Salivary duct carcinoma with rhabdoid features: Report of 2 cases with immunohistochemical and ultrastructural analyses

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ABSTRACT: Background. Salivary duct carcinoma with rhabdoid features is extremely rare.
Methods. We report 2 cases of salivary duct carcinoma with rhabdoid features treated at our institution.
Results. Case 1 was a 44-year-old Japanese man who had swelling in the left parotid region. This tumor consisted of residual pleomorphic adenoma and widely invasive carcinoma, which showed a diffuse growth pattern by atypical rhabdoid cells. Case 2 was a 66-year-old Japanese man who had swelling of the right cervical region. This submandibular tumor was also composed of both residual pleomorphic adenoma region and invasive adenocarcinoma components, whereas some metastatic lesions were purely composed of rhabdoid cells. Such cells were strongly and diffusely positive for cytokeratins (CKs), gross cystic disease fluid protein-15 (GCDP), and androgen receptor (AR). Case 1 was also positive for Her-2 and p53.
Conclusion. Both patients were diagnosed with carcinoma ex pleomorphic adenoma and their carcinomatous components were composed of salivary duct carcinoma with rhabdoid features, which is a highly aggressive tumor, similar to salivary duct carcinoma. © 2013 The Authors. Head & Neck published by Wiley Periodicals, Inc. Head Neck 36: E28–E35, 2014
KEY WORDS: salivary duct carcinoma, rhabdoid cells, carcinoma ex pleomorphic adenoma, immunohistochemistry, ultrastructure

INTRODUCTION
Salivary duct carcinoma is a high-grade neoplasm known to histologically resemble high-grade ductal carcinoma of the breast,2 and includes several variants, for example, sarcomatoid,3–4 invasive micropapillary,5 mucin-rich,6–8 and low-grade9–11 variants. Among such salivary duct carcinoma variants, sarcomatoid salivary duct carcinoma is extremely rare.2–3 Henley et al2 were the first to report 3 cases of sarcomatoid salivary duct carcinoma. Previously reported cases, which were diagnosed as carcinosarcoma of the salivary glands, may include sarcomatoid salivary duct carcinoma cases.3–12 Rhabdoid cells have been seen in several neoplasms in other organs, including renal tumor,19 brain tumor,20 gastric carcinoma,21,22 lung carcinoma,23 and breast carcinoma.24 In salivary gland neoplasms, however, such cells are not seen frequently. Nagao et al25 reported on 8 cases of sarcomatoid salivary duct carcinoma and, in their report, only 1 case was composed of rhabdoid cells as sarcomatoid components of sarcomatoid salivary duct carcinoma.

We report 2 cases of salivary duct carcinoma with rhabdoid features as carcinomatous components of carcinoma ex pleomorphic adenoma of major salivary glands that were examined immunohistochemically. Written informed consent was obtained from the patients for publication of this case report and any accompanying images.

CASE REPORTS
Case 1: Clinical findings
The patient was a 44-year-old Japanese man who had been suffering from swelling of the left parotid region. The left neck also began to show swelling. He was admitted to our hospital 9 years later. On MRI scan, a 40 × 42 × 70 mm irregularly shaped mass with low intensity on T1 and T2 resonance imaging was seen in the left parotid gland (Figure 1A). Total parotidectomy was performed under the clinical diagnosis of parotid gland carcinoma (cT4aN2bM0). After the operation, radiotherapy (60 Gy) was performed but, after 8 months, multiple bone metastases were found.

Case 1: Pathological findings
Macroscopically, case 1 showed an ill-defined grayish-white mass in the left parotid gland. A whitish elastic-hard nodule was seen in this main mass (Figure 2A).
Histologically, the nodule showed hyalinized stroma with a few spindle cells, which showed marked elastosis with Elastica van Gieson stain and, partly, myxoid stroma with spindle or stellate-shaped myoepithelial cells. This nodule was considered residual pleomorphic adenoma (Figure 2B). In the peripheral transitional area, atypical ductal cells with eosinophilic cytoplasm were observed, which were similar to salivary duct carcinoma cells (Figure 2C). The main tumor was a widely invasive carcinoma, which consisted of the diffuse proliferation of rhabdoid cells with ovoid eosinophilic cytoplasm, eccentric nuclei, marked cellular atypia, and 1 or 2 large nucleoli (Figure 2D). Such rhabdoid cells were nonadhesive. Although approximately 3% of the tumor consisted of the cribriform pattern growth of atypical ductal cells, which was considered to be salivary duct carcinoma, 97% of the tumor was composed of atypical rhabdoid cells. In the periphery of pleomorphic adenoma, a transitional zone was observed between them (Figure 2E). Numerous lymph node metastases were also seen histologically; 6 metastatic lesions showed solid growth patterns, similar to the primary carcinoma, whereas 4 lesions showed noncoherent rhabdoid cells with eosinophilic cytoplasm, eccentric nuclei, and marked cellular atypia (Figure 3D). The proportion of such rhabdoid cells was approximately 40% in the metastatic lesions, although no component of rhabdoid cells was seen in the primary tumor.

The biopsy specimen of the gastric lesion was diagnosed as the conventional type of well-differentiated tubular adenocarcinoma.

Immunohistochemical analysis (case 1 and 2)

Immunohistochemical examination was performed with an Autostainer (DakoCytomation, Dako, Carpinteria, CA). Antibodies used in this study and their results are summarized in Table 1. We used Image J (National Institutes of Health, Bethesda, MD) to estimate the percentage of Ki-67-positive tumor cell rates.

In both cases, rhabdoid cells showed diffuse positivity for pan-cytokeratin (CK), CK7, epithelial membrane antigen (EMA), gross cystic disease fluid protein (GCDFP)-15, and androgen receptor (AR; Figure 4A–4C). Moreover, such cells in case 1 were positive for Her-2, prostate-specific antigen (PSA), and p53 (Figure 4D and 4E). Although the primary carcinoma in case 2 was negative for GCDFP-15, AR, Her-2, and PSA, both this component and the rhabdoid component were strongly positive for p16.

The Ki-67 labeling index was high in both rhabdoid cell components (43.2% and 94.5% in case 1 and 2, respectively; Figure 4F), although residual pleomorphic adenoma components in both cases were very low (5.1% and 0.4% in case 1 and 2, respectively). Regarding mucin molecules, rhabdoid cells were positive for MUC1, MUC6, and CA125, especially in case 2.

In both cases, the spindle cells in the nodules were positive for α-smooth muscle actin (ASMA), calponin, CK14, vimentin, and S-100 protein; therefore, these cells
were neoplastic myoepithelial cells and this nodule was considered to be preexisting pleomorphic adenoma. In case 1, the inner atypical ductal cells in the transitional area were positive for GCDFP-15, AR, Her-2, pan-CK, and EMA (Figure 5A); whereas the spindle cells around such ductal cells were positive for ASMA, calponin, CK14, p63, and S-100 protein (Figure 5B); therefore, this lesion was considered as an in situ component of salivary duct carcinoma. The invasive component of the primary tumor in case 2 showed positivity for pan-CK and EMA, and focal positivity for epidermal growth factor receptor (EGFR) and p63. This lesion was considered adenocarcinoma, not otherwise specified (NOS).

Rhabdoid cells in both cases were negative for all myoepithelial markers, such as ASMA, calponin, CK14, p63, and S-100 protein.

We finally diagnosed case 1 as “salivary duct carcinoma with rhabdoid features ex pleomorphic adenoma” and case 2 as “poorly differentiated adenocarcinoma, NOS, carcinoma ex pleomorphic adenoma” and “metastatic salivary duct carcinoma with rhabdoid features and adenocarcinoma in the cervical lymph nodes.”

FIGURE 2. Histological findings of case 1. (A) The tumor showed a hyalinized nodule in the central area and an invasive component around it (whole mount hematoxylin-eosin stained section). (B) Part of the hyalinized nodule (B area in A: hematoxylin-eosin stain: original magnification ×200) showed spindle-shaped neoplastic myoepithelial cells in the myxoid stroma, which showed the typical histology of pleomorphic adenoma. (C) Atypical ductal carcinoma in situ component (C area in A: hematoxylin-eosin stain: original magnification ×200) was observed in the peripheral portion of pleomorphic adenoma. (D) Invasive component (D area in A: hematoxylin-eosin stain: original magnification ×200) showed the diffuse growth of noncoherent, ovoid-shaped, carcinoma cells. (D: inset: hematoxylin-eosin stain: original magnification ×400) Carcinoma cells showed eccentric nuclei, marked cellular atypia and 1 large nucleolus with eosinophilic cytoplasm. (E) Typical histology of salivary duct carcinoma, which consisted of irregular-shaped atypical glands with eosinophilic cytoplasm, relatively large cytoplasm and marked nuclear atypia, was seen at the invasive component (E area in A: hematoxylin-eosin stain: original magnification ×200).
By electron microscope, tumor cells were variably sized, ovoid, or cuboidal. Tumor cells contained the condensation of intermediate filaments, dilated segments of rough endoplasmic reticulum, and mitochondria (Figure 6). Tumor cells frequently lacked the adherence junction and tight junction. The nuclei of tumor cells frequently showed irregular shapes with dense nuclear membranes.

**DISCUSSION**

Conventional salivary duct carcinoma histologically mimics ductal carcinoma of the breast with a high nuclear grade and a solid, cribriform, or papillary type.\(^1\) In 2000, Henley et al\(^2\) reported 3 cases of salivary duct carcinoma that contained malignant spindle cell elements; the authors designated these neoplasms “sarcomatoid salivary duct carcinoma.” In 2004, Nagao et al\(^3\) reported 8 cases of sarcomatoid salivary duct carcinoma, and they used the term sarcomatoid salivary duct carcinoma to designate biphasic neoplasms with or without heterologous differentiation, provided that the carcinomatous components fulfilled the criteria for conventional salivary duct carcinoma. Nineteen sarcomatoid salivary duct carcinoma cases have been reported in the literature.\(^2–4,25\) Previous cases diagnosed as “carcinosarcoma” or “sarcomatoid carcinoma” might have been sarcomatoid salivary duct carcinoma.\(^3,12–18,25\) Usually, sarcomatoid salivary duct carcinoma consists of both malignant epithelial cells and malignant spindle cells, whereas Nagao et al\(^3\) proposed that sarcomatoid salivary duct carcinoma is composed of both epithelial and mesenchymal components, which contain heterologous elements, such as osteosarcomatous, chondrosarcomatous, giant cell tumor–like, and rhabdoid tumor-like elements. In their report, only 1 case that contained rhabdoid cells was described with 1 figure; therefore, to the best of our knowledge, the present cases are the second to be reported. Histologically, both of our cases showed diffuse proliferation of rhabdoid cells, which were similar to those of case 1 (hematoxylin-eosin stain: original magnification \(\times 200\)).

**FIGURE 3.** Pathological findings of case 2. (A) Macroscopically, the main tumor showed cystic change (asterisks) and included a yellowish nodule (black arrows). The tumor expanded from the right submandibular gland (white arrows). (B) Low magnification view of histology indicated a hyaline nodule, which was a preexisting pleomorphic adenoma (arrows), and the invasive component around the nodule, which was accompanied with cystic change (asterisk; whole mount hematoxylin-eosin stain section). (C) Invasive component showed sheet-like growth of adenocarcinoma cells with central necrosis (hematoxylin-eosin stain: original magnification \(\times 200\)). (D) Part of the metastatic lesions showed diffuse proliferation of rhabdoid cells, which were similar to those of case 1 (hematoxylin-eosin stain: original magnification \(\times 200\)).
TABLE 1. Antibodies used in this study and their results.

| Antigen | Clone | P/M | Source | Case 1 | Case 2 |
|---------|-------|-----|--------|--------|--------|
| pan-CK  | AE-1/3 | M   | Signet Laboratories (Denham, MA, USA) | (myo)+ | + + |
| EMA     | E29   | M   | Progen Biotechnik (Geiderberg, Germany) | -     | - + |
| CK7     | 0V-TL12/30 | M | DakoCytopsation (Carpinteria, CA, USA) | -     | - + |
| LMWK    | CAMS5.2 | M | Becton Dickinson (San Jose, CA, USA) | (myo)f+ | + + |
| CK14    | LL002 | M | Chemicon International (Temecula, CA, USA) | (myo)+ | - + |
| CK5/6   | D5/16B4 | M | DakoCytopsation (Carpinteria, CA, USA) | (myo)+ | (myo)f+ |
| p63     | 4A4   | M | LAB Vision (Fremont, CA, USA) | (myo)f- | - f+ |
| calponin| CALP  | M | DakoCytopsation (Carpinteria, CA, USA) | (myo)f- | - N.D. |
| S-100 protein | P | DakoCytopsation (Carpinteria, CA, USA) | (myo)- | - |
| vimentin| V9    | M | DakoCytopsation (Carpinteria, CA, USA) | (myo)- | (myo)f- |
| GCDFP-15| NCL-GCDFP-15 | M | Novocast Laboratories (Newcastle upon Tyne, UK) | - | + + |
| AR      | AR441 | M | DakoCytopsation (Carpinteria, CA, USA) | -     | - + |
| ER      | SP-1  | M | Thermo Scientific (Fremont, CA, USA) | -     | - + |
| PgR     | 1A6   | M | Novocast Laboratories (Newcastle upon Tyne, UK) | -     | - + |
| Her-2   | P     | DakoCytopsation (Carpinteria, CA, USA) | -     | 2+ |
| EGFR    | NCL-L-EGFR | M | Novocast Laboratories (Newcastle upon Tyne, UK) | f+ | - |
| PSA     | P     | DakoCytopsation (Carpinteria, CA, USA) | -     | - - |
| CEA     | CEM10 | M | TeKaRa Bio (Shiga, Japan) | f+ | - |
| CA125   | 0C125 | M | DakoCytopsation (Carpinteria, CA, USA) | p+ | f+ |
| CA19-9  | 116NS199 | M | DakoCytopsation (Carpinteria, CA, USA) | p+ | f+ |
| MUC1    | NCL-Mab695MUC1 | M | Novocast Laboratories (Newcastle upon Tyne, UK) | (SDC)p+ | + |
| MUC2    | NCL-CLPS6MUC2 | M | Novocast Laboratories (Newcastle upon Tyne, UK) | - | - |
| MUC4    | 1G8   | M | Santa Cruz Biotechnology (Santa Cruz, CA, USA) | - | - |
| MUC5AC  | CLH2MUC5AC | M | Novocast Laboratories (Newcastle upon Tyne, UK) | (SDC)f- | + |
| MUC5B   | P     | Santa Cruz Biotechnology (Santa Cruz, CA, USA) | (SDC)f- | + |
| MUC6    | CLH5MUC6 | M | Novocast Laboratories (Newcastle upon Tyne, UK) | (SDC)p+ | f+ |
| p16     | 484   | M | DakoCytopsation (Carpinteria, CA, USA) | N.D. | N.D. |
| p53     | DO-7  | M | DakoCytopsation (Carpinteria, CA, USA) | f+ | + |
| Ki-67   | MIB-1 | M | DakoCytopsation (Carpinteria, CA, USA) | 5.1% | 43.2% |

Abbreviations: PA, pleomorphic adenoma; SDCRF, salivary duct carcinoma with rhabdoid feature; AdC, adenocarcinoma; CK, cytokeratin; LMWK, low-molecular-weight keratin; EMA, epithelial membrane antigen; GCDFP, gross cystic disease fluid protein; AR, androgen receptor; ER, estrogen receptor; PgR, progesterone receptor; EGFR, epidermal growth factor receptor; PSA, prostate-specific antigen; CEA, carcinoembryonic antigen; CA, carbohydrate antigen; -, negative; f+, focally positive; p+, partially positive; +, positive; +++, diffusely and strongly positive; (myo), myoepithelial cells.

Six of the 19 sarcomatoid salivary duct carcinoma cases (32%) reported in the literature arose from a preexisting pleomorphic adenoma. These occurrence rates are higher than those reported (up to 14%) for conventional salivary duct carcinomas in the review by Barnes et al.27 Salivary duct carcinomas arising in a preexisting pleomorphic adenoma might be more prone to undergo sarcomatoid change than de novo salivary duct carcinomas; however, as only a small number of cases have been examined, additional case studies are necessary to determine whether this is true. On the other hand, the carcinomatous elements of carcinoma ex pleomorphic adenoma showed immunopositivity for AR of 50% to 100%.28–30 and such elements in carcinoma ex pleomorphic adenoma might show salivary duct carcinoma–like features, especially in in situ lesions in pleomorphic adenoma. In our cases, case 1 had an in situ lesion, which was similar to high-grade ductal carcinoma in situ of the breast: the ductal component, which was positive for GCDFP-15 and AR, was circumscribed by neoplastic myoepithelial cells of residual pleomorphic adenoma.28–30

Salivary duct carcinoma with rhabdoid features must be distinguished from several other neoplasms. The differential diagnosis includes collision tumors and hybrid carcinomas.31,32 Collision tumors are the fusion of 2 malignant neoplasms arising from independent topographic sites and they lack a transitional zone, which is commonly found in sarcomatoid salivary duct carcinoma.31 In our cases,
case 2 lacked the transitional zone in metastatic lesions, but these lesions were entirely different from metastasis from gastric cancer. A hybrid carcinoma consisting of salivary duct carcinoma and myoepithelial carcinoma has been reported rarely. Myoepithelial carcinoma can exhibit spindle cell morphologic features, whereas the hyaline type of myoepithelial carcinoma frequently shows plasmacytoid features; therefore, this tumor should be distinguished from salivary duct carcinoma with rhabdoid features. However, myoepithelial carcinoma is usually positive for ASMA, calponin, p63, and S-100 protein, but is usually negative for GCDFP-15 and AR and/or Her-2. Plasmacytoid cells are frequently seen in pleomorphic adenoma and myoepithelioma, but these cells in such tumors lack cellular atypia, such as large and irregular-shaped cytoplasm, marked nuclear atypia, and marked swelling of nucleoli. Moreover, plasmacytoid cells are positive for myoepithelial markers, but negative for GCDFP-15, AR, and/or Her-2, which are markers of salivary duct carcinoma. Metastatic tumors from distant sites should be distinguished from salivary duct carcinoma with rhabdoid features. Malignant rhabdoid tumors arising from the kidney and brain are positive for vimentin, whereas gastric, lung, and breast carcinomas with rhabdoid cells are also positive for both vimentin and CK. In our cases, the carcinoma cells were positive for CK but not vimentin, so they were not exactly “rhabdoid cells”; therefore, we diagnosed them as salivary duct carcinoma cells with rhabdoid features. Although the patient in case 2 also had gastric cancer, this lesion was histologically conventional differentiated adenocarcinoma, different from the metastatic lesions seen in the cervical lymph nodes.

Histologically, salivary duct carcinoma with rhabdoid features is also similar to nonepithelial tumors, such as malignant lymphoma, malignant melanoma, and rhabdomyosarcoma, because of the features of noncohesive cells,
when such a tumor is seen in metastatic lesions. However, salivary duct carcinoma with rhabdoid features cells were negative for lymphoma markers, such as CD45 (leukocyte common antigen), CD20, CD5, and/or CD30 (data not shown). They were also negative for melanoma markers, such as S-100 protein, HMB45, or Melan-A, and for skeletal muscle markers such as myogenin, myoD1, and desmin (data not shown); therefore, salivary duct carcinoma with rhabdoid features should be distinguished from malignant lymphoma, malignant melanoma, and myogenic sarcomas.

In the 19 sarcomatoid salivary duct carcinoma cases, this neoplasm affected 12 men and 7 women, who had a mean age of 63.4 years (range, 37–86 years). The tumors developed preferentially in major salivary glands, especially the parotid glands. Among sarcomatoid salivary duct carcinoma cases and even salivary duct carcinoma cases that have been already reported, only 1 case showed salivary duct carcinoma with rhabdoid features. Even in other salivary gland carcinomas, there has been no description that the tumor cells showed rhabdoid features. One case of salivary duct carcinoma with rhabdoid features, which was reported by Nagao et al., showed no evidence of disease 18 months after initial diagnosis and had no preexisting pleomorphic adenoma. Our patients are alive with disease and both cases arose from preexisting pleomorphic adenoma; therefore, the outcome and origin of salivary duct carcinoma with rhabdoid features remain unclear.

In conclusion, salivary duct carcinoma with rhabdoid features as a carcinomatous element of carcinoma ex pleomorphic adenoma is exceedingly rare. Immunohistochemically, rhabdoid cells were positive for GCDFP-15, AR, CKs, and EMA, which were useful markers for the diagnosis of salivary duct carcinoma with rhabdoid features.

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