Correlation of Carcinoembryonic Antigen Content with Carboxylesterase Activity in Benign and Malignant Human Tissues

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INTRODUCTION

The observation that CE3 activity was present in purified preparations of CEA(s) (6) stimulated inquiry into the possible biological role of CEA. This study was undertaken to compare the amounts of CEA and CE activities in malignant and nonmalignant human tissue extracts.

MATERIALS AND METHODS

Normal, primary, and metastatic tissues were obtained at operation or autopsy, frozen within 1 hr, and stored at —60°. They were also examined histopathologically.

Extraction. After thawing, the tissues were cleaned of fat and necrotic materials, suspended in 2 volumes of deionized water, homogenized at low speed for 20 min in a VirTis flask surrounded with ice cold water, and centrifuged at 4500 × g for 20 min. The supernatants were mixed with an equal volume of 1.2 M PCA, stirred for 10 min at room temperature, and centrifuged at 4500 × g for 20 min. The PCA supernatants were dialyzed for 60 hr against 20 changes of deionized water and were lyophilized to dryness. The lyophilized powders were stored at −20° until analyzed for esterase and CEA activities. Before analysis, the lyophilized PCA extracts were dissolved in 0.05 M borate buffer, pH 8.4, to a concentration of 25 mg/ml (except for liver metastasis of colon carcinoma where the concentration was 100 mg/ml). Ten-μl samples of these solutions were assayed for CEA. Some metastatic tissue samples had to be diluted 10- to 100-fold to obtain measurable results in the CEA assay.

CEA Quantification. The CEA activity in the tissue extracts examined was quantitated by the Hansen zirconyl-gel radioimmunoassay (3) using the standard reagents supplied by Hoffman-LaRoche, Inc., Nutley, N. J. Standard curves and sample dilutions were made in Versene buffer. Five and 10 μl of various tissue extracts of appropriate concentrations in duplicate were added to 10 ml of Versene buffer. The values of CEA obtained at the end of the radioimmunoassay procedure were calculated per mg protein in the extract.

Preparation of Anti-CEA Antiserum. Anti-CEA antiserum was prepared by immunization of a goat with 1 mg of CEA in 0.5 ml PBS (0.15 M, pH 7.4) and 0.5 ml complete Freund’s adjuvant. Serial i.m. injections were continued until an antiserum titer greater than 1:256 was obtained. This antiserum was then absorbed with PCA extracts of normal tissues (liver, colon, and serum) as previously described (1) or by recycling affinity chromatography (CNBr-Sepharose coupled with normal tissue components). When the absorbed antiserum was then assayed in double immunodiffusion (Ouchterlony), counterimmunoelectrophoresis, and immunoelectrophoresis against normal and tumor tissue extracts and purified CEA, only 1 line with identity to CEA was seen. The γ fraction of this antiserum for use in immunochromatographic assay was obtained as described earlier (7).

Esterase Activity Estimation. The esterase activity in the tissue preparations was determined using both p-NPA and α-NA as substrates (β-NA could be used interchangeably with α-NA) in chemical and immunochromatographic assays.

Immunochemical Assay. The immunochromatographic assay used was that of Kaminski and Dubois (4) with some modifications. In this assay, 0.2 ml of tissue extract was added to 0.2 ml of monospecific anti-CEA (1:5) antiserum in sets of duplicate tubes. The tubes were incubated for 30 min at 37° and...
refrigerated at 4° for 60 hr. Tubes were centrifuged and each precipitate was washed 2 times with PBS and finally was suspended in 0.1 ml of 0.05 M sodium phosphate:0.15 M NaCl buffered at pH 7.3. To this suspension was added 1.8 ml of substrate solution and the reaction mixture was incubated for 15 min at 37°. (For example, to make substrate solutions, 186 mg of α-NA and β-NA were dissolved separately in 30 ml of absolute ethanol, and 0.2 ml of this solution was further diluted to 20 ml with 0.05 M PBS at pH 7.3). The reaction was stopped by adding 0.1 ml of 1 N HCl. The absorbance of each tube was read at 350 nm (unclear solutions were clarified by centrifugation). Blanks were (a) substrate solution alone and (b) suspension of immunoprecipitate without substrate. At 330 nm, the absorbance of the esterase was approximately 15 to 20% of that of the reaction product.

Chemical Assay. The chemical assay of Verpoorte et al. (9) was modified to use p-NPA, α-NA, and β-NA (100 mM) as substrates at room temperature and in a 0.05 M PBS at pH 7.0. In this assay, 10 μl of 100 mM substrate solution made in absolute ethanol and 0.3 ml of 0.05 M PBS at pH 7.0 were added in a 3-ml cuvet to 2.7 ml of deionized distilled water and mixed thoroughly with a microglass spatula. To this were added 1 to 10 μl of tissue extracts of appropriate concentrations in aliquots at 5-min intervals. These were also mixed thoroughly, and the absorbance was read at 348 nm. Complete hydrolysis of substrates was achieved within 20 min by various tissue extracts. Further addition of extracts did not change absorbance. Blanks consisted of cuvets containing (a) all reagents except substrate solution and (b) substrate solution that had no reactivity with CEA (6). From ΔA (final A – blank A) the amount of substrate hydrolyzed per min and then per mg of protein was calculated.

Protein Estimation. Protein content of each of these tissue extracts was determined using the Folin phenol method (5). All the values for CEA and CE were calculated per mg protein.

RESULTS

The results indicated that measurable amounts of carboxylesterase activity were present in PCA extracts of human tissues. The CE activities of normal human tissues, primary, and metastatic tumor tissues were compared with CEA activities of the same tissues (Table 1). Of the normal tissues tested, colon had the highest CEA and CE activities (82 to 152 ng/mg protein and 90 to 350 units/mg protein, respectively). All of the primary carcinomas (especially the gastric carcinoma) had higher CEA and CE activities than did the normal pancreas and liver. Normal colon tissues had higher CEA levels than did the primary cancers of the lung, liver, and pancreas. Esterase activities of normal colon were comparable to the esterase activities of these 3 tumors. The CEA activity (100 to 42,000 ng/mg protein) and CE activities (102 to 855 units/mg protein) observed in the metastatic tumors were uniformly higher than those observed in the primary lung, liver, and pancreatic tumors.

When CE activities, measured chemically and immunochemically by hydrolysis of p-NPA and α-NA, were directly compared with the CEA activities of the individual tissue extracts significant correlations (r = 0.938 to 0.980) were seen (Chart 1).

Table 1
Quantitation of CEA and CE activities in PCA extracts of various human tissues

<table>
<thead>
<tr>
<th>Tissues</th>
<th>CEA activityb (ng/mg protein)</th>
<th>Chemical assay</th>
<th>Immunochemical assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-NPA</td>
<td>α-NA</td>
<td>p-NPA</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>13</td>
<td>26</td>
<td>21</td>
</tr>
<tr>
<td>Liver</td>
<td>16</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>Colon (2)</td>
<td>82,152</td>
<td>225,350</td>
<td>90,136</td>
</tr>
<tr>
<td>Primary carcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatoma</td>
<td>40</td>
<td>188</td>
<td>46</td>
</tr>
<tr>
<td>Bronchial</td>
<td>67</td>
<td>200</td>
<td>81</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>70</td>
<td>260</td>
<td>167</td>
</tr>
<tr>
<td>Gastric</td>
<td>1,080</td>
<td>570</td>
<td>470</td>
</tr>
<tr>
<td>Liver metastasis from:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esophageal cancer</td>
<td>100</td>
<td>275</td>
<td>102</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>980</td>
<td>777</td>
<td>369</td>
</tr>
<tr>
<td>Bronchial cancer</td>
<td>11,400</td>
<td>812</td>
<td>613</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>42,000</td>
<td>855</td>
<td>805</td>
</tr>
</tbody>
</table>

a One sample of each type of tissue was tested except for normal colon (2 specimens).
b These values represent the average of multiple analyses of the individual tissues.
c Chemical assay, 1 unit = 1 ng substrate hydrolyzed per min; immunochemical assay, 10 units = ΔA 330 nm of 0.02.
d All primary carcinomas were poorly differentiated adenocarcinomas; the hepatic primary was a mixed hepatocellular/cholangiolitic type. All were widely disseminated except the gastric primary, which was T4 N1 M0 classification (Staging System for Carcinoma of the Stomach: American Joint Committee for Cancer Staging and End Results Reporting, June 1971).
CEA and CE activities tended to be highest in the metastatic tissues and lowest in the normal tissues. Our previous studies (6) using purified preparations of CEA suggested that CE activity was associated with the CEA molecule. The demonstration of a significant correlation between CEA and CE activities in the tissues studied supports our previous hypothesis that esterase activity is either a biological activity of CEA or is derived from an enzyme(s) closely associated with CEA.

Using isoelectric focusing, Young and Bittar (11) reported tissue esterase activity in a variety of human cancers (Hodgkin's disease, lymphosarcoma, and adenocarcinoma of the colon and breast) and in nonneoplastic tissues obtained from cancer patients (liver, kidney, spleen, brain, and red blood cells). The fact that esterase activity occurred in sarcomas, which are not typically associated with marked CEA production, is of interest. Their studies indicated that acetylenecetases (esterases resistant to the inhibitory effects of E 600 and 10 M urea) were prominent in the febrile Hodgkin's patients, colon cancer patients, and some breast cancer patients, while carboxylesterases (esterase activity eliminated by exposure to E 600) were prominent in the afibrile lymphosarcoma patients and in nonneoplastic tissues (liver, kidney, and spleen) obtained from cancer patients. They further suggested that, in histiocytic neoplasms, lysosomal esterases probably constitute a significant proportion of the esterase activity.

The localization of CEA as a membrane secretion (glycocalyx) (2) and CE on the surface of epithelial cells (8) has been reported. Vladutiu et al. (10) have also reported the localization of carboxylesterase in the lysosomal membranes. However, further studies on the localization of the CE activity associated with CEA in malignant and nonmalignant human tissues using immunofluorescence and immunoperoxidase techniques will provide useful information.

The biological significance of these observations remains to be determined. The epithelial and connective tissue elements of tumors with both CEA and CE activity need to be analyzed separately. Biochemical and immunochemical methods should be applied to further fractionation of CE and CEA from active tissue extracts to determine whether they can be separated. The total esterase content of malignant tissues may actually be higher since some esterase activity may be lost during PCA extraction. Quantitation of CE in non-PCA-extracted tissue extracts should be made to see if any esterase activity is lost during PCA extraction. Due to insufficient amounts of available tissues, we were unable to do this in the present study. Analysis of peripheral blood samples of patients with high circulating levels of CEA should be undertaken to determine if there are correspondingly high circulating levels of CE.

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