COMPARATIVE IMMUNOFLUORESCENCE STUDY OF CARCINOEMBRYONIC ANTIGEN IN PSEUDOMUCINOUS CYSTADENOMA OF THE OVARY AND ADENOCARCINOMA OF THE LARGE INTESTINE

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The localization of carcinoembryonic antigen (CEA) in a pseudomucinous cystadenoma of the ovary and an adenocarcinoma of the large intestine was studied by the indirect immunofluorescence method. CEA was found to be concentrated in the membranes and basal part of the epithelial cells in both types of tumor.

KEY WORDS: carcinoembryonic antigen; pseudomucinous cystadenoma of the ovary; adenocarcinoma of the large intestine; immunofluorescence analysis.

Carcinoembryonic antigen (CEA) was first described as a systemic cancer antigen characteristic of the entodermal embryonic anlage [6, 7]. However, more recently CEA was found in small quantities in healthy and tumor tissues of both entodermal and nonentodermal origin [1, 11-14]. CEA was found by immunofluorescence analysis on the surface of tumor cells [2, 8, 10] and, in particular, in the glycocalyx of superficial cancer cells of the large intestine [9].

The object of the present investigation was to compare, by immunofluorescence analysis, the histomorphological structures of a pseudomucinous cystadenoma of the human ovary and carcinoma of the human large intestine, both associated with biosynthesis of CEA. The choice of pseudomucinous cystadenoma was determined by earlier observations [3] by the writers, who found CEA in malignant and benign ovarian tumors.

EXPERIMENTAL METHOD

Tumor tissue was obtained during operations in the Department of Obstetrics and Gynecology of the Faculty of Internal Medicine, Second Moscow Medical Institute, and in the Research Laboratory of Proctology, Academy of Medical Sciences of the USSR. All samples of tumor tissue were subjected to careful histomorphological analysis, from which the type of carcinoma of the large intestine and ovary was diagnosed. Samples of normal tissues were obtained at autopsy on persons dying from accidental injury.

Pieces of tissue (3-5 mm) were fixed in absolute alcohol with 1% acetic acid for 15-22 h at 4°C. Subsequent treatment of the tissue was carried out by Sainte-Marie's method [14]. Sections were cut on the MPS-2 microtome for paraffin sections. The thickness of the sections used from treatment with antibodies was 6-8 μ. The sections were dewaxed before immunochemical treatment.

The indirect method of immunofluorescence analysis was used. The sections were treated with pure antibodies of the first (antibodies against CEA) and second (antibodies against rabbit γ-globulins) orders obtained by the use of an immunosorbent prepared on the basis of glutaraldehyde [5].

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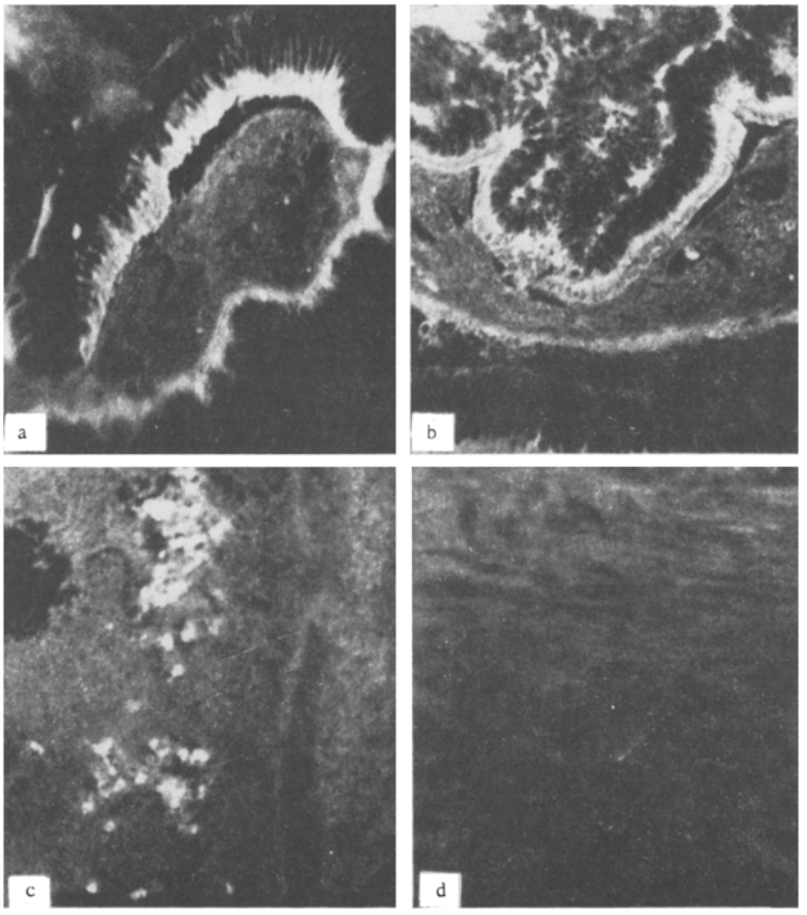

Fig. 1. Immunofluorescence analysis of CEA in sections of pseudomucinous cystadenoma of the ovary (200 ×): a, b, c) sections through pseudomucinous cystadenoma of the ovary treated with antibodies against CEA and with fluorescent second-order antibodies; d) section through the same cystadenoma treated with fluorescent second-order antibodies (control).

TABLE 1. Content of CEA in Tissue Extracts of Pseudomucinous Cystadenoma of the Ovary and Adenocarcinoma of the Large Intestine

Pathohistological diagnosis	Number of tumors	Number of CEA-positive results	Titer
Pseudomucinous cystadenoma of the ovary	14	12	1:4—1:128
Adenocarcinoma of the large intestine	10	10	1:4—1:64
Metastasis of carcinoma of large intestine in ovary	1	1	1:128
Total	25	23	1:4—1:128

The sections were studied in the ML-2B luminescence microscope, using a system of filters to excite secondary fluorescence. The specimens were photographed on KN-3* film. The following controls were used: 1) incubation of the sections with labeled second-order antibodies without preliminary treatment with specific antibodies against CEA; 2) treatment of the sections with antibodies against β_{1G}-globulin, a nonspecific antigen for that particular tissue, followed by treatment with second-order antibodies; 3) treatment of sections of normal liver and spleen, i.e., tissues not containing CEA; 4) treatment of sections of tumor tissue with antibodies against CEA exhausted by the pure CEA preparation.

Semiquantitative determination of CEA in tissue extracts of tumors of the ovaries and large intestine was carried out by titration using a standard test system by the method of Khramkova and Abelev [4].

* The microscopic investigations were carried out in the Institute of Human Morphology, Academy of Medical Sciences of the USSR. The authors are grateful to V. M. Barabanova and A. T. Mikhailov for help with the work.

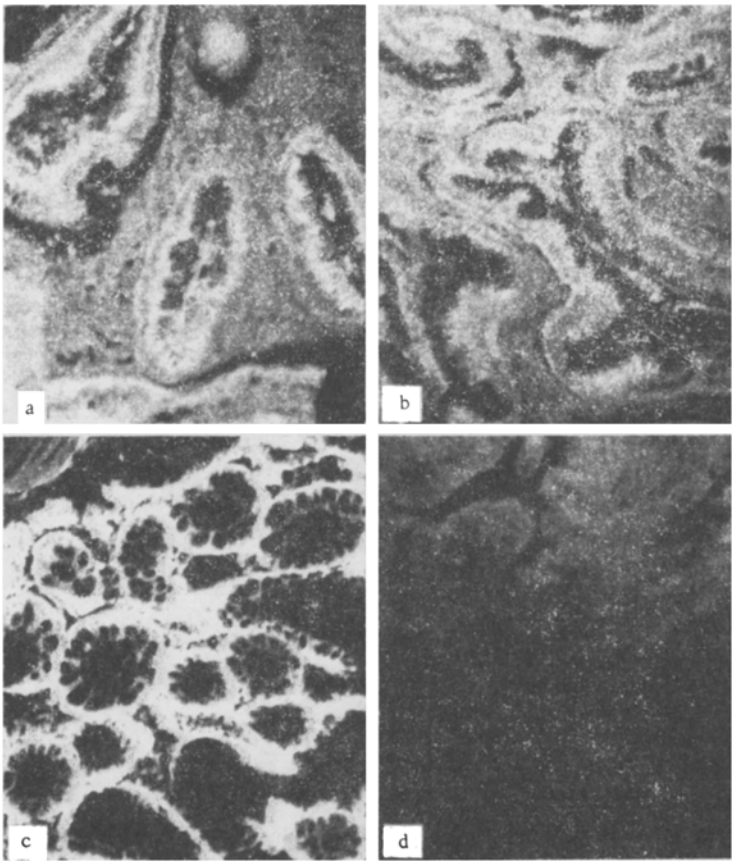

Fig. 2. Immunofluorescence analysis of CEA in sections of adenocarcinoma of the large intestine (200 ×): a, b, c) sections through adenocarcinoma of large intestine (rectum), treated with antibodies against CEA and fluorescent second-order antibodies; d) section through the same adenocarcinoma of the large intestine treated with second-order antibodies (control).

EXPERIMENTAL RESULTS

As Table 1 shows, the content of CEA in individual samples of tumors of both the ovaries and the large intestine varied within approximately identical limits (1 : 4-1 : 128). By semiquantitative determination of CEA it was possible to obtain quite comparable results of immunofluorescence analysis of CEA in the tumor tissues studied.

In sections of the pseudomucinous cystadenoma of the ovary treated with specific antibodies against CEA bright fluorescence of the epithelial cells was observed, especially in their basal part (Fig. 1a, b). Fluorescence of the contents of the cyst was not present in the sections, although individual brightly fluorescent groups of cells could be observed against the dark background (Fig. 1c). The discovery of CEA in the contents of the cystadenoma can evidently be explained by the presence of desquamated CEA-secreting epithelium in them.

A similar picture was observed during immunofluorescence analysis of CEA in sections through the adenocarcinoma of the large intestine. As Fig. 2 shows, bright fluorescence was observed in the basal part of the tumor cells and adjacent membranes. It is interesting to note that bright fluorescence of the membranes and of the basal part of the cells also was found in nearby areas of epithelium of normal glands (Fig. 2c). No background fluorescence could be seen in any of the control investigations, evidence of the high specificity of the immunohistochemical reactions. The results of this immunofluorescence analysis of CEA in the tissue of carcinoma of the large intestine agree with data obtained previously by other workers [2, 8, 10].

The results of these investigations show that CEA is concentrated in the membranes and basal part of the epithelial cells both in carcinoma of the large intestine and in pseudomucinous cystadenoma of the ovary.

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