Morphological, Biological, Biochemical, and Karyotypic Characteristics of Human Pancreatic Ductal Adenocarcinoma Capan-2 in Tissue Culture and the Nude Mouse

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ABSTRACT

Human pancreatic ductal adenocarcinoma Capan-2, derived from a 56-yr-old male Caucasian, has been studied in both tissue culture and the nude mouse. In tissue culture, tumor cells showed epithelial-like features, whereas in the nude mouse, the tumor grew as a well-differentiated adenocarcinoma, resembling histopathologically the original neoplasm. Ultrastructurally, the neoplastic cells showed characteristics of ductal epithelium. The isozyme phenotypic profile of Tumor Capan-2 was determined in eight genetically determined loci, and chromosome studies showed a hypotetraploid pattern with a number of morphological and numerical changes. Carcinoembryonic antigen was produced in trace amounts, and lactate dehydrogenase was represented only by Isoenzyme 5, regardless of environmental conditions. The characteristics of Capan-2 tumor make it a valuable addition to the small number of available pancreatic tumor lines in studies aiming at clarifying certain aspects of the biology of this type of malignancy.

INTRODUCTION

The usefulness of the nude mouse as an experimental model in cancer research is becoming increasingly evident. This is primarily due to its ability to sustain a large number of human neoplasms following transplantation of established tumor cell lines and primary xenografts removed at surgery. The fact that nude mouse transplanted human tumors preserve their morphological and biological integrity through successive transplant generations would facilitate an in-depth study of various factors which may determine tumor biological behavior and tumor response to various therapeutic modalities.

With these objectives in mind, efforts have been made by various investigators to establish in vitro and in vivo lines of adenocarcinomas of the human exocrine pancreas. The importance of these efforts becomes evident considering that pancreatic cancer is an extremely aggressive tumor ranking fourth as a cause of cancer deaths, exceeded only by cancer of the lung, large bowel, and breast (1). More than 21,000 cases are diagnosed each year (2) with a 5-yr survival rate less than 1% (3).

As of today, only a small number of established pancreatic tumors have been reported (4-11), and fewer have been adequately characterized. Furthermore, of these only occasional cases have been studied in the nude mouse.

As a continuation of our efforts to establish and characterize a series of ductal adenocarcinomas of the human pancreas encompassing a wide spectrum of histopathological and biological features, we report our studies on Tumor Capan-2 which was derived from a pancreatic adenocarcinoma of ductal origin. Tumor Capan-2 has been studied in tissue culture and the nude mouse with regard to growth characteristics, histopathological appearance, ultrastructural morphology, and production of biological markers such as LDH, and CEA. The karyotype of the line and its isozyme phenotypic profile were also evaluated.

MATERIALS AND METHODS

Cell line Capan-2 was established at Sloan-Kettering Cancer Center, Rye, NY, by Dr. J. Fogh and J. D. Loveless from a pancreatic adenocarcinoma of a 56-yr-old male Caucasian. The patient underwent total pancreatectomy and cholecystectomy, partial gastrectomy and large and small bowel omentectomy, and splenectomy. The tumor involved the head of the pancreas and infiltrated the muscularis of the duodenal wall distal to the ampulla and the peripancreatic fibroadipose tissue posteroinferiorty. Histopathologically the tumor was characterized as “carcinoma of the head of the pancreas, ductal type.” Preoperatively, serum CEA was 7.3 ng/ml; a-1 fetoprotein was negative. The patient's ABO blood group was B positive. Postoperatively the patient received chemotherapy; he died 6 yr and 9 mo later.

At the present time cell line Capan-2 is maintained in RPMI-1640 medium containing 15% fetal calf serum and supplemented with penicillin (100 IU/ml) and streptomycin (100 μg/ml) (GIBCO). Tumor cells grow in monolayer and are passed every 3 to 4 wk. The culture is free of Mycoplasma contamination (Mycoplasma medium; GIBCO).

Plating Efficiency. Plating efficiency was determined by plating 2.5 × 104 viable cells in 25-cm2 tissue culture flasks using RPMI-1640 medium. Fifteen hr later, the medium was discarded, and the attached cells were collected following trypsinization and counted. The experiment was run in triplicate.

Cell Doubling Time. Cell doubling time was determined by counting the number of viable cells from freshly trypsinized monolayers. Sixteen 25-cm2 tissue culture flasks, each receiving 5 × 104 cells, were used. Cell countings, in duplicate, were performed at 24-h intervals for 8 days. Throughout the entire procedure cell viability was determined by means of the trypan blue exclusion method.

Determination of Human LDH and CEA. The Corning agarose universal electrophoresis system (Corning, Palo Alto, CA) was used to differentiate and quantitate human LDH isozymes in tissue culture media, cell extracts, and plasma of tumor-bearing mice. Human and mouse isozymes were separated on the basis of different electrophoretic mobilities (9, 12, 13), and quantitation was done on a colorimetric densitometer (Helena Quick Scan, Jr.; Helena Laboratories, Beaumont, TX). CEA assays were performed on the plasma of tumor-bearing mice by the Abbott CEA-EIA diagnostic kit (Abbott Laboratories, North Chicago, IL.).

Enzyme Phenotypic Profile. The genetically determined enzyme phenotypic profile of cultured cells was determined according to the method of Halton et al. (14).

Cytogenetics. Cytogenetic analysis, including G banding, on cultured tumor cells was done by the methods described by Peterson et al. (15) and Seabright (16).

Nude Mice. Female athymic mice of Swiss background from our nude mouse colony were maintained in a laminar air flow clean room under controlled temperature and humidity.

Collection of Mouse Plasma Samples. For the determination of
Fig. 1. In A, cell line Capan-2 grown in tissue culture is characterized by large epithelial-like cells with large, oval, round, or indented nuclei; chromatin clumping; and basophilic granulated cytoplasm. Phase contrast, × 200 (passage 16). B, tumor cell line Capan-2 grown in the nude mouse, fourth transplant generation. Microscopically, the neoplasm is made up of duct-like structures lined with tall columnar epithelial cells showing mild nuclear pleomorphism, hyperchromasia, and occasional mitoses. Stratification of neoplastic cells is also observed. H & E, × 250.

Table 1 Enzyme phenotypic profile of Tumor Capan-2

<table>
<thead>
<tr>
<th>Cell line</th>
<th>PGM1*</th>
<th>PGM3</th>
<th>ESD</th>
<th>Me-2</th>
<th>AK-1</th>
<th>GLO1</th>
<th>G6PD</th>
<th>LDH</th>
<th>Phenotype frequency</th>
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<tr>
<td>Capan-2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>B</td>
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<td>1</td>
<td>1-2</td>
<td>1</td>
<td>2</td>
<td></td>
<td>A</td>
<td>0.000236</td>
</tr>
</tbody>
</table>

* PGM, phosphoglucomutase, EC 2.7.5.1; ESD, esterase D, EC 3.1.1.1; Me-2, malate dehydrogenase, EC 1.1.1.40; AK-1, adenylate kinase, EC 2.7.4.3; GLO1, glyoxylase 1, EC 4.4.1.5; G6PD, glucose-6-phosphate dehydrogenase, EC 1.1.1.49; LDH, EC 1.1.1.27.

Fig. 2. Growth curve of Capan-2 tumor grown s.c. in the nude mouse (fourth transplant generation). Points, mean of 6 animals, bars, S.D. Arrow, time of transplantation.

Human LDH and CEA present in the plasma of tumor-bearing mice, blood was drawn by orbital venipuncture with heparinized microcapillary tubes. Plasma was separated by centrifugation and stored at −80°C until used.

Determination of LDH in tissue culture growing cells was done as follows. Cultured cells were harvested, washed 3 times with serum-free medium, and packed by centrifugation. To the packed cells Tris-HCl:EDTA buffer was added in the ratio of 1:1 (v/v, 0.05 M Tris-HCl:0.001 M disodium EDTA). Cells suspended in buffer were disrupted by freezing-thawing in dry ice:acetone and centrifuged to remove insoluble matter, and the supernatant containing the cytosol was used for LDH determination. To determine the presence of LDH released into the culture medium, triplicate cultures growing in 25-cm² flasks were used. At approximately 75% culture confluency the culture medium was removed, monolayers were washed repeatedly with serum-free medium, and then incubated for 24 h with the same serum-free medium. Twenty-four-h culture media were collected, centrifuged to remove particulate matter, and concentrated to 100 x using a miniconcentrator unit (Amicon Corp., Lexington, MA). Concentrated media were assayed for LDH immediately or were kept frozen at −80°C until used.

Solid Tumors in Nude Mice. For injection into nude mice cultured tumor cells were dispersed with 0.5% trypsin:0.2% EDTA in Hanks' balanced salt solution (GIBCO) and adjusted to 1 x 10⁸ viable cells/0.2 ml. Cell viability was determined by the trypan blue dye exclusion test. Initially, s.c. tumors were established by giving the animals injections of 1 x 10⁸ viable tumor cells. In subsequent experiments, tumor propagation was accomplished by transplanting s.c. through a skin incision in the anterior lateral thoracic wall a small piece of tumor measuring approximately 6 mm³ (17). The size of nude mouse-grown tumors was measured in 2 dimensions with calipers, and tumor volume was calculated weekly by using the following formula (18):
Fig. 3. Electron micrograph of Tumor Capan-2 grown in tissue culture (A). The neoplastic cells are characterized by surface microvilli, interdigitation of cell membranes, and tight junctions connecting adjacent cells. The cytoplasm contains a Golgi complex, short profiles of smooth and rough endoplasmic reticulum, mitochondria, few free ribosomes, and occasional mucigen granules. Uranyl acetate-lead citrate, × 7100. Electron micrograph of Tumor Capan-2 grown in the nude mouse (B). The apical plasma membrane shows well-developed surface microvilli. The cytoplasm contains short profiles of smooth and rough endoplasmic reticulum, mitochondria, few free ribosomes, and occasional mucigen granules. Adjacent cells show infoldings of the basal cytoplasm with the presence of terminal bars, tight junctions, and well-developed desmosomes. Uranyl acetate-lead citrate, × 9150.

Tissue Culture Characteristics. Tumor Capan-2 grown in tissue culture was composed of cells exhibiting epithelial-like morphological characteristics. They were ovoid or round characterized by round, oval, or indented nuclei with prominent nucleoli, chromatin clumping, and moderately basophilic cytoplasm (Fig. 1). Histochemical studies for cytoplasmic mucin were positive. The plating efficiency of the cultured cells was 55%, and doubling time was calculated at 96 h.

For transmission electron microscopy, tumor tissue was cut into fragments 0.5- to 1-mm thick and fixed in cold 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4, immediately after removal. They were embedded in LX-112 resin, and thin sections were prepared with a Leitz ultramicrotome, stained with uranyl acetate-lead citrate, and examined in a Philips EM-301 electron microscope. Studies of Tumor Capan-2 in tissue cultures were performed on passages 14 through 25, cytogenetic studies were on passages 16 and 20, and nude mouse growth characteristics were followed for a period of 10 successive transplant generations (1 through 10).

RESULTS

Tissue Culture Characteristics. Tumor Capan-2 grown in tissue culture was composed of cells exhibiting epithelial-like morphological characteristics. They were ovoid or round characterized by round, oval, or indented nuclei with prominent nucleoli, chromatin clumping, and moderately basophilic cytoplasm (Fig. 1). Histochemical studies for cytoplasmic mucin were positive. The plating efficiency of the cultured cells was 55%, and doubling time was calculated at 96 h.

The allozyme phenotypic profile of Capan-2 tumor was studied at eight genetically determined loci, and comparison was made with HeLa cells. The results of these studies are presented in Table 1.

Tumor Growth in Nude Mice. Transplantation of 1 × 10⁶ viable tumor cells s.c. in nude mice resulted in tumor growth having a latency period, the time between transplantation and first positive evidence of tumor growth, of 4 to 6 wk. To further establish the growth characteristics of nude mouse-grown tumors, solid pieces of growing tumors measuring approximately 0.3 x 0.2 cm were transplanted s.c. as described in “Materials and Methods.” The follow-up of many consecutive transplant generations showed a consistently reproducible growth pattern (Fig. 2).

Microscopically, s.c. growing tumors exhibited characteristics of a well-differentiated adenocarcinoma, similar to that of the original neoplasm. They were made up of glandular structures supported by fibrovascular stroma. The neoplastic cells were tall columnar, showing mild nuclear polymorphism, hyperchromasia, and occasional mitoses. Stratification of neoplastic cells was also observed (Fig. 1). The cytoplasm was amphophilic or slightly basophilic. Histochemical studies revealed cytoplasmic mucin in the form of fine granules occupying the apical portion of the cytoplasm. The tumor invaded its pseudocapsule with evidence of micro- and macroinvasion. Criteria for invasion were the same as described previously (19).

Ultrastructurally, the characteristics of tumor cells growing in both tissue culture and the nude mouse were those of ductal epithelium (20, 21) and, with the exception in the amount of intracytoplasmic mucin, showed close similarity to those reported for Capan-1 and SW-1990 tumor lines (8, 9). The apical plasma membrane consisted of well-developed microvilli. The cytoplasm contained short profiles of smooth and rough endoplasmic reticulum, well-developed mitochondria, and few free

Length × (width)² × 0.4
under different environmental conditions. LDH expressed entirely at LDH-5 was present in measurable amounts in both tissue culture media and cell extracts. The ability of tumor cells to produce LDH continued in the nude mouse where it was detected over a wide range of tumor burden. Fig. 4 shows the LDH isozyme profile under varying experimental conditions, and the existing relationship between tumor weight and circulating LDH in the nude mouse is given in Fig. 5.

Studies of CEA production showed only trace amounts circulating in the serum of tumor-bearing mice.

Chromosome Studies. In Tumor Capan-2, chromosome counts ranged from 45 to 68 with most chromosome counts being in the hypotetraploid range (Table 2). Most of the chromosomes were trisomic with the exception of chromosomes 14, 15, and 21. Chromosome 14 was absent in 4 of 6 karyotypes and monosomic in 2 of 6. Chromosome 15 was monosomic in 4 of 6 karyotypes, absent in 1, and 2 copies of it were present in only 1 karyotype. Monosomic, also, was chromosome 21 in most of the karyotypes. There were 5 identifiable marker chromosomes. The marker m1 was a 1pq, m2 was an i(14), m3 was a translocation between 12q and 6q arms, m4 was a chromosome 9 with part of another chromosome translocated to the q arm, and m5 appeared to be an 11p-q-. In addition, 4 to 7 unidentifiable marker chromosomes were present in all karyotypes studied (Fig. 6).

DISCUSSION

There are two main concerns regarding tumors propagated in tissue culture or the nude mouse: tumor integrity and tumor biological stability. Studies of glucose-6-phosphate dehydrogenase identified Tumor Capan-2 as type B, thus confirming its origin from a Caucasian patient. Furthermore, the enzyme phenotypic profile made it distinct from any other tumor line, providing at the same time a reliable internal control against any accidental inter- or intraspecies contamination. Cultured Capan-2 cells preserved their epithelial character regardless of the number of passages, and in the nude mouse the tumor exhibited a well-differentiated pattern similar to that of the original tumor. We have followed Capan-2 through a number of successive transplant generations without any evidence of changes in growth pattern and histopathological appearance, an observation we have reported for a number of nude mouse-grown human tumors of different histogenetic backgrounds (8, 9, 13) and which has been confirmed in more recent reports (22, 23).

With regard to cytogenetic studies, of interest was the observation that all chromosomes were of human origin showing a consistent pattern which also reflected both quantitative and qualitative changes in a number of karyotypic profiles. In addition, marker chromosomes 1 to 5 were present in all karyotypes examined. It is of interest that marker 1p has been reported previously for another pancreatic adenocarcinoma line (20). Whether, however, the observed morphological and numerical changes relate to those of other pancreatic carcinomas is not clear at the present time. The number of pancreatic carcinomas in which detailed chromosome analysis has been performed is very small (9, 10, 24), thus precluding any generalization of the reported findings. It is hoped that, as more cases are studied, analysis of accumulated data may permit the distinction between primary and secondary karyotypic changes.

As in previous cases, LDH was selected as a tumor marker because: (a) it is present in all human cells; (b) qualitative and quantitative determinations are easy to perform; and (c) the

**Table 2** Chromosome frequency distribution of cell line Capan-2

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>10</th>
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<td>Tumor Cells</td>
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**Fig. 4.** LDH isoenzyme electrophoretogram. Pattern 1 represents the human isoenzyme profile. The slower migrating band (extreme left) is human LDH-5. Other visible bands are, from left to right, human LDH-4, -3, -2, and -1. Pattern 2 shows the mouse LDH isoenzyme profile. Note that LDH-5, which is the dominant LDH isoenzyme in the mouse, occupies a position between human LDH-4 and LDH-3. Pattern 3 represents the LDH profile of plasma of nude mouse-bearing Tumor Capan-2. In this pattern only human LDH-5 is recognized. Arrows, point of origin.

**Fig. 5.** Relationship between tumor weight and circulating human LDH in the nude mouse-bearing Tumor Capan-2. Points, single animal. U/L, units/liter.

**Fig. 6.** Diagram of karyotype showing the arrangement of chromosomes with respect to banding patterns. Chromosomes shown are those that were frequently identified in the Capan-2 karyotypes (80 cases).

**Table 2** Chromosome frequency distribution of cell line Capan-2

<table>
<thead>
<tr>
<th>No. of metaphases</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>10</th>
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<tr>
<td>No. of metaphases</td>
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<td>1</td>
<td>2</td>
<td>1</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>No. of chromosomes precisely counted</td>
<td>46</td>
<td>60</td>
<td>66</td>
<td>67</td>
<td>68</td>
<td></td>
</tr>
</tbody>
</table>

ribosomes. Typical mucigen granules were also seen in a number of cells. Adjacent cells showed extensive infoldings of the basal cytoplasm with the presence of terminal bars, tight junctions, and well-developed desmosomes (Fig. 3).

Production of LDH and CEA. Our studies indicated that tumor cell line Capan-2 preserved the ability to elaborate LDH...
difference in electrophoretic mobility between human and mouse LDH makes possible their separation and quantitation. This latter property makes LDH a useful marker in identifying the human origin of any tumor grown in the nude mouse, and under certain conditions, it may be used as an approximate indicator of tumor burden. It is worth noting that Tumor Capan-2 was represented exclusively by LDH-5 regardless of the environmental conditions (Fig. 4). This, most probably, accounts for the high correlation coefficient between the amount of LDH in the plasma of tumor-bearing mice and tumor burden (Fig. 5). It should be recalled here that mouse LDH-5, which is the dominant mouse LDH isozyme, moves electrophoretically between human LDH-4 and LDH-3 (Fig. 4), and in a mixture of mouse and human LDHs, broad overlapping of isozymes of different origin does occur in this region. This is probably one of the obstacles in successfully correlating the amount of circulating human LDH and tumor burden in tumors elaborating the entire LDH isozyme spectrum (9, 13). Capan-2 tumor is the second pancreatic adenocarcinoma of ductal origin we are reporting as elaborating only LDH-5 (8). Whether this represents a characteristic of this type of malignancy is currently under investigation in our laboratory.

CEA associated with pancreatic adenocarcinomas has been reported in pancreatic cancer patients (24) and in pancreatic cancer lines in vivo and in vitro (9, 25, 26). Capan-2 tumor produces CEA, although in very small quantities. This, undoubtedly reflects the degree of its differentiation and correlates well with the clinical history of the patient from whom the tumor originated.

In conclusion, Capan-2 tumor derived from a human pancreatic adenocarcinoma of ductal origin shows histopathological, biological, and biochemical characteristics similar to that of the original tumor. It grows well in tissue culture and the nude mouse and exhibits a distinct enzyme phenotypic profile. The low amount of CEA produced, most probably, reflects its well-differentiated character. In addition Capan-2 presents the unique feature of elaborating only LDH-5, an observation made previously on another pancreatic carcinoma line (8). In view of these characteristics Tumor Capan-2 may contribute in improving our understanding of certain aspects of the biology of human pancreatic cancer, and it may be used, along with other well-characterized pancreatic carcinoma lines, in furthering our knowledge of response to treatment of this type of malignancy (27, 28).

REFERENCES


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