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ADENOMA-CANCER SEQUENCE USING CARCINO-EMBRYONIC ANTIGEN IN COLORECTAL ADENOMA WITH EARLY INVASIVE CARCINOMA

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SUMMARY

Immunoperoxidase staining for carcinoembryonic antigen (CEA) was performed on colorectal adenoma with early invasive carcinoma in order to assess its potential diagnostic value. Using formalin-fixed, paraffin-embedded tissues and an immunoperoxidase technique, we have attempted to demonstrate CEA in 10 cases of adenoma with early invasive carcinoma and in adjacent benign mucosa. CEA was demonstrated in adenocarcinoma, in adenoma, and in glycocalyx of surface epithelial cells in normal mucosa. Adenocarcinoma tissues could not readily be identified by immunoperoxidase staining for CEA because the findings had shown a remarkable similarity between adenocarcinoma and adenoma. These findings on adenoma seem to support the view that adenocarcinoma may occur in adenoma and that these polyps have malignant potentiality. As is generally accepted, an adenoma is a precancerous lesion of colorectal cancer. Therefore, the concept of an adenoma-cancer sequence in the colorectal bowel is supported.

INTRODUCTION

The carcinoembryonic antigen (CEA) was discovered by Gold and Freedman in 1965.[4,5] Detection of CEA in serum was initially regarded as diagnostically valuable for colorectal carcinoma and has come to be widely used as a tumor marker to monitor the treatment of gastrointestinal malignancies. Immunohistochemical techniques employing

enzyme-coupled antigen-antibody reactions have greatly improved possibilities to localize antigens in tissues. This method now permits the demonstration of many tumor markers at the tissue level. Immunohistochemical localization of CEA in colorectal tissues has been expected to serve for the diagnosis of malignant and premalignant lesions. However, as CEA was demonstrated in the normal colon, it was claimed to be of little or no value in discriminating benign and malignant lesions.[1,8] Using an immunoperoxidase procedure, and formalin-fixed and paraffin-embedded tissues, we attempted to demonstrate CEA in colorectal adenoma with early invasive carcinoma. The purpose of this paper is to demonstrate the potential diagnostic value of CEA for differentiating adenocarcinoma from adenoma.

Materials and Methods

Surgical specimens of adenoma with early invasive carcinoma were removed from 10 patients with mucosal polyps in the colorectal bowel. Tissues were fixed in 10% formalin solution overnight, dehydrated, and embedded in paraffin. Sections were cut at 4 μm thickness for hematoxylin and eosin, periodic acid Schiff (PAS), and Mayer's mucicarmine stainings. For immunohistochemistry, the sections were stained with anti-CEA -antiserum (Dako PAP Kit) by using the peroxidase-anti-peroxidase (PAP) method.

The steps involved in the immunoperoxidase method for CEA are as follows: (1) Place in 60℃ incubator 30 min. (2) Deparaffinize and hydrate in distilled water. (3) Treat with hydrogen peroxidase for 10 min in a 37 ℃ incubator. (4) Wash in distilled water. (5) Wash in Tris buffer pH 7.4, using three cycle changes of 3 min each. (6) Treat with normal swine serum for 40 min in a 37 ℃ incubator. (7) Tap off excess. (8) Treat with anti-CEA-antibody (Dako) for 2 h in a 37 ℃ incubator. (9) Wash in Tris buffer pH 7.4, using three cycle changes of 5 min each. (10) Treat with swine anti-rabbit for 40 min in a 37 ℃ incubator. (11) Wash in Tris buffer pH 7.4, using three cycle changes of 3 min each. (12) Treat with PAP for 40 min in 37 ℃ incubator. (13) Wash in Tris buffer pH 7.4, using three cycle changes of 3 min each. (14) Treat with amino -ethylcarbazole (AEC) as substrate solution for 40 min at room temperature. (15) Wash in distilled water. (16) Counterstain with Mayer's hematoxylin for 5 min. (17) Wash in distilled water. (18) Mount with glycerol gelatin (Sigma).

Results

In the adenoma with early invasive carcinoma, both adenoma (Fig. 1) and adenocarcinoma (Fig. 2) tissues were strongly and positively stained with the anti-CEA-antiserum. There were no significant differences between adenoma and adenocarcinoma with regard to be immunostainability for CEA. No characteristic pattern of stainability could be found either in the adenocarcinoma or the adenoma. There was no particular localization of CEA in the cytoplasm of adenoma and adenocarcinoma tissues. Nuclei were stained with hematoxylin, but were not stained for CEA. The correlation among tumor

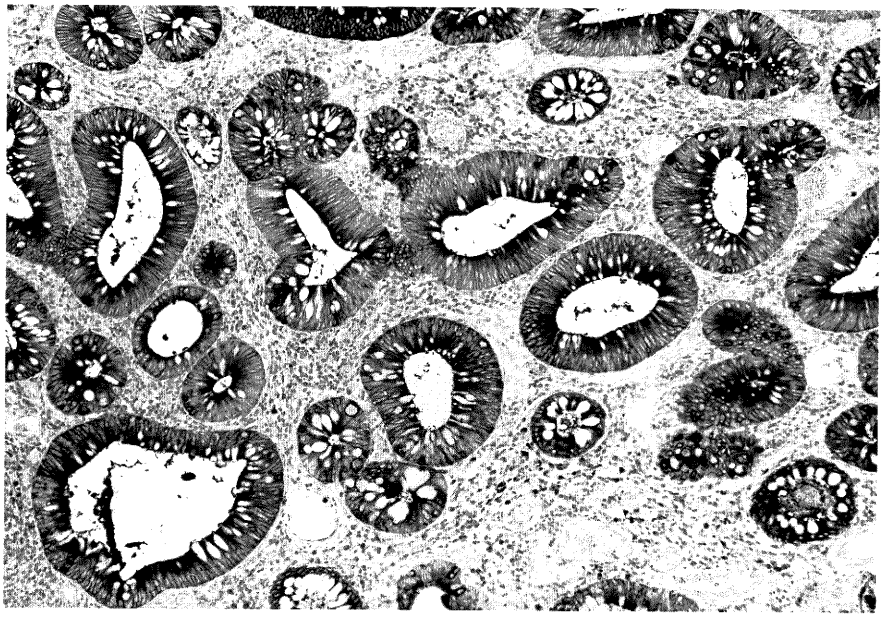

Fig. 1 Adenoma showing strong immunoreactivity for CEA. PAP method. ×100.

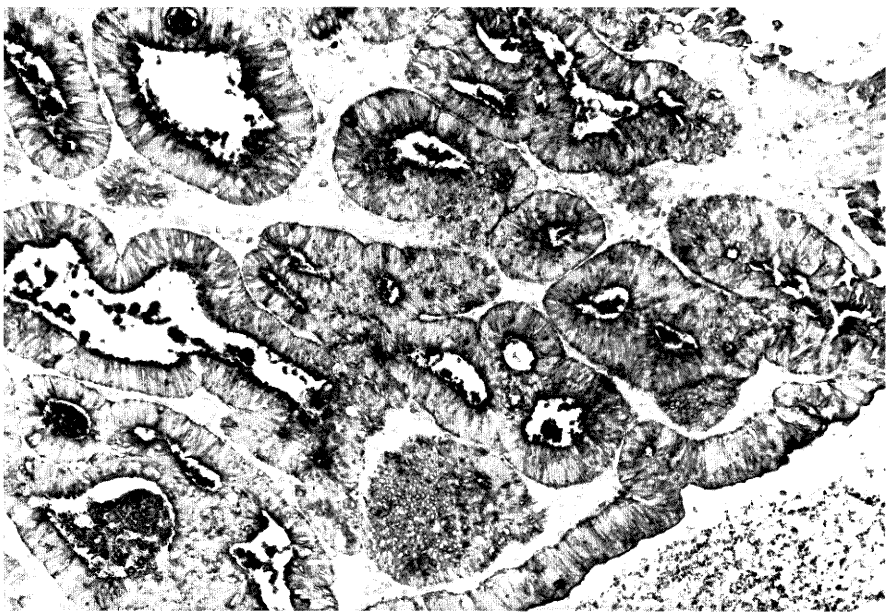

Fig. 2 Adenocarcinoma showing strong immunoreactivity for CEA. PAP method. ×100.

size, range invasion, and degree of differentiation was not admitted.

In our experience, the normal colorectal bowel, revealed CEA-like immunoreavtivity only in the glycocalyx of surface epithelial cells.

Discussion

One of marked advances in surgical pathology is the application of immunohistochemical techniques, and particularly the immunoperoxidase method for the study of antigens preserved in paraffin-embedded sections of formalin-fixed tissue specimens. Numerous reviews stress the diagnostic value of these methods as adjuncts to histological diagnosis.

There has been considerable variation in the reports of CEA localization in colorectal tissues. We found that CEA localization was similar in adenocarcinoma and in adenoma tissues after examining 10 cases of colorectal adenoma with early invasive carcinoma. Many reports on the immunohistochemical localization of CEA in colorectal tissues are optimistic about its potential value in the diagnosis of malignant and premalignant lesions.[2,3,6,7] On the other hand, other investigators describe CEA localization in normal colonic mucosa by immunofluorescence and immunoperoxidase techniques and also demonstrate it by ultrastructural localization within the cytoplasm and glycocalyx, thus they claim that CEA detection has little or no value in discriminating between benign and malignant lesions.[1,8]

For antigen preservation within the tissue, influential factors are autolysis of tissue prior to fixation, method of fixation, inadequate fixation. Furthermore, excessive heat used in the paraffin process may also influence their results. Despite these variations, CEA immunoreactivity was demonstrated in the normal and noncancerous colorectal tissues.[7] Yet the mechanism of CEA immunoreaction in normal colorectal mucosa of surface epithelial cells is not clear. The present finding indicates that CEA immunoreactivity is the glycocalyx of the epithelial cells. The most plausible explanation may be that CEA is a component of the glycoprotein coating of the apical membrane of normal cells. Therefore, the occurrence of CEA immunoreactivity can be raid the dual: in the cytoplasm of cancerous cells and in the glycocalyx of normal epithelial cells.

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