Polymorphous Low Grade Adenocarcinoma of Minor Salivary Gland Origin in the Oral Floor; Report of a case with an immunohistochemical study and an analysis of proliferative activity

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A case of polymorphous low grade adenocarcinoma (PLGAC) of minor salivary gland in the oral floor, an extremely rare location for PLGAC, is reported. The histopathology of in this case was characterized by cytological uniformity in a variety of morphological growth patterns, including tubular, solid, trabecular, papillary-cystic, and single file areas. Immunohistochemically, most of the tumor cells were positive for keratin, vimentin, and S-100 protein. Some tumor cells were positive and variably immunoreactive for muscle actin, epithelial membrane antigen (EMA), and carcinoembryonic antigen (CEA). The number of argyrophilic nucleolar organizer regions (AgNOR) is considered to be a reflection of the tumor proliferation rate. The numbers of AgNOR per nucleus in the tubular, solid, and papillary-cystic morphological growth patterns were 1.80 ± 0.08, 1.87 ± 0.04, and 1.78 ± 0.03, respectively.

There was no correlation between the number of AgNOR and the growth pattern.

Key words: polymorphous low grade adenocarcinoma, oral floor, immunohistochemistry, proliferative activity, AgNOR

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Introduction

Polymorphous low grade adenocarcinoma (PLGAC) is a recently described minor salivary gland neoplasm, usually arising intraorally, primarily in the palate(1). The term polymorphous low grade adenocarcinoma was first used by Evans and Batsakis in 1984(2) and first listed in the new classification of salivary gland tumors of the World Health Organization (WHO) published in 1991(1). It has previously been called lobular carcinoma(3,4), terminal duct carcinoma(5,6), and low grade papillary adenocarcinoma(7). PLGAC is an unusual tumor that occurs in the minor salivary glands, particularly in the palate, and has a relatively good prognosis when compared with other salivary gland adenocarcinomas(8,9). This variation in characteristics underlies its histology.

This report describes its histopathological and immunohistochemical characterizations and examines its tumor cell proliferative activity by the sensitive argyrophilic nucleolar organizer region (AgNOR) staining method(10) in a study of a case of oral floor PLGAC.

Case Report

A 79-year-old Japanese woman had noticed swelling in her right oral floor about two months prior to admission; it had progressively increased in size and pain. The patient was admitted on June 13th, 1995, to the Department of Oral and Maxillofacial Surgery at Sapporo Medical University Hospital. At the time of admission, the swelling was an elastic hard, slightly movable mass, 50×25mm, in the right region of the oral floor mucosa with large ulceration. Computerized tomography (CT) revealed a 50×25 mm solid mass near the mandible in the oral floor, which had not invaded into the mandible. It was an ovoid with smooth delineation and a nonuniform density. Physical examination and laboratory data showed no abnormal findings. The patient’s medical history was remarkable only for controlled hypertension and a premature ventricular beat. An initial biopsy suggested adenocarcinoma arising from the minor salivary glands. Surgical resection of the tumor with regional lymph nodes was performed on July 6th, 1995.

The tumor did not adhere to the surrounding connective
tissues. She has been alive and well without any clinical evidence of tumor recurrence or metastasis for a period of six months after the operation.

Materials and Methods

For light microscopy, resected specimens were fixed in 10% neutral buffered formalin, embedded in paraffin according to conventional procedures, and then stained by hematoxylin-eosin (HE) for light microscopic examination.

For immunohistochemistry, paraffin-embedded tissue serial sections, cut at about 4 μm, were prepared, and the streptavidin biotin peroxidase complex method was applied using a HISTOFINE(M) SAB-PO Kit (Nichirei Co., Tokyo, Japan). Primary antibodies used were antikeratin, anti-vimentin, Anti-S-100 protein, anti-smooth muscle actin(m-actin), anti-epithelial membrane antigen(EMA), anti-carcinoembryonic antigen(CEA), and anti-glial fibrillary acid protein(GFAP), as shown in Table 1.

For the AgNOR histochemical staining, a modification of the method of Yekeler et al.(10) was employed using a blue toning solution containing ferric chloride, potassium hexacyanoferrate(II), and oxalic acid. The number of AgNOR dots per about 1,000 random nuclei were counted in 3 to 5 microscopic fields of each main histological pattern type which included solid, tubular, and papillary-cystic patterns. The average number of AgNOR dots per type were determined.

Results

Pathological Findings

Macroscopically, the cut surface of the tumor revealed a solid lesion with areas showing necrosis and hemorrhage as well as encapsulation.

Microscopically, it had a polymorphous appearance, with solid, tubular, trabecular, papillary-cystic, and single file patterns but no cribriform pattern (Fig.1a-e).

Low-power microscopy suggested a well-circumscribed tumor; however, high-power microscopy showed invasive growth extending into the surrounding connective tissue. Perineural, intraneural, and intravascular invasions of
the tumor were seen (Fig. 2a). The stroma was composed of a densely eosinophilic hyalinized fibrous connective tissue. There was no area of chondromyxoid differentiation reminiscent of pleomorphic adenoma. The tumor cells, which were small to medium in size, were predominantly uniform and cuboidal to columnar with clear cytoplasm and indistinct cell borders. They had uniform ovoid to spindle-shaped nuclei with small and inconspicuous nucleoli and without marked cellular atypia. No mitotic figures were observed (Fig. 2b).

**Immunohistochemical Findings**

Immunohistochemical staining patterns are summarized in Table 2. Keratin was partially positive in the solid, tubular, and papillary-cystic patterns (Fig. 3a). Vi

mentin and S-100 protein are constantly positive in all histological patterns (Fig. 3b, 3c). The S-100 protein stain
Fig. 3: Immunohistochemical staining of PLGAC

a. Keratin was partially positive in the tubular pattern.

b. Vimentin is moderately or strongly positive in the cytoplasm of most tumor cells in the papillary-cystic pattern.

c. S-100 protein shows cytoplasmic and nuclear positivity.

d. Actin was positive in peripheral cells of the solid pattern.

e. EMA was partially positive in the ductal lumina in tubular structures.

f. CEA was positive in the ductal lumina in the tubular structure.

Fig. 4: Sensitive AgNOR staining method of PLGAC. Almost all cells contain only one or two AgNOR in very faintly stained nuclei.

reacted with cytoplasm and/or nuclei (Fig. 3c). EMA was partially positive in the ductal lumina proliferating in tubular structures, but not positive in the other areas (Fig. 3e). CEA was variably immunoreactive (Fig. 3f).

Actin was positive in peripheral cells of the solid pattern, but not positive or only weakly positive in the other patterns (Fig. 3d). No GFAP immunoreactivity was seen in any histological pattern.

**Sensitive AgNOR Histochemical Findings**

AgNOR were visible as well-defined dots of varying sizes and shapes inside and outside the nucleoli of the tumor cell nuclei (Fig. 4). The mean number of AgNOR per nucleus in the tubular, solid, and papillary-cystic growth patterns were $1.80 \pm 0.08$, $1.87 \pm 0.04$ and $1.78$
± 0.03, respectively. There was no correlation between the number of AgNOR and any growth pattern.

Discussion

Polymorphous low grade adenocarcinoma (PLGAC) is a minor salivary gland neoplasm recently described by Evans and Batsakis in 1984(2). The second edition of Histological Typing of Salivary Gland Tumours by the WHO in 1991(1), including salivary duct carcinoma, epithelial-myoepithelial carcinoma, and polymorphous low grade adenocarcinoma, and numerous others, have been separated from the adenocarcinoma of the carcinoma of the salivary gland. PLGAC has been established as a distinct type of minor salivary gland adenocarcinoma with characteristic clinico-pathologic findings. Despite the microscopical evidence of invasion and the disturbing neurotropism, the prognosis is good. There is a local recurrence in about 20% of the cases, but regional and distant metastases are uncommon(1). In the anatomic distribution in the oral cavity of 75 cases identified in the Registry of Salivary Gland Pathology at the Armed Forces Institute of Pathology, the palate was the most frequent site of occurrence(58.5%). In descending order of frequency, the other intraoral sites include the lip(18.7%), cheek(16%), tongue(1.3%), floor of mouth (1.3%), pharynx(1.3%), and other(1.3%)(9). Shigematsu et al. reviewed some 130 cases of PLGAC in salivary gland found in the literature. The palate was the most common site(62.3%), followed by buccal mucosa(16.2%), lip(4.6%), retromolar pad(3.8%), parotid gland(3.1%), tongue(2.3%), nasal cavity(2.3%), and other(5.4%)(11). Only a few cases of PLGAC originating in the minor salivary gland of the oral floor, including our case, have been described(8,11).

Histologically, PLGAC is characterized by its morphologic variability, cytologic blandness, and infiltrative growth pattern. The present case had a variety of growth patterns, including solid, tubular, trabecular, papillary-cystic, and single file patterns, but not the cribriform pattern, which is one of the most common microscopic patterns. Perineural, intraneural, and intravascular invasion of the tumors was noted. The origin for PLGAC is proposed to arise from the intercalated duct system, according to the light microscopic, immunohistochemical, and ultrastructural findings showing derivation from both ductal and myoepithelial cell lines (9). The immunohistochemical data of our case mostly indicated its neoplastic myoepithelial nature.

The importance of distinguishing what will be referred here as PLGAC lies in its clinical behavior. While it is generally recognized that adenocarcinoma of the salivary glands is a high grade malignant tumor with aggressive behavior and consequently a poor prognosis, PLGAC appears to follow a relatively indolent course(9).

The most difficult tumor to differentiate histologically from PLGAC is adenoid cystic carcinoma(ACC)(9,13). Histopathologically, the fascicular area and papillary structures are confined to PLGAC. Cytologically, the tumor cells of ACC are generally more basoloid and lack the eosinophilic cytoplasm and vesicular nuclear chromatin which are so typical of PLGAC(8,12). The nuclei of ACC are usually more hyperchromatic and more angular than those of PLGAC. The cytoplasmic staining of PLGAC is eosinophilic to amphophilic, whereas that of ACC displays a very pale to clear staining. EMA and CEA stained the true luminal cells of ACC in equal proportion and intensity. However, in PLGAC, the staining qualities of EMA and CEA were dissimilar to those of ACC. Both luminal and nonluminal cells stained positively for EMA and CEA. Reactivity to EMA was seen in more than 90% of the tumor cells, and reactivity to CEA was markedly variable. It usually ranges from less than 15% to 75% of tumor cells staining positive(13). However, in other reports, EMA immunoreactivity has been found to be negative(14) and weakly positive(our case, 15).

The nucleolar organizer regions(NORs) contain loops of ribosomal deoxyribonucleic acid with ribosomal ribonucleic acid genes that may be qualitatively and quantitatively related to such variables as the cell proliferation rate, cell ploidy, transcriptional activity, and tumor malignancy potential(16). Fujita et al.(17), Ishii et al.(18), and Freitas et al.(19) have reported AgNOR in malignant salivary gland tumors. However, they did not specifically analyze the proliferative activity in each of the histological patterns composing the tumors of PLGAC. In our study, the proliferative activity of the pathological patterns of PLGAC was investigated using the AgNOR histochemical staining method(10), which is more sensitive for detecting NOR dots than former methods. In this present case, there was no correlation between the number of AgNOR and any specific growth pattern. Freitas et al. reported the mean AgNOR numbers in ACC were 1.5(tubular), 2.4(cuboidal), and 2.2(solid) and that of PLGAC was 1.5(19). Fujita et al. reported that the mean AgNOR numbers of ACC were 2.07(tubular), 2.67(glandular), and 3.29(solid) and that of PLGAC was 3.37(17). Ishii et al. reported the mean AgNOR number of ACC was 2.63 and that of PLGAC was 3.19(18). In this present case, the mean AgNOR number of PLGAC was about 1.80. The mean AgNOR number of PLGAC has varied in reports comparing it with ACCs. One reason is that PLGAC has not been fully investigated by AgNOR histochemical methods until recently. The proliferative activity of a total of only 9 PLGAC cases in four reports, including ours, has been investigated by the AgNOR method(17-19). If a significant difference in the mean number of AgNOR between ACC and PLGAC is found, it will be a very useful method for distinguishing ACC from PLGAC.

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References

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