Tumor-associated Antigen(s) from Granulosa Cell Carcinomas of the Ovary

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SUMMARY

These studies indicate that granulosa cell and granulosa-theca cell cancers of the ovary contain two common tumor-associated antigenic components that are apparently absent from normal ovaries and other histologically similar normal organs. The tumor antigens were immunologically distinguishable from the tumor antigens of epithelial ovarian cystadenocarcinomas but were indistinguishable from the tumor antigens of squamous cell carcinoma of the cervix.

The granulosa cell carcinoma antigens were extractable with 0.6 M perchloric acid, and the antigenic activity was contained in a single absorbance peak when fractionated by gel filtration. Extracts of the malignant granulosa cell tumors, the tumor antigenic material isolated by gel filtration, and the serum of the patients bearing these tumors all appeared to contain carcinoembryonic antigen by a Hansen-type radioimmunoassay. However, immunological testing by direct Ouchterlony reaction and counter-electrophoresis failed to detect any carcinoembryonic antigen in the tumor extracts. Also, the precipitin-inhibition studies showed a clear difference between carcinoembryonic antigen and granulosa cell cancer antigens.

INTRODUCTION

During recent years, increasing evidence has accumulated indicating that various human neoplasms possess tumor-associated antigens. Much of this work has been stimulated by the description by Gold and Freedman (8) of CEA in embryonic digestive tissue and in entodermlarly derived neoplasms.

Several studies (1, 4, 6, 7, 10, 11) have suggested the presence of tumor-specific (associated) antigens from serous cystadenocarcinoma of the ovary and squamous cell carcinoma of the cervix. These studies further suggest that the demonstrable antigen(s) is common to tumors of the same histological type. It has also been shown that serous and mucinous cystadenocarcinomas of the ovary are immunologically indistinguishable by Ouchterlony double-diffusion, precipitin-inhibition (2), and leukocyte migration-inhibition techniques (4), in that they share a common tumor antigen that is not detectable in other tumors of the reproductive tract. Serous and mucinous cystadenocarcinomas of the ovary also possess an additional tumor antigen that does cross-react with other gynecological cancers (2). The tumor antigens of serous and mucinous cystadenocarcinoma of the ovary have been shown to be immunologically unrelated to CEA (1).

In this communication we describe a tumor antigen(s), extracted from granulosa cell ovarian cancers, that is different from the major, distinct ovarian cystadenocarcinoma antigen(s) (2) but is immunologically identical to the tumor antigen(s) of cervical carcinoma. The antigen(s) also appeared to cross-react with anti-CEA sera in radioimmunoassay testing but not enough to be identifiable as CEA by double-diffusion and counter-electrophoresis techniques.

MATERIALS AND METHODS

Surgical specimens of normal and cancerous tissues were obtained from the operating room immediately after removal and kept frozen at —20°. Autopsy specimens of normal liver and kidney were collected within 3 to 4 hr of death. All specimens were examined histologically.

Extraction Procedure. Buffered-saline extracts of 1 histologically characteristic granulosa cell and 1 granulosa-theca cell cancer of the ovary were prepared according to the method previously described for serous cystadenocarcinoma of the ovary (1). In addition to buffered-saline extraction, these tumors were extracted with 0.6 M perchloric acid (1). Twelve normal ovaries were separately pooled for extraction. Buffered-saline extracts of other individual specimens to be tested (summarized in Table 1) were similarly prepared.

Preparation of Antisera. Groups of 3 rabbits were given injections of buffered-saline extracts of the granulosa cell cancer and of buffered-saline extracts of pooled normal ovaries. The antitumor sera and the anti-normal ovary sera thus produced were serially absorbed with pooled human sera and red cells representing all the major blood groups, with homogenates of pooled normal ovaries, and with sediments of human liver and kidney. Two ml of each antisera were serially adsorbed with 1 ml of pooled normal human sera, with 0.5 ml of washed, packed red cells, and with pooled normal ovary homogenate obtained from 2 g of tissue. Twenty % homogenates of normal human liver and
Tissue extracts

<table>
<thead>
<tr>
<th>Tissue Extracts</th>
<th>No. of Specimens Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous cystadenocarcinoma</td>
<td>12</td>
</tr>
<tr>
<td>Mucinous cystadenocarcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Squamous cell carcinoma of cervix</td>
<td>8</td>
</tr>
<tr>
<td>Primary colon cancer</td>
<td>2</td>
</tr>
<tr>
<td>Metastatic colon cancer to liver</td>
<td>1</td>
</tr>
<tr>
<td>Normal cervix</td>
<td>10 (pooled)</td>
</tr>
<tr>
<td>Normal fallopian tube</td>
<td>2 (pooled)</td>
</tr>
<tr>
<td>Normal endometrium</td>
<td>2 (pooled)</td>
</tr>
<tr>
<td>Normal colon</td>
<td>2 (pooled)</td>
</tr>
</tbody>
</table>

Kidney were prepared in phosphate-buffered saline, pH 8.0, and then 10 ml of each were centrifuged at 40,000 x g for 30 min. These high-speed sediments, rather than the whole homogenates, were used for adsorption of the above-described antisera. After adsorption, the sera were clarified by centrifugation at 100,000 x g for 1 hr, and the clear supernatants were concentrated to the original volume of antisera by freeze drying. Further details of immunization procedure, harvesting of sera, and absorptive techniques have been described previously (1).

**Fractionation of Tumor Antigen.** Fractionation of the perchloric acid-soluble material of granulosa cell tumor of ovary was performed with gel filtration columns (1.5 x 90 cm) of Sephadex G-200; particle size, 40 to 120 μm. The column was packed with Sephadex and equilibrated by washing with 0.05 M sodium phosphate buffer, pH 5.5, containing 0.1 M NaCl. Lyophilized perchloric acid-soluble extract of granulosa cell cancer was dissolved in this buffer and applied to the column. A flow rate of about 10 ml/hr was maintained throughout the elution procedure. The effluent samples were analyzed for protein and nucleic acid spectrophotometrically at 280 and 260 nm. According to the elution profile, tubes were pooled and each fraction was concentrated by membrane dialysis and tested by Ouchterlony technique against the appropriate antisera.

**Immunodiffusion.** Immunodiffusion experiments were carried out on microscope slides in 1% agar buffered with pH 7.4 barbital (Γ/2 = 0.15) containing 0.02% sodium azide as preservative. The desired patterns were cut in the gel plates, and antibody wells were filled with 30 μl of antisera; antigen wells were filled with 30 μl of antigen. The protein concentration of the tissue extracts was 10 mg/ml unless otherwise specified.

**Precipitin-Inhibition Technique.** Precipitin-inhibition studies were done with Miles immunodiffusion discs containing 0.9% agarose in a borate-0.9% NaCl solution buffer system, pH 8.5 (Γ/2 = 0.175), 0.01% Merthiolate. In the precipitin-inhibition reaction, each well was filled with 150 μl of test material (antigen, or 50 μl of antisera and 100 μl of antigen in combination). The protein concentration of the tissue extracts was 40 mg/ml unless otherwise specified.

**Radioimmunoassay.** CEA assay was performed by the Hansen method, as previously described (5).

**Counter Electrophoresis.** The agar plate was prepared as described by Cawley (3), and the modified technique of Gocke and Howe as described by Teague (15) was used.

RESULTS

In the immunodiffusion studies, the absorbed antitumor sera formed 2 precipitin bands against the granulosa cell cancer extract (Chart 1A), whereas absorbed anti-normal...
ovary sera failed to develop any precipitin bands against this tumor extract. The granulosa-theca cell tumor reacted identically to the granulosa cell tumor against absorbed anti-granulosa cell tumor sera (Chart 1B). Further absorption of the absorbed antisera with an excess of pooled normal ovarian tissue components did not eliminate the 2 bands. Absorption of the antisera with either granulosa cell tumor extract or granulosa-theca cell tumor extract completely inhibited precipitin band formation (Chart 2A). When the absorbed antitumor sera were reacted against tissue extracts from normal ovary, cervix, endometrium, and fallopian tube, no precipitin band was obtained (Chart 1B).

**Fractionation of Tumor Antigen(s).** The tumor-associated antigen(s) was found to be soluble in 0.6 M perchloric acid. The elution profile of the perchloric acid-soluble material from the granulosa cell cancer from a Sephadex G-200 column is presented in Chart 3. The void volume of the column was 54 ml. Immunodiffusion tests showed (Chart 1C) the specific antigenic activity to be associated with the 1st peak just after the void volume of the column. When assayed for CEA activity, the buffered-saline extracts, crude perchloric acid extracts, and G-200 column fraction of the perchloric acid-soluble material all produced marked inhibition in the radioimmunoassay test system, indicating cross-reactivity with CEA. CEA values as calculated from the standard inhibition curve showed 24.0 ng CEA/mg protein of buffered-saline extracts and 64.5 and 70.8 ng CEA/mg of perchloric acid extracts and G-200 column fraction of the perchloric acid-soluble material, respectively. The blood specimens from the 2 patients with granulosa cell cancer were also found to contain high levels of CEA by radioimmunoassay (19.0 and 11.0 ng/ml).

When the absorbed rabbit anti-tumor sera were tested by direct Ouchterlony reaction (Chart 1D) against a buffered-saline extract of colon carcinoma and against both buffered-saline and perchloric acid extracts of a metastatic colon cancer, known to contain CEA, no precipitin band was formed.

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**Chart 3.** Elution profiles of granulosa cell cancer perchloric acid-soluble extract from Sephadex G-200 column.

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**Chart 2.** Examples of precipitin-inhibition reactions done with absorbed anti-granulosa cell cancer sera and various tumor extracts. A, center well: granulosa cell cancer extract; peripheral wells: 1, absorbed antisera + granulosa cell cancer extract; 2, absorbed antisera + granulosa-theca cell cancer extract; 3, absorbed antisera + colon cancer extract; 4, absorbed antisera + colon cancer perchloric acid-soluble extract; 5, absorbed antisera + metastatic colon cancer extract; 6, absorbed antisera + metastatic colon cancer perchloric acid-soluble extract. B, center well: granulosa cell cancer extract; peripheral wells: 1, absorbed antisera + granulosa cell cancer extract; 2, absorbed antisera + squamous cell carcinoma of cervix extract; 3, absorbed antisera + squamous cell carcinoma of cervix extract from another patient; 4, absorbed antisera + mucinous cystadenocarcinomas extract; 5, absorbed antisera + pooled serous cystadenocarcinomas extract; 6, absorbed antisera + pooled serous cystadenocarcinomas perchloric acid-soluble extract.
sera and the same tumor extracts noted above. Complete precipitin inhibition was achieved when extracts of squamous cell carcinoma of the cervix were mixed with the absorbed antisera, whereas treatment of the absorbed antisera with extracts of serous or mucinous cystadenocarcinomas of the ovary eliminated only 1 precipitin band.

**DISCUSSION**

A granulosa cell cancer and a malignant granulosa-theca cell tumor of the ovary were found to contain at least 2 tumor-associated antigens. The tumor antigens were extractable from these tumors with 0.6 M perchloric acid, and fractionation of the antigenic material by gel filtration revealed a single absorbance peak of antigenic activity. These tumor antigens were immunologically indistinguishable from the tumor antigens of squamous cell carcinoma of the cervix by direct Ouchterlony reaction and precipitin-inhibition techniques. The basis for the immunological relationship between granulosa cell cancer and squamous cell carcinoma of the cervix is not clear and has not been further studied. As has been true of all the female reproductive tract cancers we have tested (2), the malignant granulosa cell tumors showed cross-reactivity of a minor antigenic component with serous and mucinous cystadenocarcinomas of the ovary but were otherwise immunologically different.

Extracts of the malignant granulosa cell tumors, the tumor antigenic material isolated by gel filtration, and the serum of the patients bearing these tumors appeared to contain CEA by radioimmunoassay techniques. However, immunological testing by direct Ouchterlony reaction and counter-electrophoresis failed to detect any CEA in these tumor extracts. Possible interpretations of these results are that the conditions of the CEA assay used may have modified the tumor antigen(s) of malignant granulosa cell tumors so as to cross-react with CEA antigen, or there may be minute amounts of CEA present in granulosa cell carcinomas of ovary not detectable by the nonradioimmunoassay methods. Our studies do not distinguish between these 2 possibilities. Although the radioimmunoassay for CEA described by Hansen et al. (9) or by Moore et al. (13) have shown elevated levels of CEA in the plasma of patients with cancers arising from many different organ sites, immunological confirmation of the presence of CEA has rarely been made for tumors outside of the gastrointestinal tract. Sizaret and Martin (14) using immunological criteria have detected an antigen in pulmonary cancer that is identical to CEA. McNeil et al. (12) demonstrated an antigenic substance common to benign mucinous cystadenomas of the ovary and colonic cancers. It is of interest that this antigenic material was not detected in malignant ovarian adenocarcinomas by the gel-diffusion method.

**REFERENCES**


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