06.83.13

Int. J. Oral Surg. 1983: **12**: 340–343

(Key words: *antigen, carcinoembryonic; tumor, salivary gland; immunocytochemistry; cancer, oral*)

Immunocytochemical detection of carcinoembryonic antigen in salivary gland tumors

MARÍA E. ITOIZ, H. E. LANFRANCHI, R. L. CABRINI AND F. V. DOMINGUEZ

Oral Pathology Department, School of Dentistry, University of Buenos Aires and Department of Radiobiology, National Atomic Energy Commission, Buenos Aires, Argentina

ABSTRACT — The immunoperoxidase method was applied for carcinoembryonic antigen (CEA) detection in biopsy specimens of salivary gland tumors. 9 out of 10 adenoidcystic carcinomas revealed a strong and abundant reaction in tumoral glands. 10 other specimens of pleomorphic adenomas showed weak staining in the areas of epithelial proliferation. Normal glands adjacent to the tumor mass revealed a weak but constant reaction on the luminal border. As in other types of gland tumor, the quantitative estimation of CEA production by salivary gland tumors may be useful in the monitoring of recurrencies.

(Received for publication 13 December 1982, accepted 5 March 1983)

The limited specifity of immunoassay of carcinoembryonic antigen (CEA) is still under discussion. However, levels of CEA detected in plasma are at present widely used as a tumor marker and in the monitoring of patients after surgery, chemotherapy or radiation therapy[1,8].

The immunoperoxidase reaction rendered possible the localization of CEA in tissue sections processed for histopathology. This technique has been used in a great variety of biopsy specimens of malignancies as well as in premalignant and benign lesions and in normal tissues. In the earlier reports of GOLDENBERG *et al.*[2,3] a consistent reactivity of malignant tumors of the gastrointestinal tract, lung, ovary and cervix, was found. Other different neoplasms and inflammatory lesions were later found to be positive for CEA staining[6]. Among the benign tumors tested, only those from the gastrointestinal tract and ovary cystoadenoma showed some degree of CEA staining. Normal tissues in these series appeared to have minimum CEA amounts detectable by the current immunohistochemical method, with the exception of the colon and small intestine[2-4,10].

The presence of the antigen is not constant in all cases of a positive-type tumor. Even among colon adenocarcinomas, a certain % of negative cases was found. Positive cases usually produced increments of serum CEA levels which correlate with progression

of the disease. Due to this fact, GOLDENBERG *et al.*[2,3] proposed the immunoperoxidase method as a useful tool not only for detection of new sites of antigen production but also as an easy way to select those cases in which regular and repeated plasma CEA assays are required.

Little information is available on CEA production in adenoid cystic carcinomas and pleomorphic adenomas of salivary glands. The only work found in the literature is a comprehensive analysis of different types of tumor carried out by MCDICKEN & SCOTT[7] who reported a positive CEA staining in 19 out of 32 pleomorphic adenomas and in 8 out of 13 adenoid cystic carcinomas. Among these latter tumors, only one case revealed a strong reactivity.

In the present study, the immunohistochemical detection of the antigen was tested in 10 cases of adenoid cystic carcinomas and 10 pleomorphic adenomas. Although within the same tendency, our series seem to show a rather more consistent and stronger reaction in the adenoid cystic carcinomas than that reported in the aforementioned paper.

Material and methods

Specimens from salivary gland tumors obtained by biopsy or surgical excision during the last 5 years and available as formalin-fixed and paraffin-embedded blocks, were used. 10 cases of adenoid cystic carcinomas and 10 pleomorphic adenomas which contained adjacent normal glands in the same block were selected for this study.

The immunoperoxidase procedure generally conformed with the initial descriptions of PRIMUS *et al.*[9]. Tissue sections were treated with 1.5% hydrogen peroxide to eliminate endogenous peroxidase and the 3-step method was employed using the following sequence: (1) rabbit anti-human CEA antiserum from Dako immunoglobulins Ltd. N.Y. (1/1.000) for 12 h at 4 C; (2) goat anti-rabbit IgG (1/500), 30 min at room temperature; (3) rabbit peroxidase-antiperoxidase complex, from Cappel Lab. Inc. P.A., 30 min at room temperature; (4) diaminobenzidine reaction to reveal sites of peroxidase binding. Adjacent control sections of each case were simultaneously processed by replacing the first antiserum by normal rabbit serum and by rabbit anti-CEA previously adsorbed with purified CEA. Sections of a selected case of colon carcinoma were included as a positive control in every staining run.

Results

The morphologically normal glands in all the specimens studied showed some degree of reactivity on the secretory surface of acinar and duct cells (Fig. 1).

A distinct but patchy reaction was seen in all cases of pleomorphic adenoma. Cellular areas of duct proliferation were positive, the reaction product being mainly localized in the cell cytoplasm and on the apical surface. Areas of chondroid or mixoid metaplasia were devoid of antigen (Fig. 2).

9 out of the 10 adenoid cystic carcinomas showed a strong reaction for CEA in all areas of tumoral proliferation. With the exception of the non-reactive case, the amount of reaction product was much greater in adenoid cystic carcinomas than in pleomorphic adenomas. The central lumen of tumoral ducts of adenoid cystic carcinomas was occupied by strongly reactive material (Fig. 3). In the tumoral areas in which H-E stain revealed a compact cellular proliferation without a clear duct differenti-

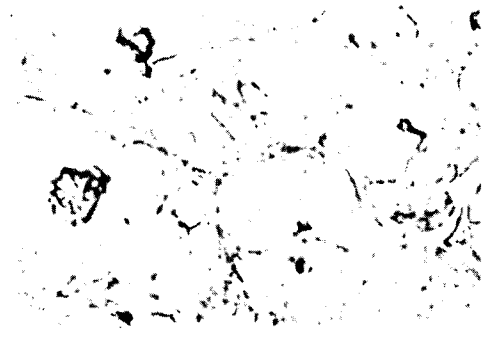

Fig. 1. CEA staining of normal salivary glands adjacent to an adenoid cystic carcinoma. A distinct reaction is seen in the luminal border af acinar cells. ×270.

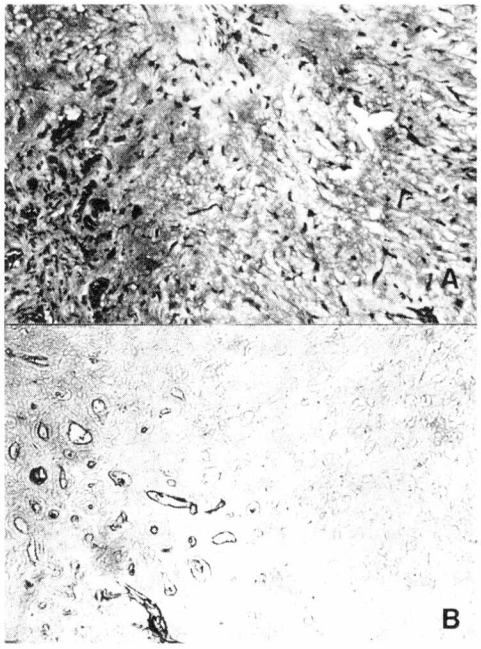

Fig. 2. (A) Haematoxilin and eosin stained section of a pleomorphic adenoma with ductal proliferation (left) and fibro-mixoid areas. (B) CEA stained section of the same case shown in A. Positive reaction in the ductal proliferation areas. Mesenchymal components were devoid of staining. ×110.

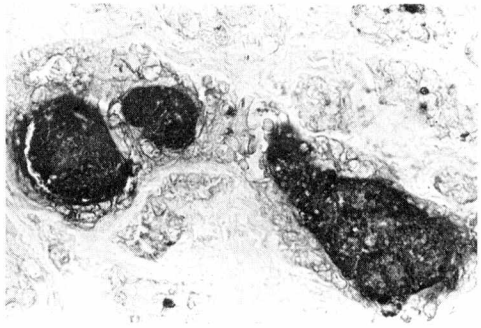

Fig. 3. Intense CEA reaction on the intraglandular deposits of adenoid cystic carcinoma glands. Intracytoplasmic reaction is also evident in tumoral cells. ×230.

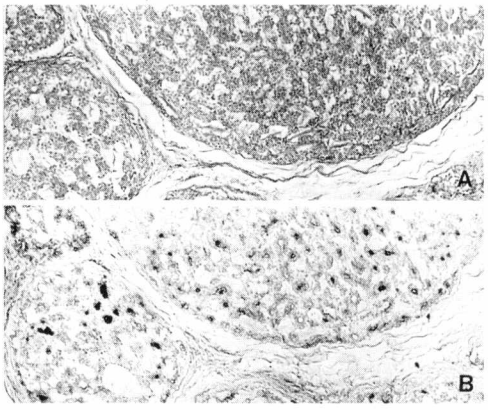

Fig. 4. (A) Compact cellular proliferation of an adenoid cystic carcinoma. H-E stain. (B) Corresponding CEA stained section showing a distinct reaction of the apical cell surface. ×30.

ation, the CEA preparations revealed a double line of reaction, thus indicating the existence of a secretory surface (Fig. 4).

All negative control preparations were devoid of staining, with the exception of varying degrees of non-specific background staining or a weak reaction of inflammatory cells due to an incomplete elimination of the endogenous peroxidase.

Discussion

The normal salivary glands appeared to be a site of CEA production. Although the subjective evaluation of the intensity of staining is rather difficult, the reaction of "normal" salivary glands in all specimens under study seemed to be similar or even stronger than that observed by McDicken & Scott[7] in specimens of non-diseased salivary glands or by Goldenberg *et al.*[3] in only 4 out of 20 samples of normal colon using a similar histochemical technique. These authors did not find positive staining in 35 further specimens of different types of normal tissue. Wagener *et al.*[10], however, reported a constant reaction in normal colon. However, they employed a more sensitive technique.

It is also feasible that the "normal"

glands, being adjacent to tumoral proliferation, might have undergone some early metabolic changes, as was suggested by MACSWEEN & ANDERSON[5], who found an increased production of CEA in suspensions of apparently normal cells adjacent to other different tumors.

Another question arises with regard to the specificity of the immunohistochemical reaction. It is known that salivary mucin is a glycoprotein containing A and B blood groups, which can give a false positive reaction in stained preparations.

The anti-CEA serum used in the present investigation, as specified by its suppliers, was previously adsorbed with blood antigens A and B and with human plasma. Moreover, we found no reaction when preparations were treated with a serum previously adsorbed with the CEA antigen. These facts suggest that within the limits of the immunohistochemical methods, we demonstrated the presence of CEA molecules.

In our series, adenoid cystic carcinomas showed much stronger staining and a greater amount of reaction than that observed in pleomorphic adenomas. This fact seems to be consistent with findings reported for tumors raised in other tissues in which the most agressive types revealed the strongest reactivity. However, MCDICKEN & SCOTT[7] did not observe the same differences in their series of salivary gland tumors. Additional studies of other tumor series would be necessary to clarify this point.

Whether the increased production of CEA in salivary gland tumors can be correlated with CEA levels in serum or saliva, is an interesting topic for further investigation. If this were so, the biochemical CEA test would appear as an accessory tool in monitoring patients after surgical or radiation treatments, once the immunohistochemical staining had proved that the primary tumor is a CEA producer.

Acknowledgements: The authors thank Mrs. Sara Orrea for excellent technical assistance.

Referenees

1. GOLDENBERG, D. M.: Introduction to the international conference on the clinical uses of carcinoembryonic antigen. *Cancer* 1978: **42**: 1397 1398.
2. GOLDENBERG, D. M., SHARKEY, R. M. & PRIMUS, F. J.: Carcinoembryonic antigen in histopathology: immunoperoxidase staining of conventional tissue sections. *J. Natl. Cancer Inst.* 1976: **57**: 11 22.
3. GOLDENBERG, D. M., SHARKEY, R. M. & PRIMUS, F. J.: Immunohistochemical detection of carcinoembryonic antigen in conventional histopathology specimens. *Cancer* 1978: **42**: 1546 1553.
4. ISAACSON, P. & JUDD, M. A.: Immunohistochemistry of carcinoembryonic antigen in the small intestine. *Cancer* 1978: **42**: 1554 1559.
5. MACSWEEN, J. M. & ANDERSON, G. H.: The quantitative relationship of carcinoembryonic antigen to tumor cells of different organs. *Cancer* 1977: **40**: 808 812.
6. LAURENCE, D. J. R. & NEVILLE, A. M.: Foetal antigens and their rôle in the diagnosis and clinical management of human neoplasms. *Brit. J. Cancer* 1972: **26**: 335 355.
7. MCDICKEN, I. W. & SCOTT, J.: The presence and distribution of carcinoembryonic antigen in tumors of human minor salivary glands. *J. Oral. Path.* 1981: **10**: 296 303.
8. MEEKER, W. R.: The use and abuse of CEA test in clinical practice. *Cancer* 1978: **41**: 854 862.
9. PRIMUS, F. J., WANG, R. H., SHARKEY, R. M. & GOLDENBERG, D. M.: Detection of carcinoembryonic antigen in tissue sections by immunoperoxidase. *J. Immunol. Meth.* 1975: **8**: 267 276.
10. WAGENER, C., CSASZAR, H., VOTOVIC, V. & BREUER, H.: Highly sensitive method for the demonstration of carcinoembryonic antigen in normal and neoplastic colonic tissue. *Histochemistry* 1978: **58**: 1 11.

Address:

Maria E. Itoiz
Departamento de Radiobiologia
Comisión Nacional de Energía Atómica
Avda. del Libertador 8250
1429 Buenos Aires
Argentina