Establishment and Characterization of Two Human Pancreatic Cancer Cell Lines Tumorigenic in Athymic Mice

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ABSTRACT

Two human pancreatic cancer lines, RWP-1 and RWP-2, have been established from 2 patients with primary pancreatic cancer metastatic to the liver. The patients' tumors, the xenografted tumors, and tumors obtained by inoculation of nude mice with cultured RWP-1 and RWP-2 cells are all moderately-well-differentiated ductal cell adenocarcinomas. Ultrastructural analysis supports the tissue histopathology findings. Xenografts of RWP-1 tumors double every 10 days, whereas the doubling time of RWP-2 xenografts is 22 days. Both tumors contain mucin. RWP-1 and RWP-2 cells have a doubling time in culture of 45 hr and form colonies in soft agar. RWP-1 cultures appear to be morphologically heterogeneous; two distinct epithelial cell types can be identified. RWP-1 and RWP-2 have modal chromosome numbers of 64 and 62, respectively. Appreciable levels of carcinoembryonic antigen, 1090 and 414 ng/10⁶ cells, respectively.

INTRODUCTION

Carcinoma of the pancreas is the fourth most common cause of cancer death in men, and in women 75 years old or more; the 5-year survival rate is less than 2% (31, 41). Biological models are needed for the study of pancreatic cancer, as much as little is known about the etiology, detection, biology, or efficacious treatment of this neoplasm. Cell lines and xenografts of human pancreatic cancer are necessary for study of the biological properties and therapeutic response of this tumor that could lead to effective approaches to treatments against this disease. In recent years, there have been a few reports describing human pancreatic cancer cell lines, including PANC-1, Hs 766 T, MIA-PaCa-2, HGC-25, COLO 357, QCP-1, and GER (1, 9, 14, 17, 19, 25, 30, 34, 35, 44, 51). We report here the establishment of 2 new human pancreatic carcinoma cell lines in tissue culture which have also been successfully xenografted into nude mice. Characterization of the cell lines and the corresponding xenografted carcinomas is described.

Portions of this work have been presented in preliminary form (13).

MATERIALS AND METHODS

Source of Material

Tumor materials, designated RWP-1 and RWP-2, were received as liver biopsies containing neoplastic tissue. RWP-1 was removed from a 57-year-old Caucasian woman. Histologically, it was classified as a liver metastasis from a primary duct cell adenocarcinoma of the head of the pancreas. RWP-2 originated from the liver biopsy of a 40-year-old Caucasian woman and was classified as a metastasis of a moderately-well-differentiated duct cell adenocarcinoma of pancreatic origin.

Heterotransplantation into Athymic Mice

The tissue was cut into 1-mm cubes and implanted s.c. into both flanks of athymic, nude mice. Athymic, nude mice bearing the nu/nu genotype on an outbred Swiss background are bred and maintained at the Roger Williams Cancer Center Animal Care Facility. After 3 and 5 weeks for RWP-1 and RWP-2, respectively, tumors had reached appreciable size (10 x 10 mm) and were at that time excised for serial transplantation and histological examination. For the past year, the 2 tumor lines have been serially transplanted in nude mice every 6 to 8 weeks, or when tumor diameter reached 12 to 15 mm. To study tumor growth, 8 fragments of the first transplant generation of each line were implanted into the flank region of nude mice, and the tumor length and width were measured twice weekly with callipers. Tissue for establishment of cell cultures of both lines was obtained from the primary xenografts from implantation of the original biopsy materials (5).

Cultivation in Vitro

Xenograft tissue was washed in 0.9% NaCl solution containing penicillin (100 units/ml), streptomycin (100 μg/ml), and Fungizone (2.5 μg/ml) (all from Grand Island Biological Co., Grand Island, N.Y.). This material was then minced, small pieces of tumor tissue were removed and placed in Falcon No. 3013 tissue culture flasks (Falcon Plastics, Oxnard, Calif.), and tissue fragments were allowed to attach 1 hr before addition of culture medium. The remainder of the tumor mince was placed into a 75-ml Wheaton graduated trypanizing flask (Wheaton Scientific, Millville, N.J.) containing 20 ml of enzyme solution for enzymatic dispersal of tumor cells. The enzyme solution used was a mixture of the following components in Hanks' balanced salt solution at pH 7.2: trypsin (1 mg/ml) (Difco Laboratories, Detroit, Mich.); Dispase neutral protease Grade II (0.5 mg/ml); DNase Grade II (10 μg/ml); and collagenase (1 mg/ml) (all from Boehringer Mannheim, Indianapolis, Ind.). This enzyme solution was sterilized by filtration through a 0.2-μm Nalgene filter (Syrbon Corp., Rochester, N.Y.). After exposure to enzyme solution for 1 hr, single-cell suspensions of RWP-1 or RWP-2 were transferred to tubes containing fetal calf serum, and cells were centrifuged and resuspended in RPMI 1640 serum-free medium supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), Fungizone (2.5 μg/ml), and gentamicin (20 μg/ml) (Schering.

1 This work was supported by Grants CA13943, CA20892, and CA30035 and by grants from the Veterans Administration and the Rhode Island Foundation.

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Received July 23, 1981; accepted April 13, 1982.
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Corporation, Kenilworth, N. J.) and buffered with 10 μM N,N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, 10 μM tris(hydroxymethyl)methylglycine, and 0.075% sodium bicarbonate solution. Aliquots of the resuspensions were then added to flasks containing RPMI 1640 with antibiotics and serum supplements.

Two modifications of RPMI 1640 growth medium were used for initiation of RWP-1 and RWP-2 cell lines in vitro. RPMI with added buffers prepared as above and supplemented with 10% fetal calf serum plus 10% dialyzed fetal calf serum was designated as "RPMI-SC." "RPMI-SK" was a modification of L-15-D (Leibovitz) with RPMI 1640 in place of the L-15 medium in a "detoxification" base (29), supplemented with 10% fetal calf serum and 10% dialyzed fetal calf serum. Media were changed every 48 hr.

The elimination of any murine fibroblasts originating from the stromal component of the xenograft tumor tissue was accomplished in both cultures by brief exposures to 0.06% trypsin/0.02% EDTA. This procedure was repeated for at least 5 consecutive days or until no further fibroblast growth could be observed. Once the tumor cells adapted to the in vitro environment, both cell lines were maintained on RPMI-SC growth medium. Both lines are routinely passed at 1:2 splits.

RWP-1 and RWP-2 cultures were examined for Mycoplasma contamination, and no Mycoplasma growth could be detected. The direct fluorescent antibody technique was also performed on both cell culture lines to detect the presence of 10 common viral contaminants (26). No viral antigen contaminants could be detected. Cells were removed from culture dishes with trypsin:EDTA.

In Vitro Growth Characteristics

Growth Rates, Growth in Agar, Saturation Densities. These growth properties were determined according to methods published previously from our laboratory (11, 12).

Plating Efficiencies. These results were obtained by plating 1 × 10^6 cells in 35-mm dishes; 16 hr later, unattached cells were washed away, and the cultures were trypsinized and counted. By this time, all cells that will adhere to the plastic surface have attached, but the vast majority have not yet had time to replicate. Colony-forming efficiency was determined by statistical counting of all patches of cells growing from those single cells that attached.

Chromosome Analysis. Exponentially growing cultures of both the RWP-1 and RWP-2 cell lines were harvested for chromosome preparation and analysis using standard cytogenetic procedures. Giemsa banding of the chromosomes was performed, and 21 metaphases for each cell line were selected for analysis.

CEA Analysis. CEA assays were performed on 3-day-old spent RPMI-SC medium from actively growing cultures and on fresh complete medium with exposure to cultured cells. All assays were performed immediately by radioimmunoassay using the Roche Kit and procedure manual (Hoffman-LaRoche Inc., Nutley, N. J.) in the Roger Williams General Hospital Toxicology Laboratory under the direction of Dr. I. Diamond. The cells remaining in each flask were harvested using trypsin:EDTA and were enumerated. The final percentage of viable cells was determined by trypan blue dye exclusion. Values are reported as ng CEA per 10^6 cells.

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Nude mice, 6 to 10 weeks old, were used as hosts for tumor cell injections. Cells in culture were trypsinized, and the cell suspensions were centrifuged (5 min at 1400 rpm in a Sorval GLC-1 centrifuge). The pellet was then washed twice, and the cells were resuspended in 0.9% NaCl solution to give a density of 5 × 10^6 and 2.5 × 10^6 cells/ml for RWP-1 and RWP-2, respectively; 0.2 ml of final cell suspension was injected s.c. into the flanks of nude mice.

Enzyme Analysis

Cells and spent media for each enzyme assay were harvested from 3 sets of 4 confluent 100-mm Falcon No. 3003 tissue culture dishes. The monolayers were washed twice with 0.9% NaCl solution to remove fetal calf serum and were then incubated further with serum-free RPMI 1640 containing antibiotics and buffers. After 48 hr, the spent medium was pooled, centrifuged at 1500 × g for 10 min, and, where necessary, concentrated up to 100-fold by ultrafiltration on Minicon-B15 sample concentrators or on an YM-10 membrane (both from Amicon Corp., Lexington, Mass.). The monolayers were washed once with 0.9% NaCl solution and loosened by scraping with a rubber policeman, the cells were collected by centrifugation at 1500 × g for 5 min. Tumors were implanted s.c. in nude mice and allowed to grow from 4 to 6 weeks. Three tumors were harvested, finely minced, and assayed separately for each enzyme. Both tumors and cells were suspended in 0.9% NaCl solution or assay buffer (5 ml/g, wet weight). The minced tumor initially was dispersed in a Dounce homogenizer. Enzymes were then extracted from the tumor or cell suspension by intermittent sonication over ice at Mark 6 for 60 sec with the microprobe of a Sonicator/Cell Disruptor (Heat Systems-Ultrasonics, Inc., Plainville, N. Y.). Homogenates were centrifuged at 15,000 × g for 15 min in an Eppendorf No. 5412 microfuge. These supernatants were assayed for enzymes within 2 hr of extraction. All extractions and centrifugations were carried out at 4°.

LDH (EC 1.1.1.27), ALP (EC 3.1.3.1), and G-6-PDH (EC 1.1.1.49) were assayed using reagent kits (Bio-Dynamics/DMC, Division of Boehringer Mannheim) according to the manufacturer's instructions. LDH was assayed in spent medium or 0.9% NaCl extracts at 37° using the Single Vial LDH-L 10 Kit No. 158224. After sonication to release bound ALP (23), this enzyme was assayed in 0.9% NaCl extracts or media concentrated 25-fold, using the optimized ALP Reagent Set No. 123854 at 30°. Aliquots were also heated at 56° for 10 min before assay to test for the heat-labile isoenzyme (16, 47). G-6-PDH was assayed in 0.9% NaCl extracts or media concentrated 25-fold using the G-6-PDH Reagent Set No. 124672 at 37°. Where appropriate, enzyme standards (Precipath E. No. 126799; Bio-Dynamics) which contain 155 milliunits LDH per ml, 391 milliunits ALP per ml, 27 milliunits G-6-PDH per ml, and 125 milliunits amylase per ml were included in these assays. For the amylase assay, cells from twice 100-mm dishes were pooled and extracted together with 1 ml of phosphate buffer (0.05 M phosphate buffer containing 0.9% NaCl, pH 7.0). In addition, spent medium from these cultures was concentrated 100-fold for the assay. Amylase (EC 3.2.1.1) was determined using amylopectin azure (Calbiochem-Behring Corp., La Jolla, Calif.) as substrate (40).

To test for trypsin (EC 3.4.21.4) or chymotrypsin (EC 3.4.21.1), the cells were harvested, and the medium was concentrated as described for the amylase assay. Tumors and cells were extracted in 0.01 M Tris-HCl, pH 8.5, containing 0.9% NaCl and 0.1% Triton X-100 (w/v). After activation with 0.5% enterokinase (Sigma Chemical Co., St. Louis, Mo.), esterase activity was assayed with tosyl-L-arginine methyl ester (Aldrich Chemical Co., Milwaukee, Wis.) or benzoyl-L-tyrosine ethyl ester (Sigma) (24). Triton X-100 (0.2% w/v) was added to the buffer used to dissolve the benzoyl-L-tyrosine ethyl ester (39). Purified trypsin and chymotrypsin obtained from Sigma were used as standards in these assays.

Protein in all homogenates was measured by the method of Lowry et al. (32). All enzyme activities are expressed as milliunits/mg protein in the original homogenate, where 1 milliunit is defined as that amount of enzyme that converts 1 nmol substrate to product per min.

Histopathology

For light microscopy, the xenografts of RWP-1 and RWP-2 tumors were fixed in 10% phosphate-buffered formalin, embedded in paraffin, sectioned at 4 to 6 μm and stained with hematoxylin and eosin. Sections of selected blocks were also stained by the following methods: periodic acid–Schiff before and after diastase treatment; Mayer's mucicarmine; and alcian blue at pH 2.5.
For electron microscopy, 1-mm cube tissue fragments were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 3 hr at 4°, washed in 3 changes of buffer, postfixed in 1% osmic acid in buffer, dehydrated in graded ethanol and propylene oxide, and embedded in Epon. One-μm sections stained with aqueous toluidine blue were used for selection of appropriate microscopic fields. Ultrathin sections cut on an LKB ultramicrotome were stained with 6.0% uranyl acetate and Reycn's lead citrate and examined in an RCA EMU 4C electron microscope.

RESULTS

Establishment of Cell Lines. In vitro cultivation of cancer cells from both xenografted tumor lines was performed using traditional methods (18, 45) at the time of first passage in the nude mouse (see "Materials and Methods"). While the techniques of explanting and preparation of single-cell suspensions by enzyme treatment (2) proved to be successful for both lines, differences in growth requirements were observed. After 5 days in culture, cells began to grow out from the RWP-1 explants, whereas enzymatic dispersal of the RWP-1 tumor yielded a suspension of cells that were 80% viable but unable to replicate upon attachment to the plastic surface. In contrast, monolayer cell cultures were established by both techniques with the RWP-2 xenograft. Differences in nutritional requirements were also apparent in our initial culture attempt in that RWP-1 cells grew only in medium containing a detoxification base (RPMI-SD; see "Materials and Methods") and not in RPMI-SC (without detoxification components), whereas RWP-2 cells grew in RPMI-SC and not in RPMI-SD. Eight weeks after the initiation of our RWP-1 culture, the cells were weaned from the detoxification medium and are now cultured in RPMI-SC.

Murine fibroblasts contaminated both primary pancreatic carcinoma cell cultures, but they were present to a greater degree in the RWP-2 cell culture. All mouse stromal material was successfully removed by repeated exposure of the cultures to low concentrations of trypsin-EDTA. Human pancreatic tumor cells have proved to be much less sensitive to detachment by this technique than did murine fibroblasts, enabling us to establish cultures of both human pancreatic carcinoma cell lines that contain only the epithelial cancer cells.

In Vitro Growth Properties. The RWP-1 cell line grows with a typical epithelial morphology on plastic as well as glass surfaces (Fig. 1). There appear to be 2 major morphologically distinct cell types in our RWP-1 cultures. One has a polygonal shape with abundant cytoplasm, possesses a large, somewhat indistinct nucleus with 1 to 4 nucleoli, and shows many cytoplasmic connections between the cells. The second consists of slightly elongated epithelial cells which tend to form swirls around the smooth, well-demarcated boundaries of the polygonal cell component. The elongated cells contain much less basophilic cytoplasm than do the polygonal cells, and they have a well-defined central nucleus with 2 prominent nucleoli. Occasional giant cells containing numerous intracellular inclusions and vacuoles appear to form a minor component of this cell line.

The RWP-2 cell line appears morphologically homogeneous (Fig. 2). Colonies growing on the surface of culture dishes maintained well-defined boundaries until near confluence; RWP-2 cells do not grow on glass. Monolayers of RWP-2 cells contain small, polygonal cells with clear, basophilic cytoplasm. The nuclei are well defined and contain 1 or 2 nucleoli. After confluence, moderate piling up of the cells occurs. Large, floating colonies that appear viable by dye exclusion are also produced. However, these floating colonies lose their ability to reestablish themselves as a monolayer culture and fail to grow in suspension.

The growth characteristics of RWP-1 and RWP-2 are summarized in Table 1. The doubling times for the 2 lines are quite similar, about 45 hr. Cells from both lines grow in soft agar, although the cloning efficiencies are not high (1.4 and 1.8% for RWP-1 and RWP-2, respectively, with 1 x 105 cells seeded). The plating efficiency in plastic dishes for RWP-1 is 54%, whereas it is 95% for RWP-2 (1 x 105 cells plated). At this inoculum, the colony-forming efficiencies from cells that attach are 19 and 18% for RWP-1 and RWP-2, respectively. The saturation densities for RWP-1 and RWP-2 are 1.2 and 2.9 x 105 cells/sq cm, respectively.

Quantitative analysis for CEA in 3-day-old spent medium from cultures of RWP-1 and RWP-2 cells in late log growth showed CEA at levels of 1090 and 414 ng/106 cells, respectively. Fresh RPMI-SC medium containing 10% fetal calf serum and 10% dialyzed fetal calf serum gave a CEA value of only 5 ng/ml by the same analytical technique.

This RWP-1 cell line is karyotypically as well as morphologically heterogeneous. The modal number of chromosomes for RWP-1 is 64 with a range of 59 to 66. One subpopulation contains a morphologically distinct and abnormal chromosome, which appears to be the result of a translocation. The other

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Doubling time (hr)</th>
<th>Saturation density (x10^6 cells/sq cm)</th>
<th>Plating efficiency on plastic (%)</th>
<th>Colony-forming efficiency on plastic (%)</th>
<th>Cloning efficiency in agar (%)</th>
<th>Modal chromosome no.</th>
<th>CEA production (ng/10^8 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RWP-1</td>
<td>45</td>
<td>1.2</td>
<td>54/10^6</td>
<td>19/10^6</td>
<td>1.4/10^6</td>
<td>64</td>
<td>1090</td>
</tr>
<tr>
<td>RWP-2</td>
<td>46</td>
<td>2.9</td>
<td>95/10^6</td>
<td>18/10^6</td>
<td>1.8/10^6</td>
<td>62</td>
<td>414</td>
</tr>
</tbody>
</table>

Cell suspensions in growth medium or agar-containing growth medium were plated in 35-mm culture dishes. The various parameters were determined as described in "Materials and Methods." Duplicate dishes were analyzed in all experiments. In the determination of doubling times, saturation densities, and plating and cloning efficiencies (on plastic and in agar) for both cell lines, the cell numbers determined on a given day for each of 2 duplicates did not differ by more than 5%. Medium conditioned by cells from either cell line for 72 hr was assayed for CEA; medium not exposed to cells had a CEA background level of 5 ng/ml as determined by radioimmunoassay.
subpopulation does not contain the marker. Cells containing the marker chromosome tend to have lower chromosome numbers (mode of 59, comprising 38% of total spreads). Those metaphases without the marker chromosome contain higher chromosome numbers (mode of 64, comprising 62% of all cells observed). The modal number of chromosomes for RWP-2 is 62 with a range of 57 to 62. Analysis indicates the appearance of two 1p— and two 1q— chromosomes in all metaphases observed. Occasionally, third and fourth marker chromosomes are found either in combination or separately. Histograms of chromosome number and distribution for both lines are shown in Chart 1.

Tumorigenicity of Cultured Tumor Cells. Both cell lines, RWP-1 and RWP-2, are tumorigenic in nude mice. Inoculation of nude mice with $1 \times 10^7$ RWP-1 cells results in a detectable tumor after 8 weeks. RWP-1 tumors from injected cells grow to small nodules, and then growth slows down significantly. Histologically, the RWP-1 xenografts resemble the original patient's tumor (Figs. 3 and 4). Nude mice, given injections of $5 \times 10^7$ RWP-2 cells, develop tumors after 2 to 5 weeks; unlike the RWP-1 nodules, these tumors grow progressively and can attain a weight of over 500 mg. Histological appearance of the RWP-2 xenografts from injected cells closely resembles that of the original human tumor (Figs. 5 and 6).

Propagation of Heterotransplants and Their Growth Properties in Nude Mice. The nude mouse xenografted tumors, which grew from implants of the original human biopsies, have been maintained continuously as transplantable xenografts in nude mice. Both of these xenografted tumors are now in their sixth passage in vivo. The implant of RWP-1 grows faster than that of RWP-2. RWP-1 has a doubling time of 10 days, whereas RWP-2 has a doubling time of 22 days (Chart 2). RWP-2 implanted carcinomas reach a maximum weight of about 500 mg, similar to the tumor size obtained from inoculation of nude mice with cultured RWP-2 cells. In contrast, RWP-1 implanted xenograft tumors grow to a larger size, up to 2 g, whereas inoculation of nude mice with cultured RWP-1 cells produces only small nodules.

Enzyme Analysis. Amylase, trypsin, and chymotrypsin, enzymes normally secreted by pancreatic acinar cells, are not detectable in cultured RWP-1 or RWP-2 cells or in spent medium concentrated 100-fold (Table 2). Low levels of amylase are present in the extracts prepared from mouse xenografts (Table 2), but the levels are the same for both tumors, and it is likely that this activity represents mouse serum amylase and not enzyme from the tumors. Some esterase activity toward tosyl-L-arginine methyl ester was detected in the RWP-2 tumor.

<table>
<thead>
<tr>
<th>Source of enzyme</th>
<th>ALP</th>
<th>LDH</th>
<th>G-6-PDH</th>
<th>Amylase</th>
<th>Trypsin</th>
<th>Chymotrypsin</th>
</tr>
</thead>
<tbody>
<tr>
<td>RWP-1 Nude mouse xenograft</td>
<td>5.89 ± 1.40a</td>
<td>1383 ± 147</td>
<td>81.9 ± 7.2</td>
<td>3.52 ± 0.09</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cells in culture</td>
<td>3.79 ± 0.06</td>
<td>597 ± 62</td>
<td>29.8 ± 1.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>48-hr spent medium</td>
<td>833 ± 47</td>
<td>Trace</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RWP-2 Nude mouse xenograft</td>
<td>15.38 ± 3.05</td>
<td>2039 ± 114</td>
<td>24.0 ± 2.8</td>
<td>3.65 ± 0.44</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cells in culture</td>
<td>10.00 ± 1.24</td>
<td>710 ± 112</td>
<td>58.7 ± 3.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>48-hr spent medium</td>
<td>0.65 ± 0.04</td>
<td>130 ± 7</td>
<td>Trace</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: Activities in cells and tumors are expressed as milliunits per mg total protein, where 1 milliunit is the amount of enzyme that converts 1 nmol of substrate per min under the conditions of the assay. Activities in spent culture media are expressed as milliunits released by $1 \times 10^7$ cells in serum-free 48-hr culture medium.

Mean ± S.D. for triplicate determinations in 3 separate experiments.
Not detectable.
Human Pancreatic Cancer Cell Lines

extract. However, it could not be reduced by incubation with 250 Kunitz Inhibitor Units of Trasylol, an amount sufficient to inhibit completely four times that amount of pure trypsin. This esterase activity does not, therefore, indicate the presence of trypsin.

Appreciable levels of intracellular enzymes, ALP, G-6-PDH, and LDH are detectable in both tumors and cultured cells (Table 2). The RWP-2 cells have higher levels of each of these enzymes than do the RWP-1 cells and, whereas LDH is found in spent media from both cell lines, little or no ALP or G-6-PDH is released by the cells into the culture medium during 48 hr of growth. The ALP is stable on heating; no loss of activity was observed after incubating extracts at 56° for 10 min. Whereas both human and murine LDH isoenzymes are found in extracts of xenografted RWP-1 and RWP-2 tumors, only human isoenzymes are present in the cultured cells. The values in Table 2 are total LDH levels in each extract.

Histopathology. The xenografts of RWP-1 and RWP-2 and the original tumors from the patients appear morphologically similar by light microscopy (Figs. 3 to 6). Both RWP-1 and RWP-2 can be classified as moderately differentiated ductal cell adenocarcinomas (8). The xenograft tumors are composed of closely approximated ductal or glandular structures (Figs. 7 and 8). Lumina are surrounded by one to several layers of cuboidal to columnar cells; orderly polarization of cells around the lumina is more frequently seen in RWP-1 than in RWP-2. Nuclei are round to oval and tend to be located in the basal portion of the cells, particularly in RWP-1. Nuclear chromatin density is variable, and nucleoli are usually conspicuous. The mucicarmine, periodic acid-Schiff, and alcian blue preparations show mucosubstance in the cytoplasm of many but not all cells. Some mucus-containing cells have characteristics of goblet or signet ring cells (Fig. 9). Accumulations of mucin are present in gland lumina and line the luminal cell borders. Mucin is more abundant in RWP-1 than in RWP-2.

Ultrastructurally, RWP-1 and RWP-2 resemble each other closely (Figs. 9 to 12). The cells are polarized around lumina, generally with their long axes perpendicular to the luminal surfaces. Round to oval nuclei with smooth or slightly indented membranes tend to be located in the more basal portion of the cells. Lateral cell surfaces have interdigitations of variable complexity and are bound by small desmosomal junctions. Apical cell surfaces have numerous stubby microvilli associated with glycocalceal bodies and core rootlets (33) (Fig. 11, inset). The cytoplasm contains profiles of ribosome-studded endoplasmic reticulum; free ribosomes; round, ovoid, or irregular mitochondria; small Golgi apparatus and smooth-walled vesicles; and sometimes fine filamentous material. The cells corresponding to goblet cells by light microscopy show, in their apical portion, numerous membrane-bound vesicles containing flocculent material. These vesicles fuse with each other and with the apical cell membrane. The flocculent material is also present in the lumina. No zymogen granules are found, and there are no cells with characteristics of islet cells.

DISCUSSION

The 2 human pancreatic adenocarcinoma cell lines (RWP-1, RWP-2) described herein were established in tissue culture directly from nude mouse xenografts of the primary biopsy material. The resulting xenografts are similar histologically to the primary human biopsies and to each other; each is moderately well differentiated, and both produce mucin and give rise to duct-like structures. RWP-1 and RWP-2 are representative of the histological class of pancreatic carcinomas that accounts for 80% of pancreatic cancer encountered by the clinician. This degree of morphological differentiation is not commonly observed among other currently available human pancreatic cancer cell lines that also give rise to tumors in nude mice. One other human pancreatic cancer cell line, COLO 357, has been described by Morgan et al. (34) as a cell line established from a metastatic adenocarcinoma with well-differentiated mucin-containing ducts, but as yet this cell line has not been reported to give rise to nude mouse tumors. Most of the other human pancreatic cancer cell lines established in culture and available for study have been derived from tumors classified as poorly differentiated or undifferentiated carcinomas such as PANC-1, MIA PaCa-2, and GER (20, 30, 51). Of the dozen or so available pancreatic carcinoma cell lines, only the culture established by Grant et al. (20) is also being carried as a xenograft from the initial tumor implantation. Five of these lines (PANC-1, HGC-25, HS 766T, CaPan-1, and SW-850) do produce tumors when injected into either immunosuppressed or immunodeficient hosts (1, 14, 17, 30, 35). In contrast, RWP-1 and RWP-2 not only are tumorigenic in the athymic host from cultured cells but also are carried both as continuously cultured cells and as xenografted tumor lines in the nude mouse from the original biopsy implantation. RWP-1 and RWP-2 cells have unusually low colonizing ability in semisolid medium (<2%) in contrast to most of the other pancreatic cancer cell lines which grow to a much greater degree in agar-containing growth medium (1, 51).

The RWP-1 cell line appears heterogeneous by 2 criteria, its morphology in culture and its karyotype. RWP-1 cultures contain 2 distinct epithelioid cell types; one subpopulation has a significantly larger cell volume than does the second. As yet, we have no indication that the RWP-2 cell line exhibits similar heterogeneity. We have had experience with 2 different heterogeneous carcinomas; subpopulations of neoplastic cells have been isolated from a spontaneously occurring mouse mammary tumor (12) and from a human colon carcinoma (11). Several studies with these systems, which illustrate the significance of intraneoplastic diversity, have been presented in other reports from our laboratories (7, 10). The demonstration that a human pancreatic cancer cell line is heterogeneous and that the cloning of the parent line to obtain the subpopulations responsible for the variation would contribute to our understanding and investigation of this clinically important neoplasm. It would also contribute to our understanding of the phenomenon of tumor cell heterogeneity, which is of critical importance in experimental and clinical oncology (4, 15, 22, 27, 28, 36-38, 42, 43, 46). We therefore are currently attempting to isolate the 2 subpopulations that are observed in our RWP-1 cultures.

In our search for markers of pancreatic cancer as well as of heterogeneity, we have tested our cell lines for several enzymes and CEA (6). RWP-1 and RWP-2 cultured cells and xenografted tumors have high levels of G-6-PDH, an enzyme of the pentose phosphate pathway, which may play a regulatory role in the synthesis of ribose 5-phosphate in cancer cells (48). This enzyme is commonly found in pancreatic and other cancer cells, probably as a result of an increased capacity for nucleotide synthesis (30, 49, 51). We also have detected the presence

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of ALP and LDH in both of our cell lines. Although most other available pancreatic cancer cell lines contain G-6-PDH, only 3 laboratories have reported the presence of ALP or LDH (1, 20, 51). While ALP and G-6-PDH were not released by either cell line during 48 hr of monolayer culture, appreciable amounts of LDH did accumulate in the medium. LDH release is associated with cell death (3) and, since these cells were grown in serum-free medium in order to collect material free of fetal calf serum proteins, it is probable that some cell death did occur, allowing the release of LDH into the medium. Both of our cell lines produce significant amounts of CEA. Of all the other human pancreatic cancer cell lines reported, only 3 have been shown to produce detectable levels of CEA (25, 30, 34). The fact that we found no evidence of trypsin or chymotrypsin in the media

In summary, 2 new human pancreatic cancer cell lines have been established. These lines are maintained in tissue culture and as xenografted tumors in nude mice. Morphological, ultrastructural, and biochemical characterization of both cell lines indicates that, unlike most of the currently available lines, RWP-1 and RWP-2 are representative of the most common type of human pancreatic carcinoma. As such, they should be a significant contribution to investigators studying human pancreatic cancer.

ACKNOWLEDGMENTS

We wish to thank Ann Richardson for her superb assistance in the preparation, staining, and banding of the chromosomes and in the analysis of the karyotypes.

REFERENCES

Human Pancreatic Cancer Cell Lines


Fig. 1. Photomicrograph of living RWP-1 cells in monolayer culture. Phase optics, × 200.

Fig. 2. Photomicrograph of living RWP-2 cells in monolayer culture. Phase optics, × 200.

Figs. 3 and 4. RWP-1. Similar histological pattern of moderately-well-differentiated ductal adenocarcinoma of original patient's tumor (Fig. 3) and nude mouse xenograft tumor (Fig. 4). H & E, × 63 (original magnification).

Figs. 5 and 6. RWP-2. Similar histological pattern of moderately-well-differentiated ductal adenocarcinoma of original patient's tumor (Fig. 5) and nude mouse xenograft tumor (Fig. 6). H & E, × 63 (original magnification).

Figs. 7 and 8. RWP-1 (Fig. 7) and RWP-2 (Fig. 8) xenograft tumors in nude mice. Ductal and glandular differentiation with intracellular and intraluminal mucin. H & E, × 160 (original magnification).

Figs. 9 and 10. Electron micrographs of RWP-1 xenograft tumor in nude mouse.

Fig. 9. Cells radially arranged around lumen with basally placed nuclei and microvillus apical cell borders. × 8,580.

Fig. 10. Intracytoplasmic mucin granules and mucin-filled goblet cell (top right). × 11,100. Inset, microvilli with glycocalyceal bodies and core rootlets. × 38,400.

Figs. 11 and 12. Electron micrographs of RWP-2 xenograft tumor in nude mouse.

Fig. 11. Tumor cells radially arranged around lumen, microvillus apical cell borders. × 5,800.

Fig. 12. Apical portion of cells showing microvilli with glycocalyceal bodies and core rootlets. Junctional complex is moderately well developed. × 15,800.
Establishment and Characterization of Two Human Pancreatic Cancer Cell Lines Tumorigenic in Athymic Mice

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