Retinoic Acid-Binding Protein in Human Breast Cancer and Dysplasia

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ABSTRACT—Seventy-five specimens of human breast tissue were checked for the presence of cellular retinoic acid-binding protein (cRABP). Fifty-two percent of the primary carcinomas and 43% of the dysplastic breast lesions (stage III) contained detectable amounts of cRABP, whereas no cRABP was found in normal tissue. Sucrose gradient centrifugation and electrophoresis on agarose were used for analysis of the presence of cRABP. The cRABP of human origin (normal uterus and neoplastic mammary tissue) differed in its mobility in agarose electrophoresis from that of rat testis cRABP—J Natl Cancer Inst 61: 1375-1378, 1978.

Retinol (vitamin A alcohol) is necessary for normal growth and differentiation of epithelial cells. Recent reports show that retinol and synthetic derivatives may prevent preneoplastic changes caused by carcinogens in epithelial cells (1-4). RA exhibits similar properties (5, 6) as do synthetic analogs of RA (7, 8). Specific binding proteins in a manner similar to that found for steroid hormones

Assay for cRABP.—Aliquots, 250 or 500 μl, of the supernatant fraction were incubated at 4° C for 4 hours in the dark with an equal volume of [3H]RA (10⁻⁷ M) in the assay buffer (50 mM Tris-HCl, pH 7.5). The incubation was carried out in the presence or absence of a 200-fold excess (2X10⁻⁵ M) of unlabeled RA. The unbound ligand was removed by treatment of the solution with 250 or 500 μl of a dextran-coated charcoal suspension (0.25% charcoal and 0.025% dextran in 50 mM Tris-HCl buffer, pH 7.5).

Sucrose gradient sedimentation.—Aliquots, 200 μl, of the supernatant fractions were layered on linear 5-20% sucrose gradients and centrifuged at 4° C for 16 hours at 50,000 rpm.

Agarose electrophoresis.—For the electrophoresis the method described by Wagner (15) was used. Agar was replaced by agarose because agarose has lower electroosmosis. We prepared slab gels by heating 1 g agarose in 100 ml of 50 mM Michaelis buffer, pH 8.2. The clear solution was allowed to cool to 50° C and subsequently poured in a mold containing a glass plate (9.9X8.4 cm) to support the gel. Ten wells (50 μl) along the center line were punched out after the gel had formed. The glass plate containing the gel was placed on a brass plate coated with Teflon, and the temperature was lowered to 0° C by a circulating cooling mixture. We obtained electrical contacts by placing buffer-soaked filter paper at the long ends of the slabs. In the chamber was 100 mM Michaelis buffer, pH 8.2. The test solution was freed from unbound [3H]RA by the above charcoal method and introduced in the center wells (2X50 μl/sample). Electrophoresis was performed immediately to avoid diffusion and run at 120 mA and 230 V for 90 minutes at 4° C. For analysis of the distribution of radioactivity, we sliced the gel into squares (9X3 mm). Only the anodic part of the plate was checked for specific RA binding. The gel slices were dissolved in Instagel and counted for radioactivity. We identified the peaks by running [3H]RA and

Abbreviations used: cRABP = cellular retinoic acid-binding protein; HSA = human serum albumin; RA = retinoic acid.

1 Received December 20, 1977; revised June 19, 1978; accepted June 23, 1978.
2 Supported by the F. Hoffmann-La Roche Foundation and F. Hoffmann-La Roche, Ltd., Basel, Switzerland.
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HSA plus [3H]RA simultaneously with tissue extracts incubated with radioactively labeled ligand. For the mixing experiment, a crude tumor extract (4 mg protein/ml) was mixed with a partially purified rat testis cRABP preparation (1.1 mg protein/ml) and analyzed as described above.

**RESULTS**

Sucrose gradient sedimentation of breast tissue samples incubated with [3H]RA revealed two peaks of...
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1.00
0.80
0.60
0.40
0.20

C

1.00
0.80
0.60
0.40
0.20

C

FRACTION NUMBER

TEXT·FIGURE 3.—Mixing expt with extracts from rat testis and human breast cancer. Agarose electrophoresis was used to analyze for cRABP. A: cRABP from rat testis (1.1 mg protein/ml). B: cRABP from human breast cancer (4.6 mg protein/ml). C: Analysis of the mixed solutions from rat testis and human breast cancer. Samples run in absence (O—O) and presence (●—●) of 200-fold excess unlabeled RA. Arrows indicate start of electrophoretic migration.

radioactivity sedimenting at 2S and 4.6S (text-fig. 1A). The binding at 2S represented the cRABP inasmuch as this peak could be abolished by a 200-fold excess of unlabeled RA. This inhibition was specific for RA

inasmuch as a similar excess of retinol showed no inhibition. According to Sani and Hill (16), the 4.6S peak represented nonspecific binding to serum albumin. The pattern in text-figure 1A was the one encountered in most tissue samples analyzed. Text-figure 1B exhibits a profile typical of a biopsy sample with a very high concentration of cRABP, whereas the tissue sample shown in text-figure 1C contained no detectable amounts of cRABP. The criterion for the presence of a specific RA binding was the abolishment of the 2S peak by an excess of unlabeled RA.

Text-figure 2A demonstrates the sedimentation pattern and text-figure 2B shows the electrophoretic migration of cRABP obtained from a mammary tumor. With both methods, the peak characteristic for cRABP was very prominent. The peak at fraction 11 (text-fig. 2B) disappeared completely with an excess of unlabeled RA. The radioactivity found in fractions 3-5 represented a mixture of free and unspecifically bound ligand to HSA.

On agarose electrophoresis, cRABP of human origin did not migrate to the same position as did the rat testis cRABP.

Text-figure 3A demonstrates the electrophoretic profile for cRABP obtained from rat testis, and text-figure 3B shows the electrophoretic profile obtained for cRABP from human breast tumor. The maximum radioactivity in the rat testis material for cRABP was found in fraction 7 as compared to fraction 11 of the human sample (text-fig. 3B). The mixing experiment suggested that cRABP from the two sources was different (text-fig. 3C). Such a difference went undetected by sucrose gradient centrifugation of the rat testis and the human tumor extracts. The electrophoretic migration pattern of cRABP (text-fig. 3B), obtained from a primary carcinoma, was not restricted to human breast cancer. Human uterus extracts, which contain the RA-binding protein (17), incubated with [3H]RA and subjected to agarose electrophoresis, showed the ligand specifically bound also at fraction 11 (results not shown). Furthermore, the electrophoretic profile of extracts from human breast tumors in stage MII (proliferation of ductules in breast tissue plus intraductal epithelial proliferation) was the same as in the primary carcinomas (results not shown).

Table 1 shows the distribution of cRABP in breast dysplasias, with different proliferative activities, and in primary carcinomas. In 52% of the carcinomas, cRABP

Table 1.—Distribution of cRABP in dysplastic and neoplastic human mammary tissue

<table>
<thead>
<tr>
<th>Type of tissue, stage*</th>
<th>No. of biopsies</th>
<th>No. of tissue extracts containing cRABP</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI-F</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MI</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MII</td>
<td>14</td>
<td>6</td>
<td>43</td>
</tr>
<tr>
<td>CA</td>
<td>29</td>
<td>15</td>
<td>52</td>
</tr>
</tbody>
</table>

* Classified according to the criteria in (18); MI-F, simple dysplasia with extensive fibrosis; MI, simple dysplasia; MII, proliferative dysplasia; and CA, primary carcinoma.
activity was detected. Also, 43% of the specimens with pronounced epithelial proliferation (stage MI) contained cRABP. Neither of the 16 samples graded MI-F (fibrosis and atrophy of the breast tissue) nor MI (proliferation of ductules in breast tissue) exhibited detectable amounts of cRABP.

**DISCUSSION**

These results add more information to our understanding of the distribution of cRABP in the different stages of breast tumor formation. In contrast to Ong et al. (13), we found cRABP in only 52% of the invasive carcinomas examined. Therefore, cRABP cannot necessarily be called an obligatory product of carcinoma formation. Also, we detected the binding protein in less than half of the breast lesions of proliferative dysplasia (stage MII), which are considered as being possibly precancerous. None of the tissue specimens graded MI-F, which was considered normal tissue, contained cRABP (table 1).

Retinoids obviously influence the differentiation of epithelial cells (6, 8). Attempts to elucidate their mode of action in relation to the occurrence of steroid receptors in breast carcinomas are in progress in this laboratory. These studies and those concerning the adenyl cyclase protein kinase system as biochemical markers (19, 20) may provide a better understanding of the many factors influencing the development of human breast tumors.

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