Acinar Cell Carcinoma of the Rat Pancreas Grown in Cell Culture and in Nude Mice

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ABSTRACT

An acinar cell carcinoma of the pancreas, which was induced in a male Wistar rat by repeated injections of azasenine, was propagated in cell culture. Sheets of epithelial-like cells grew within 2 weeks and were subcultured serially. Initially, acinar cell carcinoma in the culture medium produced a high level of amylase, but the secretion ceased rapidly as cells began to proliferate. Only negligible amounts of trypsinogen and chymotrypsinogen were detected in cell homogenates at passages 7 and 9. The chromosome distribution ranged from hypodiploid to hyperdiploid. When cultured cells were transplanted s.c. into nude mice, palpable tumors appeared within 4 weeks and could be transplanted serially. Histological examination of the tumor showed poorly differentiated carcinoma without acinar structures. Tumor homogenate contained amylase, trypsinogen, and chymotrypsinogen, and the electron microscopic examination revealed that many tumor cells contained zymogen-like granules. These results indicate that pancreatic acinar cell carcinoma in cell cultures, in which there was no differentiated function, can be activated to synthesize tissue-specific enzymes when transplanted into nude mice by yet undefined factors present in the host animals. The cell line and transplantable tumors of pancreatic acinar cell carcinoma may be useful in the analysis of the biological behavior of this type of tumor and in the study of the control mechanisms of the synthesis of tissue-specific products in cells.

INTRODUCTION

Acinar cell carcinoma has been recognized as a distinct histopathological entity of human pancreatic tumors (3–6, 9, 20, 31). It is generally regarded as rare, and the reported incidence varies between 1 and 15%, depending upon different surveys. One possible reason for the reported low incidence of acinar cell carcinoma is the failure of recognition, by ordinary histological examination, of the acinar cell origin of poorly differentiated tumors.

Recent development of several animal models of pancreatic carcinogenesis indicates that certain chemical agents preferentially induce neoplastic lesions of the acinar cell type (12, 16, 17, 28). Among them, azasenine has been shown to induce multiple acinar cell carcinoma in rats (17), and the experimental system offers a unique opportunity to investigate the biology of acinar cell carcinoma of the pancreas. During the past year, we attempted to grow cells in vitro from azasenine-induced acinar cell carcinomas. Although cells of epithelial morphology were successfully cultured, the differentiated functions of these cells as acinar cells of the pancreas were extremely low. However, resurgence of the functional activities, as measured by pancreatic enzyme synthesis, can be induced upon transplantation of cultured cells into nude mice.

MATERIALS AND METHODS

Source of Tumor Tissue. Male Wistar rats (Hilltop Laboratories, Scottdale, Pa.), weighing 130 to 150 g at the beginning of the experiments and maintained on laboratory chow (Ralston Purina Co., St. Louis, Mo.), were treated with multiple i.p. injections of azasenine (Cabaliochem, La Jolla, Calif.). Azasenine was given at a dose of 20 mg/kg of body weight twice a week for 2 months, and then once a week for a total of 22 injections. With this experimental regimen, at between 14 and 18 months, acinar cell carcinomas of the pancreas (Fig. 1) developed in 13 of 16 rats. One of these tumors was used in the present experiment.

Cell Culture. One of the induced tumors, measuring 0.9 cm in the greatest dimension, and well demarcated from the main body of the pancreas, was used as a source of cell culture. By sterile technique, the tumor was resected into a Petri dish containing Williams Medium E (GIBCO) supplemented with 12% fetal bovine serum (GIBCO), 2 mM glutamine, penicillin (100 μg/ml), streptomycin (100 μg/ml), and Fungizone (0.25 μg/ml). A fragment of the tumor tissue, 5 x 5 x 5 mm, was minced into pieces less than 0.5 mm. The remainder of the tumor was used for histological examination. Multiple pieces of the minced tumor as well as possible spilled cells in the Petri dish were transferred into 25-sq cm Falcon tissue culture flasks (Falcon Plastics, Oxnard, Calif.) with 10 ml of the culture medium and then incubated at 37° in 5% CO₂ and humidified atmosphere. The outgrowth of epithelial cells began 2 days after plating, and the culture medium was changed every 2 to 3 days. Six weeks after the initial plating, confluent cultured cells were dissociated with 0.25% trypsin in Hanks' balanced salt solution without calcium and magnesium (GIBCO) and were subcultured. Subsequent subcultures were made every 4 to 5 weeks. Cell growth rates were determined after every 3 passages by seeding 2 x 10⁵ cells/flask and by counting daily by hemocytometer the number of cells in triplicate flasks.

Chromosome Counts. The chromosome numbers of cultured cells were determined at the ninth passage. Cultures in a midlogarithmic growth phase were treated for 4 hr with 0.05% Colcemid solution. After treatment, cells were trypsinized, and

1 Supported in part by Research Grant CA 20116 from the National Cancer Institute through National Pancreatic Cancer Project and by Grant AM 06334 from the National Institute of Arthritis, Metabolism, and Digestive Diseases.
2 To whom requests for reprints should be addressed.
3 H. Shinozuka and S. L. Katyal. Cellular characterization of acinar cell nodules of the rat pancreas induced by chemical carcinogens, manuscript in preparation.

The abbreviation used is: GIBCO, Grand Island Biological Co., Grand Island, N. Y.
metaphase smears were prepared and stained with Giemsa (30); 100 cells in metaphase were analyzed.

Transplantation. Cells at the tenth passage were transplanted into nude mice and syngeneic rats. After dissociation of cultured cells by trypsin, cells were washed twice in the culture medium, and 5 to 6 x 10⁶ cells in the medium were inoculated s.c. into the backs of young Wistar rats (50 to 60 g) and nude mice (BALB/c nu/nu, 6 weeks old; GIBCO Animal Resource Laboratory, Madison, Wis.).

Histology. The original tumor which served as the source of cell culture, the cells in the culture, and the tumors growing on nude mice were examined by both light and electron microscopy. For light microscopy, tissues were fixed in Stieve's solution and the sections were stained with hematoxylin and eosin. Cells in culture were fixed in situ with 70% methanol and were subsequently stained with Wright's stain. For electron microscopy, the tissues or cells were fixed in 1% osmium tetroxide in phosphate buffer, dehydrated in a graded series of ethanol, and embedded in Epon-Araldite. Thin sections were stained with uranyl acetate and lead citrate and were examined with a Philips EM300 electron microscope.

Enzyme Assays. Tissues were homogenized with 9 volumes of ice-cold 10 mM Tris-HCl buffer, pH 8.5, containing 0.9% NaCl solution and 0.1% Triton X-100 (22). Homogenization was performed with a Model PT10 Polytron (Brinkman Instruments, Inc., Westbury, N. Y.) at Setting 2 for 1 min. Cells in culture were harvested and washed twice with 0.25 M sucrose and were sonicated in 1 to 2 ml of the above buffer with a Branson Sonifier (microtip, Setting 6 for 1 min). Amylase was determined with reduced volumes of reagents on 0.2 ml of suitably diluted aliquots of sera, culture medium, and the tissue or cell homogenates, according to the methods previously described (22). Phosphate buffer (20 mM), pH 6.9, containing 6 mM NaCl was used as the diluting and incubating buffer. For the trypsinogen and chymotrypsinogen assays, the homogenates were diluted 1-, 10-, 100-, and 200-fold with 10 mM Tris buffer, pH 8.5, containing 0.1% Triton X-100 and 0.9% NaCl solution. Suitable aliquots of diluted homogenates were incubated with an equal volume of 1% enterokinase (Miles Laboratories) in the above buffer for 2 hr at 37°C (21). Activated homogenates (0.1 or 0.2 ml) were used for the assay of trypsin with p-toluenesulfonyl-L-arginine methyl ester, and the assay of chymotrypsin with n-benzol-L-tyrosine ethyl ester, according to the methods previously described (21). Units of enzyme activity, per g of pancreas and tumor tissue and total cells in cultures, were expressed as mg of maltose released per min for amylase and μmol of the substrates hydrolyzed per min for trypsin and chymotrypsin, respectively. Proteins were measured using the method of Lowry et al. (19).

RESULTS

The tumor of the pancreas used as a source for cell cultures in the present experiment was a moderately well-differentiated acinar cell carcinoma (Fig. 2). It was composed of tightly packed epithelial cells forming distinct acinar structures, and the cells contained abundant zymogen granules readily identifiable by electron microscopy (Fig. 3). There were areas of loss of acinar structure which were replaced by sheets of epithelial cells. Mitosis was frequent. On the whole, the tumor had a relatively small amount of connective tissue stroma.

When pieces of the tumor were incubated in the culture medium, the outgrowth of epithelial cells began 2 days after the plating and became confluent by 4 to 5 weeks. The results of the determinations of amylase content in the medium are shown in Chart 1. Significant amounts of amylase were secreted in the medium during the first 24-h period; thereafter, however, the amounts of amylase secreted in the medium declined rapidly. The fresh culture medium containing 12% fetal bovine serum used in these studies and the same medium obtained from hepatoma cell culture were tested and found to be negative for amylase.

Abundant epithelial cell growth was evident by the seventh day of the culture, at which time there were no detectable amounts of amylase in the medium. The first-passage cells grew to confluence in 2 weeks. After 3 to 4 passages, the cultured cells grew as monolayers and exhibited typical epithelial cell morphology characterized by sheets of polygonal cells with abundant cytoplasm (Figs. 4 and 5). Occasional giant cells were observed. Electron microscopy of the cultured cells showed flattened cells containing moderate amounts of rough endoplasmic reticulum and well-developed Golgi apparatus. No zymogen granules were detected (figures not shown). Longer incubation periods produced focal areas of multilayered growth. The population-doubling times of the cells, determined at the seventh and ninth passages, were 18.9 and 18.2 hr, respectively. Chromosome analysis was done at the ninth passage, and the results are shown in Chart 2. The chromosome distribution was wide and ranged from hypodiploid to hypotetraploid, with a model number of 41.

Acinar cells in culture at the 10th passage were transplanted onto 2 nude mice and onto 6 young syngeneic rats. In nude mice, palpable tumors began to appear in 3 to 4 weeks at all 4 sites injected. Subsequent transfer of the tumor was 100% successful. No tumors developed in rats during the 4-month observation period following the s.c. injection of the cultured cells. The histological examination of the transplanted tumors from nude mice revealed poorly differentiated carcinoma composed of sheets of large epithelial cells admixed with connective tissue stroma (Fig. 6). No acinar structures were evident. The tumors invaded underlying musculature and the skin, and central necrosis of the tumor mass was evident.

The enzyme contents of the tumors and the pancreas of the host nude mice, determined at the second- and the third-generation transplants, are shown in Table 1, together with the results obtained from the cells in culture. Amylase, trypsinogen,
of rough endoplasmic reticulum. In some cells, there were detected. The presence of amylase could not be demonstrated cells, low bevels of trypsinogen and chymotrypsinogen were than those in the pancreas of the host animals. In cultured dilations of the cisternae of endoplasmic reticulum which were examined (Fig. 7). Cancer cells contained irregularly shaped zymogen granules in approximately one in every 20 to 25 cells tumors, although the enzyme levels were considerably lower and chymotrypsinogen were consistently demonstrable in the pancreas In cell culture at the ninth passage. A total of 100 cells were counted.

Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Amylase (units/mg protein)</th>
<th>Trypsinogen (units/mg protein)</th>
<th>Chymotrypsinogen (units/mg protein)</th>
<th>Protein (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas of nude mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>18.36</td>
<td>0.803</td>
<td>5.710</td>
<td>117.6</td>
</tr>
<tr>
<td>2</td>
<td>21.60</td>
<td>1.051</td>
<td>6.911</td>
<td>124.3</td>
</tr>
<tr>
<td>Tumors on nude mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.08</td>
<td>0.034</td>
<td>0.031</td>
<td>151.3</td>
</tr>
<tr>
<td>2</td>
<td>0.085</td>
<td>0.017</td>
<td>0.067</td>
<td>100.8</td>
</tr>
<tr>
<td>Tumor cells in culture</td>
<td></td>
<td>0.0085 ± 0.003</td>
<td>0.011 ± 0.011</td>
<td>6.83 ± 1.85</td>
</tr>
</tbody>
</table>

*Expressed per g of pancreas and tumors and as total protein for the tumor cells in culture.*

**DISCUSSION**

In the present study, we demonstrated that cells from the azaserine-induced acinar cell carcinoma of the rat pancreas can be successfully propagated into cell culture, which later can be serially transplanted into nude mice. Although the cells in culture grew with typical epithelial morphology, their functional activities, as determined by the secretion of amylase into the medium, ceased within a relatively short period of time. The levels of trypsinogen and chymotrypsinogen also become marginal when cells remained in culture. It is generally observed that loss of tissue-specific properties occurs upon explanation of cells in vitro (2, 13, 14, 15, 26, 32), and the maintenance or the expression of functional properties of cells in vitro is highly dependent on the culture conditions and on the nutrient environment (2, 7, 15, 25, 29). In addition, there are several reports which demonstrate enhanced differentiation of poorly differentiated carcinoma when they were explanted into culture conditions (1, 8, 27). It was hypothesized that the capacity to differentiate beyond the level attained in vivo appears to depend on the environmental factors present in culture, such as the presence of solid-liquid interface in culture (1). The culture medium used in the present study was Williams Medium E, which was originally designed to culture adult liver cells and is considered to be one of the enriched media (33). It has been shown that, in cultured hepatoma cells, Williams Medium E supported a higher level of differentiation in cells than a minimum medium such as Eagle’s minimum essential medium (11). It is possible that, in our cultures of acinar cell carcinoma, some additional factors such as hormones and/or other nutrient components may be necessary for the expression and maintenance of the pancreatic acinar cell function.

In this regard, it is extremely interesting to note that cultures of acinar cell carcinoma, upon transplantation into nude mice, resumed limited functional activities. We chose 3 enzymes for the identification of the acinar cell functions, namely, amylase, trypsinogen, and chymotrypsinogen. While the detection of amylase may be considered as nonspecific, the detection of trypsinogen and chymotrypsinogen is highly specific for the pancreatic acinar cells. The demonstration of the latter 2 enzymes in the transplanted tumor, although considerably less than those of the normal pancreas, would allow us to conclude that the tumors are indeed pancreatic acinar cell carcinomas. The electron microscopic study of transplanted cancer cells further supports our conclusion. Some tumor cells contained mature zymogen granules and some osmiophilic material in the cisternae of endoplasmic reticulum, which can be considered to be precursors of the secretory enzymes or proenzymes. The presence of moderately well-developed rough endoplasmic reticulum and Golgi apparatus are also compatible with the cell type of acinar cell origin. It is reasonable to assume that host nutritional and/or hormonal factors present in nude mice favored the resurgence of the functional activities of cultured cells. Another possibility is that the development of connective tissue stroma in the tumors growing on nude mice may facilitate enhanced differentiation of tumor cells, analogous to the effects of mesenchyme on pancreas rudiment (10, 24). However, the precise mechanism of controlling factors influencing the state of differentiation of cancer cells is not yet known.

The demonstration of the acinar cell nature of poorly differentiated carcinomas when transplanted to nude mice illustrates the difficulty in classifying poorly differentiated carcinomas of human pancreas. Even though a majority of human pancreatic carcinomas are considered to be ductal in origin, our observations indicate that undifferentiated carcinomas may be of acinar origin.
Two recent reports indicate successful transplantation of experimentally induced acinar cell carcinomas of the pancreas into syngeneic animals; one being induced by nafenopin and the other by azaserine (18, 23). In both cases, the transplanted tumors maintained their functional activities as acinar cells during the early stages of transplant generation. Transplantable ducal adenocarcinomas of the pancreas induced by N-nitroso-bis(2-oxopropyl)amine have also been described recently (27). Availability of these tumors and the comparative analysis of tumor cells grown in vivo and in vitro may become instrumental in further elucidating the biological behavior of pancreatic carcinomas and in analyzing the control mechanisms of synthesis and secretion of tissue-specific products of cells.

ACKNOWLEDGMENTS

We wish to express our thanks to Dr. B. Lombardi for his critical discussion during the course of this study, to Dr. S. F. Pan for her assistance in cytogenetic analysis, to Dr. S. Boggs for the use of the nude mice facilities, and to M. Nefores for her excellent technical assistance.

REFERENCES


Fig. 1. Gross appearance of one of the typical acinar cell carcinomas of the pancreas induced in a rat by repeated injections of azaserine. The tumor (arrows) is a nodular mass which is protruding from the main body of the pancreas (P). Spleen (S) is attached to the pancreas.

Fig. 2. Light microscopic histological appearance of the acinar cell carcinoma of the pancreas, portions of which were used in the present experiment. The tumor is composed of tightly packed epithelial cells maintaining, in many areas, acinar structures. The cytoplasm contained readily identifiable zymogen granules. Note the relatively scanty connective tissue stroma. H & E × 110.

Fig. 3. Electron micrograph of the acinar cell carcinoma of the pancreas shown in Fig. 2. Tumor cells contain abundant zymogen granules (Z) and form distinct lumens (L); n, nucleus. Uranyl acetate-lead citrate, × 8,400.

Fig. 4. Epithelial cell culture derived from the acinar cell carcinoma of the pancreas 7 days after the seventh passage. Many cells are polygonal and have abundant cytoplasm. Wright’s stain, × 110.

Fig. 5. Higher magnification view of cultured cells from the acinar cell carcinoma of the pancreas. Abundant granular cytoplasm and ovoid nuclei with prominent nucleoli can be seen. Wright’s stain, × 380.

Fig. 6. Light microscopic histological appearance of the transplanted tumor growing on a nude mouse. The tumor is composed of sheets of epithelial cells with a moderate amount of connective tissue stroma. Carcinoma cells vary in size with abundant cytoplasm and pleomorphic nuclei. No acinar formation is evident. H & E, × 240.

Fig. 7. Electron micrograph of the transplanted tumor on a nude mouse. Portions of 3 cancer cells are shown, one of which shows the presence of zymogen granules (Z), nucleus (n); cell membrane (cm). Uranyl acetate-lead citrate, × 9,600.

Fig. 8. Electron micrograph of a portion of the cytoplasm of transplanted acinar cell carcinoma cells. Note dilated cisternae of rough endoplasmic reticulum (arrows) containing osmiophilic materials of different density (ρ). Uranyl acetate-lead citrate, × 25,000.

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