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A case of nasopharyngeal clear cell carcinoma diagnosed by molecular analysis

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ABSTRACT
Clear cell carcinoma (CCC) in the salivary gland is a rare malignant neoplasm and is hardly located in the nasopharynx. An 18-year-old female presented with a 3-year history of nasal congestion. Endoscopic examination revealed a reddish round-shaped mass with a smooth surface in the posterior wall of the nasopharynx. An en bloc resection of the tumor was performed by endoscopic surgery. Preliminary pathological diagnosis made by histopathological examination was mucoepidermoid carcinoma. However, FISH analysis for MAML2-split was negative but that for EWSR1-split was positive. In addition, EWSR1-ATF1 fusion transcript was detected in the RT-PCR analysis. The final pathological diagnosis of CCC was made on the basis of these findings. Since EWSR1-ATF1 fusion is not observed in other salivary gland tumors, identifications of this unique fusion gene by FISH and RT-PCR are useful tools to confirm the diagnosis of CCC.

1. Introduction
Clear cell carcinoma (CCC) in the salivary gland is a rare malignant neoplasm that predominates in the minor salivary glands of the palate, base of the tongue, and floor of the mouth. CCC also has been reported in parotid and submandibular glands, larynx, orbital content, and very occasionally nasopharynx [1]. Most of the patients with CCC are in their sixth decade of life with a slight female predominance, and CCC accounts for <1% of all salivary gland tumors. Presentation varies according to the location of the tumor but they are often painless, submucosal asymptomatic mass [1]. CCC is characterized by low-grade malignancy with occasional metastatic spread and the prognosis with this neoplasm is generally favorable. Locoregional and distant metastatic rates are reported in 15–25% cases at the time of presentation in the literature [1].

Histopathologically, CCC was first described in 1994 by Milchgrub et al. as a unique entity made up of clear cells that form nests and cords in hyalinized stroma. Initially, CCC was called as hyalinizing clear cell carcinoma (HCCC) but was renamed as CCC in the new edition of WHO classification of Head and Neck in 2017. Since CCC has a wide differential diagnosis including other clear-cell containing tumors, such as epithelial–myoepithelial carcinoma, mucoepidermoid carcinoma (MEC), and myoepithelial carcinoma, it is very difficult to distinguish CCC from other clear-cell containing tumors based on the histopathological examination [1]. However, recently, consistent expression of Ewing sarcoma breakpoint region 1-activating transcription factor 1 (EWSR-ATF1) fusion protein has been reported in CCC. The EWSR1 rearrangement by fluorescence in situ hybridization (FISH) presented in 18 of 22 (82%) cases of CCC [2] suggested its potential role in the pathological diagnosis of CCC.

In this article, we report a case of nasopharyngeal CCC in an 18-year-old female, which was first histopathologically diagnosed as MEC and then turned out to be CCC by gene testing positive for both EWSR1 rearrangement by FISH and EWSR1-ATF1 fusion gene by the RT-PCR analysis.
2. Case report

An 18-year-old female presented with a 3-year history of nasal congestion. Endoscopic examination revealed a reddish round-shaped mass with a smooth surface in the posterior wall of the nasopharynx (Figure 1). Contrast computed tomography (CT) revealed a low-contrast mass and magnetic resonance imaging (MRI) revealed a $23 \times 27$ mm mass at the roof of the nasopharynx with iso-high density on T1-weighed image and heterogenous enhancement with high density on T2-weighed image (Figure 1). There was no evidence of lymph node or distant metastases.

Although the lesion was not well enhanced in CT and MRI, considering the patient’s age and location of the tumor, we listed juvenile angiofibroma of the nasopharynx first in the differential diagnosis. Thus, we performed angiography for artery embolization of feeding artery prior to the surgical extirpation. However, no significant candidate for embolization of feeding artery was observed. Surgery was performed by transnasal endoscopic approach under general anesthesia. MEC was used for the pathological diagnosis of frozen section of the biopsy specimen at the beginning of the surgery, therefore we expanded the extent of resection. Tumor was resected in an en bloc manner with nasopharyngeal mucosa of superior and posterior wall as well as Rosenmuller fossa. Prevertebral fascia was included in the surgical specimen. Principally, tumor was grasped by forceps through the right nasal cavity and was visualized by VISERA ELITE II\textsuperscript{R} video system (Olympus CO., Tokyo) and dissected using Coblator\textsuperscript{R} Surgery System (Procise\textsuperscript{TM} EZ-VIEW, Smith & Nephew plc, London) through the left nasal cavity. Total operating time was 3 h and 38 min and intraoperative blood loss was 30 ml.

Postoperative course was uneventful and the patient was discharged 5 days after surgery. Although surgical margin was close to cancer, considering the young age of patient and relatively favorable prognosis of this disease, no adjuvant therapy such as radiotherapy or chemotherapy was administered. Patient has been followed up for 12 months with no evidence of disease.

3. Pathological findings

The tumor was $3.2 \times 2.5 \times 2.5$ cm in size. Microscopically, interstitial desmoplastic change and edematous change was observed. The tumor was formed of sheets, nests, and cords of tumor cells with clear cytoplasm. The nuclei of tumor cells were small and rare mitotic figures were identified. The stroma consisted of fibrous tissue with edematous change (Figure 2A). The number of cystic components was $<20\%$. Perineural invasion or vascular invasion was not present, however, small necrosis existed and the tumor cells infiltrated the tumor margins. Immunohistochemical staining was positive for Periodic acid-Schiff (PAS) (Figure 2B), cytokeratin and p63, and negative for calponin. MIB1 index was 5%. Initially, MEC intermediate grade was suspected, and thus, to confirm the pathological diagnosis of MEC, we performed FISH analysis using formalin-fixed, paraffin-embedded specimen for analyzing MAML2 elsewhere [3]. However, an interphase FISH analysis revealed no split signals for MAML2 (Figure 3). Since CCC shows similar pathological features of intermediate grade MEC, we performed FISH analysis for EWSR1 rearrangement, which has been specifically

Figure 1. Preoperative imaging. Endoscopic examination revealed a round-shaped red tumor with a smooth surface located on the posterior wall of the nasopharynx (left). MRI revealed a $23 \times 27$ mm mass at the roof of the nasopharynx with iso-high density on a T1-weighed image and virtual heterogenous enhancement with high density on T2-weighed image (center & right).
identified in CCC but not in MEC [2,4,5]. Split signals of EWSR1 rearrangement were detected in the tumor cells (Figure 3). To further confirm the EWSR1 rearrangement, EWSR1-ATF1 fusion transcript was detected by RT-PCR analysis [6] (Figure 4). Finally, the present tumor was diagnosed as CCC of the minor salivary glands. Written informed consents were obtained from the patient and her parents for this case report.

4. Discussion

In this article, we reported the youngest case of CCC arising in the nasopharynx of the adolescent. Identification of specific chromosome rearrangement was quite helpful for differential diagnosis. Surgical extirpation was successfully performed by endoscopic approach.

MAML2 rearrangement has been reported as a characteristic and specific abnormality for MEC and found in 34–81% of all MEC cases. In addition, recent studies indicated that this unique fusion gene is associated with a quite favorable clinical course, suggesting its clinical value as a reliable prognostic factor [3]. Thus, we examined the presence of MAML2 rearrangement by FISH in the present case, resulting in negative split. On the other hand, EWSR1-ATF1 fusion gene was originally described in conventional clear cell sarcomas of tendons and aponeurosis and have recently also been encountered in angiomatoid fibrous histiocytomas as well as in a few cases of soft tissue myoepithelial tumors [2,4,5]. According to the study of Antonescu et al. [4], EWSR1-ATF1 fusion
has been found in 87% of CCC and is not detected in any of the morphological mimic including MEC. To our knowledge, no other specific fusion genes or proteins have been reported for MEC or CCC.

Since MEC and CCC show confusingly similar histopathological features, characteristic fusion genes, CRTC1/CRTC3-MAML2 in MEC and EWSR1-ATF1 in CCC, can be useful for the differential diagnosis. There is a report of high-grade salivary MEC with EWSR1-POU5F1 [7]. Thus, confirmation of EWSR1 rearrangement by FISH alone may not be the hallmark of CCC by itself to distinguish this lesion from MEC, while there is no additional report on high-grade salivary MEC with EWSR1-POU5F1. However, there is no report on salivary carcinoma other than CCC with EWSR1-ATF1 fusion gene, which was confirmed in the present case by sequencing of RT-PCR product [5,6].

From the clinical point of view, CCC arising in the nasopharynx is exceptionally rare. According to the recent literature review consisting of 111 reported cases with CCC in head and neck [1], oral cavity is the most common site (n = 64) followed by oropharynx (n = 36), and CCC in the nasopharynx was observed only in four cases. To date, including the present case, only 14 cases have been reported in English and Japanese literature (Table 1) [2,4–6,8–14]; among them, details were provided in 10 of 14 cases. Nine patients were female and only one patient was male; among these, our case was the youngest. Five patients were treated by surgery with radiotherapy and/or chemotherapy, four patients were treated by surgical resection alone, and one patient was treated by chemotherapy alone. Among nine patients with follow-up information, recurrence was observed only in one patient, but four times over 12 years [8].

The recommended treatment is surgical resection. The effect of adjuvant radiotherapy and chemotherapy remains unknown. For patients with positive margins, re-resection should be attempted to obtain negative margins or, if this is unfeasible, adjuvant radiotherapy should be considered. According to the literature review by Albergotti et al. [1], patients with head and neck CCC treated by surgical resection without documented positive margins and without evidence of metastasis at the time of resection had a 10 in 49 (20.4%) risk of any recurrence, and only one patient died with the disease. Positive but not close margin (<2 mm) was associated with recurrence (50.0% vs 0.0%; p = .12). In the present case, we employed an endoscopic approach for resection since the patient was adolescent female and low-grade malignancy was expected. Although enough safety margin was not obtained due to the anatomical complexity of nasopharynx, as the patient is still young and is at the risk of radiation-induced cancer [15], she cannot be treated by postoperative radiation at present. In addition, only 3 of 82 reported patients died with the disease [1]. Thus, we decided to carefully follow up the patient without adjuvant radiotherapy or chemotherapy.

The overall risk of any recurrence of CCC in head and neck occurred in 22 of 111 (19.8%) and the average time for recurrence was 42.1 months (range = 6–180 months) [1]. Tumor necrosis, lymph node metastasis and positive margins but not primary sites have been reported as significant factors to predict recurrence. As above mentioned, recurrence may occur more than 10 years after the initial treatment [8]. Thus, long-term follow-up is required for patients with CCC, while most of the reported patients have been alive without disease [1].

### 5. Conclusions

Although we initially diagnosed intermediate grade MEC based on pathological features and immunohistochemical studies, we finally diagnosed the case as CCC.

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**Table 1. Reported cases of nasopharyngeal CCC.**

| No. | Author (year) | Age | Sex | Symptom | EWSR1 | Tx   | Recurrence     |
|-----|---------------|-----|-----|---------|-------|------|---------------|
| 1   | Tang (1995)   | 51  | Female | N.S.  | N.S. | S + RT | Three times |
| 2   | Cheng (2008)  | 63  | Female | N.S.  | N.S. | S + RT | No           |
| 3   | Wang (2010)   | 57  | Male | N.S.  | N.S. | S + CT | No           |
| 4   | Antonescu (2011) | 77 | Female | N.S.  | Split-i | S + RT | No           |
| 5   | Shah (2013)   | N.S. | N.S. | N.S.  | N.S. | S + CT | No           |
| 6   | Bilodeau (2013) | N.S. | N.S. | N.S.  | Split-i | N.S.  | No           |
| 7   | Bilodeau (2013) | N.S. | N.S. | N.S.  | Split-i | N.S.  | No           |
| 8   | Bilodeau (2013) | N.S. | N.S. | N.S.  | Split-i | N.S.  | No           |
| 9   | Ceballos (2013) | 38 | Female | Stuffy nose | Split-i | S + RT + CT | No       |
| 10  | Dosemame (2015) | 22 | Female | Stuffy nose | Split-i | CT    | No           |
| 11  | Fukuda (2015) | 63  | Female | Asymptomatic | Split-i | S    | No           |
| 12  | Nakano (2015) | 27  | Female | Hearing loss | Split-i | S    | No           |
| 13  | Teranishi (2017) | 69 | Female | Asymptomatic | Split-i | S    | No           |
| 14  | Our Case (2017) | 18 | Female | Stuffy nose | Split-i | S    | No           |

N.S.: not stated; S: Surgery; RT: radiotherapy; CT: chemotherapy.
by identifying EWSR1 rearrangement by FISH and EWSR1-ATF1 fusion by RT-PCR. Since EWSR1-ATF1 fusion is not observed in other salivary gland tumors, FISH and RT-PCR are useful tools to confirm the diagnosis of CCC in salivary glands.

**Disclosure statement**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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