Establishment and characterisation of two cell lines with different grade of differentiation derived from one primary human pancreatic adenocarcinoma

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Received September 10 / Accepted November 7, 1991

Summary. From a liver metastasis of a human pancreatic adenocarcinoma, we have established cell lines for studying the cell biology of this tumor. We obtained two cell lines with different morphological, chromosomal and functional properties. One of them, named PaTu 8988s, revealed a solid growth in nude mouse xenografts with cells exhibiting only occasional polar organisation of the cytoplasm. In general, no apical or basolateral plasma membrane domains could be distinguished and the sparse organelles were randomly distributed throughout the cytoplasm. Secretory products, such as mucin, were weakly stained histochemically or were completely absent. Transglutaminase (TGase) activity used as a marker for cellular differentiation was low in these cells. The other cell line, named PaTu 8988t, grew tumors composed of tubular structures when injected subcutaneously into nude mice. Cells were polarized with distinct apical and basolateral plasma membranes and the cytoplasmatic organelles were arranged with the nucleus in the lower part of the cell, while the apical cytoplasm contained the Golgi complex and numerous secretion granules. A high content of mucin was stained histochemically and transglutaminase activity was ten times higher than in PaTu 8988s. Comparing the chromosome number per metaphase plate, both cell lines showed a major peak, with 45–55 chromosomes per metaphase plate in PaTu 8988s and about 110–120 chromosomes per metaphase plate in PaTu 8988t. When the two cell lines were injected intravenously into the tail vein of nude mice, only PaTu 8988s developed metastases localized exclusively in the lung, whereas PaTu 8988t produced no metastases in any organ. We conclude, that two cell lines exhibiting different grades of differentiation as well as a different potency to metastasize can be established from the same primary tumor, and that these cell lines represent a suitable model for further study of the cell biology of human pancreatic adenocarcinoma.

Key words: Pancreatic adenocarcinoma – Cell lines – Human – Transglutaminase – Differentiation

Introduction

Histopathologic studies in the past have demonstrated that human pancreatic adenocarcinoma, a usually fatal tumor develops from the duct system in the pancreas (Klöppel and Fitzgerald 1985). Three major grades of differentiation have been distinguished in primary tumors using criteria of glandular differentiation, nuclear size, anaplasia and mitotic activity. The grades of differentiation correlate with the growth kinetics of tumors transplanted into nude mice and also with prognosis (Klöppel et al. 1986; Kern et al. 1987).

These findings could indicate differential biological behaviour among tumors of different grades of malignancy which are however poorly, understood. To study these differences at the cellular and molecular level, model systems are needed which preserve the characteristics of the primary tumors. The induction of a pancreatic duct-cell tumor in experimental animals was successful only in the golden syrian hamster using the carcinogen N-nitrosobis(2-oxopropyl)amine (BOP) (Scarpelli and Rao 1978; Scarpe1li et al. 1984). Although some similarities between the BOP-tumors and human pancreatic adenocarcinomas have been shown comparing their reactivity with several antibodies (Matsuzaki et al. 1989; Takiyama et al. 1990), it remains difficult to assess whether this model is useful for studying human tumor biology. Alternatively, a number of human cell lines have been established in the past 15 years (for review see McIntyre et al. 1986) but only one study with different clones representing different stages of differentiation has been reported so far (Kim et al. 1989). We have therefore attempted to established cell lines with different degrees of differentiation and report in the present study on two such lines generated from the same primary tumor. These lines not only differ in their cytological features but also in their metastatic potential.
Materials and methods

Primary tumor. The cell lines were established from a liver metastasis of a nonresectable tumor from a 64-year-old female patient. The tissue was excised during a palliative operation involving a bilo-digestive anastomosis and gastroenterostomy in August 1985. The collected tissue samples were further processed for light microscopy, electron microscopy and cell culture.

Establishment of the cell lines. The tumor tissue was cut into small pieces of about 1 mm³ and were explanted into 25 cm² cell culture flasks (Becton and Dickinson, Heidelberg, FRG). Explants were covered with Dulbeccos modified Eagles medium (DMEM: Flow Laboratories, Meckenheim, FRG) containing 10% fetal calf serum (Gibco, Karlsruhe, FRG) 10% horse serum (Gibco, Karlsruhe, FRG), 60 mg/l penicillin (Seromed, Berlin, FRG) and 10 mg/l streptomycin (Seromed, Berlin, FRG) and were cultivated at 37°C in a humidified chamber equilibrated with 2% CO₂. After 2 months, fibroblasts and tumor cells had migrated out of the explants and the first passage could be performed. Trypsination of cells with a solution containing 0.25% trypsin (Seromed, Berlin, FRG) and 5 mM EGTA (Serva, Heidelberg, FRG) was performed under microscopic observation. Since fibroblasts were detached earlier than the tumor cells, the nonadherent cells were rinsed with a large portion of fibroblasts could be removed by suction, while tumor cells were still adherent. The second passage was done 2 months later and again fibroblasts were removed as described above. The time between the passages decreased with 1 week per passage until the cells could be split about every 4 days. At this point the serum concentration of the medium was reduced to 5% fetal calf serum and 5% horse serum. Penicillin and streptomycin were substituted by gentamycin (Gibco, Karlsruhe, FRG) at a concentration of 50 µg/ml.

After the fourth passage, fibroblasts were hardly detected and the first pool of cells was frozen for storage. Continuously passaged cells, now designated PaTu 8988s (s for solid), had reached the 200th passage in November 1990. Thus, controlled observation was performed using increasing concentrations of acetone and, after equilibration with 100% acetone, the samples were dried in a Balzer critical point dryer (Balzer, Fürstenstum Lichtenstein). Cells were then sputtered with gold using the sputter-coater S 150 (Edwards, Frankfurt, FRG) and examined in an ISI-SX-30 scanning electron microscope (Leitz, Wetzlar, FRG).

Transmission electron microscopy

For electron microscopic investigations tissue samples were cut into small pieces of about 1 mm³ with a razor blade and were fixed by immersion in a mixture of 2.5% formaldehyde, 2.5% glutaraldehyde and 0.05% picric acid in 0.067 M cacodylate buffer at pH 7.4 for 2 h (Ito and Karnovsky 1968). Cells in culture were washed with phosphate-buffered saline (PBS) at 37°C and were fixed for 30 min in the same fixative diluted 1:2 with the cacodylate buffer. After fixation, the cells were rinsed with cacodylate buffer and were carefully scraped off with a rubber policeman. The transfusion cultures were fixed with the same procedure but the cells were processed when attached to the membrane and embedded in an orientated position. Standard procedures for dehydration and embedding in Epon were employed. Thin section were stained with uranyl acetate and lead citrate and examined in an EM 109 electron microscope (Zeiss, Oberkochem, FRG).

Preparation of metaphase chromosomes. Cells in the logarithmic phase of growth were incubated in a medium containing 5 µg/ml Colcemid (Gibco, Karlsruhe, FRG) for 5 h. They were then trypsinized as described above and subsequently incubated for 45 min in a 1% sodium citrate solution at 37°C. A mixture of acetic acid/ethanol (1:4) was freshly prepared and 1 volume of this solution was mixed with 9 volumes of the cell suspension. After 2 min cells were spun down, half of the supernatant was discarded and the same volume of acetic acid/ethanol solution was added for resuspension of the cells. After repeating this procedure three times the whole supernatant was discarded and the cells were resuspended in 5 ml acetic acid/ethanol solution; this solution was stored at -20°C.

Cleaned microscope slides were cooled to 4°C and a drop of the cell suspension was pipetted on the slide from the height of about 30 cm. Immediately after application the cells were spread by shortly heating the slide and stained with a Giemsa-solution. Metaphase plates were examined and photographed with a Zeiss Photomicroscope (Zeiss, Göttingen, FRG). The number of chromosomes per metaphase plate was counted on the prints of the photographs. In a single experiment about 65 metaphase plates were evaluated.

Immunohistology. Immunohistological analysis of the expression of cytokeratins was performed in cultured cells using the monoclonal antibody anti-cytokeratin-pan (Boehringer, Mannheim, FRG) reacting with a common domain of all human cytokeratins. Cells were cultivated on 8-chamber Lab Tek™ slides (Nunc, Naperville, USA) until they were semi confluent. They were washed three times...