A Carcinofetal Antigen Located on the Membrane of Cells from Rat Intestinal Carcinoma in Culture

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SUMMARY

Heteroantisera were produced in rabbits by immunization with cultivated cells from rat intestinal carcinomas. The sera were made specific by in vivo absorption in syngeneic rats. On immunofluorescence, these sera recognized a membrane-associated antigen common to five different intestinal carcinomas and to fetal intestine. The antigen was not detected in noncancerous adult intestine, nonintestinal fetal tissues, or three nonintestinal tumor cell lines.

INTRODUCTION

In addition to the tumor-specific transplantation antigens, usually characteristic of an individual cancer, tumor-associated tissue-specific antigens have been described in a range of human tumors (1, 3, 7, 9) or chemically induced animal tumors (14). Such antigens are usually present in the corresponding fetal tissues. A carcinofetal antigen, apparently specific to cancerous or fetal intestinal cells of the rat, is described in this study.

MATERIALS AND METHODS

Animals and Tumors. Three inbred strains of rats were used: BN and Lewis strains, initially supplied by the Centre de Selection des Animaux de Laboratoire, Orléans-la-Source, France; and BD-IX strain, donated by Professeur H. Druckrey, Freiburg, Germany. Colonies of these strains have been maintained by brother-sister mating in our laboratory. Induction of intestinal carcinomas by DMHN, or MNNG and the obtaining of transplantable lines of these tumors have been previously reported (10–12). Lines DHB, DHD, and DHE (DMH-induced colonic carcinomas in BD-IX rats), NGA (MNNG-induced duodenal carcinoma in BN rats), and LGA (MNNG-induced duodenal carcinoma in Lewis strain rats) have been used in this work.

Cell Culture of Rat Intestinal Carcinomas. Cell cultures of the 5 above-mentioned transplantable lines have been initiated by trypsinization of the grafted tumors. Pure epithelial-like colonies (Fig. 1) were obtained by selectively removing fibroblasts by repeated 10-min incubations in 1% disodium EDTA in phosphate-buffered saline. The cells grew as monolayers in 75-sq cm Falcon tissue culture flasks in Ham's F10 medium, supplemented with 10% fetal calf serum and 10% normal human serum. They were serially passaged after 0.5% trypsin dissociation. Injections of cultivated cells s.c. grew as adenocarcinomas in syngeneic rats. Cultures obtained between the 3rd and the 15th passage were used in this work. Other culture lines have been obtained from the Department of Biochemistry, University of Dijon, France (line 6FR, 10th passage, normal hepatocytes of Wistar rats); Institut de Recherches Scientifiques sur le Cancer, Villejuif, France (line LF, 422nd passage, dimethylaminoazobenzene-induced hepatoma, in Wistar rats); and Department of Tumor Biology, Karolinska Institutet, Stockholm, Sweden (neurinoma GE3, 44th passage; glioma 290T, 22nd passage; both lines obtained from BD-IX rats treated with ethylnitrosourea).

Specific Antisera. Rabbits were immunized by tumor cells DHD or DHE trypsinized from cell cultures. They received 3 i.p. injections (1 to 6 × 10⁶ cells/injection) at 15-day intervals and were bled 10 days after the last injection. The specificity of the antisera was obtained by in vivo absorption (8). BD-IX rats received 0.1 ml of rabbit antiserum per kg i.p. and were bled by cardiac puncture 16 hr later. According to the tumor cell line used for immunization, rat sera will be referred to as anti-DHD or anti-DHE sera. Control sera were prepared from BD-IX rats bled 16 hr after i.p. injection of 10 ml of normal rabbit serum per kg.

Indirect Immunofluorescence. Target cells were prepared by mechanical dissociation of tissues (normal colon, lymph nodes, fetal tissues) or trypsinization of cell cultures. In this case, cells were always used 4 to 6 hr after enzyme treatment. Undiluted antiserum (50 μl) was added to 5 × 10⁴ target cells. After 20 min incubation at room temperature, cells were washed twice in Ham’s F10 medium. Fluorescein isothiocyanate-conjugated anti-rabbit Ig sheep serum (Institut Pasteur, Paris, France) was added (50 μl) for 20 min. Cells were washed 3 times, put in suspension in 100 μl of buffered glycerol:Ham’s F10 medium, 1:1 (v/v), and examined for membrane fluorescence using a Leitz Orthoplan fluorescence microscope. The ratio of cells demonstrating membrane fluorescence to living cells was determined by 2 independent observers. The dead cells showing intracellular fluorescence were disregarded; their percentage was...
always low (less than 10%) for lymphocytes or cells prepared from culture, and was usually higher (up to 50%) in freshly prepared adult or fetal intestinal cells.

RESULTS

The results of the indirect fluorescence tests are given in Table 1. All 5 intestinal cancer lines gave a positive membrane fluorescence reaction (Fig. 2) with both anti-DHD and anti-DHE sera but not with control serum. The percentage of stained cells and the intensity of the reaction were, however, weaker with duodenal lines LGA and NGA than with colonic lines DHB, DHD, and DHE. The reactivity of anti-DHD and anti-DHE sera with homologous cancer cells could be removed by absorption with other tumor cell lines, suggesting that a common antigenic specificity was being detected (Table 2).

No fluorescence was found when anti-DHD or anti-DHE sera were tested on normal adult colonic cells, lymphocytes, or hepatocytes (Table 1). However, intestinal cells from 18- and 21-day-old fetuses showed a positive reaction, whereas nonintestinal fetal tissues (liver or cells from the cephalic end) gave negative results. No fluorescence was found on dissociated cells from 14-day-old fetus, but the intestinal tube could not be isolated, and fluorescence on the few intestinal cells could have been obscured by the bulk of negative nonintestinal cells. The intestinal cells of a 2-day-old newborn rat were also negative. Anti-DHD and anti-DHE sera did not react with cells from nonintestinal cancer lines (hepatoma LF, neurinoma GE 3, or glioma 290 T).

DISCUSSION

The results of this study demonstrate that rat intestinal carcinomas from 5 different lines contain a common membrane-associated antigen. The presence of this antigen does not depend on the rat strain (BD-IX, Lewis, or BN), the carcinogenic agent used to induce the tumor (DMH or MNNG), or the location of the cancer in the intestine (duodenal or colic). Heteroantibodies formed against a cancer line can be absorbed out by cells from another line, establishing the immunological identity of the antigen(s)

Fig. 1. Phase-contrast photograph of cell culture of rat intestinal carcinoma, line DHD. × 425.

Fig. 2. Membrane immunofluorescence of intestinal tumor cell (line DHD) with anti-DHE serum. × 1330.
Carcinofetal Antigen of Rat Intestinal Carcinoma

Table 1
Membrane fluorescence on various cancer, normal, or fetal cells (ratio of cells demonstrating membrane fluorescence to living cells)

<table>
<thead>
<tr>
<th>Target cells</th>
<th>Control serum (%)</th>
<th>Serum anti-DHD (%)</th>
<th>Serum anti-DHE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonic carcinoma DHB*</td>
<td>1</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Colonic carcinoma DHD*</td>
<td>1</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Colonic carcinoma DHE*</td>
<td>1</td>
<td>94</td>
<td>97</td>
</tr>
<tr>
<td>Duodenal carcinoma NGA*</td>
<td>1</td>
<td>41</td>
<td>36</td>
</tr>
<tr>
<td>Duodenal carcinoma LGA*</td>
<td>2</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>14-day-old fetus, all tissues*</td>
<td>0</td>
<td>NT</td>
<td>0</td>
</tr>
<tr>
<td>18-day-old fetus, intestinal cells*</td>
<td>0</td>
<td>NT</td>
<td>100</td>
</tr>
<tr>
<td>18-day-old fetus, liver cells*</td>
<td>0</td>
<td>NT</td>
<td>0</td>
</tr>
<tr>
<td>18-day-old fetus, cells from cephalic part*</td>
<td>0</td>
<td>NT</td>
<td>100</td>
</tr>
<tr>
<td>20-day-old fetus, intestinal cells*</td>
<td>0</td>
<td>NT</td>
<td>100</td>
</tr>
</tbody>
</table>

a Obtained by trypsinization of cell culture.
b Freshly dissociated tissue.
t NT, not tested.

Table 2
Absorption of anti-DHD and anti-DHE sera by colonic cancer lines

<table>
<thead>
<tr>
<th>Serum</th>
<th>Absorption</th>
<th>Target cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-DHD</td>
<td>None</td>
<td>DHB 88 DHD 84 DHE 78</td>
</tr>
<tr>
<td>Anti-DHD</td>
<td>DHB, 4 x 10^3 cells/ml</td>
<td>0 0 NT</td>
</tr>
<tr>
<td>Anti-DHD</td>
<td>DHE, 2 x 10^3 cells/ml</td>
<td>NT* 1 2</td>
</tr>
<tr>
<td>Anti-DHE</td>
<td>None</td>
<td>95 95 92</td>
</tr>
<tr>
<td>Anti-DHE</td>
<td>DHB, 4 x 10^3 cells/ml</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Anti-DHE</td>
<td>DHE, 7 x 10^3 cells/ml</td>
<td>NT 1 5</td>
</tr>
</tbody>
</table>

* NT, not tested.

Present on different intestinal tumors. The antigen is present on intestinal cells of 18- and 20-day-old fetuses but is not found on adult intestinal cells, suggesting a carcinofetal specificity. Nevertheless, it cannot be excluded that this specificity could be only apparent: trace amounts of the antigen on the surface of adult intestinal cells could be insufficient for detection by immunofluorescence, although they could be detected by more sensitive methods. Furthermore, absorption experiments with fetal intestine should be performed before accepting the complete cross-reactivity between fetal and cancer antigen(s). This absorption should require cultivated intestinal fetal cells, inasmuch as preparations from tissues are usually contaminated by dead or nonepithelial cells.

In addition to carcinofetal specificity, the antigen also demonstrates a tissue specificity. It is not detectable on nonintestinal fetal or tumor cells (hepatoma, glioma, neurinoma). Thus it has the properties of a tissue type-specific antigen (7). The antisera described in this work appear, therefore, to have a cancer and tissue specificity not clearly demonstrated in heteroantisera prepared by more conventional methods. Antisera obtained by immunization of rabbits by soluble extracts of rat intestinal tumors and then absorbed with normal tissue extracts revealed 3 tumor-associated antigens (F. Martin et al., submitted for publication). Two of the antigens are present in trace amounts in mucous cells of the normal digestive tract, the 3rd antigen is located in polymorphonuclear cells infiltrating the tumor and is probably identical to the rat colon carcinoma antigen reported by Garmaise et al. (5). The better specificity of the sera described in the present study could depend on the immunization method, using living cells from culture, or on the in vivo absorption procedure. A drawback of the in vivo absorption is the low titer of antisera, due to a dilution of rabbit antibodies in rat serum; from Hersey’s data (8), the dilution factor can be estimated to be approximately 10 under the conditions used in this work.

With respect to tissue specificity, the carcinofetal antigen of rat intestinal tumor differs from the oncofetal antigens described by Thomson and Alexander (14) on the surface of sarcoma cells and in early embryos and the fetal antigen found by Baldwin et al. (2) in rat hepatomas and sarcomas. The rat intestinal carcinofetal antigen could be rather similar to the antigen(s) detected on cancerous and fetal cells of the human colon by lymphocytotoxicity (4, 6) or delayed cutaneous hypersensitivity reaction (9). However, further experiments are needed to know whether the rat antigen, like the human one(s), can induce an immunological reaction in the tumor-bearing host.

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