ORAL DISEASES

Oral Diseases (2011) 17, 462–468 doi:10.1111/j.1601-0825.2010.01781.x

www.wiley.com

REVIEW ARTICLE

The cellular and molecular mechanisms of bone invasion by oral squamous cell carcinoma

E Jimi[1,6], H Furuta[1,2], K Matsuo[3], K Tominaga[4,6], T Takahashi[5], O Nakanishi[2]

[1]*Division of Molecular Signaling and Biochemistry, Department of Bioscience;* [2]*Division of Dental Anesthesiology, Department of Control of Physical Functions;* [3]*Division of Oral Pathology, Department of Bioscience;* [4]*Division of Maxillofacial Diagnostic and Surgical Science;* [5]*Division of Oral and Maxillofacial Reconstructive Surgery, Department of Oral and Maxillofacial Surgery;* [6]*Center for Oral Biological Research, Kyushu Dental College, Manazuru, Kokurakita-ku, Kitakyushu, Fukuoka, Japan*

Oral squamous cell carcinomas (SCCs) are malignant tumours that frequently invade the mandibular bone and bone invasion is a common clinical problem. Recent studies have revealed that bone resorption by osteoclasts is an important step in the process of bone invasion by oral SCCs. However, the cellular and molecular mechanisms of bone invasion by oral SCCs remain unclear. Oral SCCs invade the mandibular bone through an erosive, mixed or infiltrative pattern that correlates with clinical behaviours. The expressions of interleukin (IL)-6, IL-11, tumour necrosis factor (TNF) α and parathyroid hormone-related protein (PTHrP) were higher in the infiltrative pattern than in the erosive pattern. These cytokines lead to receptor activator of NF-κB ligand (RANKL) expression or osteoprotegerin (OPG) suppression not only in oral SCC cells but also in cancer stromal cells to induce osteoclastogenesis. Taken together, oral SCCs provide a suitable microenvironment for osteoclastogenesis to regulate the balance of RANKL and OPG. In this review, we introduce recent advances in the knowledge of the cellular and molecular mechanisms, by which oral SCC invades mandibular bone based on the recent findings of our lab and others.

Oral Diseases (2011) **17**, 462–468

Keywords: oral squamous cell carcinomas; bone invasion; IL-6; RANKL

Introduction

Oral squamous cell carcinoma (SCC) represents 1–2% of all human malignancies in Japan (Okamoto *et al*, 2000). However, oral SCC is the sixth most common cancer and more than 300 000 new cases are diagnosed each year worldwide (Choi and Myers, 2008). It is estimated that 7000 patients die from this type of cancer each year. Malignant tumours of the oral cavity account for the majority (36%) of the newly diagnosed cases, followed by larynx cancer, hypopharynx cancer and all head and neck cancers (more than 90% of which are squamous cell carcinoma) (Muir and Weiland, 1995; Uchida, 1998; Otoh *et al*, 2005). At least 95% of oral SCC cases occur in individuals aged 40 years or older and SCC occurs twice as often in men as in women. Men are affected more often than women because, in most countries, men tend to be heavier users of tobacco and alcohol. The most common sites for squamous cell carcinoma are the tongue and gingiva. A malignant tumour of epithelial origin, SCC has a regional distribution that affects the biological activity of the neoplasm. The behaviour of SCC depends on its site of origin and each anatomical site has its own particular spread pattern and prognosis (Muir and Weiland, 1995; Uchida, 1998; Otoh *et al*, 2005).

Oral SCC usually presents first as slightly elevated surface lesions with erythema (Sokolosky *et al*, 1986; Müller and Slootweg, 1990). The lesions, termed erythroplasia, should be biopsied. These early red lesions are asymptomatic and may be either carcinoma *in situ* or invasive carcinoma. One-third of lesions are pure white; they are known as leukoplakia but only 10% of them are carcinoma *in situ* or invasive carcinoma. Tender, painful lesions are usually suggestive of perineural invasions. When lesions become palpable masses, symptoms such as a vague persistent sore throat or ear infection occur. The clinical features may vary according to the affected intraoral subsite. In more advanced cases, dissemination to the ipsilateral submandibular and jugulodigastric nodes are common, and the patient may present with a mass in the neck (Sokolosky *et al*, 1986; Müller and Slootweg, 1990; Stoeckli *et al*, 2002). When lymph node or remote bone and organ metastases

Correspondence: Eijiro Jimi, Division of Molecular Signaling and Biochemistry, Department of Biosciences, Kyushu Dental College, 2-6-1 Manazuru, Kokurakita-ku, Kitakyushu, Fukuoka 803-8580, Japan. Tel: +81-93-285-3047, Fax: +81-93-582-6000, E-mail: ejimi@kyu-dent.ac.jp
Received 20 August 2010; Revised 13 October 2010; accepted 31 October 2010

are associated with an early oral primary lesion, a second, more advanced primary upper aerodigestive or lung cancer is often responsible for the metastases (Sokolosky *et al*, 1986).

Squamous cell carcinomas of the gingiva are the second most common carcinoma of the oral cavity next to those of the tongue (Muir and Weiland, 1995; Uchida, 1998; Otoh *et al*, 2005). Unlike lingual carcinomas, gingival carcinomas easily invade underlying jawbone. Gingival SCCs are thus among the very rare human neoplasms that directly invade the bone. In particular, the extension of oral SCC into mandibular bone classifies the tumour as stage IV and is considered an indicator of poor prognosis (Shah and Lydiatt, 1999). Advanced oral SCC has a high mortality and treatment is complicated by disruption of speech and swallowing after surgical resection. Patients with mandibular invasion should be treated surgically but the extent of mandibular resection required remains controversial. Resection of the mandibular bone leads to physical damage and frequently results in psychological problems for patients (Totsuka *et al*, 1991a,b).

Although some controversy still exists, the bone destruction associated with invasion is thought to be mediated by osteoclasts rather than directly by the carcinoma (Guise and Mundy, 1998). Invasion of the maxilla or mandible by oral SCC is currently ascertained only by radiological inspection. In some patients, the tumour causes invasive resorption of the mandible; in others, it is in contact with the mandible but does not show invasive progression into the mandible. Therefore, radiographic diagnosis alone is inadequate and the development of a new diagnostic technique that allows accurate estimation of the extent of mandibular invasion is needed (Totsuka *et al*, 1991a,b).

Recently, techniques utilizing molecular biology, cancer genetics and cancer biology have been developed (Stephenson, 2000) and used to generate new data on the cellular and molecular mechanisms of bone invasion by oral SCC. Therefore, in this review, we first summarize the histological patterns of bone invasion by oral SCC. We next describe recent advances in knowledge of the cellular and molecular mechanisms, by which oral SCC invades mandibular bone based on the recent findings of our lab and others. Finally, we discuss possible molecular therapeutic targets for oral SCCs.

Histological patterns of bone invasion by oral squamous cell carcinoma

Squamous cell carcinoma is thought to arise from keratinizing or malpighian epithelial cells (McGregor and MacDonald, 1988, 1993). The hallmark of squamous cell carcinoma is the presence of keratin, or 'keratin pearls', on histological samples. These are well-formed desmosome attachments and intracytoplasmic bundles of keratin tonofilaments. Morphologically, squamous cell carcinoma is variable and may appear as plaques, nodules, or verrucae that may be scaly or ulcerated and white, red, or brown (McGregor and MacDonald, 1988, 1993).

Recent studies have shown that once the oral SCC has invaded the mandible, it may progress through the bone in an erosive, mixed or infiltrative histological pattern (Totsuka *et al*, 1991b; Slootweg and Muller, 1989; Carter *et al*, 1983). The erosive pattern of bone invasion is marked by a broad pushing front, a sharp interface between tumour and bone, osteoclastic bone resorption and fibrosis along the tumour front and an absence of bone islands within the tumour mass (Figure 1a). In contrast, the infiltrative pattern of bone invasion is characterized by nests and projections of tumour cells along an irregular front, residual bone islands within the tumour and haversian system penetration (Figure 1b) (Müller and Slootweg, 1990; Totsuka *et al*, 1991a). SCC nests are located within stromata with polyhedral fibroblasts. The stroma is myxoid with abundant delicate reticulin fibres and few collagen fibres (Ito *et al*, 2003). Cases exhibiting features of both patterns are described as having a mixed pattern. The histological pattern of mandibular invasion seems to correlate with the clinical behaviour. In fact, the infiltrative lesions are more likely to have primary, regional and distant recurrence. According to Wong's report, the 3-year disease-free survival in the infiltrative pattern was 30% and that in the erosive pattern was 73% (Wong *et al*,

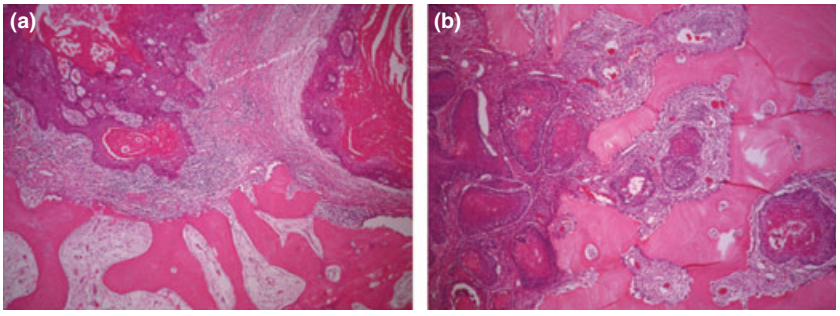

Figure 1 Representative samples of gingival SCC with erosive (**a**) and infiltrative patterns (**b**). The surgically resected samples were decalcified and stained with haematoxylin and eosin (H&E). (**a**) The erosive pattern of bone invasion is marked by a broad pushing front, a sharp interface between tumour and bone, osteoclastic bone resorption and fibrosis along the tumour front and an absence of bone islands within the tumour mass (×40). (**b**) The infiltrative pattern of bone invasion is characterized by nests and projections of tumour cells along an irregular front, residual bone islands within the tumour and haversian system penetration (×40)

2000). It has been suggested that the erosive pattern of oral SCC extends in a more predictable fashion and may be treated with marginal mandibulectomy rather than segmental resection. Therefore, the identification of these distinct histological patterns raised the possibility that each pattern may exhibit a different behaviour, which questions previous assumptions that mandibular invasion presents a universally ominous sign. Furthermore, the postoperative pathological determination of the bone invasion pattern provides important prognostic information and therefore should be routinely commented on by pathologists reviewing cases with mandibular invasion (Wong *et al*, 2000).

The molecular mechanisms regulating osteoclast differentiation and function

The osteoclast, which is the sole bone-resorbing cell, is a unique polykaryon, whose activity in the context of the osteoblast dictates skeletal mass. The differentiation and activation of osteoclasts are closely regulated. Osteoclastic bone resorption consists of several steps: the proliferation of osteoclast precursors and hematopoietic cells, differentiation of progenitors into mononuclear prefusion osteoclasts (pOCs), fusion of pOCs into multinucleated osteoclasts, formation of the clear zone (actin ring) and ruffled border (activation) and apoptosis. It has been proposed that osteoblasts or bone marrow stromal cells are involved in osteoclastogenesis through a mechanism involving cell-to-cell contact with osteoclast precursors. This hypothesis was proved by the discovery of a new member of the TNF ligand family: receptor activator of nuclear factor-κB ligand (RANKL) (Suda *et al*, 1999; Nakashima *et al*, 2003; Boyle *et al*, 2003).

Osteoclastogenesis is regulated by a complex signalling system that involves three essential molecules: RANKL, its receptor (RANK) and its decoy receptor osteoprotegerin (OPG). RANKL is expressed in osteoblasts and bone marrow stromal cells. RANKL and macrophage colony-stimulating factor (M-CSF) can induce osteoclastogenesis in the absence of osteoblasts suggesting that RANKL and M-CSF are both crucial for osteoclast development. RANK is expressed as a transmembrane heterotrimer on the surface of hematopoietic osteoclast progenitors, mature osteoclasts, chondrocytes and mammary gland epithelial cells. Interactions between RANKL and RANK have been shown to induce differentiation from osteoclast precursors to osteoclasts. Osteoblasts and stromal cells also synthesize osteoprotegerin. Thus, a balance between the expression levels of RANKL and osteoprotegerin is crucial for regulating osteoclast differentiation and function (Figure 2) (Suda *et al*, 1999; Nakashima *et al*, 2003; Boyle *et al*, 2003). This notion is supported by the finding that the targeted disruption of RANKL and OPG in mice results in severe osteopetrosis due to the absence of osteoclasts and osteoporosis by an increased number of osteoclasts, respectively. Furthermore, mice lacking RANK have a complete block of osteoclast development that can be rescued by the transplantation of bone marrow cells from wild-type mice, indicating that RANK-deficient mice have an intrinsic defect in osteoclast function (Nakashima *et al*, 2003; Boyle *et al*, 2003).

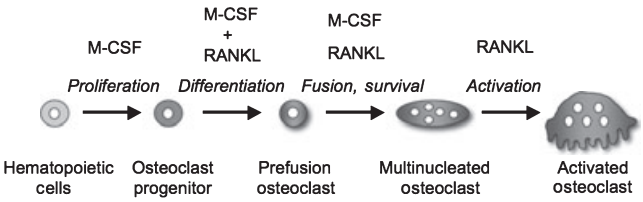

Figure 2 The differentiation pathway of osteoclast progenitors into functionally active osteoclasts. Osteoclastic bone resorption consists of multiple steps: the proliferation of osteoclast precursors and hematopoietic cells, differentiation of progenitors into mononuclear prefusion osteoclasts (pOCs), fusion of pOCs into multinucleated osteoclasts, clear zone (actin ring) and ruffled border formation (activation) and apoptosis. RANKL is the sole factor for both osteoclast differentiation and function

The cellular and molecular mechanisms regulating osteoclast differentiation and function by oral SCC

The role of cytokines released from oral SCC cells on osteoclast formation

Recent studies have established that bone resorption by osteoclasts is an important step in the process of bone invasion and metastasis in several malignancies (Guise and Mundy, 1998). Once cancer cells invade the bones, they proliferate inhibiting their apoptosis as the bone is a storehouse of variety of cytokines and growth factors and thus provides an extremely fertile environment for cell growth (Guise and Mundy, 1998). Carter *et al* (1983) demonstrated that oral SCC cells produced prostaglandins E_2 and F_2, which are involved in osteoclastic bone resorption. Several *in vitro* and animal experiments using human oral SCC cells have shown that tumour cells produce several cytokines, such as transforming growth factor (TGF)-β, interleukin (IL)-1β, IL-6, IL-11, TNFα and parathyroid hormone-related protein (PTHrP) (Guise and Mundy, 1998). Recently, Shibahara *et al* (2005) histopathologically classified 38 specimens of lower gingival SCC into an invasion group (23 cases) and a non-invasion group (15 cases) and then examined protein expression of TGF-β, IL-1α/β, IL-6, IL-11, IL-18, TNFα and PTHrP using immunohistochemical techniques. The invasion group showed a high level of expression of IL-6, IL-11, TNFα and PTHrP. In contrast, expression of TGF-β, IL-1α/β and IL-18 was not different between these two groups suggesting that various cancer-derived cytokines, such as IL-6, IL-11, TNFα and PTHrP, play important roles in bone invasion by oral SCC (Shibahara *et al*, 2005).

IL-6 is an important cytokine that stimulates osteoclastic bone resorption by inducing RANKL expression in osteoblasts (O'Brien *et al*, 1999). More importantly, several reports have revealed that the serum levels of IL-6 are elevated in patients with head and neck SCC and the elevation correlates with clinical outcomes such as radioresistance, recurrence and survival (Jabońska

et al, 1997; Chen *et al*, 1999; Duffy *et al*, 2008). Furthermore, PTHrP, which was originally identified as a factor responsible for humoral hypercalcemia of malignancy (Burtis *et al*, 1987), is synthesized by many malignant tumours, including oral SCC (Shibahara *et al*, 2005). Although previous reports have suggested the importance of IL-6 and PTHrP in oral SCC-induced bone resorption, few studies have investigated their roles in osteoclastogenesis induced by oral SCC. Therefore, Kayamori *et al* (2010) recently performed microarray analysis using 43 human oral SCC specimens and found that many of the specimens highly expressed PTHrP; but a few overexpressed IL-6 mRNA. However, the expression of IL-6 was observed in both cancer cells and host stromal cells. In the same study, conditioned medium (CM) from oral SCC cell lines stimulated RANKL expression in stromal cells and osteoclastogenesis. Antibodies against PTHrP and IL-6 receptor suppressed RANKL expression in stromal cells and osteoclastogenesis induced by cancer cell-derived CM. These data suggest that oral SCC provides a suitable microenvironment for osteoclastogenesis not only by producing IL-6 and PTHrP but also by stimulating stromal cells to synthesize IL-6 (Figure 3) (Kayamori *et al*, 2010).

RANKL/RANK system in osteoclastic bone resorption

The RANKL/RANK signalling pathway also regulates bone invasion and bone metastasis by several types of human cancer, such as breast cancer (Thomas *et al*, 1999; Bendre *et al*, 2003), prostate cancer (Brown *et al*, 2001), myeloma (Sezer *et al*, 2003; Yaccoby *et al*, 2004) and oral SCC (Nagai *et al*, 2000). It has been reported that cancer cells expressing RANKL were able to induce osteoclastogenesis even in the absence of other accessory cells (Nagai *et al*, 2000; Farrugia *et al*, 2003). On the other hand, not all cancers express RANKL; and cell-to-cell contact between cancer cells and host cells does not always lead to RANKL expression (Giuliani *et al*, 2001; Ohshiba *et al*, 2003).

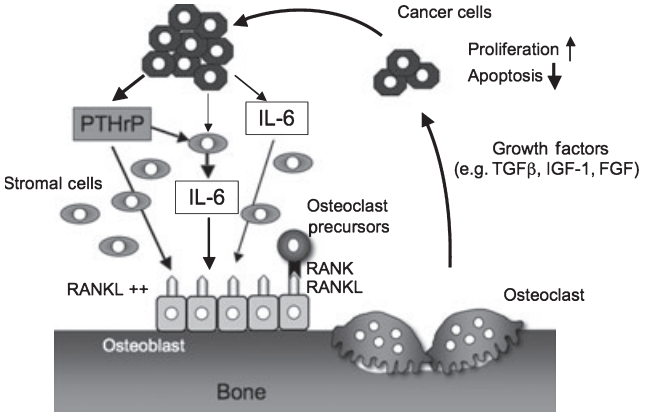

Figure 3 Schematic representation of the role of IL-6 and PTHrP in oral SCC-induced osteoclast formation. Bone is a storehouse of various growth factors, including insulin-like growth factor and transforming growth factor β. IL-6 and PTHrP produced by oral SCC cells and IL-6 produced by stromal cells induce fibroblasts/osteoblasts to synthesize RANKL and they subsequently induce osteoclast formation. These growth factors are released into the bone microenvironment in active forms as a consequence of osteoclastic bone resorption and they stimulate cancer cell proliferation and inhibit apoptosis

A histological analysis of cancer stromal cells shows that they comprise various types of cells, including fibroblasts, myofibroblasts, endothelial cells and inflammatory cells (Ito *et al*, 2003; Ishikuro *et al*, 2008). These cells and their products play crucial roles in establishing the tumour microenvironment, which regulates the proliferation, survival, invasion and metastasis of cancer cells. The fibrous stroma intervened between the invading cancer nests and the resorbing bone surface (Figure 1). These results strongly indicate that stromal cells regulate osteoclast formation induced by oral SCCs. As described previously, IL-6 and PTHrP released from oral cancer cells induced osteoclastogenesis through RANKL expression in stromal cells (Kayamori *et al*, 2010).

We also have examined the molecular mechanisms, by which oral SCCs regulate osteoclastogenesis using BHY cells. BHY cells were derived from a highly differentiated human squamous cell carcinoma from the lower alveolus. The original tumour of BHY cells was highly invasive to the mandibular bone and the muscle layer of the oral floor and grew aggressively at the primary site (Kawamata *et al*, 1997). We have shown that although BHY cells, which were highly invasive into mandibular bone when inoculated into the masseter in nude mice (Okamoto *et al*, 2000), expressed RANKL on their cell surface, they failed to induce osteoclastogenesis in cocultures of mouse bone marrow cells (BMCs) and BHY cells (Tada *et al*, 2005). However, adding BHY cells to a coculture of mouse primary osteoblasts (POBs) and BMCs markedly induced osteoclastogenesis in the absence of osteotropic factors. To further analyse possible mechanisms of tumour cell-induced osteoclastogenesis in our coculture system, we examined the expression level of mouse and human RANKL, OPG and GAPDH using species-specific PCR primers in cocultures of POBs and BHY cells. Neither mouse nor human RANKL expression was changed in cocultures of POBs with BHY cells. Adding BHY cells suppressed mouse OPG mRNA expression and protein production by POBs. This is consistent with the finding that BHY cells do not enhance osteoclastogenesis in cocultures of BMCs and POBs from OPG-deficient mice, suggesting that cell-to-cell contact of BHY cells and osteoblasts downregulates OPG expression in osteoblasts. To further determine whether this holds true *in vivo*, we examined the relationship between osteoclasts and SCC cells in mandibular bone lesions caused by invasive oral SCC in five patients. The immunohistochemical analysis showed a reduction of OPG expression in stromal cells in osteolytic lesions when compared with normal lesions. These results strongly indicate that induction of RANKL expression or reduction of OPG expression in cancer stromal cells is more important for osteoclast formation than the expression of RANKL in oral SCC cells.

Despite the importance of oral SCC cells for osteoclastogenesis, the role of its mechanisms in osteoclast

function, such as multinucleation, survival and pit-forming activity, is not fully understood. Therefore, we examined the effect of oral SCCs on osteoclast function using BHY cells. Purified osteoclasts rapidly died due to spontaneous apoptosis, but M-CSF, IL-1 and RANKL prevented the apoptotic cell death of purified osteoclasts (Jimi *et al*, 1995, 1999). We therefore examined the effects of BHY cells and BHY-CM on the survival of osteoclasts. Both BHY cells and conditioned medium from BHY cells supported the survival of osteoclast survival by suppressing Bim expression, a member of the BH (Bcl-2 homology) three-only family of pro-apoptotic proteins. Enriched osteoclasts (purity was $<70\%$) did not have pit-forming activity and adding osteoblasts or RANKL induced their pit-forming activity (Jimi *et al*, 1996; Jimi *et al*, 1999). Therefore, we examined the effects of BHY cells and BHY-CM on the pit-forming activity of enriched osteoclasts. Adding BHY cells but not the BHY-CM induced pit-forming activity by osteoclasts and adding OPG abrogated the activity (Tada *et al*, 2009). Taken together, oral SCC cells regulate not only osteoclast formation but also function (Figure 4).

Growth factors released from bone matrix stimulates tumour cell growth

Bone is a good environment for the progression of bone invasion. It has been suggested that oral SCC cells release soluble factors that activate osteoclast differentiation and function directly or indirectly via osteoblasts (Figure 3) (Guise and Mundy, 1998). During bone destruction, osteoclasts release various growth factors, including insulin-like growth factor (IGF-1), fibroblast growth factor (FGF) and TGF β that are deposited into mineralized bone matrix (Figure 3). These growth factors enhance the local microenvironment surrounding the tumour cells, which express receptors for these growth factors and stimulate tumour cell proliferation and inhibit their apoptosis (Partridge *et al*, 1996; Papadimitrakopoulou *et al*, 2006; Goda *et al*, 2010). This cycle, called the 'vicious cycle', has been proposed to explain tumour development in bone (Guise, 2009).

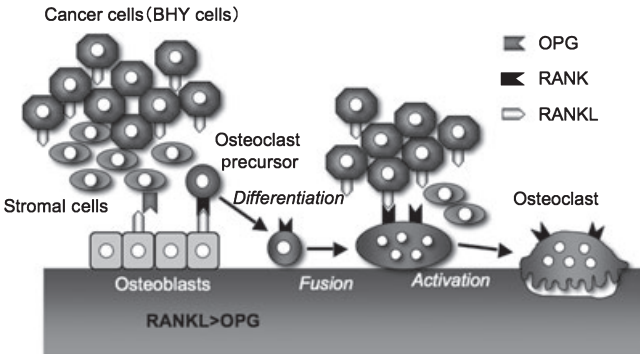

Figure 4 Schematic representation of the role of the RANKL/RANK system on oral SCC-induced osteoclast differentiation and function in BHY cells. BHY cells induce fibroblast stromal cells/osteoblasts to synthesize RANKL or suppress OPG expression. This subsequently induces osteoclast formation and bone resorption activity. BHY cells also directly modulate osteoclastic function such as survival, multinucleation and bone resorption activity

Conclusions and Perspectives

The presence of mandibular invasion is an important criterion when considering surgical intervention. However, it is not yet possible to preoperatively determine from clinical findings whether bone invasion has taken place or ascertain the degree of malignancy from cytological evidence obtained by biopsy. This underscores the urgent need for more accurate analysis (Totsuka *et al*, 1991a,b).

Oral SCCs invade mandibular bone through an erosive, mixed or infiltrative pattern and poor prognosis is highly correlated with the infiltrative pattern (Totsuka *et al*, 1991b; Slootweg and Muller, 1989; Carter *et al*, 1983). Among cancer-related cytokines, IL-6, IL-11, TNFα and PTHrP are more highly expressed in the infiltrative pattern than in the erosive pattern (Shibahara *et al*, 2005). Furthermore, IL-6 is produced in oral SCC cells and cancer stromal cells to induce RANKL expression in conjunction with PTHrP in stromal cells and osteoclastogenesis (Kayamori *et al*, 2010). Although there is still controversy about whether oral SCCs expressing RANKL or stromal cells expressing RANKL contribute more to osteoclastogenesis, oral SCC provides a suitable microenvironment for osteoclastogenesis to regulate the balance of RANKL and osteoprotegerin (Tada *et al*, 2005; Kayamori *et al*, 2010).

In the past few decades, a great deal of progress has been made in our understanding of the pathogenesis of oral SCC. In addition, new approaches have been developed in the fields of molecular biology, cancer genetics and cancer biology to examine the cellular and molecular mechanisms of bone invasion by oral SCCs. Using these techniques, the specific molecular events involved in bone invasion of this cancer are gradually being clarified. The development of targeted approaches (e.g. IL-6, RANKL) to oral SCC analysis will yield an accurate estimation of bone invasion and contribute to the development of target therapy for oral SCC.

Recently, the RANKL inhibitor denosumab, a human monoclonal antibody against human RANKL has been developed and is being tested in the clinic. A phase 1 clinical trial was conducted with denosumab in 54 patients with multiple myeloma and breast cancer with radiographically evident bone lesions (Body *et al*, 2006). The inhibition of osteoclast differentiation and function by blocking RANKL/RANK constitutes a potentially novel approach to maintaining skeletal integrity. Indeed, blocking RANKL/RANK with sRANK or OPG successfully prevents the development of bone invasion induced by oral SCCs.

Acknowledgements

All protocols for the present experiments were reviewed and approved by the Kyushu Dental College Research Ethics Committee, Fukuoka, Japan (Approval number 09–17). Eijiro

Jimi designed the study, performed the literature review, prepared the initial and final versions of the paper and submitted the document. Hiroyuki Furuta performed the literature review and reviewed the intermediate drafts. Kou Matsuo prepared pathohistology images and reviewed the intermediate drafts. Kazuhiro Tominaga and Tetsu Takahashi provided clinical samples and reviewed the intermediate drafts. Osamu Nakanishi reviewed the intermediate and final versions of the paper. We declare no conflict of interest. This work was supported by a grant-in-aid from Kyushu Dental College Internal Grants (to E.J., K.M., K.T., T.T., and O.N.).

References

Bendre M, Gaddy D, Nicholas RW, Suva LJ (2003). Breast cancer metastasis to bone: it is not all about PTHrP. *Clin Orthop Relat Res* **415**(Suppl.)**:** S39–45.

Body JJ, Facon T, Coleman RE *et al* (2006). A study of the biological receptor activator of nuclear factor-κB ligand inhibitor, denosumab, in patients with multiple myeloma or bone metastases from breast cancer. *Clin Cancer Res* **12:** 1221–1228.

Boyle WJ, Simonet WS, Lacey DL (2003). Osteoclast differentiation and activation. *Nature* **423:** 337–342.

Brown JM, Corey E, Lee ZD *et al* (2001). Osteoprotegerin and rank ligand expression in prostate cancer. *Urology* **57:** 611–616.

Burtis WJ, Wu T, Bunch C *et al* (1987). Identification of a novel 17,000-dalton parathyroid hormone-like adenylate cyclase-stimulating protein from a tumor associated with humoral hypercalcemia of malignancy. *J Biol Chem* **262:** 7151–7156.

Carter RL, Tsao SW, Burman JF, Pittam MR, Clifford P, Shaw HJ (1983). Patterns and mechanisms of bone invasion by squamous carcinomas of the head and neck. *Am J Surg* **146:** 451–455.

Chen Z, Malhotra PS, Thomas GR *et al* (1999). Expression of proinflammatory and proangiogenic cytokines in patients with head and neck cancer. *Clin Cancer Res* **5:** 1369–1379.

Choi S, Myers JN (2008). Molecular pathogenesis of oral squamous cell carcinoma: implications for therapy. *J Dent Res* **87:** 14–32.

Duffy SA, Taylor JM, Terrell JE *et al* (2008). Interleukin-6 predicts recurrence and survival among head and neck cancer patients. *Cancer* **113:** 750–757.

Farrugia AN, Atkins GJ, To LB *et al* (2003). Receptor activator of nuclear factor-κB ligand expression by human myeloma cells mediates osteoclast formation *in vitro* and correlates with bone destruction *in vivo*. *Cancer Res* **63:** 5438–5445.

Giuliani N, Bataille R, Mancini C, Lazzaretti M, Barille S (2001). Myeloma cells induce imbalance in the osteoprotegerin/osteoprotegerin ligand system in the human bone marrow environment. *Blood* **98:** 3527–3533.

Goda T, Shimo T, Yoshihama Y *et al* (2010). Bone destruction by invading oral squamous carcinoma cells mediated by the transforming growth factor-β signalling pathway. *Anticancer Res* **30:** 2615–2623.

Guise TA (2009). Breaking down bone: new insight into site-specific mechanisms of breast cancer osteolysis mediated by metalloproteinases. *Genes Dev* **23:** 2117–2123.

Guise TA, Mundy GR (1998). Cancer and bone. *Endocr Rev* **19:** 18–54.

Ishikuro M, Sakamoto K, Kayamori K *et al* (2008). Significance of the fibrous stroma in bone invasion by human gingival squamous cell carcinomas. *Bone* **43:** 621–627.

Ito M, Izumi N, Cheng J *et al* (2003). Jaw bone remodeling at the invasion front of gingival squamous cell carcinomas. *J Oral Pathol Med* **32:** 10–17.

Jabońska E, Piotrowski L, Grabowska Z (1997). Serum Levels of IL-1b, IL-6, TNF-a, sTNF-RI and CRP in patients with oral cavity Cancer. *Pathol Oncol Res* **3:** 126–129.

Jimi E, Shuto T, Koga T (1995). Macrophage colony-stimulating factor and interleukin-1 α maintain the survival of osteoclast-like cells. *Endocrinology* **136:** 808–811.

Jimi E, Nakamura I, Amano H *et al* (1996). Osteoclast function is activated by osteoblastic cells through a mechanism involving cell-to-cell contact. *Endocrinology* **137:** 2187–2190.

Jimi E, Akiyama S, Tsurukai T *et al* (1999). Osteoclast differentiation factor acts as a multifunctional regulator in murine osteoclast differentiation and function. *J Immunol* **163:** 434–442.

Kawamata H, Nakashiro K, Uchida D, Harada K, Yoshida H, Sato M (1997). Possible contribution of active MMP2 to lymph-node metastasis and secreted cathepsin L to bone invasion of newly established human oral-squamous-cancer cell lines. *Int J Cancer* **70:** 120–127.

Kayamori K, Sakamoto K, Nakashima T *et al* (2010). Roles of interleukin-6 and parathyroid hormone-related peptide in osteoclast formation associated with oral cancers: significance of interleukin-6 synthesized by stromal cells in response to cancer cells. *Am J Pathol* **176:** 968–980.

McGregor AD, MacDonald DG (1988). Routes of entry of squamous cell carcinoma to the mandible. *Head Neck* **10:** 294–301.

McGregor AD, MacDonald DG (1993). Patterns of spread of squamous cell carcinoma to the ramus of the mandible. *Head Neck* **15:** 440–444.

Muir C, Weiland L (1995). Upper aerodigestive tract cancers. *Cancer* **75:** 147–153.

Müller H, Slootweg PJ (1990). Mandibular invasion by oral squamous cell carcinoma. Clinical aspects. *J Craniomaxillofac Surg* **18:** 80–84.

Nagai N, Kyakumoto S, Sato N (2000). Cancer cells responsible for humoral hypercalcemia express mRNA encoding a secreted form of ODF/TRANCE that induces osteoclast formation. *Biochem Biophys Res Commun* **269:** 532–536.

Nakashima T, Wada T, Penninger JM (2003). RANKL and RANK as novel therapeutic targets for arthritis. *Curr Opin Rheumatol* **15:** 280–287.

O'Brien CA, Gubrij I, Lin SC, Saylors RL, Manolagas SC (1999). STAT3 activation in stromal/osteoblastic cells is required for induction of the receptor activator of NF-κB ligand and stimulation of osteoclastogenesis by gp130-utilizing cytokines or interleukin-1 but not 1,25-dihydroxyvitamin D_3 or parathyroid hormone. *J Biol Chem* **274:** 19301–19308.

Ohshiba T, Miyaura C, Inada M, Ito A (2003). Role of RANKL-induced osteoclast formation and MMP-dependent matrix degradation in bone destruction by breast cancer metastasis. *Br J Cancer* **88:** 1318–1326.

Okamoto M, Hiura K, Ohe G *et al* (2000). Mechanism for bone invasion of oral cancer cells mediated by interleukin-6 *in vitro* and *in vivo*. *Cancer* **89:** 1966–1975.

Otoh EC, Johnson NM, Olasoji HO, Danfillo IS, Adeleke OA (2005). Intra-oral carcinomas in Maiduguri, north-eastern Nigeria. *Oral Dis* **11:** 379–385.

Papadimitrakopoulou VA, Brown EN, Liu DD *et al* (2006). The prognostic role of loss of insulin-like growth factor-binding protein-3 expression in head and neck carcinogenesis. *Cancer Lett* **239:** 136–143.

Partridge M, Kiguwa S, Luqmani Y, Langdon JD (1996). Expression of bFGF, KGF and FGF receptors on normal oral mucosa and SCC. *Eur J Cancer B Oral Oncol* **32B:** 76–82.

Sezer O, Heider U, Zavrski I, Kuhne CA, Hofbauer LC (2003). RANK ligand and osteoprotegerin in myeloma bone disease. *Blood* **101:** 2094–2098.

Shah J, Lydiatt WM (1999) Buccal mucosa, alveolus, retromolar trigone, floor of mouth, hard palate, and tongue tumors. In: Thawley SE, ed. *Comprehensive Management of Head and Neck Tumors*. 2nd edn. WB Saunders: Philadelphia, pp. 686–693.

Shibahara T, Nomura T, Cui NH, Noma H (2005). A study of osteoclast-related cytokines in mandibular invasion by squamous cell carcinoma. *Int J Oral Maxillofac Surg* **34:** 789–93.

Slootweg PJ, Muller H (1989). Mandibular invasion by oral squamous cell carcinoma. *J Craniomaxillofac Surg* **17:** 69–74.

Sokolosky M Jr, Bouquot JE, Graves RW (1986). Esophageal carcinoma metastatic to the oral cavity. *J Oral Maxillofac Surg* **44:** 825–827.

Stephenson J (2000). Researchers buoyed by promise of targeted leukemia therapy. *JAMA* **283:** 317–321.

Stoeckli SJ, Pfaltz M, Steinert H, Schmid S (2002). Histopathological features of occult metastasis detected by sentinel lymph node biopsy in oral and oropharyngeal squamous cell carcinoma. *Laryngoscope* **112:** 111–115.

Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ (1999). Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. *Endocr Rev* **20:** 345–357.

Tada T, Jimi E, Okamoto M, Ozeki S, Okabe K (2005). Oral squamous cell carcinoma cells induce osteoclast differentiation by suppression of osteoprotegerin expression in osteoblasts. *Int J Cancer* **116:** 253–262.

Tada T, Shin M, Fukushima H *et al* (2009). Oral squamous cell carcinoma cells modulate osteoclast function by RANKL-dependent and -independent mechanisms. *Cancer Lett* **274:** 126–131.

Thomas RJ, Guise TA, Yin JJ *et al* (1999). Breast cancer cells interact with osteoblasts to support osteoclast formation. *Endocrinology* **140:** 4451–4458.

Totsuka Y, Usui Y, Tei K *et al* (1991a). Results of surgical treatment for squamous carcinoma of the lower alveolus: segmental vs. marginal resection. *Head Neck* **13:** 114–20.

Totsuka Y, Usui Y, Tei K *et al* (1991b). Mandibular involvement by squamous cell carcinoma of the lower alveolus: analysis and comparative study of histologic and radiologic features. *Head Neck* **13:** 40–50.

Uchida Y (1998). Statistical and epidemic studies oral cancer diagnosed and treated by oral surgeons in Japan. *Jpn J Oral Biol* **7:** 16–26.

Wong RJ, Keel SB, Glynn RJ, Varvares MA (2000). Histological pattern of mandibular invasion by oral squamous cell carcinoma. *Laryngoscope* **110:** 65–72.

Yaccoby S, Wezeman MJ, Henderson A *et al* (2004). Cancer and the microenvironment: myeloma-osteoclast interactions as a model. *Cancer Res* **64:** 2016–2023.