CHAPTER 28

Cell Lines of the Human and Rodent Exocrine Pancreas

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Continuous cell lines established from tumors of pancreatic origin provide a valuable resource as surrogates for normal cells for studies of the biology of specific cell types of the pancreas. Studies of the human pancreas are difficult because fresh human pancreatic specimens are rarely obtainable, and the quality of those that become available is often poor. The exocrine pancreas produces high levels of degradative enzymes that are believed to be responsible for the rapid decline in the viability of tissue obtained postmortem or from surgery. Although pancreatic tissues are easily obtained from experimental animals, informative studies of the individual cell types of the pancreas are still difficult because of the complex cellular composition that includes acinar cells, islets, duct cells, and stromal cells. The latter include smooth muscle, nerve, connective tissue, and vascular cells. Although isolation and primary culture of acinar, ductal, and islet cells have been reported (13,25,49,82) each of these cultures is still contaminated with other cell types. There is also variability in the molecular and biochemical properties from one preparation to another. The cultures are technically difficult to prepare and they are often difficult to maintain in long-term culture.

Thus established cell lines provide an important source of cells of pancreatic origin for experimental analysis. When used under appropriate conditions, they are particularly useful for the biochemical characterization of tumor markers or markers for specific normal cell types. They can be used for studies of metabolism, gene expression, mechanisms of gene regulation (transcriptional, translational, and post-translational), and tumor cell biology. Because the environment of the cells can be precisely controlled in culture, the effects of various extracellular factors (e.g., hormones, chemotherapeutic agents, and differentiating agents) on the proliferation, morphology, and metabolic activity of the cell can be examined. In addition, it is possible to study the interactions of different cell types with one another.

Several pancreatic adenocarcinoma cell lines have been established and their biological properties examined. Tables 1 and 2 provide a list of published human, mouse, hamster, and rat pancreatic adenocarcinoma cell lines. Most of these cells were established from tumors displaying a ductal morphology. This is not surprising, because most human pancreatic tumors (90%) are adenocarcinomas, with 75% of these being ductal in appearance at the light-microscopic level (12). However, considerable controversy exists regarding the actual cell of origin for these exocrine carcinomas because both ductal and acinar cells of the normal pancreas are capable of forming tubular arrangements, and thus acinar cells cannot be excluded as possible cells of origin for at least some of these adenocarcinomas (4).
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<sup>a</sup> The appropriate or presumed phenotype of the cell line is indicated as du (ductal), ac (acinar), an (anaplastic), or un (unknown).

<sup>b</sup> Available from the American Type Culture Collection.

<sup>c</sup> The histology was described as adenosquamous.

Because pancreatic duct cells of different species vary in their biochemical and molecular properties, the human pancreatic adenocarcinoma cell lines are obviously better models of human pancreatic duct cells than the animal lines are. However, one disadvantage of human cell lines is that for in vivo studies they must be transplanted into immunosuppressed animals such as nude or SCID mice. An advantage of the animal cell lines is that they can be transplanted into the species and strain of origin of the tumor, which allows the study of host-tumor interactions. In this chapter we summarize some of the better characterized ductal and acinar cell lines of
human and rodent origin and explain how they have been and can be used to study specific aspects of the pancreas.

**DUCTAL CELL LINES**

The currently available cell ductal lines are of human or rodent origin, with most being from humans. With the exception of RP-J and RP-F344-1 (Table 2), they were all derived from pancreatic adenocarcinomas with ductal morphology as determined at the level of light microscopy. This is perhaps the only feature used to place these cell lines into one group, as this expanding body of cell lines has not been systematically characterized. A summary of antigentic and biochemical markers that distinguish pancreatic duct, acinar, and islet cells has been presented by Githens (*this volume*). Studies of some of the human “ductal” cell lines have demonstrated significant variability in the expression of the different biochemical markers. For example, carbonic anhydrase II, a gene that is expressed in pancreatic duct cells, is easily detected in some cell lines, such as MDA-Panc-3, Capan-1, CAPAN-2, and BxPC-3, but it was not detected in PANC-1 (22). The RNA for the human pancreatic MUC-1 apomucin is expressed in HPAF, Colo357, SW979, Capan-1, and Capan-2, but not in PANC-1 or Hs 766T (42).

The best characterized human cell lines are Capan-1, Capan-2, BxPC-3, PANC-1, HPAF, and Colo357. Capan-1 and Capan-2 are probably the most differentiated in phenotype, as both express mucins and carbonic anhydrase II. Of particular interest is the dome formation observed in Capan-1, which is believed to be a morphological expression of transepithelial fluid transport (44). PANC-1 tends to display fewer ductal features than the other human ductal cell lines.

Four cell lines have been established from ductal adenocarcinomas arising in Syrian golden hamsters (8, 20, 67, 86), and other lines have been established from mouse tumors (11, 43) (Table 2).

**ACINAR CELL LINES**

Cell lines derived from acinar cell carcinomas are much less common than those established from ductal tumors. The best characterized pancreatic acinar cell line is AR42J (30). It was established from a transplantable tumor line derived from a primary carcinoma of the exocrine pancreas of a rat treated with azaserine (53). AR42J cells elaborate exocrine enzymes characteristic of pancreatic acinar cells (30), and the mRNAs for amylase, chymotrypsin, and trypsin were calculated to be roughly 10.5%, 1.5%, and 0.078%, respectively, of the total poly(A)+RNA content of these cells. These levels are 42%, 18%, and 0.9%, respectively, of the amounts present in adult rat pancreas and 68%, 33%, and 1.5% of the amounts present in the "embryonic differentiated state" of the rat pancreas, respectively (27). AR42J may be useful for identifying additional cis-acting regulatory elements of these and other acinar cell-specific genes as well as in the analysis of trans-acting factors that interact with the cis-acting regulatory elements to modulate the expression of these genes (5).

When AR42J was established, a second cell line, AR41P, was also established by passaging supernatant
culture fluids obtained 24 hr after the initial seeding of the minced tumor fragments that gave rise to AR42J. AR42J produces higher levels of exocrine enzymes than AR41P, and AR42J forms tumors in athymic nude mice while AR41P does not (30).

Of the human pancreatic tumor cell lines, only one, HPCYO, has been established from an acinar cell carcinoma (94). This is not surprising, because only about 2% of the human pancreatic adenocarcinomas are classified as acinar cell type on the basis of histological examination at the level of light microscopy. HPCYO produces trypsin but not amylase. A second line, Colo357, a human pancreatic adenocarcinoma cell line established from a metastatic lesion in a celiac axis lymph node, was found to contain detectable levels of trypsin, elastase, and chymotrypsin, but histologically the lymph node was observed to contain neoplastic foci of well-differentiated mucin-containing ducts. The reason for this is not clear.

REGULATION OF GROWTH AND SECRETION

Both ductal and acinar cell lines are useful in studying pancreatic growth regulation, which is the result of a balance between positive and negative signals. It is influenced by an assortment of hormones, growth factors, and their respective receptors (for recent reviews related to this subject, see refs. 51 and 85). Pancreatic cell lines arise from tumors, in which a disruption in normal growth regulation has occurred. These cell lines have proved to be useful models for studying the abnormal interactions of hormones and growth factors and their receptors at the cellular and molecular level in cancer and have enhanced our understanding of how these interactions are involved in the pathogenesis of this disease. In addition, these studies have also contributed to our understanding of these interactions in the normal pancreas.

The rat pancreatic acinar cell line AR42J has been the focus of numerous receptor studies. Receptors for cholecystokinin (CCK) (41,48,69), gastrin (68), bombesin (50), somatostatin (89), insulin (58), substance P (91), epidermal growth factor (EGF) (80), vasoactive intestinal peptide (VIP) (81), and the pituitary adenylate cyclase-activating peptides (7,65) have partially been characterized in these cells. Basic fibroblast growth factor has been found to stimulate growth as well as an increase in ornithine-decarboxylase activity in AR42J (14).

The growth effects of various gastrointestinal regulatory peptides on human pancreatic adenocarcinoma cell lines have been most extensively examined in MiaPaCa-2 and PANC-1. Liehr et al. (47) observed that EGF and insulin stimulated growth of these lines, but bombesin, secretin, VIP, somatostatin, CCK₈, and CCK₉ did not. To assess growth effects, the cells were preincubated in 10% fetal bovine serum-supplemented medium without the peptide, and then incubated in serum-free medium containing the peptide. Beauchamp et al. (3) observed that EGF and insulin caused a concentration-dependent increase in anchorage-independent growth in MiaPaCa-2 but not in PANC-1. The growth medium they used was not supplemented with serum. Liebow et al. (46) studied the effects of EGF and somatostatin on MiaPaCa-2 and observed that EGF did not stimulate growth in serum-containing medium but significantly increased growth in serum-free medium. This stimulatory effect could be entirely eliminated by the addition of somatostatin.

Transforming growth factor (TGF)-α gene expression has been the focus of recent studies of pancreatic adenocarcinoma because it has been observed in a number of cancer cell lines and has a growth-stimulatory effect (15). The TGF-α protein has structural homology to EGF, and it is thought that its interaction with the cell is mediated through the EGF receptor (55). Korc et al. (37) demonstrated that the EGF receptor gene displayed an enhanced expression in four human pancreatic adenocarcinoma cell lines. They determined that this enhanced expression was not due to gene amplification. However, three of these cell lines, T3M4, PANC-1, and Colo357, were found to have structural alterations on chromosome 7, where the gene for the EGF receptor resides. The fourth cell line, UACC-462, was found to contain multiple copies of chromosome 7. Later studies demonstrated that TGF-α RNA is expressed by several pancreatic adenocarcinoma cell lines (3,75). Three of these lines, T3M4, PANC-1, and Colo357, as discussed above, overexpress EGF receptor. This provides evidence for an autocrine loop that would give tumor cells a growth advantage in the process of tumor development and progression.

Other growth factors that have been found to be expressed in the human pancreatic adenocarcinoma cell lines MiaPaCa-2 and PANC-1, and therefore may contribute to the pathogenesis of the disease, include bFGF, platelet-derived growth factor B (c-sis), TGF-β₁, and TGF-β₃. TGF-β₂ was detected in PANC-1 but not in MiaPaCa-2, and EGF was not detected in either cell line (3).

Treating of AR42J cells with CCK results in an increase in their amylase secretion. Glucocorticoids enhance the secretion by increasing the number of CCK receptors (48). At least two types of CCK receptors (A and B) have been identified in AR42J (41,72). They have the same affinity for CCK₈ but different affinities for gastrin. Gastrin stimulates growth through the B receptor.

Studies of the effects of CCK on the growth of human pancreatic carcinoma cell lines in culture have had conflicting results. As mentioned above, Liehr et al. (47) did not observe growth effects of CCK₈ or CCK₉ on MiaPaCa-2 or PANC-1 in culture. In contrast, Smith et al. (76,77) observed that (Thr⁴Nle³)CCK₉, CCK₈, and
CCK₉₉ stimulated growth of SW1990, PANC-1, Mia-PaCa-2, BxPC-3, RWP-2, and CAPAN-2. They observed that the CCK-induced cell proliferation of SW1990 was inhibited by proglumide, a CCK-receptor antagonist. Frazier et al. (23) have observed stimulation of MDAPanc-3 growth by CCK₈.

Edwards et al. (19) determined that desglugastrin, secretin, and CCK₈ can stimulate the growth of the human pancreatic ductal tumor cell line WD PaCa in tissue culture as assessed by [³H]thymidine uptake. Townsend et al. (87) observed that when H2T cells were injected into hamster cheek pouches, neither cerulein (a CCK analogue) nor secretin stimulated growth of these cells when injected intraperitoneally into the hamster. When both were administered together, a significant increase in the weight and DNA content of the pancreatic adenocarcinoma was observed.

DIFFERENTIATION AND METAPLASIA

Two pancreatic cell lines were established in separate experiments by placing a well-differentiated transplantable acinar cell carcinoma (DSL-6) derived from a Lewis rat into culture (52,54). Although the cultured tumor cells initially produced amylase, production of exocrine enzymes ceased after 1 to 2 weeks. The cultured cells were tumorigenic in Lewis rats. When grafted into syngeneic rats, one cell line (DSL-6A/C) produced solid tumors with a high percentage of fibrous tissue surrounding duct-like structures (Fig. 1) (see Longnecker, this volume), and the second cell line (DSL-6B/C) yielded tumors that grew with a mixed phenotype, with squamous, mucinous, and poorly differentiated glandular areas (adenosquamous). In each case, the altered phenotype persisted when the tumors were returned to culture and then reimplanted into rats.

The original tumor had a high number of CCK receptors as measured by a radioligand-binding assay, but DSL-6A/C lacks the receptors. DSL-6B/C has not been evaluated for the presence of receptors. Electron microscopy showed duct-like cells without zymogen granules and with little rough endoplasmic reticulum. Immunohistochemical studies of the cell lines and the regrafted tumors demonstrated expression of several ductal markers including cytokeratin 19. These studies provided strong support for the hypothesis that duct-like carcinomas can arise from neoplastic pancreatic acinar cells in rats. These tumor cells have lost acinar cell differentiation and acquired ductal markers.

A cell line useful for the study of differentiation is the human cell line HPAF. It was established from the ascites fluid of a patient with pancreatic adenocarcinoma by Metzgar et al. (56). Cultures of these cells were heterogeneous, ranging from small mononuclear cells to large multinucleate cells. The reactivity of the individual cells to two monoclonal antibodies, DU-PAN-1 and DU-PAN-2, was also heterogeneous. These antibodies were generated using HPAF cells as the antigenic stimulus.

Five morphologically distinct clones of HPAF cells have been isolated (34). Of these, two clones representing distinctly different stages of differentiation, CD11 and CD18, were further characterized. Both clones display the same isozyme patterns as HPAF. CD11 was more differentiated than CD18 on the basis of the following characteristics. (a) The doubling time for CD11 (42 hr) was greater than for CD18. (b) In the absence of collagen, CD18 formed colonies in soft agar, while CD11 did not. When collagen was used, both clones formed colonies in soft agar, but the percentage of efficiency was greater for CD18. (c) When grown on a monolayer, ultrastructural studies revealed that rough endoplasmic reticulum (RER) was abundant in CD11 and was generally associated with well-developed Golgi complexes. In contrast, RER was less frequent in CD18, with free ribosomes scattered in the cytoplasm of CD18.

Both CD11 and CD18 formed tumors after injections of the cloned cells into nude mice. The CD11 tumor contained an abundance of well-differentiated ductal structures. The tubular structures consisted of well-polarized columnar cells surrounded by connective tissue. In contrast, CD18 had the morphology of poorly differentiated tumors, with solid nests of isoprismatic cells that lacked cellular polarization and that consequently did not form luminal spaces. In CD11 tumors, DU-PAN-2 was expressed on the apical membranes of cells and in the lumina of ductal structures. However, this antigen was not detected on most sections of tumors derived from CD18 cells. Circulating DU-PAN-2 was detected in the sera of nude mice bearing CD11 tumors, and the amount of antigen correlated well with the size of tumor. In contrast, DU-PAN-2 antigen was not detected in the sera of mice bearing CD18 tumors of comparable size.

The morphological differences in xenografts from the two clones were most evident at the ultrastructural level. CD11 xenografts were composed of well-polarized cells, while those of CD18 lacked distinct polarity. The apical cytoplasm of CD11 was full of secretory granules, while they were rare in CD18, and the RER and Golgi complexes were well developed in CD11 but not in CD18.

MUCIN GENE EXPRESSION

PC-1, a hamster pancreatic ductal carcinoma cell line, was established from a pancreatic ductal carcinoma induced in a Syrian golden hamster by N-nitrosobis(2-oxypropyl)amine (BOP) (20). When PC-1 is injected into homologous hamster pancreas, tumor formation occurs, allowing one to study its behavior both in vivo and in vitro. The blood group related antigens A, B, H, Le⁺, Le⁻, and Le⁻, which are commonly expressed in human pancreatic cancers, were studied in this cell line and found to be expressed both in cultured cells and in trans-
FIG. 1. Phase contrast photomicrographs of MDA-Panc-3 (a), BxPC3 (b), Capan-1 (c), and CFAC (d) growing in monolayer. ×200.
plantaible tumors. These findings suggest that PC-1 may serve as a useful model for studies that focus on mucins in pancreatic cancer, their potential usefulness in immunodiagnosis and immunotherapy, and their role in tumor progression.

CYSTIC FIBROSIS

CFPAC-1 is a pancreatic adenocarcinoma cell line that serves as a model for studying the electrolyte impairment in cystic fibrosis. Cystic fibrosis is an autosomal recessive disorder. Approximately 70% of the mutations in cystic fibrosis patients are a specific deletion of three base pairs that results in the loss of a phenylalanine residue at amino acid position 508 of the cystic fibrosis gene product (33,66). One of the major clinical symptoms associated with the disorder is pancreatic exocrine insufficiency from impairment of electrolyte transport by epithelial cells. (see Lebenthal, this volume). CFPAC-1 was established from a well-differentiated pancreatic adenocarcinoma that developed in a cystic fibrosis patient who was homozygous for the common three-base-pair deletion (70). The cell line will be a useful tool for further biochemical and physiological investigation of the cystic fibrosis defect in the pancreas.

ONCOGENES AND TUMOR SUPPRESSOR GENES

The first example of oncogene activity in human pancreatic adenocarcinoma was identified by transformation of NIH3T3 cells with DNA isolated from the human pancreatic adenocarcinoma cell line A1165 and was found to be due to the human homologue of K-ras (10). In a similar study using Panc-1, K-ras was once again identified as the transforming gene (59). Since then it has been determined that mutations in codon 12 of K-ras occur frequently in adenocarcinomas of the exocrine pancreas (1,74). Using mutation-specific synthetic oligonucleotides, Smit et al. (74) determined that the mutations are predominantly G-T transversions, in contrast to the predominant K-ras mutations in colorectal carcinomas, which are predominantly G-A transitions. The T3M4 pancreatic adenocarcinoma cell line is an exception, having a mutation at codon 61 of K-ras (28).

Barton et al. (2) demonstrated that abnormalities in the p53 tumor suppressor gene are common in formalin-fixed and fresh human pancreatic adenocarcinoma specimens as well as pancreatic adenocarcinoma cell lines. They screened a series of pancreatic adenocarcinoma cell lines for mutations in the p53 gene by immunohistochemistry, immunoprecipitation, and direct sequencing of polymerase chain reaction-amplified DNA from regions that have been considered to be "hot spots" for p53 mutations. They determined that seven of 13 cell lines displayed immunohistochemically detectable levels of p53. By immunoprecipitation, p53 was only detected in five of the seven that were immunohistochemically positive and none of those that were negative. Mutant p53 genes were detected in all five of the cell lines that were positive for p53, while the other lines did not display mutations in the regions examined. In each case, the mutations were point mutations in p53 exons 5, 6, 7, or 8.

In colorectal carcinoma it is apparent that carcinogenesis results from a series of genetic alterations (21,78, 90) manifested phenotypically by the adenoma-to-carcinoma sequence (64). While there is no clear-cut evidence for an adenoma-to-carcinoma sequence in human pancreatic adenocarcinoma, it is presumed that these tumors arise as a result of multiple genetic events. That mutations in the p53 tumor suppressor gene and mutations at codon 12 of the K-ras gene are both common occurrences in human pancreatic adenocarcinomas support this view. Further support comes from the studies of Yamada et al. (92), who established the human pancreatic adenocarcinoma cell line PSN-1. Like the primary tumor from which it arose and the two metastatic lymph nodes, PSN-1 displayed a 50-fold amplification of the c-myc gene and a threefold to sixfold amplification of an activated K-ras with a point mutation causing a GGT-to-CGT change at codon 12. In addition, the cell lines displayed an increase in the levels of c-myc and K-ras transcripts.

In the studies of Yamada et al. (92) and Fujii et al. (24), the identification of point mutations in oncogenes in both a cell line and the tumor from which it is derived is important because it verifies that the mutations did not arise from the culturing process. Fujii et al. (24) examined 10 primary hamster pancreatic ductal adenocarcinomas induced by BOP and in each case detected GGT-to-GAT point mutations at codon 12 of the K-ras gene. The same point mutation was detected in two monoclonal cell lines, PC-1 and PC-1-0, established from BOP-induced hamster pancreatic tumors that were subcutaneously implanted into hamsters. C1-3 and C1-7, two clonal cell lines, derived from PC-1, were also found to carry the GGT-to-GAT point mutation. The mechanism for the sequence specificity of these mutations is unknown.

GENERAL CARE OF PANCREATIC CELL LINES

While the morphological and biological differences between different pancreatic cell lines determine how they will be used, they all require the same monitoring. Records should be kept of the passage number of cell lines, and cells should be frozen back at the different passage levels. Cells in culture should be monitored routinely to determine if changes have occurred. These changes can
occur as a result of alterations in culture conditions, out-
growth of a subpopulation of cells, genome plasticity, contamination with infectious agents, or contamination with another cell line. In addition, the same cell cultures can display differences in their properties at different levels of confluency.

To monitor cultures, photographs should be taken under the different culture conditions being used to document the morphological appearance of the cells. Different levels of confluency should be photographed since morphological changes occur as cultures begin to reach confluency. Figure 1 depicts the variation in morphology that can be observed among different cell lines grown in monolayer. Karyotype analysis, isozyme analysis, or both should be performed on cell cultures as they reach higher passage levels and at any point in time when the identity of the cell line is in question (62). The cultures should be routinely tested for contamination by mycoplasma, other bacteria, yeast, mold, and viruses.

Analyzing a cell line’s karyotype and isozyme patterns at the earliest possible time point after establishing the line sets a base line against which one can monitor subsequent changes. A record of these characteristics at increasing passages verifies the authenticity of the cell line. Both karyotypes and isozyme patterns can be used to distinguish human cell lines from those of other species. However, to determine if a cell line has become contaminated with another line of the same species, it may be necessary to perform isozyme analysis (6,29,62,63).

The karyotypes of many of the established pancreatic adenocarcinoma cell lines including Colo357 (57), Panc-1 (45), BxPC-3 (83), and Capan-2 (38) have been reported. These lines are all aneuploid. MDAPanc-3 is unusual, because it has a stemline chromosome number of 43 (23). Marker chromosomes, distinct from those found in the normal human karyotype, are always found. Multiple karyotypes are always seen within a cultured cell population, but usually there are one or more predominant populations. With increasing passages, the karyotype may begin to change as one cell population begins to outgrow another or as the culture undergoes genetic changes in vitro.

Because tumors commonly have abnormal karyotypes, it is difficult if not impossible to determine whether the altered karyotype of a cell line is the same as that of the tumor from which it arose or whether it is a result of changes in the genome that occurred after placing the tumor tissue in culture. This is due to the technical difficulties of performing karyotype analysis of solid tumors. Chromosomal alterations can also be induced by radiation treatment and chemotherapy. Examples were described above where specific changes in the cell lines (point mutations in K-ras and amplification of c-myc) were also found in the primary tumor (24,92). This demonstrated that the alterations in these oncogenes were not artifacts of the culturing process.

Care should also be taken to identify the origin of tumors that arise in animals as a result of introduction or implantation of cultured cells. Upon placing these tumors back into culture, the resulting cells may be found to be of the same origin as the host or a mixture of host and implanted cells. If the host cells are normal cells, they will almost certainly die out; but in rare cases, the host cells have been found to be transformed and could be propagated in long-term culture (6,62).

An example of outgrowth of a subpopulation of cells in a culture is the HPAF cell line described by Metzgar et al. (56). The culture was heterogeneous in early passage, but after passage 20, a large multinuclear cell type became predominant. As discussed above, cloning of early passage cells gave rise to several isolates with distinct properties.

While cloning will help resolve this problem, it is possible that some cell types can only be grown when cocultured with another cell type, making cloning impossible. Also, there may be situations in which one cell type continues to give rise to another cell type, and this may vary, depending on culture conditions. In summary, careful observation and documentation are required for reliable use of cultured cells.

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