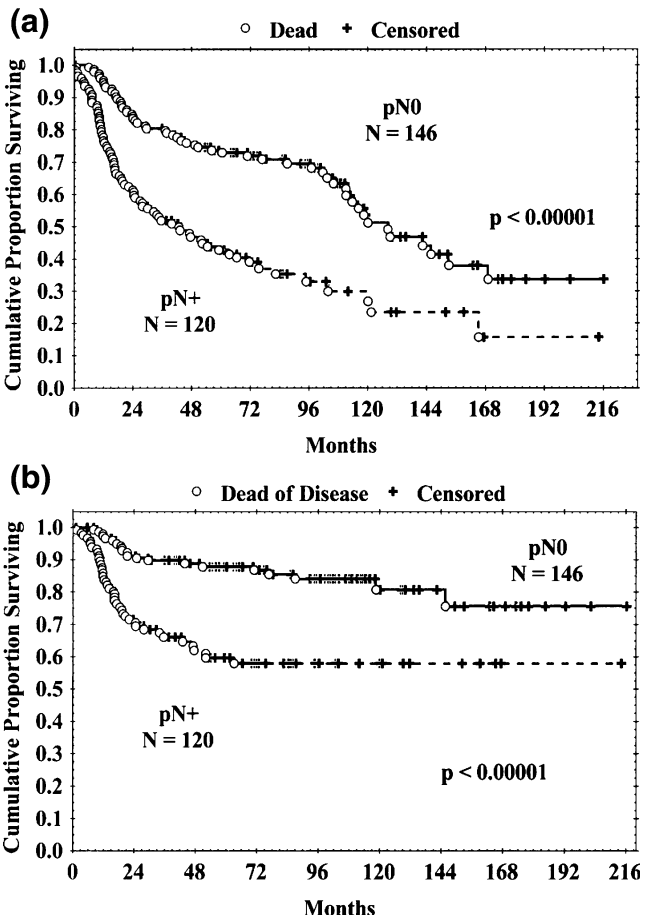

Fig. 3 Impact of lymphatic metastases on treatment outcomes. (**a**) Overall survival rate and (**b**) disease-specific survival rate of patients with SCCOT. *pN0* node negative group; *pN+* node positive group. Used with permission from [19]

histological examination, also has been reported in small-volume metastatic disease [37]. The incidence of ECS was reported to be histopathologically documented in approximately 60% of patients with cervical metastases, and generally it is thought to be related to nodal size. If a lymph node is larger than 3 cm in diameter, it has a 75% chance of having ECS [14]. In our retrospective study of 266 patients with SCCOT who underwent surgical treatment of the primary lesion and neck dissection, we found that the overall and disease-specific survival rates were 50 and 65% for pN+/ECS− patients and 30 and 48% for pN+/ECS+ patients, respectively (Fig. 4). Further evaluation of this cohort of patients revealed no difference in survival rates due to extent of extracapsular spread outside the lymph node capsule [20]. These findings suggest that intensive regional and systemic adjuvant therapy may be indicated for SCCOT patients with ECS. Other reports, however, did not identify ECS as a definitive prognostic factor [38–40].

While on occasion ECS may be detected by a diagnostic imaging procedure [41], pathologic analysis of a neck dissection specimen remains the only definitive method of identifying the presence of ECS. Although ECS occurs mainly in larger lymph nodes, we identified ECS in 19% of patients with clinical N0 disease who were found to have pathologically N+ lymph nodes [42]. Multiple nodal involvement, large nodal size, and fixation of nodes are other nodal features associated with poor prognosis [43].

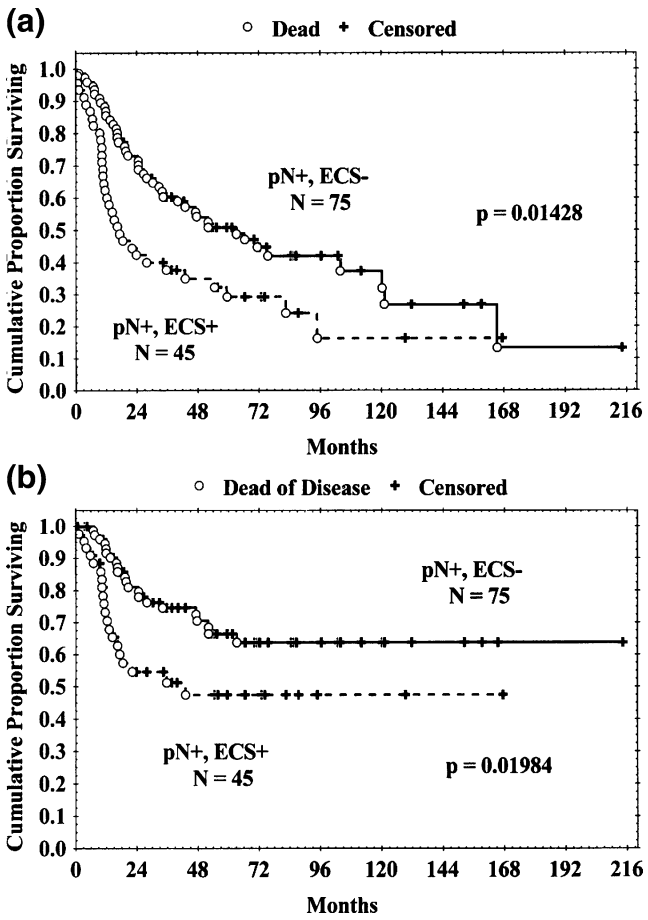

Fig. 4 Impact of extracapsular spread on treatment outcomes. (**a**) Overall survival rate and (**b**) disease-specific survival rate of patients with SCCOT. Used with permission from [19]

2.3 Risk factors for the development of Distant Metastasis (DM) of SCCOT and the significance to outcomes

Distant metastasis is another mechanism of treatment failure in patients with SCCOT. The incidence of distant metastasis from HNSCC regions varies from 5 to 24% in clinical reports [44] and is higher than 40% in some autopsy examinations [45]. The 5-year survival rate for patients with oral or oropharyngeal cancers who develop distant metastasis is 21% [6]. The occurrence of distant metastases without previous lymph node metastasis is very rare in SCCOT, which is consistent with a stepwise model of disease progression [46]. In addition, the risk of distant metastatic disease is more related to the burden of nodal disease than to the size or stage of the primary tumor. N stage, histopathologic evidence of lymphatic or vascular invasion, and ECS are all associated with increased risk of DM [47]. The risk of DM is less than 10% for N0–N1 disease and rises to approximately 30% for patients with N2 and N3 disease [48, 49]. The diagnosis of DM typically occurs 9–12 months after initial tumor identification, and in 84% of cases, it occurs within the first 2 years [46, 50]. Lungs and bone are the sites most often involved with DM in SCCOT, and skeletal and hepatic metastases occur less commonly. A retrospective study of 727 patients with head and neck cancer found that distant metastatic spread to the lungs occurred in 83.4% of the cases with DM, followed by spread to the bones in 31.3% [50]. In patients with advanced-staged HNSCC or locoregional failure, the risk of distant metastasis is approximately 10%. For the diagnosis of distant metastatic disease, positron emission tomography (PET) or computed tomography (CT) of the lungs is recommended [46].

3 Clinical evaluation for metastatic disease and staging

Because the extent of cervical lymph node metastasis is highly significant in determining the prognosis and choice of treatment in patients with SCCOT, early and reliable detection of cervical lymph node metastases is needed. Patient history and physical examination remain essential components in the staging of SCCOT. The fine-needle aspiration cytology (FNAC) technique, which is the least invasive pathologic examination method, remains the standard of care for evaluation of a neck mass in patients

at high risk of SCCOT. Generally, a histologic examination of completely removed lymph nodes is necessary to make a definitive pathologic diagnosis of cervical lymph node disease.

Both CT and magnetic resonance imaging (MRI) may be used in determining the extent of the primary SCCOT tumor, regional lymph node status, and the presence of distant metastatic disease, thereby providing important staging information [51–53]. The accuracy of CT and MRI in the assessment of cervical lymph nodes depends to a large extent on the criteria defined for lymph node metastases [42, 54]. In these imaging examinations, a node diameter of more than 10 mm, a round shape, clustering in groups, and the detection of non-contrast-enhanced areas within lymph nodes or necrotic areas are the most reliable criteria used for determining the presence of lymph node metastases. However, it may be difficult to see these finding in small lymph nodes [42, 55]. CT seems to be better than MRI for the assessment of necrotic areas in lymph nodes. Close et al. found that CT identified lymph node metastasis in 67% of cases of oral cavity and oropharyngeal carcinoma with clinically negative neck disease, and several studies have shown a greater than 80% accuracy rate for CT identification of pathologic neck disease [36, 56–58]. Extracapsular spread is characterized by irregular lymph node edges and the absence of fine fatty layers on CT/MRI [54], as well as the infiltration of adjacent structures.

Recently, ultrasonography (US) has been used for the examination for neck disease that is not palpable clinically, and it provides the advantage of not exposing the patient to additional radiation. When the technique is combined with FNAC, the specificity of US-guided cytology can approach 90% [59]. On sonograms, cervical lymph node metastases generally appear as low-echogenic, round or bean-shaped structures, with a diameter of more than 10 mm [60]. US also may be helpful in the assessment of major vascular invasion. In staging examinations, all enlarged lymph nodes should be suspect, because lymph nodes with a diameter of less than 10 mm are still found to be involved with cancer, some with extracapsular spread [17]. In spite of improved technical equipment, extracapsular spread in small lymph nodes is only rarely identified conclusively with US.

Positron emission tomography (PET) is also becoming an increasingly popular tool for the identification of primary, recurrent, and metastatic disease [61, 62]. CT and MRI can be fused with PET images for co-registration of morphologic and metabolic images to known tumorous masses and thus increase the benefits [63].

In spite of advances in these diagnostic imaging procedures, all are limited in their ability to detect smaller tumor volumes. The incidence of micrometastases that cannot be detected by any imaging technique is as high as 25% [64]. Therefore, on the basis of imaging criteria alone, it is still difficult to determine to what extent the neck should be included in the primary treatment scheme to avoid possible undertreatment or overtreatment.

Staging of SCCOT is important for determining prognosis and proper treatment of the disease. The clinical staging of SCCOT follows the TNM classification system from the American Joint Committee for Cancer (AJCC; Tables 1 and 2) [65]. The TNM system is based on the assessment of three components: T is the extent of the primary tumor, N is the absence or presence and extent of

Table 1 TNM staging of cancer of the oral cavity

Cancer stages	
Primary Tumor (T)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma *in situ*
T1	Tumor 2 cm or less in greatest dimension
T2	Tumor more than 2 cm but not more than 4 cm in greatest dimension
T3	Tumor more than 4 cm in greatest dimension
T4 (lip)	Tumor invades through cortical bone, inferior alveolar nerve, floor of mouth, or skin of face, i.e., chin or nose
T4a	(oral cavity) Tumor invades adjacent structures (e.g., through cortical bone, into deep [extrinsic] muscle of tongue [genioglossus, hyoglossus, palatoglossus, and styloglossus], maxillary sinus, skin of face)
T4b	Tumor invades masticator space, pterygoid plates, or skull base and/or encases internal carotid artery
Regional Lymph Nodes (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension
N2	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension; or in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension; or in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
N2a	Metastasis in a single ipsilateral lymph node more than 3 cm but not more than 6 cm in greatest dimension
N2b	Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension
N2c	Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
N3	Metastasis in a lymph node more than 6 cm in greatest dimension
Distant Metastasis (M)	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Superficial erosion alone of bone/tooth socket by gingival primary is not sufficient to classify a tumor as T4.

Table 2 Definition of TNM and stage grouping for cancer of the oral cavity

Stage groupings			
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1	N1	M0
	T2	N1	M0
	T3	N1	M0
Stage IVA	T4a	N0	M0
	T4a	N1	M0
	T1	N1	M0
	T2	N2	M0
	T3	N2	M0
	T4a	N2	M0
Stage IVB	Any T	N3	M0
	T4b	Any N	M0
Stage IVC	Any T	Any N	M1

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regional lymph node metastasis, and M is the absence or presence of distant metastasis [65]. This is a clinical staging system that is defined by the anatomic extent of the tumor. Although the AJCC staging system is useful for standardizing the description of SCCOT, it does not take into account other important factors known to affect survival and outcome, such as performance status, nutritional status, immune status, and pathological status such as the presence of ECS. However, because lymph node status is a significant predictor of survival outcomes, accurate staging is crucial to identifying SCCOT patients who may benefit from adjuvant therapy [36].

4 Treatment of cervical metastases

More than 30% of patients with SCCOT can be expected to have cervical lymph node metastases, even in cases of clinically node-negative disease [25, 32, 66–68]. Options for treatment depend on the size and location of the primary tumor, lymph node status, the presence or absence of distant metastases, the patient's ability to tolerate treatment, and the patient's preferences. The standard treatment for SCCOT in the United States is surgery.

4.1 Elective neck management versus observation

If cervical lymph node metastases are diagnosed, the neck should be treated. However, the treatment of SCCOT patients with clinically negative neck disease is controversial. There are two options for management of the neck in early-stage SCCOT. One is elective neck dissection and the other is observation, or a wait-and-see policy. The surgical treatment of the neck has a therapeutic benefit as well as a diagnostic/staging benefit for patients. Surgery removes metastatic deposits in lymph nodes and lymphatic vessels within the neck, serves to determine the extent and pathologic staging of disease, and provides prognostic evidence, such as ECS [69]. It is generally acceptable to perform elective lymph node dissection when the risk of occult metastases is estimated to be greater than 20% [70]. Dissection of lymph node levels I to III, also known as the supraomohyoid neck dissection [71], has been commonly used in the management of the clinically node negative neck in patients with SCCOT because levels I and II are the main sites of metastasis of SCCOT and levels IV and V only rarely harbor nodal metastatses [29, 72, 73]. However, surgical neck management may be unnecessary in some SCCOT patients with very early-stage, thin lesions, as no real benefit in survival has been demonstrated compared with delayed neck dissection for lymph node metastases [74]. Management of the neck with observation requires careful follow up with highly reliable imaging [75]. Some studies have reported a higher survival rate with observation than with elective neck dissection [74, 76, 77].

The potential for overtreatment of the clinically negative neck with lymph node dissection has increased the use of sentinel lymph node (SLN) biopsy. The SLN concept is based on the theory of orderly progression of tumor cells within the lymphatic system [78–80]. The SLN is the first lymph node to which the lymphatic system flows and, therefore, the first to receive metastatic tumor cells from the primary tumor site [81]. In breast cancer and malignant melanomas, SLN biopsy is already a part of treatment protocols [78–80]. SLN biopsy may have a role in reliable staging procedures in the clinically negative neck in SCCOT. Although some have reported the overall sensitivity of SLN biopsy as >90% in HNSCC [82, 83], it is not yet possible to say whether the results of SLN identification in SCCOT are consistent and reliable. Additional prospective studies will need to be performed in order to determine the utility of this method for staging the neck in patients with SCCOT.

4.2 Radiotherapy

The elective treatment of clinically node-negative disease in the neck can be accomplished radiotherapeutically as well as surgically. External-beam radiation therapy has cure rates similar to those of patients with early-stage SCCOT [84]. Retrospective studies have also shown better control of neck disease when neck irradiation is added to surgery [85–

88]. However, this treatment is associated with sequellae and complications, including xerostomia, mucositis, and osteoradionecrosis, as well as second primary tumor development. Also, the use of radiation to treat SCCOT may preclude its later use in a population that is at relatively high risk for developing second primary tumors in the head and neck region. Radiotherapy may be given as a single treatment or as an adjunct to surgery, and it is often used as a postoperative surgical adjuvant in patients with node-positive disease, ECS, perineural invasion, or positive or close margins [89]. There is evidence that postoperative radiotherapy to the neck improves local-regional control [90], and for patients with high-risk pathology following surgery, postoperative radiotherapy may significantly improve disease control above survival [91–93].

4.3 Therapeutic neck management

If cervical lymph node metastasis is apparent at presentation, treatment of the neck is mandatory. The radical neck dissection (RND) or modified radical neck dissection (MRND) has been the standard treatment for the therapeutic management of clinically positive cervical lymph node metastasis. However, RND may be an unnecessarily extensive procedure, and there has been a gradual shift toward procedures that are more function-sparing. Increasing evidence in the literature indicates the efficacy of selective neck dissection and MRND in the treatment of positive lymph nodes in the neck [94–96]. In our retrospective study of 220 patients with SCCOT who underwent surgical treatment of the primary lesion and neck dissection, there were no significant differences in the overall and disease-specific survival rates of patients with advanced SCCOT treated with selective neck dissection versus those in the MRND treatment group (Fig. 5) [97]. However, regional disease control was better in the group that had more aggressive surgery, and some patients with extensive nodal disease may benefit from more aggressive treatment of the neck [97]. Prospective randomized clinical studies of outcomes of selective neck dissection for clinically node positive necks will be needed to resolve the issue regarding the optimal extent of neck dissection in N+ patients.

4.4 Role of chemotherapy in high-risk disease

Although chemotherapy is not a curative single modality in SCCOT, it has an important adjunctive role. It is usually used adjunctively or palliatively in cases of very large, unresectable lesions or distant metastasis. Adjuvant chemotherapy has also been reported to improve the rate of organ preservation [98–101]. Many studies report that chemotherapy does not increase overall survival in head and neck cancer patients. However, a statistically significant improvement is seen in disease-free survival with the use of adjuvant chemotherapy [98–104]. Licitra et al. [100] suggested the potential benefits of neoadjuvant chemotherapy in oral cavity cancers. Laramore et al. [105] reported that HNSCC patients with high-risk pathologic features (at least two positive neck nodes, ECS, and/or positive surgical margins) improved more from adjuvant chemotherapy than those in the low-risk group in terms of both tumor control and survival. In addition, for patients with high-risk, advanced stage (III–IV) disease that is expected to relapse, chemotherapy combined with radiotherapy has been shown to be beneficial [106–112]. Platinum compounds and fluorouracil are of the most commonly used chemotherapy agents. These compounds, particularly cisplatin, are also strong radiosensitizers.

5 Biology of SCCOT metastases

The process of metastasis is very complex, and the genetic and biochemical determinants remain incompletely understood in most cancers, including SCCOT. The development of metastasis involves the generation of new blood and lymph vessels (lymphangiogenesis), growth, requiring breakdown of the extra-cellular matrix (ECM), escape from immune surveillance, transport to other sites with adhesion, subsequent invasion of the organ that hosts the metastasis, and tumor proliferation at the secondary site. For successful invasion and metastasis, other “hallmark capabilities” of cancer are also needed, including autonomy in growth signaling, evasion of apoptosis, unresponsiveness to growth inhibitory signaling, limitless replication, and angiogenesis [113].

Angiogenesis, the generation of new blood vessels, plays an important role in the proliferation of primary tumors by maintaining a supply of oxygen and nutrients that supports tumor growth and metastasis [114]. While many factors have been implicated in promoting angiogenesis, vascular endothelial growth factor (VEGF) is one of the most potent mediators of tumor angiogenesis, inducing endothelial cell proliferation, migration, and survival and capillary tube formation [115, 116]. VEGF promotes tumor angiogenesis in HNSCC and many other tumor types [117, 118]. The VEGF family has six members: VEGF(-A), VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placenta growth factor [119]. VEGF(-A) plays essential roles in vasculogenesis and angiogenesis. Although its mechanism is still unclear [120], lymphangiogenesis plays an important role in tumor metastasis, as recent data suggest that the lymphatic system is involved in the metastatic spread of several human cancers, including HNSCC [121–123]. VEGF-C, VEGF-D, and their receptor on the lymphatic endothelium, VEGFR3,

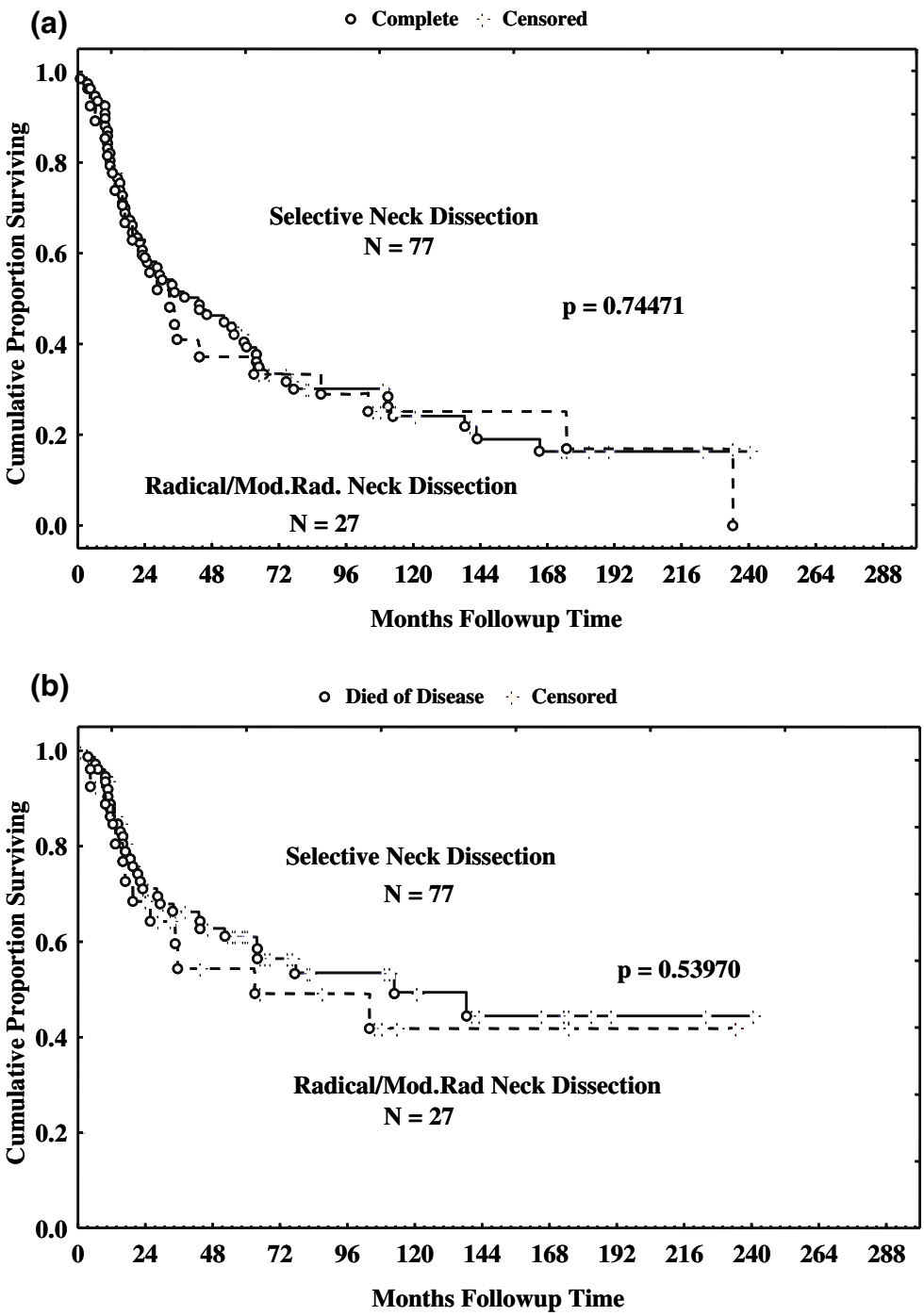

Fig. 5 (**a**) Overall survival of the elective neck treatment group (*solid line*) and radical neck treatment group (*dotted line*) and (**b**) disease-specific survival of the elective neck treatment group (*solid line*) and radical neck treatment group (*dotted line*)

are thought to control lymphangiogenesis. Several studies have reported that overexpression of VEGF is associated with poor prognosis and metastases in SCCOT [124–126], and VEGF-A and/or VEGF-C expression have been reported to correlate with regional lymph node metastases in SCCOT [127–129].

It is thought that the loss of normal cell–cell as well as cell–ECM contacts, are amongst the earliest events in the process of metastasis. Cadherins, proteins that span the intercellular space between two cells, are found predominantly in cells of epithelial origin and are considered tumor suppressor genes, in that loss of expression or function contributes to malignant transformation. E-cadherin, a member of this family, is a calcium-dependent protein that maintains epithelial cell adhesion and polarity [130]. E-cadherin plays a key role in junctional adherence through the binding of the extra-celluar domains of E-cadherin molecules in neighboring cells. This adhesion depends on an association with the cytoplasmic catenins. The catenins are a family of proteins including; α-, β-, and γ-catenins.

β-Catenin plays a key role in Wnt-mediated signal transduction as well as cell adhesion [131, 132]. The loss of E-cadherin/β-catenin-mediated adherens junctions is one of the most important hallmarks of the epithelial–mesenchymal transition (EMT) and tumor progression. Indeed, loss or reduction of E-cadherin expression has been shown to occur early in epithelial carcinogenesis, which correlates with the development of lymph node metastasis in SCCOT [133, 134]. Diniz-Freitas et al. [135] reported that reduced E-cadherin expression in SCCOT is associated with more aggressive tumor behavior and worse prognosis. Mechanistically, cadherins initiate a program of signal transduction that suppresses growth, proliferation, and migration [136]. The reduction of E-cadherin and β-catenin expression has been reported to be associated with regional metastasis of SCCOT [137, 138].

Degradation of the basement membrane that supports the squamous epithelium must occur for a tumor cell to invade and metastasize. Matrix metalloproteinases (MMPs) are a large family of zinc-dependent enzymes that catalyze the disassembly of ECM. MMPs facilitate tumor cell invasion and metastasis by at least three distinct mechanisms: ECM degradation; attachment to ECM components; and cellular motility. Increased MMP expression has been implicated in invasion and metastasis for numerous tumor types, including SCCOT. It has been shown that aberrant expression of MMP-9 is an early event in epithelial carcinogenesis and correlates with aggressive tumor behavior [139, 140].

EMT is an intriguing model that has been espoused to explain certain features of cancer progression, including tumor cell invasion and metastasis [141, 142]. In this process, epithelial cells lose their apical-basal polarity, cell–cell adhesion, and cytoskeletal structure [143]. Once they begin to express surface proteins that characterize mesenchymal cells, they become capable of migrating through the basement membrane and basal lamina into the extracellular space. Induction of EMT in squamous cell carcinoma drives tumor progression [144]. Identifying the mechanisms of EMT has led to a greater understanding of the molecular mechanisms of metastasis and the development of novel targets for prevention of metastasis [142]. In addition, understanding the molecular mechanisms of EMT might provide a novel strategy for cancer therapy [145].

To establish metastasis, tumor proliferation at the secondary site is required. Various growth factors and their receptors regulate tumor proliferation, including interleukin (IL)-8, transforming growth factor (TGF)-α, epidermal growth factor (EGF), and epidermal growth factor receptor (EGFR). EGFR and two of its ligands, TGF-α and EGF, are commonly expressed in head and neck cancer. EGFR is a transmembrane tyrosine kinase receptor. Signaling through this receptor leads to the activation of multiple signaling proteins that initiate a cascade of several signal pathways, including the Ras-Raf-mitogen-activated protein kinase (MAPK) pathway, the phosphatidylinositol 3-kinase (PI3K)-Akt pathway, and the signal transducer and activators of transcription (STAT) pathway, which convey the signal to promote cellular responses, such as proliferation and survival [146]. EGFR overexpression in HNSCC has been reported to be of strong prognostic value [147–152].

5.1 The role of targeting pathways of SCCOT metastases in prognosis and treatment

The Wnt/β-catenin signaling pathway is thought to play a crucial role in the development and progression of cancer [153, 154]. Wnt proteins are a large family of secreted glycoproteins with at least 19 known human family members that bind to the Frizzeled receptors [155–157] and LRP5/6 co-receptors [158, 159]. Wnt/β-catenin signaling, which is only activated when both Frizzeled and LRP5/6 receptors are complexed with Wnt ligands, plays an important role in regulating cell proliferation and differentiation [160]. β-catenin, a key downstream component of the Wnt/β-catenin signaling pathway, also plays an important role in cell–cell adhesion, and a switch between these two processes is associated with EMT and cancer development and progression.

Extracellular Wnt proteins bind to Frizzeled receptors, which in turn mediates phosphorylation of the Dishevelled protein through binding to Axin. Dishevelled inhibits phosphorylation of β-catenin by disrupting a complex consisting of adenomatous polyposis coli (APC), Axin, and glycogen synthase kinase 3-β (GSK3β). Unphosphorylated β-catenin then translocates into the nucleus and associates with the T-cell factor/lymphoid-enhancer factor (Tcf/Lef) family to form a functional transcription factor that mediates the transactivation of target genes involved in the promotion of tumor progression, invasion, and metastasis, such as c-MYC, cyclin D1, MMP7, gastrin, and ITF-2 [161–168].

In various types of carcinoma cells, Wnt/β-catenin signaling initiates proliferation, dedifferentiation, and EMT [169, 170]. In addition, it is known that Wnt/β-catenin signaling directly up-regulates MMP expression [171]. Therefore, activation of the Wnt/β-catenin signaling pathway can have a significant impact on tumor progression. Uraguchi et al. [172] demonstrated that oral squamous cell carcinomas express Wnt members and activate the signaling pathway and suggested that enhanced Wnt expression and signaling accelerate the progression of carcinomas by activating EMT and local invasiveness. Thus, the Wnt/β-catenin signaling pathway could play a central role in the progression and metastasis of SCCOT. Although our understanding of this signaling pathway has significantly improved with the identification of key

regulator proteins over the past decade, little is still known about the role of the Wnt/β-catenin signaling pathway and its principal mediator, β-catenin, in SCCOT. Further work is required to understand the detailed biology of SCCOT metastasis and to evaluate the potential for targeted therapies.

5.2 Models of SCCOT metastases

To analyze the tumor biology of regional metastases of SCCOT, we developed an orthotopic nude mouse model of SCCOT [173]. In this model, we have shown that the tumorigenicity of oral epithelial cancer cells is greater when the cells are injected into the tongue rather than under the skin of nude mice. These tumors are histopathologically similar to SCCOT primary tumors and can develop regional and distant metastases (Fig. 6). In order to generate tumor lines of increased metastatic potential, we have isolated regional metastases from cervical lymph nodes after the development of orthotopic tongue tumors and established new cell lines from them. Cervical and distant metastases were seen with greater frequency after the injection of these new cell lines. Recently, we have established luciferase (Luc)-transduced SCCOT cell lines and orthotopic animal models for studying tumor metastasis of SCCOT *in vivo*. We have introduced the Luc gene into the PBMN-I-GFP retroviral vector and produced a Luc-expressing retrovirus. The virus has been used to establish Luc-transduced stable SCCOT cell lines, and we have analyzed regional metastases of this orthotopic mouse model of SCCOT using the IVIS 200 imaging system (Xenogen Corporation, Berkeley, CA). In this way, we can monitor regional metastases by

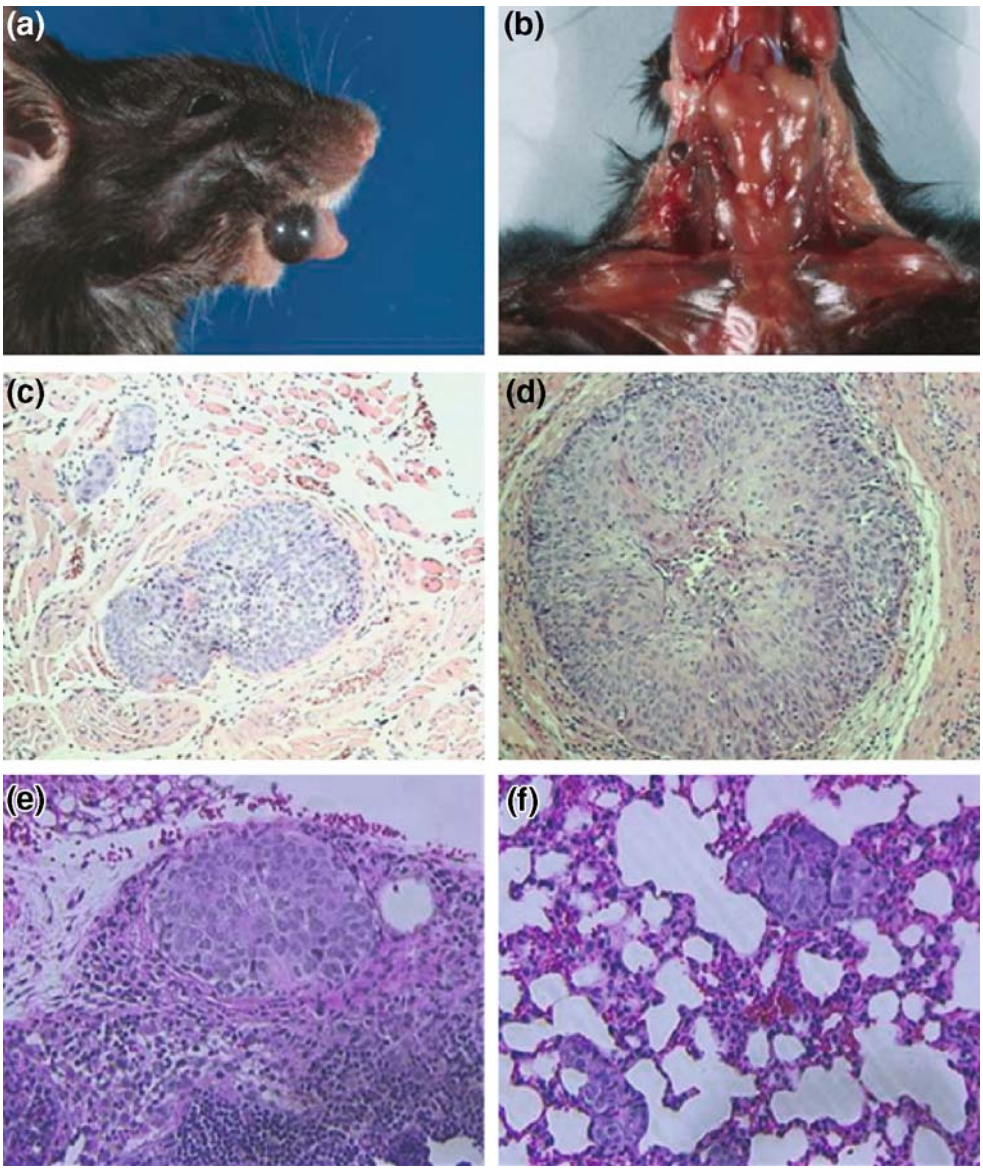

Fig. 6 (**a**) and (**b**) Regional metastasis from orthotopic sublingual implant of B16-BL6 melanoma cells. An obvious tongue tumor and bilateral metastases are seen. (**c**) and (**d**) Comparative histopathology showing the resemblance between the Tu167 human SCCOC cell line grown orthotopically in the tongue of a nude mouse and a human SCCOT tumor. (**e**) Regional metastasis of the orthotopic Tu167G-LN1 SCCOT, established with metastatic lymph nodes of the Tu167 cell line. (**f**) Distant pulmonary metastasis from the orthotopic Tu167G-LN1 SCCOT. Modified from [173]

using localized photon emission (Fig. 7) and also assess quantitatively the volume of the primary tumor and regional metastases. This technique enables us to better study regional metastasis of SCCOT *in vivo*. Although an animal model, especially one with immunosuppressed animals, cannot correlate directly with the metastatic process occurring in patients, these techniques are useful for investigating some of the cellular and molecular mechanisms of metastasis in SCCOT.

5.3 Predictive markers of nodal metastasis of SCCOT

Since lymph node metastasis is the single most reliable independent prognostic factor in patients with SCCOT, it is critical to identify patients who have cervical lymph node metastases even though they have no clinical evidence of nodal spread. In our own series, 33.5% of clinically node-negative (cN0) patients were found to have pathologically involved nodes or pN+ disease after neck dissection. While some patients with small primary tumors have cervical lymph node metastasis, others with larger tumors may have no regional lymph node involvement. As the sensitivity of preoperative imaging by CT or MRI and clinical examination is only ~70% [174], and there are no imaging studies capable of detecting micrometastasis in cervical lymph nodes, predictive markers that indicated a high risk of cervical lymph node metastasis would have a major role in determining the optimal therapeutic strategy for these patients.

Several groups have demonstrated that tumor thickness is a powerful predictive factor for occult lymph node metastasis in oral cancer [66, 133, 175–184]. Recently, Lim et al. [133] reported that patients with SCCOT tumors >4 mm thick should be considered at high risk for late cervical metastasis. Although tumor thickness has gained recognition as an important feature that is predictive of nodal metastasis, the tumor thickness cut-off value has not been definitively determined and ranges from 2 to 10 mm in different series [66, 133, 175–184]. Other histopathologic parameters that have been used to predict nodal disease include keratinization, mode of invasion, intravascular and perineural invasion, and lymphocyte infiltration [185, 186].

Recently, there has been increasing focus on the investigation of molecular markers that will predict cervical lymph node metastasis. Many molecular markers, including EGFR, VEGF, and β-catenin, have been suggested to be predictive factors for cervical lymph node metastases. P53 is a tumor suppressor gene that negatively regulates the cell cycle and serves to protect the integrity of the genome. Approximately 50% of cancers of the head and neck studied contain a mutation of p53 [187]. Studies evaluating the utility of p53 as a predictor of regional nodal disease in SCCOT have had mixed results [188–190]. Cyclin D1 is a proto-oncogene located on chromosome 11q13 [191] that is elevated in response to extra-cellular mitogens and is a rate-limiting regulator of G1-phase progression through the cell cycle [192]. Cyclin D1 has been shown to be associated with increased lymph node stage in anterior tongue cancer [193, 194]. Ki-67 is a proliferation marker, and the Ki-67 index has been reported to be correlated with poor prognosis in SCCOT [195]. With regards to angiogenesis-related factors, tumor microvessel density has been found to be correlated with regional recurrence in T1-3N0 SCCOT [196]. Podoplanin, a mucin-like glycoprotein that is important in lymphangiogenesis [197], has been found to be expressed in oral cancer and correlates with lymph node metastasis and poor clinical outcomes [198]. While there are some data to suggest that evaluating each of these factors in primary SCCOT tumor specimens could have a role in predicting

Fig. 7 Bioluminescence imaging of mice orthotopically transplanted with luciferase-transducecd OSC19 cells. The tips of the mice tongues were injected with OSC19Luc+ cells. Mice were imaged using the IVIS 200 imaging system (Xenogen Corporation, Berkeley, CA). In brief, mice were first injected intraperitoneally with 40 mg/kg luciferin (Xenogen Corporation) and were anesthetized with 2% isoflurane (Abbott Laboratories, Chicago, IL). After 9 days, all mice exhibited localized photon emission at the site of injection. On day 9, Mice 582 and 583 had no cervical lymph node metastases. By day 13, both mice showed a progression of tumor spread from the tongue to the cervical lymph nodes

nodal metastasis, none of these has been validated in larger, prospectively acquired, clinically annotated specimen sets. Thus, further investigation in this area is needed.

Recently, complementary DNA (cDNA) microarray analysis has begun to identify distinct gene expression profiles of HNSCC and to create a preliminary comprehensive database of HNSCC gene expression [199, 200]. Over 20 studies of cDNA microarray analysis of HNSCC have been reported [201]. Roepman et al. [202] investigated the molecular profiling of primary tumors from HNSCC for the potential of predicting the presence of lymph node metastasis at the time of diagnosis. They found 102 predictor genes that correctly predicted local lymph node metastases with cDNA microarray gene expression analysis of 82 squamous cell carcinoma tumor specimens of the oral cavity and oropharynx. They suggested that gene expression of the primary tumor could decipher the metastatic state. O'Donnell et al. [203] also reported a metastatic gene expression set from 18 primary tumors of patients with SCCOT. Comparative genomic hybridization (CGH), which was first developed by Kallioniemi et al. in 1992 [204], is helpful for detecting individual gains, losses, and amplifications in genetic information. Several genetic analyses of HNSCC based on CGH have been reported [205–208]. Further development of these biological investigations may provide the means for screening SCCOT patients for the presence of large numbers of genes or proteins simultaneously and should be helpful as biomarkers to predict regional metastasis of SCCOT through revealing the gene expression signature.

6 Conclusion

In this review, we have focused on the clinical significance, current treatment strategies, and mechanisms of metastasis of SCCOT. Cervical lymph node metastasis is a critical event for patients with SCCOT, as this is the most reliable predictor of poor treatment outcomes. Therefore, patients found to have node-positive SCCOT, especially with ECS, need treatment intensification to improve treatment outcomes. As neck dissection is currently required to determine the nodal status and the presence or absence of ECS, methods of determining the biologic aggressiveness of disease from analysis of the primary tumor specimen are highly desirable. Contemporary biological tools, including cDNA microarrays, array-based CGH, and integrative genomics approaches should be very useful, and prospective multi-institutional analyses of these techniques should yield important progress in this area. Through this type of work, it is anticipated that novel potential targets for therapy will emerge, which could improve survival and quality of life for patients with SCCOT.

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