Establishment and Characterization of a Human Pancreatic Cancer Cell Line (SUIT-2) Producing Carcinoembryonic Antigen and Carbohydrate Antigen 19-9

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A new tumor cell line (SUIT-2) derived from a metastatic liver tumor of human pancreatic carcinoma has been established in tissue culture and in nude mice, and maintained for over five years. In tissue culture, the cells grew in a monolayered sheet with a population doubling time of about 38.2 hr, and floated or piled up to form small buds above the monolayered surface in relatively confluent cultures. Chromosome counts ranged from 34 to 176 with a modal number of 45. Subcutaneous injection of cultured cells into nude mice resulted in tumor formation, histopathologically closely resembling the original neoplasm which had been classified as moderately differentiated tubular adenocarcinoma. Electron microscopic observation of the neoplastic cells revealed a characteristic pancreatic ductal epithelium. SUIT-2 cell line produces and releases at least two tumor markers, carcinoembryonic antigen and carbohydrate antigen 19-9, propagates even in serum-free medium, and metastasizes to the regional lymph nodes in nude mice xenografts.

Key words: Human cell line — Pancreatic cancer — Carbohydrate antigen 19-9 — Carcinoembryonic antigen

Measuring serum levels of various tumor markers such as carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), and pancreatic oncofetal antigen is considered useful both in diagnosis and in monitoring the results of treatment of pancreatic cancer, although the exact organ specificities of these tumor markers have not yet been determined. Carcinoma of the pancreas is the fourth most frequent cause of cancer death in Japan at present and its incidence is sharply increasing. Poor prognosis in management of cancer of the pancreas has been explained by difficulty in early detection of the neoplastic process, lack of effective treatment, and limited knowledge of the biological characteristics of this type of cancer. Establishment of biological models, including permanent cell lines of human pancreatic cancer, is required in order to clarify the biological characteristics of this form of cancer.

Fourteen established cell lines of human pancreatic cancer in tissue culture have been reported, though production of CEA was mentioned in only 8 reports and a CA19-9 producing cell line has not yet been reported. A new cell line of human pancreatic cancer which produces both CEA and CA19-9 has been established and designated as "SUIT-2". This nomenclature derives from "SUI" which forms part of the word pancreas in Japanese and "T" from tumor.

MATERIALS AND METHODS

Patient The patient was a 73-year-old Japanese male with advanced pancreatic cancer, involving the entire pancreas and extending into the duodenum, common bile duct, portal vein, and surrounding fibroadipose tissue at the time of an exploratory laparotomy on July 6, 1981. Peritoneal dissemination with ascites and metastases to peripancreatic lymph nodes and the liver were also observed. Biopsy specimens were obtained from the pancreas, greater omentum, and liver. Ascites was also collected for diagnosis and tissue culture. Histopathologically, the tumor was classified as a moderately differentiated tubular adenocarcinoma. CEA and CA19-9 contents in ascites preserved at -90° were estimated in January, 1986, as 181.4 ng/ml and 119,000 U/ml, respectively, by the

The abbreviations used are: CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; PBS(−), Ca2+ - and Mg2+-free phosphate-buffered saline solution; MEM, Eagle's minimal essential medium; FBS, fetal bovine serum; HE, hematoxylin-eosin; P-3, passage-3.
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methods described below. The patient's ABO blood group was A+.

**Primary Culture** A biopsy specimen of metastatic liver mass, about 0.5 g in wet weight, was minced with sharp scissors into small pieces and dispersed with 0.25% trypsin (DIFCO, Detroit), 1,000 protease units of Dispase (Godo Shusei, Tokyo), and 0.02% EDTA (Nakarai, Kyoto) in Ca²⁺- and Mg²⁺-free phosphate-buffered saline solution [PBS (−)] in order to obtain a single cell suspension. After exposure to this enzyme solution for 30 min, the cell suspension was centrifuged at 250g for 10 min. The cell pellet was suspended in 10 ml of Eagle's minimal essential medium (MEM; Nissui, Tokyo) with Hanks' balanced salt solution and then centrifuged again in the same manner. After being washed 3 times with MEM, the cells were resuspended in 10 ml of MEM containing 50% ascites of the same patient, which had previously been centrifuged at 2,000g for 20 min to remove floating cells, filtered through 0.22 μm filter units (Millex GS; Millipore, Bedford) to remove contaminating bacteria, then supplemented with penicillin (100 units/ml) and streptomycin (100 μg/ml) (GIBCO, Grand Island). Aliquots of this resuspension were then distributed into 5 plastic Petri dishes (35 mm) (Corning/Iwaki Glass, Tokyo), and cultivated at 37° in a humidified atmosphere of 5% CO₂ in air. During 5 days of primary culture, sparsely populated colonies of epitheloid cells surrounded by fibroblastic cells were observed. The growth medium was replaced every 4 to 7 days, and the supplementary ascites was gradually reduced to a final concentration of 10% in order to avoid a rapid change in the environment of the cancer cells. The elimination of fibroblastic cells was accomplished chemically by brief exposure to 0.05% trypsin in PBS(−) and mechanically by scrubbing with a silicone rubber policeman. This procedure was repeated for 7 consecutive days, or until no further fibroblastic cell growth could be observed. About one month later, epitheloid cells without contaminating fibroblastic cells had propagated to the stage of confluent culture in one Petri dish. The cells were then passed by treatment with 0.25% trypsin and 0.02% EDTA in PBS(−).

**Serial Passage and Storage** The cell line was routinely passaged by trypsinization using 0.25% trypsin in PBS(−), and maintained in growth medium, MEM supplemented with 10% fetal bovine serum (FBS) (Flow, McLean), penicillin (100 units/ml), and streptomycin (100 μg/ml). Initially, the monolayered cells were passaged at a 1:2 split, but by the 10th passage 1:8 to 32 splits were commonly made every week. During serial passages, cells of different passage numbers in growth medium containing 10% dimethyl sulfoxide (Nakarai, Tokyo) were frozen and stored in liquid nitrogen. After thawing, the stored cells were propagated in culture without a noticeable change in morphology.

**Growth Curve** A single cell suspension of 1 × 10⁷ cells, passage 3 (P-3), was plated into 35 mm plastic Petri dishes with 2 ml of growth medium, which was replaced every day. A cell count was taken each day in three dishes. Viable cells were determined by the dye exclusion method after staining with 0.1% nigrosin solution. Doubling time was determined during the exponential phase of growth.

**Chromosome Analysis** Exponentially growing cells (P-3) on cover glass were incubated with 10⁻⁷M colcemid (Nakarai) for 6 hr. After hypotonic treatment for 30 min at 37° in 0.075M KCl, the cells were fixed in a methanol:acetic acid (3:1) solution. Trypsin-Giemsa staining was performed, and one hundred metaphases were analyzed for chromosome number.

**Colony Forming Efficiency** A single cell suspension was plated into 60 mm plastic Petri dishes at concentrations of 1 × 10², 2 × 10², 5 × 10² and 1 × 10³ cells with 5 ml of growth medium, which was replaced on day 7. After 14 days, the cells were washed with PBS(−), fixed with absolute alcohol, and stained with Giemsa solution. Colonies consisting of 16 or more cells were counted under a phase-contrast microscope.

Colony forming efficiency in soft agar was also examined by the modified method of Hamburger and Salmon. In brief, the cells were plated at concentrations of 1 × 10⁴ and 1 × 10⁵ cells in 1.0 ml of growth medium containing 0.3% agar over a feeder layer of 1.5 ml of the same growth medium containing 0.5% agar. After 14 days, colonies consisting of 16 or more cells were counted under a phase-contrast microscope.

**Culture in Serum-free Medium** Cells (P-268) were cultivated with a growth medium in which supplemented FBS had been reduced gradually to a final concentration of 0.3% agar over a feeder layer of 1.5 ml of the same growth medium containing 0.5% agar. After 14 days, colonies consisting of 16 or more cells were counted under a phase-contrast microscope.

**Tumorigenicity, Passage and Growth** Congenitally athymic nude mice (BALB/c/nu/nu; CLEA Japan, Kumamoto) were propagated and maintained in a pathogen-free environment. Six- to 8-week-old nude mice were used as hosts for tumor cell injection. After trypsinization of cultured cells and centrifugation of the cell suspension at 250g for 10 min, the cell pellet was washed twice with PBS(−) and resuspended in the same solution. Tumor cells (1 × 10⁵ in 0.2 ml) were injected subcutaneously into the flanks of the nude mice. The
resulting tumors were measured with calipers and their volumes were estimated by using the following formula: \( V = \frac{L \times W \times H}{2} \) (\( V \), volume; \( L \), length; \( W \), width; \( H \), height).

Passages of the xenografts were also performed. Tumors in nude mice were removed 2 to 3 months after inoculation and washed with MEM, then minced into 2 mm cubes and passed by retransplantation with trocars into the bilateral scapular areas of 3 nude mice. The growth curve of the xenografts (P-2) was obtained by measuring 6 tumors in the 3 nude mice.

**Histopathology** For light microscopy, the xenografts were fixed in 10% phosphate-buffered formalin, embedded in paraffin, sectioned on a microtome, and stained with hematoxylin-eosin (HE), periodic acid-Schiff, and alcian blue.

For transmission electron microscopy, xenografts were cut into 1 mm cubes and fixed with 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) for 3 hr at 4°C immediately after removal. Fixed tissues were washed with PBS(-), postfixed in 1% osmic acid, dehydrated in graded ethanol and propylene oxide, and embedded in Epon. Ultrathin sections were stained with uranyl acetate and Reynold’s lead citrate and examined in a JEM 100B electron microscope (JEOL, Tokyo).

**CEA and CA19-9** A cell suspension (5×10^4 cells) was seeded into every well of a 24-well micro-plate (Corning/Iwaki Glass) with 1 ml of growth medium, which was replaced every day. The spent media from days 3, 7, and 11 were transferred into test tubes, centrifuged at 500g for 10 min to remove floating cells, and stored at -70°C. CEA assay in the spent medium was performed by enzyme-immunoassay (EIA) with an Abbott kit (DAINABOT, Tokyo) for P-26 and a Roche kit (Nippon Roche, Tokyo) for P-242, and CA19-9 assay was performed by EIA with a Centocor kit (Toray-Fuji Bionics, Tokyo) for P-242.

**RESULTS**

**Establishment of SUIT-2** About one month after primary culture of human pancreatic cancer cells was completed, epithelial cells became predominant in one dish, replacing virtually all the fibroblastic cells. The epithelial cell line, SUIT-2, thus established has undergone more than 270 passages to date.

**In vitro Growth Characteristics** Under a phase-contrast microscope, the cells in the semiconfluent culture were seen to be of 2 types: an elongated and spindle-shaped form; and a polygonal form typical of epithelial cells. In a more confluent culture, the cells floated or piled up to form small buds above the surface of the monolayer cell sheet, and

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**Fig. 1.** Microphotograph of living SUIT-2 cells in monolayer culture. Phase-contrast optics, ×200 (original magnification).
single or multiple vacuoles were occasionally seen in the cytoplasm (Fig. 1). Giant cells containing a large nucleus and vacuoles were seen sporadically.

In the exponential phase, the population doubling time in P-3 was estimated as 38.2 hr (Fig. 2). In P-26 and P-268, the doubling times were estimated as 30.9 hr and 29.1 hr, respectively. Chromosome counts ranged from 34 to 176 with a modal number of 45. Most of the numbers were in the diploid range (Fig. 3).

Colony forming efficiency was not high, being 5.3% in plastic Petri dishes and 0.18% in soft agar at a concentration of $1 \times 10^5$ cells per dish (Table I). Plating efficiency, $1 \times 10^5$ cells in 35 mm dishes, was about 62.5% in P-3.

It was confirmed that the cells could propagate with a 31.3 hr population doubling time and be passaged by the use of 0.05% trypsin solution in PBS (−) even in serum-free medium.

**Growth Characteristics of Xenografts**

Inoculation of nude mice with $1 \times 10^7$ SUIT-2 cells resulted in a detectable tumor after one week. The inoculated tumors grew to about 1,000 mm$^3$ in volume after 5 weeks. The tumors grew exponentially with a volume doubling time of 8.3 days (Fig. 2). Transplantation of xenografts into 3 mice showed an almost 100% take with a short lag phase of 7 days. Macroscopically, tumors were well encapsulated, firmly attached to the covering dermis, and exhibited no invasion into underlying tissue until the tumor volume exceeded

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**Table I. Colony Forming Efficiencies in Plastic Petri Dish and in Soft Agar**

<table>
<thead>
<tr>
<th></th>
<th>Petri dish</th>
<th>Soft agar</th>
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<tbody>
<tr>
<td>Number of cells</td>
<td>$1 \times 10^2$</td>
<td>$2 \times 10^2$</td>
</tr>
<tr>
<td>CFE* (%)</td>
<td>2.5</td>
<td>2.7</td>
</tr>
</tbody>
</table>

* CFE, colony forming efficiency.
Fig. 4. Microphotograph of a metastatic liver tumor from pancreatic cancer. HE, ×200 (original magnification).

Fig. 5. Microphotograph of a xenograft of SUIT-2 cells in a nude mouse. The histological pattern of this xenograft closely resembled that of the tumor of origin. HE, ×200 (original magnification).
1,000 mm$^3$. However, tumors larger than 2,000 mm$^3$ eventually ulcerated, invaded, and sometimes metastasized to the regional lymph nodes (2/9 mice, 22.2% or 3/18 lymph nodes, 16.7%).

Microscopically, xenografts about 1,000 mm$^3$ in volume were surrounded by a fibroblastic tissue continuous with the fibrovascular stroma. Central necrosis, 45% to 50% in area, was usual and the viable tumor was situated peripherally and/or related to stromal blood vessels. The tumor closely resembled the primary tumor of the patient, and were classified as moderately differentiated tubular adenocarcinoma with variable glandular differentiation ranging from good to poor. The neoplastic cells were either cuboidal or columnar, with low mitotic activity (Figs. 4, 5 and 6). Periodic acid-Schiff and alcian blue preparations showed mucosubstance in the cytoplasm of a majority of the cells. Some mucus-containing cells had the characteristics of signet ring cells. Accumulation of mucosubstance was also evident in the gland lumina.

The cells in the area of ductal differentiation showed microvillus processes on the luminal surface and lateral intercellular space under electron microscopic examination. The cytoplasm contained rough-surfaced endoplasmic reticuli, free ribosomes, round ovoid, and irregular mitochondria, and smooth-walled vesicles corresponding to mucin droplets. However, no secretory granule, essential to acinar cells, was found (Fig. 7). These findings indicate that the tumor cells originated in the ductal epithelium of the pancreas.

CEA and CA19-9 Release of CEA into the spent medium was less in P-242 than in P-26, while the greatest quantity of released CEA occurred on relatively confluent culture (day 11) in both passages (Table II). Maximal release of CA19-9 into spent medium in P-242, however, was achieved on day 7 (Table II).
Establishment of a cancer cell line, particularly of pancreatic adenocarcinoma cells, is often difficult, because of their low propagative ability and the contamination by rapidly growing fibroblasts in vitro. SUIT-2 cell line, however, has been continuously propagated by serial subcultures and transferred for over five years. SUIT-2 has maintained its original biological characteristics not only in morphology but also in productivity of CEA, though the population doubling time has shortened during the passages (38.2 hr in P-3, 30.9 hr in P-26, and 29.1 hr in P-268) probably as a result of adaptation to environmental changes. CEA production at various levels by cell lines of human pancreatic cancer has been described in eight reports (Table III). Although productivity of CEA cannot be easily compared in the human pancreatic cancer cell lines reported, the CEA productivity of SUIT-2 appears to be relatively low. In a previous report on gastric cancer cell lines, CEA production had a relationship to the histopathological type of the derived tumor, i.e., the CEA production in a gastric cancer cell line...

Table II. CEA and CA19-9 Contents in Spent Media

<table>
<thead>
<tr>
<th>Cells</th>
<th>Days in culture</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>day 3</td>
<td>day 7</td>
<td>day 11</td>
</tr>
<tr>
<td>P-26(a)</td>
<td>CEA(a)</td>
<td>1.1</td>
<td>1.3</td>
<td>5.7</td>
</tr>
<tr>
<td>P-242(b)</td>
<td>CEA(a)</td>
<td>0.8</td>
<td>1.2</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>CA19-9(b)</td>
<td>131</td>
<td>256</td>
<td>187</td>
</tr>
</tbody>
</table>

\(a\) P-26, SUIT-2 cells after 26 passages.
\(b\) P-242, SUIT-2 cells after 242 passages.
\(c\) Values are ng/1 x 10\(^6\) cells/24 hr.
\(d\) Values are U/1 x 10\(^6\) cells/24 hr.

**DISCUSSION**

Establishment of a cancer cell line, particularly of pancreatic adenocarcinoma cells, is often difficult, because of their low propagative ability and the contamination by rapidly growing fibroblasts *in vitro*. SUIT-2 cell line, however, has been continuously propagated by serial subcultures and transferred for over five years. SUIT-2 has maintained its original biological characteristics not only in morphology but also in productivity of CEA, though the population doubling time has shortened during the passages (38.2 hr in P-3, 30.9 hr in P-26, and 29.1 hr in P-268) probably as a result of adaptation to environmental changes. CEA production at various levels by cell lines of human pancreatic cancer has been described in eight reports (Table III). Although productivity of CEA cannot be easily compared in the human pancreatic cancer cell lines reported, the CEA productivity of SUIT-2 appears to be relatively low. In a previous report on gastric cancer cell lines, CEA production had a relationship to the histopathological type of the derived tumor, i.e., the CEA production in a gastric cancer cell line...
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Table III. Human Pancreatic Cancer Cell Lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>(Ref.)</th>
<th>Histological type of derived tumor</th>
<th>CEA in spent medium</th>
<th>Tumorigenicity in nude mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>expon.</td>
<td>station.</td>
</tr>
<tr>
<td>Capa</td>
<td>(4)</td>
<td>tub. adenoca.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PANC-1</td>
<td>(5)</td>
<td>undif. ca.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HGC-25</td>
<td>(6)</td>
<td>undif. ca.</td>
<td>+ 0</td>
<td></td>
</tr>
<tr>
<td>MIA-PaCa-2</td>
<td>(7)</td>
<td>undif. ca.</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>poorly dif. adenoca.</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>QGP-1</td>
<td>(9)</td>
<td>islet cell ca.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AsPC-1</td>
<td>(10)</td>
<td>well-poorly dif. adenoca.</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>HPAF</td>
<td>(11)</td>
<td>mod. dif. adenoca.</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>RWP-1</td>
<td>(12)</td>
<td>mod.-well dif. adenoca.</td>
<td>363.3</td>
<td>+</td>
</tr>
<tr>
<td>RWP-2</td>
<td>(12)</td>
<td>mod.-well dif. adenoca.</td>
<td>138</td>
<td>+</td>
</tr>
<tr>
<td>T3M4</td>
<td>(13)</td>
<td>tub. adenoca.</td>
<td>152.5</td>
<td>492</td>
</tr>
<tr>
<td>SW-1990</td>
<td>(14)</td>
<td>pap. adenoca.</td>
<td>7.2</td>
<td></td>
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<tr>
<td>COLO-357</td>
<td>(15)</td>
<td>adenosq. ca.</td>
<td>35.7</td>
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<tr>
<td>HPC-Y1</td>
<td>(16)</td>
<td>well dif. tub. adenoca.</td>
<td>54.3–65.7</td>
<td></td>
</tr>
<tr>
<td>SUIT-2</td>
<td></td>
<td>mod. dif. tub. adenoca.</td>
<td>1.1</td>
<td>5.7</td>
</tr>
</tbody>
</table>

a) Ref., reference number.
b) According to the original papers. tub., tubular; adenoca., adenocarcinoma; undif., undifferentiated; ca., carcinoma; dif., differentiated; mod., moderately; pap., papillary; adenosq., adenosquamous.
c) According to the original papers (values are ng/1×10^6 cells/24 hr). expon., exponential phase; station., stationary phase.
d) +, positive; −, negative; 0, not examined.
e) Tumorigenicity was examined in the cheek pouch of hamster.
f) positive, immunohistochemically positive.

line derived from well-differentiated tubular adenocarcinoma was relatively low and dependent on the growth phase of the cell, and that from poorly differentiated adenocarcinoma and signet ring cell carcinoma was high and was only slightly or not at all dependent on the growth phase of the cells. Among pancreatic cancer cell lines, however, production of CEA increased in the stationary phase in most of the cell lines regardless of their histopathological types.

CA19-9, a recently reported tumor marker, was also produced by SUIT-2. Several cell lines producing CA19-9 have been reported, but SUIT-2 is the first human pancreatic cancer cell line confirmed to produce CA19-9. In contrast with the CEA production of SUIT-2, which was maximal in relatively confluent cultures, CA19-9 released into spent medium was highest on day 7. Accordingly, the productions of CEA and CA19-9 by SUIT-2 were assumed to be independent.

Tumorigenicity in nude mice was examined in 12 of the 14 pancreatic cancer cell lines reported, with transplantation succeeding in 11 cell lines. SUIT-2 in vitro was always transplantable to nude mouse and is the only one of the human pancreatic cell lines for which metastasis to the regional lymph nodes in nude mice has been confirmed.

In addition to the unique characteristics of SUIT-2 such as production of both CEA and CA19-9 and metastases to the regional lymph nodes, it could propagate even in a serum-free medium. This latter characteristic might be essential in investigating the biological activity of pancreatic ductal adenocarcinoma.

(Received Aug. 11, 1986/Accepted Nov. 5, 1986)

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