Salivary adenoid cystic carcinoma with an early phase of high-grade transformation: case report with an immunohistochemical analysis

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Abstract

Background: The early phase of salivary gland carcinomas with high-grade transformation (HGT) is extremely rare. We reported one case of adenoid cystic carcinoma (AdCC) with early HGT, herein.

Case presentation: The patient was a 27-year-old Japanese woman who suffered from swelling of the left parotid region. Most of this tumor consisted of typical AdCC histology, whereas the central area of this tumor was composed of solid growth component by atypical cells with clear cytoplasm and marked nuclear atypia. Immunohistochemically, this area was strongly and diffusely positive for epithelial membrane antigen, p53, p16, Her-2, cyclin A and cyclin B1. The Ki-67 labeling index of this area was high, entirely different from that of AdCC area.

Conclusion: Overall, this area was an early phase of AdCC-HGT. This case is the second case of early AdCC-HGT. We discuss the development of salivary gland carcinoma with HGT.

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Keywords: High-grade Transformation, Adenoid Cystic Carcinoma, Salivary Gland, Early Phase, Immunohistochemistry

Background

The concept of dedifferentiation or high-grade transformation (HGT) in salivary gland carcinoma derived from the one of bone and soft tissue sarcomas. Cheuk et al. reported the adenoid cystic carcinomas (AdCC) with HGT in 1999 [1]. Ide et al. reported the incipient phase of AdCC-HGT [2], but the early phase of the HGT of AdCC remains unclear. We report herein one additional case showing the early phase of HGT in salivary AdCC.

Pathological findings

Macroscopically, an ill-defined grayish-white mass was observed in the left parotid gland (Figure 2). Histologically, most part of the tumor showed a cribriform growth pattern of the relatively small tumor cells, which could be diagnosed as typical AdCC (Figure 3A, B, C), whereas about 10% of the tumor was composed of the solid subtype of AdCC (grade II) (Figure 3D). In the central portion of the tumor (approximately 10% of this tumor: the size was 7 × 5 mm), an entirely different...
component from AdCC was observed, which consisted of solid and sheet-like growth by atypical cells with clear cytoplasm and marked nuclear atypia (Figure 3E). The tumor cells in this area, which were larger than typical AdCC cells, showed prominent nucleoli and irregular-shaped nuclei (Figure 3F). Such a component lacked ductal-myoepithelial differentiation. Although necrosis was seen, perineural invasion, and angiolymphatic invasion were not observed in “the sheet-like area”. There was no transitional zone between conventional AdCC areas and “the sheet-like area”. In AdCC component, periodic acid-Schiff (PAS)-positive lumens were seen, but “the sheet-like area” was negative for PAS and Alcian blue staining. The surgical margin was positive and the extraglandular extension was observed. Immunohistochemistry was performed on the deparaffinized tissue sections using the primary antibodies listed in Table 1 and the EnVision (DakoCytomation, Carpinteria, CA, USA) method. Immunostaining showed that the only true lumen of the typical AdCC portion was positive for epithelial membrane antigen (EMA), whereas “the sheet-like area” was diffusely positive for EMA. The AdCC area was positive for S-100 protein, p63, and cytokeratin (CK) 14 and alpha-smooth muscle actin, whereas “the sheet-like area” was negative for such antigens, except for the positivity for p63 and CK14 in the myoepithelial cells (Figure 4). Moreover, the AdCC area was weakly positive for Her-2 and the negativity for p16, whereas “the sheet-like area” revealed diffuse and strong positivity for such antigens. The AdCC area showed sporadic positivity for p53, cyclin A and cyclin B1, whereas “the sheet-like area” revealed diffuse and strong positivity for such antigens. The Ki-67 labeling index of the AdCC area and “the sheet-like area” showed 34.5% and 73.2%, respectively (Figure 5A). “The sheet-like area” indicated the overexpression of p53, p16, Her-2, cyclin A and cyclin B1 (Figure 5B,C,D,E), different from those of the typical AdCC.

Finally, we diagnosed this case as “AdCC with an early phase of HGT”.

Discussion
The tumor in this case consisted of three different components: lower-grade (cribriform subtype) of AdCC, solid-subtype AdCC and HGT. As three components existed separately, we believe that HGT occurs abruptly in typical AdCC. In the present case, HGT component lacked biphasic differentiation. Recently, Costa et al. reported three major criteria of AdCC-HGT [3]: 1) proliferation of tumor cells at least a focal loss of myoepithelial cells surrounding tumor nests, 2) nuclear size at least 2–3 times the size of tubular/cribriform AdCC nuclei and 3) thickened irregular nuclear membranes and prominent nucleoli in a majority of cells. The present case fulfilled at least the above 3 criteria. On the other hand, as HGT component was composed of atypical clear cells, the differential diagnosis with clear cell carcinoma, not otherwise specified (CCCNOS) or myoepithelial carcinoma is needed. However, CCCNOS is usually a low-grade malignant tumor...
with mildly nuclear atypia and hyalinized stroma, and is diffusely positive for p63 [4]. Therefore, “the sheet-like area” is not CCCNOS component. Clear cell type of myoepithelial carcinoma showed the positivity for myoepithelial markers, such as ASMA and p63 [5]. Therefore, “the sheet-like area” is not the component of myoepithelial carcinoma. Moreover, the present case is different from low-grade cribriform cystadenocarcinoma (LGCCC), carcinoma ex pleomorphic adenoma (CXPA) and other rare tumors such as an epithelioid angiosarcoma. Usually, LGCCC shows the intraductal lesions with surrounding by p63-positive myoepithelial cells without atypia [6]. The present case showed cribriform pattern, but the tumor was not intraductal lesion. The criteria of CXPA are the malignant tumor, which usually consist of salivary duct carcinoma component, arising from benign pleomorphic adenoma [7]. The present case never contained the
component of pleomorphic adenoma. Epithelioid angiosarcoma rarely occurs in the salivary gland, and tumor cells are infrequently positive for CK and p63 [8]. However, epithelioid angiosarcoma focally shows sinusoid-like spaces and is also positive for vimentin and CD31. The present case was negative for vimentin and CD31 (date not shown), and showed no findings such as sinusoid-like spaces.

HGT is recognized when a lower-grade malignant neoplasm abruptly transforms to a high-grade [9,10], in which the high-grade component is juxtaposed to the original lesion or the original line of differentiation is lost [1]. This concept should be distinguished from malignancy from arising a benign tumor such as CXPA, hybrid tumors, and transformation within a high-grade carcinoma to another high-grade pattern [11]. However, the gradually successive transition from solid-subtype AdCC to HGT was also reported [12].

Recently, Seethala et al. indicated that, in AdCC-HGT, p53 gene mutation and overexpression of cyclin D1 and Her-2 were related to the dedifferentiation process [14], the accumulation of p53 gene product and overexpression of cyclin A, cyclin B1, and Her-2 were related to such a phenomenon in the present case. Overall, “the sheet-like area” of this tumor was high-grade carcinoma, which was considered the early phase of AdCC-HGT. The HGT component might have gene mutation of p53 and p16, and moreover, amplification of the Her-2 gene, which suggested that this component had the higher-grade malignancy. Nagao et al. reported 6 cases of HGT-AdCC and suggested that the HGT process was related to p53 gene mutation and overexpression of cyclin A, cyclin B1, and Her-2 and loss of pRb gene products [10]. In HGT of AdCC, the overexpression of p16 may be related to loss of pRb gene products, not human papilloma virus infection [15]. In the genetic analyses on AdCC with HGT, the increase of c-Myc gene and a low level increase of Her-2 gene in HGT areas were observed, but the role of Her-2 in AdCC-HGT still remains unclear [16]. However, the present

### Table 1 The antibodies used in this study and their results

| Antigens | Antibody | Source | ACC | HGT |
|----------|----------|--------|-----|-----|
| EMA      | E29      | DakoCytomation (Carpinteria, CA, USA) | +   | +   | ++  |
| GCDFP-15 | NCL-GCDFP15 | Novocastra Laboratories Ltd. (New Castle Upon Tyne, UK) | -   | -   | -   |
| Her-2    | AR441    | DakoCytomation (Carpinteria, CA, USA) | -   | -   | -   |
| EGFR     | NCL-L-EGFR | Novocastra Laboratories Ltd. (New Castle Upon Tyne, UK) | -   | -   | -   |
| AR       |          | DakoCytomation (Carpinteria, CA, USA) | -   | -   | -   |
| CK5/6    | DS/16 B4 | DakoCytomation (Carpinteria, CA, USA) | +   | +   | -(myo+) |
| S-100    |          | DakoCytomation (Carpinteria, CA, USA) | +   | +   | -   |
| ASMA     | 1A4      | DakoCytomation (Carpinteria, CA, USA) | ++  | +   | -   |
| calponin | CALP     | DakoCytomation (Carpinteria, CA, USA) | +   | f+  | -   |
| CK14     | LL002    | Chemicon International (Temecula, CA, USA) | +   | p+  | -(myo+) |
| p36      | 4A4      | Lab Vision (Fremont, CA, USA) | ++  | p+  | -(myo+) |
| mammaglobin | 304-1A5 | DakoCytomation (Carpinteria, CA, USA) | -   | f+  | +   |
| p53      | DO-7     | DakoCytomation (Carpinteria, CA, USA) | f+  | p+  | ++  |
| cyclin A | NCL-CYCLN A | Novocastra Laboratories Ltd. (New Castle Upon Tyne, UK) | 10.3% | 17.3% | 34.5% |
| cyclin B1| NCL-CYCLN B1 | Novocastra Laboratories Ltd. (New Castle Upon Tyne, UK) | 0.5% | 12.2% | 50.3% |
| cyclin D1| DSC-6    | DakoCytomation (Carpinteria, CA, USA) | -   | 1.1% | -   |
| cyclin E | NCL-CYCLN E | Novocastra Laboratories Ltd. (New Castle Upon Tyne, UK) | -   | -   | -   |
| p16      | G175-405 | BD Biosciences (Franklin Lakes, NJ, USA) | f+  | p+  | ++  |
| MDM2     | SMP14    | Thermo Fisher Scientific (Cheshire, UK) | -   | -   | -   |
| Ki-67/16 | MB-1     | DakoCytomation (Carpinteria, CA, USA) | 34.5% | 49.7% | 73.2% |

ACC, adenoid cystic carcinoma area; HGT, high-grade transformation area.
EMA, epithelial membrane antigen; GCDFP-15, gross cystic disease fluid protein-15; EGFR, Epidermal growth factor receptor; AR, androgen receptor; CK, cytokeratin; ASMA, alpha-smooth muscle actin.

++, positive (20-60%); ++, diffusely positive (>60%); myo, myoepithelial cells.
case supposed that Her-2 overexpression is related to HGT at least.

Among the salivary gland carcinomas, AdCC frequently showed the HGT. Ide et al. reported that early phase of AdCC-HGT consisted of the scattered foci of anaplastic carcinoma in the co-/pre-existing low-grade AdCC [2]. In series of AdCC-HGT reported by Seethala et al., one case had only a 20% HGT component in typical AdCC and another case contained only a 10% HGT component, who died of disease [13]. As median percentage of HGT component is 70%, which is reported by Seethala et al. [13], we consider that if HGT component is less than 10%, it should be included into the early phase of AdCC-HGT. The present case contained no evidence of scattered high-grade lesions, but one small focus of high-grade carcinoma existed in the central area of the AdCC. Ide et al. do not describe on the proportion of HGT components in the main tumor [2]. Sato et al. reported the gradual progression from low-/intermediate-grade AdCC to high-grade AdCC and moreover to AdCC-HGT [11]. The dedifferentiation or the HGT of the salivary gland carcinomas might occur abruptly within the co-/pre-existing low-grade carcinoma, as seen from the

**Figure 4** The immuno-phenotypes of high-grade carcinoma, comparing with those of typical AdCC. The high-grade carcinoma component showed strong and diffuse positivity for EMA (A: immunostaining x200), whereas the typical AdCC component showed limited positivity for the true lumens in the cribriform growth area (B: immunostaining x200). The high-grade carcinoma component was negative for ASMA (C: immunostaining x200; the blood vessels were positive), whereas the peripheral cells adjacent the pseudocysts in the AdCC area were positive for ASMA (D: immunostaining x200). The high-grade carcinoma component was negative for p63 (E: immunostaining x200; the involved myoepithelial cells were positive), whereas the peripheral cells adjacent the pseudocysts in typical AdCC area were positive for p63 (F: immunostaining x200).
histological and immunohistochemical findings of the present case. In the present case, typical AdCC and HGT components were also clearly demarcated, and no findings of gradual progression were observed.

**Conclusions**

In summary the early phase of the AdCC-HGT was derived from abruptly genetic and phenotypic changes. The present case is the first report that an early phase of AdCC-HGT was related to cyclin A, cyclin B1 and p16 overexpression, in addition to p53 gene product accumulation and Her-2 overexpression.

**Consent**

Written informed consent was obtained from the patient for publication of this Case Report and any accompanying images.
Abbreviations

ADCC: Adenoid cystic carcinoma; HGT: High-grade transformation; PAS: Periodic acid-Schiff; EMA: Epithelial membrane antigen; CK: Cytokeratin; CCGCOS: Clear cell carcinoma, not otherwise specified; LGCCC: Low-grade cribriform cystadenocarcinoma; CXP: Carcinoma ex pleomorphic adenoma.

Competing interests

The author declares to have no competing interests.

Authors’ contributions

KK analyzed that data and wrote the manuscript as a main contributor. TM and TN conducted the pathological examination. All authors have read and approved the final manuscript.

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