Immunohistochemical demonstration of carcinoembryonic antigen, amylase, secretory component, immunoglobulin A and lysozyme in human minor salivary gland tumors

—A preliminary study—

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Introduction

Carcinoembryonic antigen (CEA) was discovered in 1965 by Gold and Freedman¹⁾. Subsequent immunohistological studies have confirmed that CEA is present not only in colon cancer, carcinomas of the non-digestive organ and salivary gland tumors but even in normal organs as well. Hence, many investigators have been of the opinion that CEA is not specific for cancer but rather has a tendency to increase in amount in the presence of cancer²⁾.

Immunoglobulin secreted in the salivary glands plays a major role in the local immune mechanism of the oral field as has been shown by application of immunohistological techniques and immunoelectron microscopy.

CEA and immunoglobulins have often been studied separately but seldom with regard to their correlation.

These considerations led us to undertake an immunohistological study of five secretory proteins, i.e., CEA, amylase, secretory component (SC), immunoglobulin A (IgA), and lysozyme, in order to examine changes in their localization in relation to carcinogenesis and with a view to recognizing the biological properties and functional profiles of tumors in the minor salivary gland.

Materials and Methods

Twenty-one cases of primary minor salivary gland tumors observed at the Nihon University Dental Hospital were used. Included were 11 cases of pleomorphic adenoma (PMA), 7 mucoepidermoid tumor (MET), and 3 adenoid cystic carcinoma (ACC). Histologically normal minor salivary glands attached to the resected tumors served as controls, together with normal colon mucosa and colon cancers obtained from autopsy and biopsy.

For localization of CEA (DAKO-patts) 1:10, an immunohistochemical technique using the peroxidase-antiperoxidase (PAP) method of Sternberger et al.³⁾ was applied in 5 μ m sections of formalin-fixed tissue and paraffin-embedded tissue.

Immunohistochemical examinations of amylase (Nordic) 1:20, SC (DAKO-patts) 1:20, IgA (Behling) 1:20, and lysozyme (DAKO-patts) 1:40 with PAP method in formalin-fixed and paraffin-embedded sections were processed in the same way as described above.

To increase antigenicity, the sections were incubated in 0.3% trypsin for 15 min at room temperature before treatment with goat serum⁴⁾. Control of the staining was performed by using normal rabbit serum in place of the specific antiserum or by omitting the antiserum.

Results and Discussion

CEA

The results for all normal minor salivary glands were negative. In most PMA, the luminal border of the ductal cells was weakly positive. In MET, among the 3 categories of well, moderately, and poorly differentiated, as classified by Healey et al.⁵⁾, the poorly differentiated group had a strong posi-

tive. The localization of CEA were small tubules and were situated adjacent to the lumina (Fig. 1). The clear cells at the periphery of the tubules were always negative. Cells forming cyst-like space with a typical cribriform pattern and lumen of duct-like structure had positive ACC findings (Fig. 2). In both ACC and MET with a high degree of positive in the poorly differentiated group, the CEA reaction was proportional to the degree of malignancy as reported by Ejek-

Fig. 1 Immunoperoxidase staining of CEA in mucoepidermoid tumor. CEA was present in small tubules. (Counterstain hematoxylin $\times 250$)

Fig. 2 Immunoperoxidase staining of CEA in adenoid cystic carcinoma. CEA was present in cytoplasm at the apical cell border and in the lumen of the microcyst in cribriform pattern. (Counterstain hematoxylin $\times 200$)

Fig. 3 Immunoperoxidase staining of amylase in adenoid cystic carcinoma. Intense staining of secretory material (arrows) in cribriform pattern and apparently non-neoplastic acini at the periphery of tumor. (Counterstain hematoxylin $\times 200$)

Fig. 4 Immunoperoxidase staining of IgA in mucoepidermoid tumor. IgA positive cells were present diffusely in stroma, particularly adjacent to neoplastic duct. (Counterstain hematoxylin $\times 200$)

kam⁶⁾.

AMYLASE

The localization of amylase in the neoplastic tissue corresponded to that of CEA, but it is interesting to note that whereas the normal minor salivary glands reacted positively to amylase (Fig. 3), it was negative to CEA.

IgA and SC

IgA-producing immunocytes were diffusely present in the stroma, particularly adjacent to the neoplastic duct in PMA and MET (Fig. 4) and generally widely showed a more positive reaction to SC than IgA. Aside from ACC positive for hyalinized stroma in the cribriform pattern, all other tumor cells showed negative ACC.

LYSOZYME

Tumor cells in the neoplastic tissue did not show a clear reaction to positive lysozyme and in PMA had weakly positive results similar to IgA and SC. IgA and SC were all found in the same locality as ACC. Negative lysozyme in the neoplastic tissue confirmed the report by Caselitz⁷⁾.

From our preliminary examination, it was found that CEA and amylase were different from IgA, SC and lysozyme in distribution depending on the malignancy of the tumor. It is interesting to note that amylase was found even when affected by tumor. Further accumulation of case data and analysis will be necessary in order to prove the interrelationships between the above 5 secretory proteins in salivary gland tumors⁸⁾.

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Note: While this paper was in press, Caselitz et al. observed tumor antigens in parotid neoplasms. (Caselitz, J. et al.: *J. Oral. Pathol.* 11: 374-386, 1982)

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