Yamanashi Med. J. **27**(2), 71 ～ 78, 2013

Case report

Epithelial-Myoepithelial Carcinoma of the Parotid Gland: A Case Report and Immunohistochemical Analysis

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Abstract: Epithelial myoepithelial carcinoma (EMC) is a rare salivary gland tumor consisting of inner duct-lining epithelial cells and outer clear myoepithelial cells, which is a characteristic composition of biphasic tubular structures. Here, we describe the gross, histological, and immunophenotypic characteristics of a case with typical EMC. An 87-year-old woman diagnosed with EMC of the left parotid gland was referred to our hospital, where she underwent total parotidectomy with complete excision of the lesion. Histopathological examination revealed typical features of EMC. Immunohistochemical examinations were performed for further characterization, and included potential biological markers Ki-67, p53, Her-2/neu, carcinoembryonic antigen (CEA), and Bcl-2. The epithelial cells were diffusely positive for CEA, but no other significant positive staining was identified. Although EMC is recognized as a low-grade tumor, the relatively high likelihood of local recurrence and the presence of variants with high-grade components should be considered. Immunohistochemical analysis can provide useful information for both the diagnosis and biological evaluation of this tumor.

Key Words: epithelial-myoepithelial carcinoma, salivary gland tumor, immunohistochemistry

INTRODUCTION

Salivary gland carcinomas are known to have diverse histological subtypes, and account for approximately 7% of cancers arising in the head and neck[1)]. Epithelial-myoepithelial carcinoma (EMC) is a rare salivary gland tumor that was initially reported by Donath *et al.*[2)]. Histologically, EMC is characterized by biphasic tubular structures comprising inner duct-lining epithelial cells and outer clear myoepithelial cells, and it exhibits a high degree of cellular differentiation and low-grade aggressiveness. EMC is therefore considered a clinically low-grade carcinoma showing favorable prognosis, with 5-year overall survival rates of more than 80%[3,4)]. Several histological variants overlapping with other salivary type tumors such as oncocytic and apocrine EMC have recently been recognized[5,6)]. Moreover, an increasing number of reports have described hybrid carcinoma combining EMC and dedifferentiated EMC[5,7–9)]. Such variants are relatively rare, but may cause clinical problems regarding differential diagnosis and treatment.

Here, we report a case with typical EMC of the

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Received August 23, 2011
Accepted December 13, 2012

parotid gland, and further analyze the immunohistochemical features.

CASE REPORT

An 87-year-old woman presented with a lump in the left parotid region. The lump was nontender and had been present for approximately 4 months. Although fine-needle aspiration (FNA) provided a diagnosis of class II, probably benign tumor, computed tomography (CT) demonstrated a mass lesion that had invaded into the surrounding tissues, so incisional biopsy was performed. Histological examination of the surgical specimens indicated EMC of the parotid gland, and she was referred to our hospital.

On physical examination, her ears, nose, oral cavity, oropharynx, hypopharynx, and larynx all appeared unremarkable. Neither facial nerve palsy nor palpable cervical nodes were evident. Axial T1-weighted magnetic resonance imaging (MRI) demonstrated a hypointense, homogenous mass in the left parotid gland (Fig. 1A). The lesion measured 3 × 3 × 5 cm. Axial T2-weighted

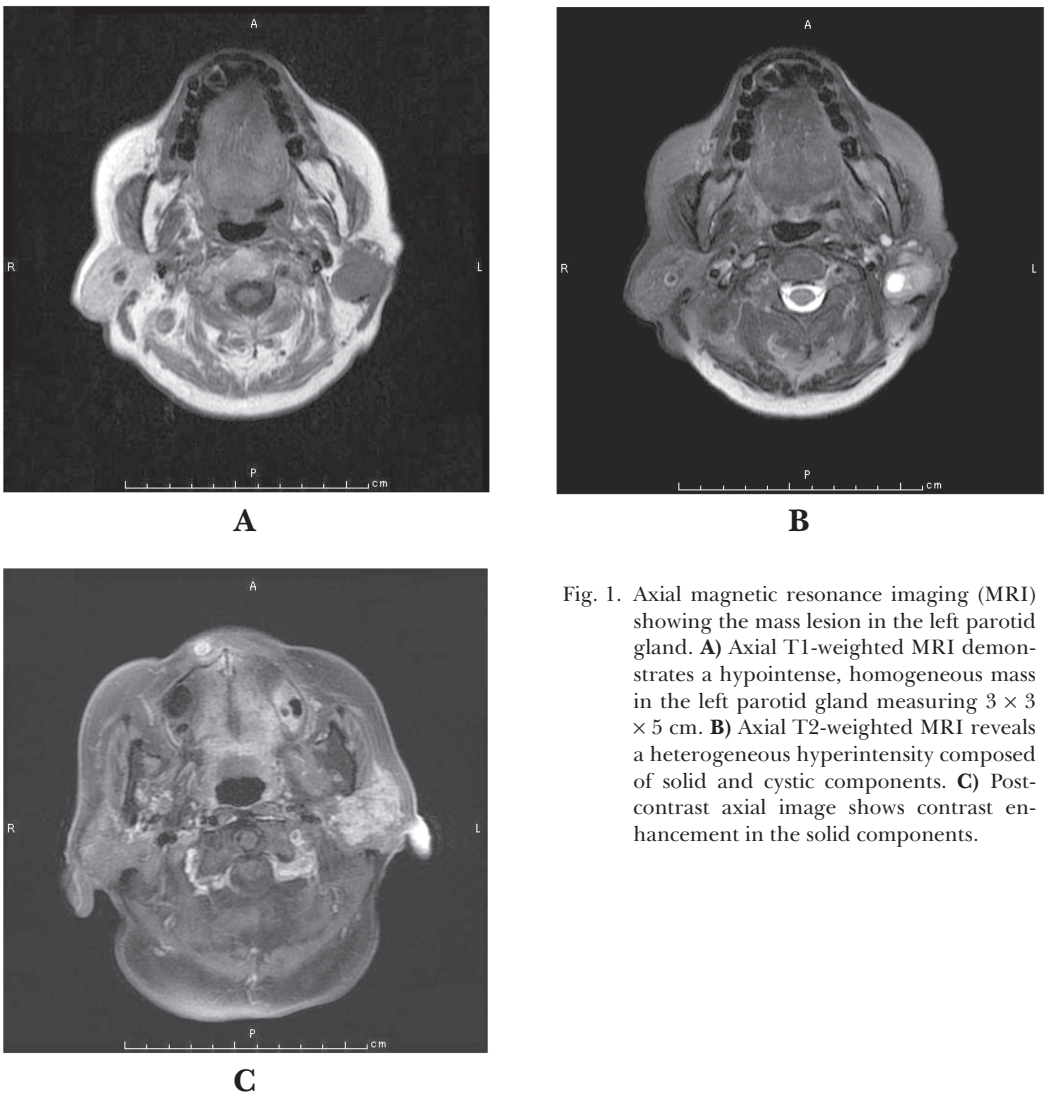

Fig. 1. Axial magnetic resonance imaging (MRI) showing the mass lesion in the left parotid gland. **A)** Axial T1-weighted MRI demonstrates a hypointense, homogeneous mass in the left parotid gland measuring 3 × 3 × 5 cm. **B)** Axial T2-weighted MRI reveals a heterogeneous hyperintensity composed of solid and cystic components. **C)** Post-contrast axial image shows contrast enhancement in the solid components.

MRI revealed a heterogeneous hyperintensity comprising solid and cystic components (Fig. 1B). Post-contrast axial imaging showed contrast enhancement in the solid components (Fig. 1C). As expected, positron emission tomography (PET) showed marked focal fluoro-deoxyglucose uptake in the left parotid gland, with the lesion showing a standardized uptake value of 6.13 (data not shown).

The patient underwent total parotidectomy with complete excision of the lesion. In this case, selective neck dissection was not performed.

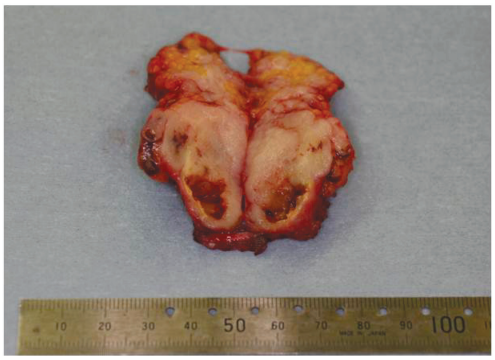

Fig. 2. The resected tumor measures 4 × 3 × 2.5 cm and contains a multinodular, well-circumscribed, tan-white tumor with a cystic area.

The surgical specimen contained a multinodular, well-circumscribed, tan-white tumor with a cystic area, measuring 4 × 3 × 2.5 cm (Fig. 2). Over the first postoperative year, the patient has done well, with no evidence of either local recurrence or cervical lymph node metastases.

Pathological Findings

The tumor component exhibited a multinodular growth pattern and was separated by incomplete dense fibrous connective tissue septa that had been invaded by tumor cells. The tumor showed characteristic double-layered duct-like structures consisting of eosinophilic ductal cells and pleomorphic or spindle-shaped myoepithelial cells with clear cytoplasm, indicating typical features of EMC (Fig. 3). The ductal cells contained eosinophilic cytoplasm and uniform round nuclei. Nuclear atypia was unremarkable. Vascular invasion was not noted, but both adipose tissue and perineural invasion were partially observed. Margin status was negative and tumor necrosis was not present.

For further characterization of this tumor, immunohistochemical examination was per-

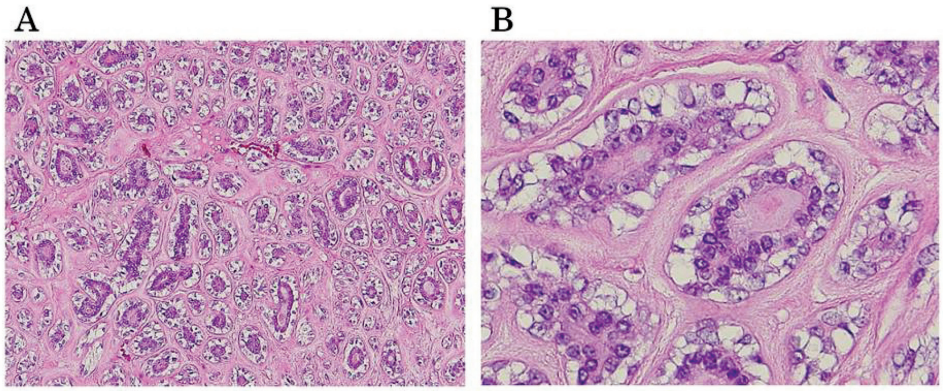

Fig. 3. Histopathological examination reveals a typical epithelial-myoepithelial carcinoma showing biphasic tubular structures composed of inner dark cells and outer clear cells. Hematoxylin-eosin stain; A, original magnification × 100; B, original magnification × 400.

Table 1. Antibodies used for immunohistochemistry

Antibody	Company	Clone	Dilution
AE1/AE3 (pan-cytokeratin)	Dako, Glostrup, Denmark	AE1/AE3	1:100
CAM5.2	Becton Dickenson, San Jose, CA	CAM5.2	1:1(prediluted)
EMA	Dako, Glostrup, Denmark	E29	1:50
p63	Dako, Glostrup, Denmark	4A4	1:25
SMA	Dako, Glostrup, Denmark	1A4	1:50
Vimentin	Dako, Glostrup, Denmark	V9	1:100
S-100 protein	Dako, Glostrup, Denmark	Polyclonal	1:500
Ki-67 (MIB-1)	Dako, Glostrup, Denmark	MBI-1	1:50
p53	Dako, Glostrup, Denmark	DO-7	1:50
Her-2/neu	Dako, Glostrup, Denmark	Polyclonal	1:200
CEA	Dako, Glostrup, Denmark	II-7	1:25
Bcl-2	Dako, Glostrup, Denmark	124	1:50

Table 2. Immunohistochemical analysis

Antibody	Epithelial cell	Myoepithelial cell
AE1/AE3 (pan-cytokeratin)	+	-
CAM5.2	+	-
EMA	f+	-
p63	-	+
SMA	-	+
Vimentin	-	+
S-100 protein	-	+
Ki-67 (MIB-1)	1%	3%
p53	-	-
Her-2/neu	-	w+
CEA	+	w+
Bcl-2	w+	-

f+, focal positive; w+, weak positive

formed. Formalin-fixed, paraffin-embedded sections were cut at a thickness of 4 μm. Antibodies, manufacturers, and dilutions are listed in Table 1. The results are summarized in Table 2. As expected, inner ductal cells were positive for several epithelial markers, including AE1/AE3 (pan-cytokeratin), CAM5.2, and epithelial membrane antigen (EMA) (Fig. 4A, B). Meanwhile, outer myoepithelial cells were positive for p63, smooth muscle actin (SMA), vimentin, and S-100 (Fig. 4C-E).

Moreover, the sections obtained from specimens were stained with the following potential biological markers: Ki-67; p53; Her-2/neu; carcinoembryonic antigen (CEA); and Bcl-2. Proliferative activity as defined using Ki-67 antibody revealed positivity in 1% of inner ductal cells and 3% of outer clear cells (Fig. 4F). Epithelial cells were negative for p53 and Her-2/neu, very weakly positive for Bcl-2, and positive for CEA.

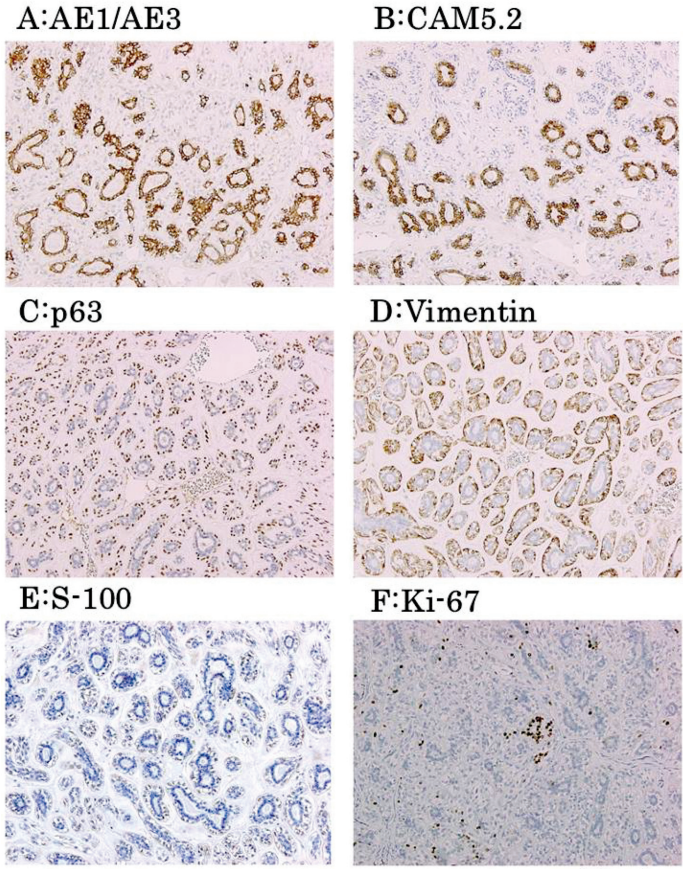

Fig. 4. Immunohistochemical examination. A, AE1/AE3; B, CAM5.2; C, p63; D, vimentin; E, S-100; F, Ki-67 (original magnification × 100).
Inner ductal cells are positive for AE1/AE3 and CAM5.2, whereas outer myoepithelial cells are positive for p63, vimentin, and S-100. Proliferative activity according to Ki-67 antibody revealed positivity in 1% of inner ductal cells and 3% of outer clear cells.

On the other hand, myoepithelial cells were negative for p53 and Bcl-2, and very weakly positive for Her-2/neu and CEA.

Discussion

Since EMC was recognized as a separate entity of salivary gland cancer in the 1991 World Health Organization classification, more than 100 cases with EMC have been described [3,4,7,10–14]. The mainstay of treatment for EMC is surgical removal with intraoperative facial nerve monitoring, but preoperative diagnosis is difficult. Although imaging studies including CT, MRI, and PET, as well as FNA, were performed in the present case, EMC was not able to be diagnosed by any of these examinations. Piscioli *et al.* suggested that imaging studies including CT and MRI may be unsatisfactory and misleading in the diagnosis of EMC [15]. On the other hand, FNA is

known to be diagnostic in most cases with parotid tumors and is always recommendable for decisions regarding treatment. In general, open incisional biopsy should not be performed as the initial diagnostic procedure because of the risk of dissemination. Gross *et al.* proposed that open incisional biopsy can be considered in only cases with certain infiltrative parotid diseases, such as lymphoepithelial or lymphoproliferative diseases[16]. In the present case, although CT suggested a malignancy, the results of FNA suggested a benign tumor. If discrepancies between imaging studies and cytomorphological examination are seen, additional intraoperative frozen section or incisional biopsy might be warranted. Avoiding inadequate resection by thorough preoperative evaluation and preparation of the patient for possible parotidectomy is important.

EMC is considered to be a low-grade malignant neoplasm because of the high degree of cell differentiation and slow infiltrative growth, but two points are of particular importance in the management of EMC. The first point is the existence of variants. EMC is defined as a biphasic neoplasm composed in varying proportions of ductal cells and clear myoepithelial cells, so a much broader histological spectrum than previously recognized is observed. As a result, several histological variants, such as an oncocytic variant, EMC with myoepithelial anaplasia, dedifferentiated EMC, and hybrid carcinoma consisting of EMC and a histologically different carcinoma component, have been reported to date[4,7,9,17]. In addition, such variants show relatively worse prognosis than previously thought.

The second point is that relatively high rates of lymph node and distant metastasis and/or local recurrence have been demonstrated. Toida *et al.* reported cervical lymph node and distance metastases in 19.6% and 9.8% of cases, respectively[10]. On the other hand, Seethala *et al.* described a recurrence rate of 36.3%[4]. Similarly, Fonseca *et al.* reported recurrences in 50% of cases studied[3]. Moreover, death due to EMC was found in 40% of patients. Thus, some cases with EMC display a poor prognosis. Seethala *et al.* described margin status, angiolymphatic invasion, tumor necrosis, and myoepithelial anaplasia as the most important factors predicting recurrence[4]. In addition to these factors, the identification of markers that can accurately predict biological behavior is urgently needed. To date, the expression of various molecules, such as Ki-67, p53, matrix metalloproteinase, and Her-2/neu, and nuclear DNA content have been investigated using immunohistochemistry and flow cytometry[11,17–19]. Ki-67 is a well-known proliferation marker, and a number of studies have demonstrated potential links between proliferative activity and clinical outcome in patients with EMC[3,18]. Interestingly, the myoepithelial cell component shows higher proliferative activity, suggesting an important role for myoepithelial cells not only in the growth of EMC, but also in the progression or dedifferentiation to high-grade carcinoma[11]. In the present case, the Ki-67 proliferation index was 3% in myoepithelial cells and 1% in epithelial cells. Expressions of 4 different markers including p53, Her-2/neu, CEA, and Bcl-2 in tumor cells were also investigated using immunohistochemistry. Although epithelial cells showed diffuse positive staining for CEA, no other significant positive staining was evident. In terms of the expression of biological potential markers, Seethala *et al.* reported that none of the cases with a Ki-67 proliferative index $<10\%$ recurred. Meanwhile, positive results for Bcl-2 were achieved in 6 of 9 tumors (66.7%) and p53 was highly expressed in only 1 dedifferentiated EMC of 13 tumors[4]. These findings suggest that our case might have relatively good biological potential. Indeed, no evidence of re-

currence has been found after 1 year of follow-up after treatment. To date, various parameters such as histopathological pattern, Ki-67 proliferative activity, p53, and Bcl-2 have been studied to predict clinical outcome in patients with salivary gland EMC, but none have shown any significant correlation with prognosis, possibly due to the small number of cases studied[3,11,17,18]. Further analysis using a sufficiently large sample should be considered to determine the most important factors.

Taken together, we have described a case of typical EMC. Neither imaging studies nor cytopathological examination suggested the diagnosis of this rare tumor. Although EMC is generally recognized as a low-grade tumor, attention must be paid to the relatively high likelihood of local recurrence after initial treatment. Moreover, presence of variants with high-grade components and the broader morphologic spectrum should also be considered. Immunohistochemical analysis can provide information that is useful for diagnosis, understanding the complex architecture of salivary gland tumors, and the biological evaluation of this tumor.

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