IMMUNOHISTOCHEMICAL STUDY OF CARCINO-EMBRYONIC ANTIGEN (CEA) IN HUMAN TUMORS USING CRUSTACIN, A CEA-SPECIFIC ONCOPRECIPITIN

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This paper gives the results of a study of human tumors by immunohistochemical methods using a CEA-specific oncoprecipitin, crustacin (CR) by comparison with the use of anti-CEA antibodies (AB). It was shown previously that CR gives a reaction of antibody type with CEA [1]. CR and AB reveal closely related determinants in embryonic and normal human tissues [5], epitopes for CR (CEA-CR) being more embryo-specific than those for AB (CEA-AB). Structural differences may perhaps exist in some of the determinants revealed with the aid of AB and CR.

The aim of the present investigation was to continue the study of specificity and characteristics of interaction of CR and AB with CEA in human tumors, taking into account the morphological and functional features of different tumors.

EXPERIMENTAL METHOD

Altogether 58 different human tumors were studied. Strains of tumors of the rectum (RPK) and colon (RTK) — RTK-9, RTK-11, RTK-12, and RPK-10 — were generously provided by Dr. E. S. Revazova (All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR). Histological sections 5-6 μ thick were cut from tissues fixed in buffered formalin and embedded in paraffin wax, by the method in [8].

CEA was determined in sections with AB and CR by the indirect histochemical method of enzyme immunoassay in the modification described previously, using the same controls [5]. Morphological and functional characteristics of the human tumors were assessed by determining organ-specific intestinal antigen (β-I-MA [2] and pepsinogen C (PGC) [3] by the indirect immunoperoxidase method. The test for β-I-MA was used in all cases, that with PGC only in cases of carcinoma of the stomach.

EXPERIMENTAL RESULTS

CEA, reacting with AB and CR, was found in epithelial neoplasms of varied genesis (Table 1). The localization of CEA-AB and CEA-CR was largely identical — mainly along the apical border of the glandular structures of the adenomas and highly differentiated adenocarcinomas (AC). In less highly differentiated tumors they were localized mainly inside the cytoplasm of cancer cells. The presence of appreciable quantities of CEA-AB was observed always in the extracellular mucus of the CEA-positive malignant neoplasms, whereas, except in tumors of the ovaries, CEA-CR was absent. Despite the undoubtedly similarity of localization of CEA-CR and CEA-AB, distribution within the cytoplasm of tumor cells with different degrees of differentiation was more characteristic of CR. Another feature distinguishing the reaction with CR is the marked zonality of the distribution, i.e., in one preparation regions both positive and negative for CR could be present. Meanwhile, all these zones differed only very slightly in the intensity of their reaction with AB.

CEA was found to be most marked in tumors of the stomach and intestine [6, 7]. However, CR revealed CEA in thse neoplasms in fewer cases and with weaker reactions (Fig. 1), whereas CR, on the other hand, reacted much more strongly than AB with CEA in tumors of the ovaries (Table 1).

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TABLE 1. Results of Immunohistochemical Study of Human Tumors with AB, CR, β-I-MA, and PGC

Neoplasm	AB	CR	β-I-MA	PGC
Carcinoma of colon and rectum	20/20	13/20	19/20	—
highly differentiated AC	9/9	6/9	8/9	—
moderately differentiated AC	5/5	5/5	5/5	—
undifferentiated AC	2/2	2/2	2/2	—
AC transplanted into nude mice	4/4	0/4	4/4	—
Adenoma of colon and rectum	2/2	1/2	2/2	—
Carcinoma of stomach	10/10	8/10	10/10	6/10
highly differentiated AC	5/5	4/5	5/5	4/5
moderately differentiated AC	3/3	2/3	3/3	2/3
undifferentiated AC	1/1	1/1	1/1	0/1
signet-cell carcinoma	1/1	1/1	1/1	0/1
Adenoma of stomach	2/2	2/2	2/2	1/2
Carcinoma of:				
esophagus	2/2	1/2	0/2	—
lungs	2/2	1/2	0/2	—
breast	3/3	3/3	0/3	—
uterus	1/2	1/2	0/2	—
uterine tubes	0/1	0/1	0/1	—
Tumors of ovaries	6/11	9/11	2/11	—
Carcinoma of thyroid gland	0/1	0/1	0/1	—
Pleomorphic salivary gland adenoma	1/1	1/1	0/1	—
Carcinoma of bladder	0/1	0/1	0/1	—

Legend. Numerator gives number of cases with positive reaction, denominator — total number of cases. Test for PGC carried out only with samples for tumors of the stomach.

The intensity of the reactions with CR and AB in intestinal tumors was only slightly dependent on the histological type and level of structural differentiation of the neoplasms, but it will be noted that CR reacted with fewer highly differentiated AC. However, the presence of reactions for β-I-MA in carcinomas with different histological structure, evidence that the tumor contains a large number of functionally differentiated cells, rules out any suggestion of the greater abundance of CEA-CR in anaplastic tumor cells and less in differentiated cells. The opposite results for determination of CEA-AB and CEA-CR in tumors of the human colon and rectum, transplanted into nude mice, indicate certain differences in the antigenic phenotype of tumor strains (Table 1).

Malignant neoplasms of the stomach which were studied contained CEA reacting with AB and β-I-MA in all cases and with PGC in some cases, so that these tumors can be regarded as carcinomas of intestinal or gastrointestinal type [3, 4]. The character of the reaction of CR and CEA in these tumors did not differ from that in tumors of the colon, and in particular, definite disparity was observed here between structural and functional differentiation. This is clear from the difference between expression of tissue markers β-I-MA and PGC compared with the abundance of reactions with CR. The localization of CEA-CR was characterized by zonality of distribution in the tissues and by predominant localization within the cytoplasm.

The reaction with CR in ovarian tumors was more marked (Fig. 2) than that with AB, and this evidently indicates that expression of the determinant revealed by CR is original in character. However, in this case also, close correlation undoubtedly exists between CEA-AB and CEA-CR, as is confirmed by the similarity of their localization in tissues of ovarian tumors.

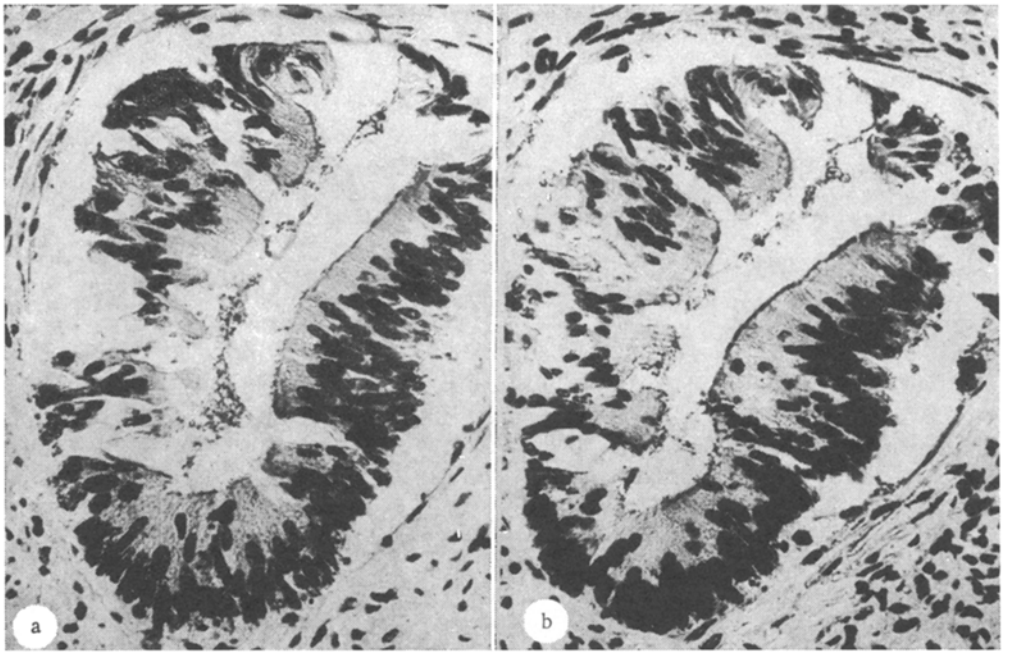

Fig. 1. AC of the colon. a) Immunoperoxidase reaction with CR reveals CEA mainly within the cytoplasm of cells of the tumor structure; b) immunoperoxidase reaction with AB reveals mainly along apical border of cells of tumor structure. Weak reaction in cytoplasm. Here and in Fig. 2: hematoxylin, 360 ×.

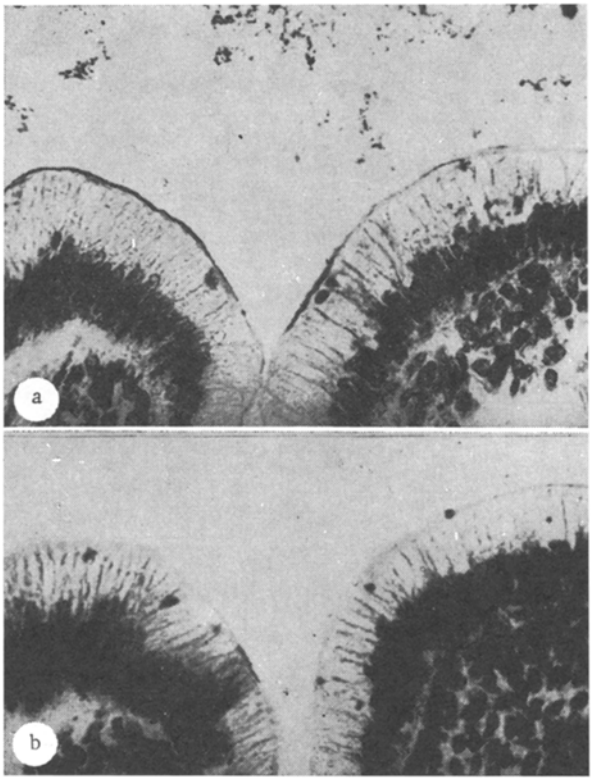

Fig. 2. Pseudomucinous cystoma of ovaries. a) Immunoperoxidase reaction using CR reveals CEA along apical border of tumor cells. Reaction present in mucus; b) negative immunoperoxidase reaction using AB against CEA.

The study of tumors with AB and CR thus confirmed the close kinship between these reagents, which reveal CEA identically as regards localization and specificity. Meanwhile, CEA-CR has undoubted distinguishing features, which are manifested as a unique kind of cellular expression mainly in the cytoplasm of tumor cells, a weaker reaction with CR in tumors of the stomach and intestine, and a stronger reaction in ovarian tumors, and also the clearly defined zonality of their tissue localization. The immunohistochemical properties of CR ob-

served in this study may be determined by structural differences in the antigenic determinants of CEA, revealed by AB and CR. CEA-CR is expressed only at certain moments of existence of this antigen, as is shown by the predominantly cytoplasmic localization of CEA-CR, the marked zonality of its distribution in the tissues, and its absence in mucus reacting with AB. Expression of CEA-CR probably reflects a certain stage of function and differentiation of the tumor cells. Meanwhile, the determinant revealed by CR in ovarian neoplasms may acquire importance of its own and become dominant and determine expression of CEA.

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