Mucoepidermoid carcinoma of the sublingual gland harboring a translocation of the *MAML2* gene: A case report

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Abstract. Among tumors of the major salivary glands, tumors in the sublingual gland are rare. Although mucoepidermoid carcinoma (MEC) represents a histological type of salivary gland tumor, it is occasionally difficult to diagnose due to its histological variation. The present study reports a case of MEC harboring a mastermind-like transcriptional coactivator 2 (*MAML2*) gene translocation in the sublingual gland. A 76-year-old Japanese woman with a mass in the left submandibular region was referred to Kurume University Hospital (Kurume, Japan). Computed tomography scans revealed that the tumor was predominantly located in the sublingual gland, and tumor resection was performed. Histologically, the tumor was composed of cells that exhibited low-grade nuclear atypia and clear and/or granular eosinophilic cytoplasm, and that were proliferating in solid patterns. Periodic acid-Schiff and alcian blue staining revealed a small number of mucinous cells in the tumor. Immunohistochemically, the tumor cells were positive for p40 and p63. Fluorescence *in situ* hybridization (FISH) analysis revealed a *MAML2* gene split. The definitive pathological diagnosis was low-grade MEC, as the case lacked any factors indicative of high-grade malignancy. To the best of our knowledge, this is the first report of MEC in the sublingual gland with *MAML2* gene translocation confirmed by FISH.

Introduction

Among tumors of the major salivary glands, tumors of the sublingual gland are rare, accounting for <0.5% of cases (1). Although mucoepidermoid carcinoma (MEC) is one of the most common malignant salivary gland neoplasms, and ~50% of MECs occur in the major salivary glands, including the parotid or submandibular gland (2,3), MEC of the sublingual gland is a relatively rare disease.

MEC is a malignant epithelial neoplasm that was first described by Stewart *et al* in 1945 (4). MEC is the most common malignant salivary gland tumor among cases reviewed at the Armed Forces Institute of Pathology since 1970 and other treatment centers in the United States (2). In addition, the frequencies are comparable to those studies from other countries (2).

MEC is histologically characterized by the presence of mucinous, intermediate and squamoid cells, while clear, columnar and oncocytic cells are less commonly observed (3,5). Typically, the pathological diagnosis of MEC is relatively easy; however, definitive diagnosis may be challenging when the tumor is composed of less common cell types (5). MEC is sub-classified into low, intermediate and high grades on the basis of its histological features (2,3,5,6).

No chemical carcinogens or oncogenic viruses are associated with MEC (6). However, prior exposure to radiation is a contributing factor for MEC (7).

Prognosis depends on clinical stage, site, grading and adequacy of surgical excision (5). Low grade tumors exhibit excellent prognoses, however; high grade tumors demonstrate metastasis to the regional lymph nodes and distant sites including lungs, bone, and brain (5).

Mastermind-like transcriptional coactivator 2 (*MAML2*) gene translocation is observed in more than half of all cases of MEC, and is a useful diagnostic marker (8,9). The present study reports a case of MEC occurring in the sublingual gland of a 76-year-old female. A review of the relevant literature is also presented.

Case report

A 76-year old Japanese woman suffering from Parkinson's disease presented at Kurume University Hospital (Kurume, Japan) in April 2016 due to a mass in her left submandibular region. Upon examination, a soft mass was palpable. Contrast-enhanced computed tomography (CT) scans (Fig. 1A and B) and a magnetic resonance imaging

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(MRI) examination (Fig. 1C and D) revealed a cystic mass originating from the sublingual gland in the floor of the oral cavity. Fine-needle aspiration cytology was performed, which revealed only inflammatory cells, including histiocytes, lymphocytes and neutrophils, with no epithelial component. The tumor was removed percutaneously.

The sublingual gland tumor was encapsulated and did not adhere to the surrounding tissue. Macroscopically, the tumor was 20 mm in diameter and contained black fluid (Fig. 2). Tissue preparation and staining were performed as follows: Paraffin-embedded tissue samples were cut at 4 μm and examined on a coated glass slides, and then labeled with antibodies using the BenchMark ULTRA (Ventana Automated Systems, Inc., Tucson, AZ, USA) and Bond-III autostainer (Leica Microsystems, Ltd., Milton Keynes, UK).

Primary antibodies were as follows: Endomysial antibody (EMA; cat no. 1504; ready to use; clone E29/EP1), S100 protein (cat no. N1573; ready to use) (both from DakoCytomation; Agilent Technologies, Inc., Santa Clara, CA, USA), mitochondria (cat no. 6280-0004; 1:500; clone AE1; Biogenesis; Morphosys AG, Poole, UK), p63 (cat no. M7247; 1:100, clone 4A4; DakoCytomation; Agilent Technologies, Inc.), and p40 (cat no. PC373; 1:500, clone 5-17; Calbiochem; Merck KGaA, Darmstadt, Germany).

For p63 and p40, BenchMark ULTRA was used. Briefly, each slide was heat-treated at 99˚C using Ventana CC1 retrieval solution (Ventana Automated Systems, Inc.) for 30 min, and incubated at room temperature with each antibody for 30 min. This automated system used the streptavidin-biotin complex method with 3,3' diaminobenzidine (DAB) as the chromogen (Ventana UltraVIEW DAB detection kit; Ventana Automated Systems, Inc.). Immunostaining with EMA, S100 protein and mitochondria were performed on the same fully automated Bond-III system using on-board heat-induced antigen retrieval with epitope retrieval solution 2 (pH 9.0) for 5 min and a Refine polymer detection system (Leica Microsystems, Ltd.). DAB was used as the chromogen in all these immunostainings.

Histological evaluation revealed that the tumor was encapsulated by fibrous tissue (Fig. 3A) and composed of cells with clear (Fig. 3B) or granular eosinophilic cytoplasm (Fig. 3C), proliferating in solid patterns. Individual cells had subtle nuclear atypia. Hyalinized stroma was observed (Fig. 3D). A small number of mucinous cells were observed in the tumor following alcian blue (Fig. 4A) and periodic acid-Schiff (PAS) staining. Diastase-resistant PAS-positive granules were observed in the cytoplasm of the tumor cells (Fig. 4B), and were interpreted as fine hyaline globules. Immunoreactivity for p63 (Fig. 4C) and p40 (Fig. 4D) was found in the tumor cells. However, S-100 protein and epithelial membrane antigen (EMA) were not detected. A positive reaction for mitochondria was observed to be scattered in individual cells, indicating that the tumor was unlikely to be oncocytoma or oncocytic carcinoma in differential diagnosis.

Since there was no indication of perineural invasion, necrosis, mitoses or anaplasticity, the tumor was considered a low-grade malignancy according the grading scale proposed by Goode *et al* (10). Although microinvasion into the surrounding fibrous capsule was observed, no vascular invasion was confirmed by Elastica van Gieson and D2-40 staining.

Fluorescence *in situ* hybridization (FISH) analysis for a *MAML2* (11q21) gene translocation was performed using a ZytoLight® SPEC MAML2 Dual Color Break Apart Probe (ZytoVision GmbH, Bremerhaven, Germany). FISH analysis detected a split signal in the *MAML2* gene, with a rate of 83% of the counted nuclei in the tumor cells (Fig. 5). Based on these findings, a final diagnosis of low-grade MEC was determined.

Following the complete surgical removal of the tumor, fludeoxyglucose-positron emission tomography (PET) revealed no other metastatic lesions. As histological evaluation demonstrated clear margins, and the MEC was determined to be a low-grade type, no additional treatment was performed. During the 6-month postoperative course, no evidence of local recurrence or distant metastasis was observed, and the patient remains relatively well thus far.

The present study was approved by the ethics committee of Kurume University (approval no. 17003) and written informed consent was obtained.

Discussion

Tumors of the sublingual gland are uncommon, and MEC of the sublingual gland is even more rare. To date, only three such cases have been reported in the English literature (11-13). In one case, bone formation was observed within the tumor (12). To the best of our knowledge, there have been no reports of MEC of the sublingual gland with confirmed *MAML2* gene translocation.

MEC is typically composed of mucinous, squamoid and intermediate cells. Clear cells, oncocytic cells and columnar cells are occasionally present and prominent (2,5). Since MEC exhibits various histological structures composed of these cell types, it is occasionally difficult to differentiate MEC from other types of tumor, such as clear cell, oncocytic and squamous cell carcinomas (2,5).

In the present case, the tumor was composed of cells with granular eosinophilic or clear cytoplasm, and a mucinous cell component was also observed in a limited area. Therefore, it was difficult to determine a diagnosis of MEC based solely on morphological observation. Immunohistochemical staining for p40 and p63, as well as mucin staining, aided the diagnosis. Immunohistochemically, the tumor cells were negative for S-100 protein, a representative myoepithelial marker. p40 and p63 (14), which are myoepithelial as well as squamous cell markers (15), were positively expressed in the tumor cells, indicating that the tumor had features of squamous and/or squamoid differentiation. The presence of mucinous cells meant that the tumor was less likely to be an oncocytic or clear cell carcinoma. However, mucinous cells are not only detected in MEC, but also in various other tumors with mucinous metaplasia (2).

As the tumor in the present case was histologically composed of cells with granular eosinophilic or clear cytoplasm, the differential diagnoses included benign and malignant tumors with oncocytic or clear cytoplasm, such as oncocytoma, acinic cell carcinoma, mammary analogue secretory carcinoma of the salivary gland, epithelial-myoepithelial carcinoma and clear cell carcinoma. The small number of mucinous cells identified in the tumor indicated the possibility of MEC rather than other salivary gland tumors; however,
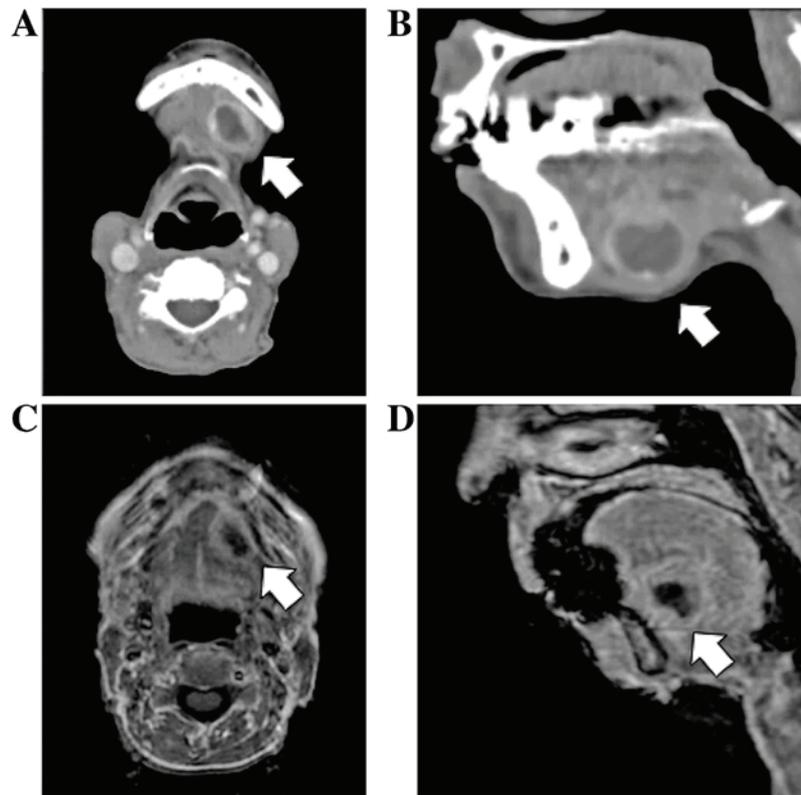
Figure 1. Imaging findings of the head and neck in a patient with sublingual gland mucoepidermoid carcinoma. (A) Axial and (B) sagittal contrast-enhanced CT scans. (C) Axial and (D) sagittal T1-weighted MRI scans. Contrast-enhanced CT and MRI scans revealed a cystic tumor lesion located in the floor of the oral cavity, growing from the sublingual gland (arrow). CT computed tomography; MRI, magnetic resonance imaging.

Figure 2. Macroscopic view of the tumor. The tumor was ~20 mm in diameter and contained black fluid.

as the tumor occurred at an unusual site, FISH analysis was performed to confirm this diagnosis. FISH analysis for a *MAML2* gene translocation is a useful supplemental method for cases where the diagnosis of MEC is uncertain.

MAML2 gene translocation is a specific gene rearrangement present in MEC tumors, and FISH analysis of the *MAML2* gene is therefore useful for the screening of MEC (8,9). Noda *et al* (8) reported that *MAML2* gene translocation was detected in ~65% of primary MECs, and that the cut-off value for the split signal in *MAML* genes is >7%. In the present case, the rate of *MAML* split signal detection was 83%,

and the tumor was therefore considered to have *MAML2* gene translocation.

The majority of MECs with *MAML2* gene translocation are histologically classified as low-grade (8), as demonstrated by the present case. *MAML2* gene translocation is typically detected in MECs that are predominantly composed of epidermoid, intermediate and mucinous cells; however, there have been a few reports of clear cell or oncocytic variants of MEC harboring a *MAML2* gene split (16,17).

The *MAML2* gene translocation is an oncogenic event that underlies the development of MEC (18). Therefore, *MAML2* gene translocation may be observed in various types of cells in MEC. Tumor cells with a granular eosinophilic or clear cytoplasm may indicate oncogenesis as the degree of *MAML2* gene translocation is high in the tumor.

Although FISH analysis for a *MAML2* gene translocation is not routinely performed to diagnose MEC, its use in unusual cases may aid in determining an accurate diagnosis. The *MAML2* gene and other molecular biomarkers have been identified to form the basis for the development of novel therapeutic strategies (19). Noda *et al* (8) reported that *MAML2* gene translocation is associated with a favorable prognosis in cases of MEC. However, a more recent study reported that *MAML2* gene status has no association with the prognosis of MEC (20). As the association between *MAML2* status and prognosis is controversial, *MAML2* status in MEC must be investigated in a larger cohort of patients in order to determine its precise roles. *MAML2* gene translocation may also aid the prediction of prognoses and selection of optimal treatments.
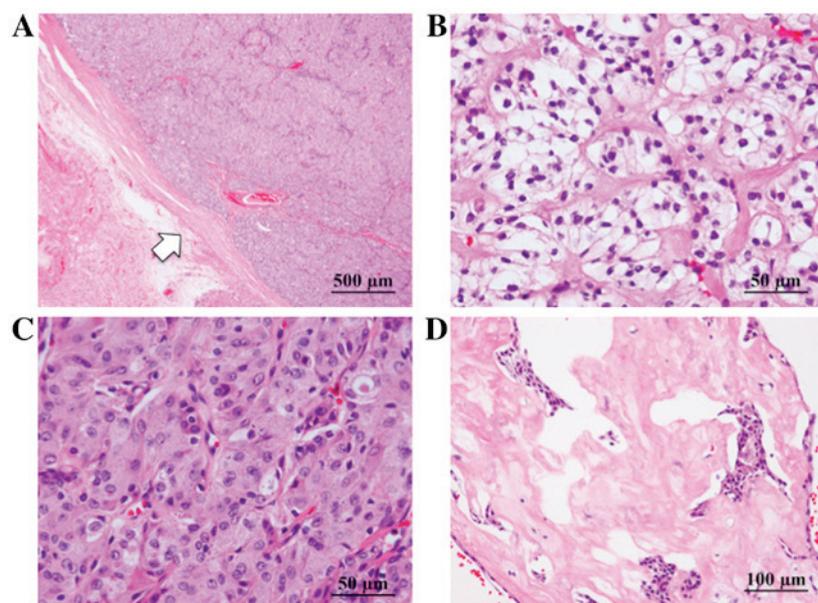
Figure 3. Pathological findings of the tumor following hematoxylin and eosin staining. (A) The tumor was encapsulated by fibrous tissue (arrow). (B-D) The tumor was composed of cells with low-grade nuclear atypia, with (B) clear or (C) granular eosinophilic cytoplasm; a solid pattern of cell proliferation was observed, along with (D) hyalinized stroma.
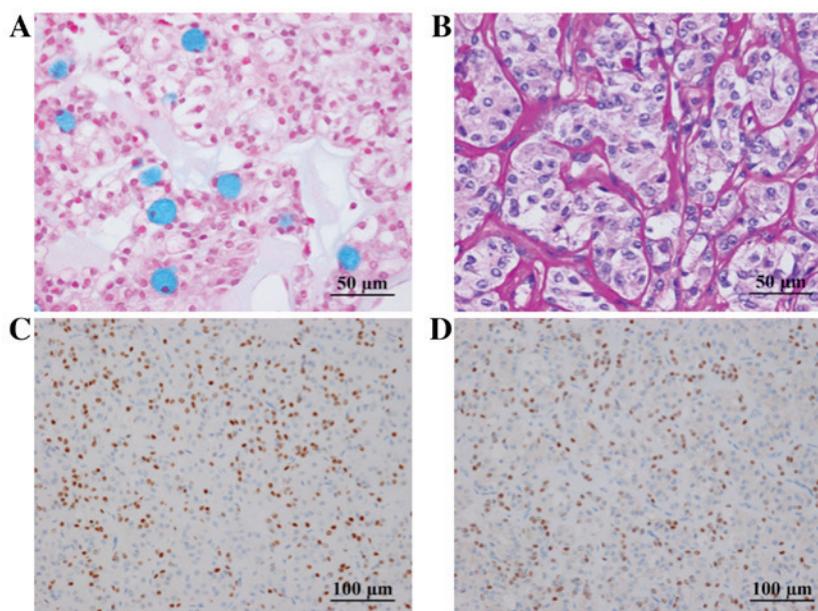
Figure 4. (A) Alcian blue staining revealed mucinous cells in the tumor. (B) Diastase-digestion PAS staining revealed diastase-resistant PAS-positive globules. The cells exhibited positive immunoreactivity for (C) p63 and (D) p40. PAS, periodic acid-Schiff.
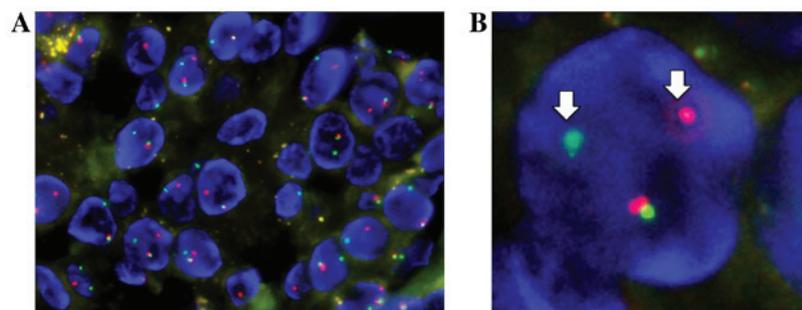
Figure 5. Fluorescence *in situ* hybridization analysis of *MAML2* gene translocation. (A) The split signals of the *MAML2* gene were present in 83% of the counted nuclei in tumor cells. (B) Arrows indicates the split signals (one green and one red) of tumor cells. *MAML*2, mastermind-like transcriptional coactivator 2.

The 5-year survival rates of low-grade and high-grade MEC are 97 and 27%, respectively (21). Therefore, it is important to determine the histological grade of this malignancy, as this influences the clinical features and prognosis (22). MEC may be classified into three grades according to its pathological features, including cystic components, perineural invasion, necrosis, mitoses and anaplasticity (2,3,5,10). As the tumor in the present case lacked any of these features, it was categorized as a low-grade malignancy.

Surgical resection of the primary tumor is the standard treatment for all grades of MEC. The majority of cases of high-grade MEC tend to metastasize, and selective neck dissection during initial surgery is therefore recommended (22). In addition, adjuvant radiotherapy is recommended in cases of high-grade MEC (22). By contrast, low-grade MEC is less aggressive; thus, surgical resection alone is usually sufficient, as in the present case. However, there is a potential risk of local metastasis in low-grade MEC, and this occurs in ~4% of cases (10). Therefore, careful clinical follow-up is recommended.

Previous reports have described the utility of PET/CT for MECs of the lung or bronchi (23,24), and one report described the assessment of low-grade MEC with high-resolution PET/CT (24). It is unclear whether FDG accumulates in low-grade MEC. However, we suggest that PET/CT combined with CT and MRI could be used as a supplemental examination, as the prognosis and the frequency of metastasis in MECs of the sublingual gland are unclear. The precise roles of PET/CT for salivary gland MECs must be investigated in a larger patient cohort.

In summary, the combined use of immunohistochemistry, FISH analysis of *MAML2* gene translocation and histological observation is a useful and reliable examination technique for the accurate diagnosis of atypical MEC. To the best of our knowledge, this is the first report of MEC of the sublingual gland with *MAML2* gene translocation confirmed by FISH.

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