NOTE

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TRANSPLANTABLE ILEAL ADENOCARCINOMAS OF ACI RATS

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Transplantation of 9 kinds of bracken-induced ileal adenocarcinomas subcutaneously in ACI rats established two transplantable strains of adenocarcinoma (73-357, 77-238). Ascitic conversion succeeded in the strain 73-357. Biological and morphological, particularly electron microscopic, characteristics of these strains are reported.

There have been reported several transplantable animal intestinal tumors.3, 7, 10, 13, 16, 17) These tumor lines are almost colorectal tumors except one jejunal adenocarcinoma.17) It is well known that rats given bracken materials develop ileal tumors frequently.2, 5) This paper reports successful transplantation of two tumors out of nine ileal adenocarcinomas induced in ACI rats by administration of several kinds of bracken materials.

Materials and Methods

A total of 9 kinds of bracken-induced tumor4.8,11) was transplanted subcutaneously in ACI rats of both sexes. They were all adenocarcinomas located within the terminal 7.0 cm of the ileum forming short papillary or polypoid projections into the lumen of the gut. The tumors invaded the submucosa, tunica muscularis, and a few invaded the serosa. Tumor line, treatment given to tumor-bearing animals, histological type, sex of tumor-bearing rat, site of primary tumor in the ileum, and number of rats used for primary transplantation are summarized in Table I.

After washing the surface of tumor in saline solution, the tumors were minced with scissors. Tissue homogenate was prepared by the use of a tissue pressor equipped with a metal sieve having 2.5 cm inside diameter. About 0.5~0.7 ml of the material was transplanted subcutaneously into the back of rats of the same sex, weighing 100~120 g. Penicillin (10^5 units) was injected immediately after the transplantation. Serial transplantation was carried out with tumor of donors near the end of their life span. In each transplantation, tumor-bearing animals were observed without further treatment until death to examine their life span except the ones which were killed to use as donors for transplantation. The intraperitoneal transplantation was also tried several times. At first, 0.5~0.7 ml of tissue homogenate from subcutaneous tumor was transplanted intraperitoneally. For serial intraperitoneal transplantation, 0.5 ml of tumor ascites, which is in the state of nearly pure culture, was usually used.

The tumors and other organs were examined histologically, using the stainings of Hematoxylin and Eosin, Alcian Blue-PAS, and Grimelius. Tissues for transmission electron microscopic observation were fixed in a mixed solution containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.5) for 2 hr, then washed several times in the buffer, and kept overnight at 4°. They were then postfixed in a phosphate-buffered 1% OsO4 solution (pH 7.4) for 2~3 hr, dehydrated through ascending concentrations of ethanol, and embedded in Quetol-651. For scanning electron microscopic observation, samples were fixed and dehydrated as above and then dried, using a critical-point method in a Hitachi HCP-1 dryer followed by gold sputtering coating in a JEE-4B/4C vacuum evaporator, and examined with a JSM-V3 scanning electron microscope.

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<table>
<thead>
<tr>
<th>Tumor line</th>
<th>Bracken materials used to induce tumor</th>
<th>Histological type</th>
<th>Sex of rat with primary tumor</th>
<th>Site in the ileum (distance in cm from the ileocecal junction)</th>
<th>No. of rats used for primary transplantation</th>
<th>No. of rats successfully transplanted</th>
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<tbody>
<tr>
<td>73-357</td>
<td>Dried powder from unprocessed matured bracken fern</td>
<td>Moderately differentiated tubular adenocarcinoma</td>
<td>Male</td>
<td>9.0</td>
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<td>73-725</td>
<td>Dried powder from unprocessed immature bracken fern</td>
<td>Well-differentiated tubular adenocarcinoma</td>
<td>Male</td>
<td>4.0</td>
<td>2</td>
<td>0</td>
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<td>73-772</td>
<td>—do—</td>
<td>Well-differentiated tubular adenocarcinoma</td>
<td>Male</td>
<td>0</td>
<td>2</td>
<td>0</td>
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<td>73-776</td>
<td>—do—</td>
<td>Well-differentiated tubular adenocarcinoma</td>
<td>Female</td>
<td>7.0</td>
<td>2</td>
<td>0</td>
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<tr>
<td>75-518</td>
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<td>4.0</td>
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<td>75-520</td>
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<td>75-570</td>
<td>—do—</td>
<td>Moderately differentiated tubular adenocarcinoma</td>
<td>Female</td>
<td>4.0</td>
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<td>0</td>
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<tr>
<td>77-238</td>
<td>Concentrated watery extract of dry powder from immature bracken fern</td>
<td>Well-differentiated tubular adenocarcinoma</td>
<td>Male</td>
<td>5.0</td>
<td>2</td>
<td>1</td>
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</table>
TRANSPLANTABLE ILEAL ADENOCARCINOMA

Results and Discussion

Two tumors (73-357 and 77-238) out of 9 adenocarcinomas were successfully established as transplantable tumor strains.

Strain 73-357: The primary tumor was a large sessile polyp at the oral 9.0-cm distance from the ileocecal junction in the terminal ileum of a rat fed a diet containing dry powder of matured bracken. Histology of this tumor was moderately differentiated tubular adenocarcinoma which did not produce mucin. Survival time was 164 days in the first generation, but it became shorter in the following transfers. Mean survival time became about 25 days in recent years (Table II). Histological appearance of serially transplanted subcutaneous tumors resembled the original one (Photo 1). Lung metastasis was observed infrequently. At the 8th transfer generation, intraperitoneal transplantation was tried in 2 recipients in which tumor ascites accumulated within 3~6 weeks after transplantation. Tumor cells in the ascites were found as free cells and cell islands. At autopsy, carcinomatous peritonitis was noted in the diaphragm, mesenteries, omentum, pancreas, and liver. Thus, an ascitic tumor strain (73-357A) was obtained (Photo 2). However, this ascitic tumor strain was lost at the 33rd generation. Again the ascitic conversion was done on the 28th transfer generation of the original subcutaneous tumor and the strain 73-357B was obtained. Microscopic appearance of the ascitic tumor strain 73-357B was similar to that of 73-357A. This ascitic tumor strain has been serially transplanted for 18 transfer generations up to date (1978). General electron microscopic features of the subcutaneously transplanted tumors were similar to those of the original one. Occasionally, well-developed tonofilaments were observed in the cytoplasm of the tumor cells of the basal portion near the basement membrane, and relatively shorter and widely spaced microvilli were seen in the apical area (Photo 3). However, microvilli of the tumor cells in ascitic form were numerous and widely distributed on the surface (Photos 4 and 5). In the cytoplasm of the ascitic tumor cells, lipid droplets were frequently observed and desmosomes were noted between the adjacent cells (Photo 5).

Strain 77-238: The primary tumor was a sessile polyp at the oral 5.0-cm distant from the ileocecal junction in the terminal ileum. Histology of this tumor was well-differentiated tubular adenocarcinoma. Peculiar histology of this tumor was the presence of enteroexocrine cells such as goblet or Paneth type cells (Photo 6). They were observed histologically as Alcian Blue-positive in goblet cells and PAS-positive in Paneth cells. Electron microscopically, Paneth type cells were detected as those having 2.1 ~0.3 μm electron-dense Paneth granules. It was common that the Paneth granules at the base were often more electron dense than those in the apex of tumor cells (Photo 7). Infrequently, a few of enteroendocrine cells with abundant electron-dense small secretory granules of 0.3~0.15 μm in diameter were

<table>
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<tr>
<th>Tumor strain</th>
<th>No. of transfer generations</th>
<th>Average survival days</th>
<th>Method for transplantation</th>
<th>Transplantation rate (%)</th>
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<tr>
<td>73-357</td>
<td>59</td>
<td>30 (161~64)</td>
<td>sc</td>
<td>100</td>
</tr>
<tr>
<td>A*</td>
<td>33</td>
<td>25 (12~58)</td>
<td>ip</td>
<td>90</td>
</tr>
<tr>
<td>B</td>
<td>18</td>
<td>20 (11~41)</td>
<td>ip</td>
<td>95</td>
</tr>
<tr>
<td>77-238</td>
<td>8</td>
<td>80 (20~198)</td>
<td>sc</td>
<td>93</td>
</tr>
</tbody>
</table>

* Regressed at the 33rd transfer generation.
sc = subcutaneous transplantation, ip = intraperitoneal transplantation.
seen mainly in the basal area near the basement membrane (Photo 8). However, these enteroendocrine-type cells were not definitely detected histologically in Grimelius-stained specimen. This transplantable tumor strain has been serially transplanted for 8 generations to the present (Table II). No metastasis was seen at any site. Since the 3rd transfer generation, ascitic conversion has often been tried, but it was not successful, resulting in formation of only tumor nodules in the peritoneal cavity.

Although 9 ileal adenocarcinomas were transplanted in this study, primary transplantation was successful in only two cases. This low transplantability is probably due to the bacterial contamination from the digestive tract as reported before.3) As far as we know, there is no ileal transplantable tumor described, whereas there have been reported several transplantable, chemically induced colorectal tumors in rats,3) and some successful xenotransplantation of human adenocarcinomas of the colon,1,12,14,15) rectum,13) and stomach9,15) into immune-deprived animals or the mutant nude mouse. Original occurrence of ileal tumors is rare, either in experimental animals or in human, but the ileum is the most preferred site in bracken carcinogenicity in rats.6) Present ileal transplantable strains may be useful for examination of the nature of lower small intestinal tumors. The enteroendocrine cells seen in the strain 77-238 seem to resemble some of entero-glucagon-producing cells from the morphology of secretory granules. In this study, the number of secretory granule-producing cells in the strain 77-238 has decreased by serial passage of transplantation. Ward et al.17) also reported that Paneth cells in the established jejunal adenocarcinoma disappeared after the 5th transfer generation. The strain 73-357 has been maintained either in ascitic or solid form, while ascitic conversion was yet unsuccessful in the strain 77-238. The transplantable tumor strains established in the present study will be a useful tool in analyzing human adenocarcinomas of the alimentary tract and in pathophysiological studies of lower small intestine.

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References


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EXPLANATION OF PLATES


Photo 2. Microscopical appearance of the ascitic tumor line 73-357A (16th generation). Tumor cells were observed as single cells and small islands. ×560.

Photo 3. Electron microscopical appearance of the subcutaneously transplanted tumor line, 73-357 (40th generation). Tono filaments (T) are located in the basal portion near the basement membrane (B). Microvilli in the apex are not well developed. ×4,600.

Photo 4. Scanning electron photomicrograph of the ascitic tumor cells grown as small groups of cells (73-357B, 15th generation). Numerous microvilli are observed on the surface. ×2,700.

Photo 5. Electron microscopic appearance of the two ascitic tumor cells attached to one another (73-357B, 15th generation). Lipid droplets in the cytoplasm of tumor cells and desmosomes between the adjacent cells are seen. A tumor cell possesses intracytoplasmic lumina equipped with microvilli. ×4,800.

Photo 6. Histology of another transplantable adenocarcinoma (77-238, 2nd generation) grown in the subcutaneous space. Paneth type cells (arrows) and other mucin-producing cells are seen. H-E. ×340.

Photo 7. Electron microscopic appearance of a Paneth-type tumor cell (77-238, 2nd generation). Electron density of the Paneth granules in the basal portion is higher than that of granules in the apical area. ×3,800.

Photo 8. A portion of the tumor strain of 77-238, 2nd generation. An enteroendocrine type cell (E) is seen in the area near the basement membrane (B). In the right lower portion of this photograph, are also seen enteroexocrine type cells. ×5,800.

H-E = Hematoxylin-Eosin stain