Derivation of Ductlike Cell Lines from a Transplantable Acinar Cell Carcinoma of the Rat Pancreas

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Two cell lines were derived from a transplantable acinar cell carcinoma that had been established from a primary carcinoma of the pancreas in an azaserine-treated Lewis rat. The cultured tumor cells initially produced amylase, but production of exocrine enzymes ceased after 1–2 weeks in culture. The cultured cells were tumorigenic in Lewis rats, and one line produced solid tumors composed of ductlike structures surrounded by dense fibrous tissue. The second cell line produced partially solid and partially cystic tumors with a mixed phenotype of squamous, mucinous, and glandular areas when it grew in vivo following regrafting. Both cell lines lost structural and immunohistochemical acinar cell markers while acquiring duct cell markers during culture and regrafting. These studies provide strong support for the hypothesis that ductlike carcinomas can arise from neoplastic pancreatic acinar cells in rats. (Am J Pathol 1993, 143:292–303)

Approximately 75% of carcinomas of the human exocrine pancreas are classified as ductal adenocarcinomas, while several other variant histological types are also considered to be of ductal origin.1 As animal models of pancreatic carcinoma have been developed, distinct species differences in the response to chemical carcinogens have become apparent. Both spontaneous and chemically induced carcinomas in rats are predominantly of acinar cell phenotype, whereas in hamsters, most carcinomas are of ductal phenotype. Accordingly, most carcinomas induced in rats by azaserine are of the acinar cell type.2 As this and other rat models were described, the question of relevance to the human was raised because of the acinar cell phenotype.

Since 80% of the mature pancreas is composed of acinar cells, it is reasonable to assume that these cells are likely to be a target for carcinogens. This is clearly the case in rat models of pancreatic carcinogenesis because early focal proliferative lesions and nodules maintain acinar cell differentiation. Acinar cell adenomas are relatively common, while proliferative lesions within ducts are virtually unknown in the rat. Some acinar cell carcinomas have a minor component with ductal phenotype.3 Bockman has described sequential changes in acinar tissue leading to the formation of tubular complexes.4 These observations suggest that tumors with a ductal phenotype may be derived from neoplastic acinar cells that have undergone metaplasia to a ductal phenotype.

The experiments described here are based on an azaserine-induced acinar cell carcinoma of the rat pancreas that in early passages was the best differentiated of six such carcinomas that have been successfully transplanted. This carcinoma was placed in culture with the goal of establishing a well-differentiated cell line that might be found useful in studies of acinar cell biology. Although the tumor cells grew in culture and initially released amylase and lipase, they failed to maintain production of exocrine enzymes. The cultured cells were tumorigenic in rats and have yielded tumors with ductal characteristics. These studies provide strong support for the hypothesis that ductlike carcinomas can sometimes arise from transformed acinar cells.

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Materials and Methods

The same basic experiment has been performed twice with minor variations. These experiments will be referred to as culture cycles 1 and 2. The alternating sequence of culture, regrafting, culture, etc is summarized in Figure 1.

Transplantable Tumor

Lewis rats were obtained from Charles River Laboratories (Wilmington, MA). A transplantable tumor designated as DSL-6 was derived in 1986 from a primary acinar cell carcinoma of the pancreas. The carcinoma developed in a male Lewis rat (DSL-101-79) that was given three intraperitoneal 30 mg/kg injections of azaserine when the rat was 2, 3, and 4 weeks of age. This rat was autopsied at 2 years of age, and a 7-mm tumor was sampled for histological evaluation and subcutaneously transplanted into Lewis rats. Histologically, the tumor was a moderately well-differentiated acinar cell carcinoma. This tumor was transplanted into three rats at first and subsequent passages. The transplanted tumors have maintained a consistent phenotype similar to that of the primary tumor through 12 passages. These transplants served as the source of cells for experiments in culture cycles 1 and 2. Aliquots of tumor have been cryopreserved at several passages and are maintained in liquid nitrogen.

Cycle I

<table>
<thead>
<tr>
<th>Tumor ID</th>
<th>Growth</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSL6A</td>
<td></td>
<td>Acinar cells</td>
</tr>
<tr>
<td>DSL6A/C1</td>
<td></td>
<td>Transient amylase secretion</td>
</tr>
<tr>
<td>DSL6A/T1</td>
<td></td>
<td>Ductal, scirrhous</td>
</tr>
<tr>
<td>DSL6A/C2</td>
<td></td>
<td>No amylase</td>
</tr>
<tr>
<td>DSL6A/T2</td>
<td></td>
<td>Ductal, scirrhous</td>
</tr>
</tbody>
</table>

Cycle II

| DSL6B     | Acinar, moderately differentiated |
| DSL6B/C1  | Transient amylase secretion      |
| DSL6B/T1  | Adenosquamous                    |
| DSL6B/C2  | No amylase                       |
| DSL6B/T2  | Adenosquamous                    |

Figure 1. Flow chart history of Cycle I, DSL-6A and Cycle II, DSL-6B cell lines in vitro and in vivo.

Cell Lines

In culture cycle 1 a cell line (DSL-6A/C1) was obtained from the DSL-6 tumor tissue taken at the eighth transplantation. Tissue was minced and plated in Waymouth's MB 752/1 medium with 10% heat-inactivated fetal bovine serum (FBS) and 0.1 mg soybean trypsin inhibitor (SBTI)/ml, penicillin, and streptomycin and incubated in 5% CO2 and air at 37 °C in a Corning tissue culture flask. SBTI was omitted from the growth medium after the initial plating. Cultures were passaged with 0.2 mmol/L EDTA containing 0.25% trypsin. In culture cycle 2 DSL-6B/C1 was developed using the same protocol.

AR42J cells from the American Type Culture Collection (Rockville, MD) were grown in Ham's F12K medium with 15% FBS.

Variation of Culture Conditions

A variety of culture conditions have been tested on established cell cultures to evaluate their effect on phenotype, both morphologically and as reflected by amylase or lipase secretion.

Medium components were tested in Waymouth's MB 752/1 medium with 10% heat-inactivated FBS (HyClone Laboratories, Logan, UT). Logsdon's medium,5 designed specifically for mouse pancreatic acinar cells, contained 100 U/ml penicillin, 100 mg/ml streptomycin, 0.5 mmol/L IBMX (isobutylmethylxanthine), 0.2 mg/ml SBTI, 1 mmol/L insulin, 1 mmol/L epidermal growth factor, 1 mmol/L carbachol, and 10 mmol/L corticosterone. All reagents and growth media were obtained from Sigma Chemical Co. (St. Louis, MO). In addition, a simpler
supplemented Waymouth's medium with 10% FBS, 10 nmol/L caerulein (a gift from Dr. R. deCastiglione, Farmitalia Carlo Erba, Milan, Italy), 10 mg/ml dexamethasone, and 0.1 mg/ml SBTI was tested. SBTI was tested alone in Waymouth's/10% FBS medium.

Several substrates were also tested. Corning cell culture flasks (Corning Glass Works, Corning, NY) were used as a control, to compare with Falcon Primaria flasks (Becton-Dickinson, Lincoln Park, NJ) and plastic flasks coated with Matrigel (Collaborative Research, Waltham, MA) or Collagen I (rat tail collagen prepared in the laboratory as described). In addition, some primary cultures were plated on Permanox slides (Nunc, Naperville, IL).

**Tumorigenicity of Cultured Cells**

Approximately $2 \times 10^7$ cultured tumor cells suspended in 0.4 ml growth medium were inoculated subcutaneously into three syngeneic weaning rats.

**Light Microscopy**

The primary tumor and transplanted tumors were fixed in Bouin's fixative or 10% neutral buffered formalin, and embedded in paraffin, and sections were stained with hematoxylin and eosin (H&E). DSL-6A/C1 and C2 cells were grown on glass coverslips or Permanox slides, rinsed, and fixed in 10% buffered formalin, followed by staining with H&E. To prepare sections of cells for comparison with tumor sections, monolayers of cells were scraped, centrifuged, fixed in buffered formalin, and embedded in paraffin for sectioning.

**Antibodies**

In the present investigation monoclonal antibodies (MAbs) and commercially available anticytokeratins (anti-CKs) which distinguished pancreatic acinar (OC.1) and ductal cells (OV-6, CK-7, CK-19) were used to characterize tumors and cell lines derived in culture. In addition to these cell-specific markers, OC.2, an acinar/ductal marker, and CK-14, a marker of squamous epithelium, were employed for some experiments. The production and characterization of MAbs have been described previously. MAb OV-6 was kindly provided by Dr. Harold Dunsford (University of Texas Medical Branch, Galveston, TX). All anti-CKs were obtained from Boehringer Mannheim (Indianapolis, IN). Some of the MAbs are not suitable for the study of fixed, paraffin-embedded tissues and could not be applied retrospectively at all stages of the study.

**Immunohistochemistry**

Indirect immunofluorescence (IIF) or immunoperoxidase analysis was carried out on cell pellets and cultured cells as previously described. Cultured cells were scraped into a pellet and either frozen in hexane chilled by a dry ice acetone bath or fixed in Omnifix (Xenetics Biomedical, Tustin, CA) or cold acetone and embedded in paraffin. For some studies, minced primary tumor tissue was plated on Permanox culture slides (Nunc), and at various intervals after plating, monolayer cultures were rinsed and fixed in cold acetone. IIF analysis was also carried out on frozen sections of azaserine-induced transplantable pancreatic tumors. Briefly, subcutaneous tumors were excised, and small blocks of tumor were frozen as described above and stored at -70°C until analyzed. Acetone-fixed serial sections were stained with MAbs followed by affinity-purified, fluorescein-conjugated, goat anti-mouse immunoglobulin (Sigma) as the secondary antibody. Non-specific staining was assessed by examining tissue sections stained with culture supernatants from P3x63Ag8 myeloma cells. Sections were examined using a Nikon Microphot FX equipped with epifluorescence and a 35-mm camera.

For some experiments, double-labeling IIF analysis of cultured cells and frozen tissue sections was carried out using MAbs in combination with isotype specific secondary antibodies conjugated to either fluoresceothiocyanate or Texas red. Photomicrographs were recorded with Ektachrome 200 film and images were printed on black and white Kodak Panalure paper. With this technique, reactive areas appear as dark staining cells.

**Electron Microscopy**

Small cubes (1-2 mm$^3$) of tumor tissue or monolayer cultures were fixed in 4% buffered glutaraldehyde. Cultures were scraped after fixation and pelleted. Both types of specimen were postfixed in osmium tetroxide and embedded for sectioning. Thin sections were examined using a Jeol 2000FX electron microscope.

**Amylase and Lipase Activity**

Amylase or lipase activity of culture medium was measured with an Olympus Demand analyzer and
Technicon Amylase reagent (Technicon Instruments Co., Tarrytown, NY) or on a Kodak Ektachem Analyzer (Kodak, Rochester, NY).

Doubling Time in Vitro

DSL-6A/C1 cells were plated in triplicate cultures at $5 \times 10^5$ cells/ml. At feeding intervals (3 or 4 days) during the next 10 days, triplicate cultures were rinsed, dissolved in 3 N KOH, and precipitated with 10% trichloroacetic acid and an aliquot used for measurement of total protein by the Bio-Rad reagent (Bio-Rad Laboratories, Richmond, CA). Doubling time was calculated graphically.

CCK Receptor Assay

Assays were performed using the method of von Schrenk\textsuperscript{13,14} and frozen samples of DSL-6A/C1 at the fifth passage level, transplanted DSL-6, and AR42J, a cell line which was isolated from DSL-1, another azaserine-induced transplantable tumor.\textsuperscript{15} Cultured cells were harvested by scraping and pelleted by centrifugation.

Regrafting of AR42J Cells

AR42J cells were harvested by scraping, and approximately $10^7$ cells were inoculated subcutaneously in male Lewis rats. Tumors developed after 2-3 weeks \textit{in vivo} and were harvested at autopsy for histological study.

Results

The histological phenotype of the first DSL-6 transplant was similar to that of the primary tumor, and tumors from serial transplantation, sometimes of cryopreserved tumor, continue to maintain an acinar cell phenotype to the present—growing as moderately to poorly differentiated acinar cell carcinomas. The primary acinar cell carcinoma did not contain

Figure 2. DSL-6, the first transplant established from a carcinoma induced by azaserine in a male Lewis rat. Acinar structures are present throughout. A few acinar lumens in the upper left corner are dilated and contain homogeneous secretion (arrows). H&E. ×158.

Figure 3. Serial tumor transplant of the DSL-6 tumor as used for initiation of cycles 1 and 2. The tumor maintains acinar phenotype. Although lobules of tumor are separated by fibrous stroma, the tumor is not truly desmoplastic. H&E. ×158.

Figure 4. DSL-6A cell line in culture at the "C" stage, i.e., after regrafting and reculture. The polygonal cells have prominent nuclei. Two mitotic nuclei are present. H&E. ×158.
ductal elements that could be identified in H&E-stained sections. The transplantable tumor line derived from this tumor maintained acinar differentiation but exhibited a microcystic growth pattern in some areas (Figure 2). The microcystic phenotype diminished with time, but acinar formation continues (Figure 3). Focal necrosis is noted centrally in large tumors. The transplanted DSL-6 tumors are not desmoplastic.

Cell Lines

In the isolation (cycle 1) of cell line DSL-6A, the early days of the culture were characterized by the production of large amounts of acid in the medium, necessitating medium changes at 1–2-day intervals. Epithelioid plaques appeared in the culture, which were not overgrown by endogenous fibroblasts. At the first subculture, 2 months later, no amylase was present in the medium. Within a month, after the next passage level, a marked change in morphology occurred that was coincident with a loss of normal stromal elements. Tumor cells that had been growing as colonies on the fibroblastoid monolayer now attached to the plastic. A continuous cell line emerged which has retained the same morphology. By 3 months in vitro, all normal fibroblastoid cells had disappeared. The doubling time was measured as 4 days on passage 7 cells. This cell line has now been carried through 18 subcultures, over a period of several years. Various passage levels have been cryopreserved.

A second cell line, DSL-6B/C1, was established using the same protocol (culture cycle 2). A similar cell line emerged as described for DSL-6A/C1. Cells were grown in Waymouth’s MB 752/1 medium with 10% heat-inactivated FBS (Way/10) with and without SBTI and, in addition, in Logsdon’s medium, which was developed specifically for murine pancreatic acinar cells. No differences were observed either in amylase secretion or in cell morphology when compared to Way/10, the medium selected for routine use.

Morphology of Cultured Cells

The early cultures in both cycles were characterized by islands of cells appearing to contain two epithelioid cell types: a denser population of smaller cells bordered the edge of the islands, which were composed of large flat cells (Figure 4). The smaller cells did not increase in number but remained at the periphery of the island until they became confluent. We have assumed that they did not represent a separate phenotype but resulted in some way from growth on a plastic substrate. In addition, apparently dead, flat plaques of cells lifted off the monolayer, suggesting the loss of differentiated cells. This morphology was exhibited as long as there were any normal fibroblasts remaining in the culture but is not present in the continuous cell line.

Enzyme Secretion of Primary Cultures

All cultures initially contained high levels of amylase activity in spent medium (>1000 units/L) collected after 4-day culture. Amylase levels quickly declined and were absent when tested several months later. None of the alternative media conditions affected the level of amylase activity, nor was any morphological change in phenotype observed by phase microscopy in those cultures compared to unsupplemented Way/10 (data not shown). Collagen I substrate provided no advantage in either growth or enzyme secretion over untreated plastic culture flasks. Cells appeared to attach 1 day sooner on Matrigel than on the other substrates or uncoated plastic, and amylase secretion was retained for a few days longer in these cultures (Figure 5). None

![Graph](image-url)
of these measurements were related to cell number or protein because the original cultures were established from minced tissue. Therefore, quantities of enzyme activity are not as significant as the length of time of their persistence in the cultures. All conditions supported cell attachment by the fourth day in vitro. Neither medium variations nor use of special substrates altered the morphological phenotype of the cells in vitro as detected by phase microscopy, nor did they support the maintenance of the acinar phenotype.

In one of the experiments described in Figure 5, a portion of the original tumor was homogenized in phosphate-buffered serum at 1:10 (w/v) dilution and assayed for amylase and lipase with the following result: amylase: 230,218 U/L; 387 U/mg tumor tissue; lipase: >4.05 U/mg tumor tissue. For comparison, cells from DSL-6A/C3 were assayed similarly. A 0.1-ml volume of packed cells, diluted to 1.0 ml in Tris buffer, produced no detectable amylase and <0.01 U lipase/ml.

**Growth of Cultured Cells in Vivo**

DSL-6A cells at the sixth passage, 8 months after the tumor was placed in culture, were regrafted subcutaneously in Lewis rats and were tumorigenic. The resulting tumors (DSL-6A/T1) were solid and very firm, suggesting a high content of fibrous tissue. Histologically this is a desmoplastic ductlike carcinoma (Figure 6), in contrast to the acinar morphology observed in the DSL-6 tumor from which the DSL-6A cell line was obtained. Following 3 months’ growth in vivo, DSL-6A/T1 tumor tissue was quickly readapted to in vitro conditions in a manner similar to that of the parent DSL-6A/C1 culture and was designated as DSL-6A/C2. DSL-6A/C2 cells did not secrete amylase at any time and expressed the ductal phenotype when they were regrafted a second time. After this passage through rats and return to culture, the cells were similar to DSL-6A/C2.

*Figure 6. DSL-6A/T1 regrafted tumor growing subcutaneously in dense fibrous tissue of a Lewis rat. Ductlike structures are present throughout. H&E, ×79.*

*Figure 7. DSL-6B cell line growing subcutaneously following regrafting. A. Dilated, mucous filled spaces partially lined by glandular epithe-lium (×104). B. Squamous area in the same tumor (H&E, ×150).*
Cells from cycle 2 (DSL-6B) were regrafted at the fourth passage. The carcinomas were partially solid and partially cystic. Histologically, the tumor presented a mixed phenotype but retained no areas of acinar cell differentiation. Some areas consisted of poorly differentiated adenocarcinoma, other areas were mucin secreting (Figure 7A), and several areas showed a stratified squamous pattern (Figure 7B). There was moderate desmoplasia in the mucinous areas. The tumor cells exhibited the same sequence of events in vitro when returned to culture as DSL-6A/C2, i.e., attachment to the flask and growth of tumor cells as small colonies among fibroblasts, which gradually disappeared. A mixture of phenotypes suggestive of secretory (signet ring cells) and squamous (pavement epithelium) cells were observed in vitro.

Regrafted AR42J cells exhibited a glandular phenotype characteristic of poorly differentiated acinar cell carcinoma. There was no desmoplasia, duct formation, mucin secretion, or squamous metaplasia (Figure 8).

**Ultrastructure**

The DSL-6 transplantable carcinoma shows ultrastructural features of acinar cells, including the formation of glandular spaces with microvilli on the luminal surface with intercellular tight junctional complexes near the lumen (Figure 9). The cytoplasm contained moderate numbers of dense membrane-bound granules consistent with zymogen, abundant free polysomes, scattered profiles of rough endoplasmic reticulum (RER), and abundant mitochondria. Microfilament bundles were prominent in some specimens.

Cells fixed for electron microscopy at passage 2, 3 months after initiation of DSL-6A/C1, lacked zymogen granules, contained free ribosomes, formed microvilli on their surfaces, and were linked by tight junctions (Figure 10). They mimic ductal cell morphology. These have been compared and found to be similar to cells obtained after the second passage through the rat, DSL-6A/C2, at the second culture passage. Cells from the second cell line, DSL-6B, were similar in culture.

Regrafted cells from the DSL-6A cell line retained several features exhibited in culture, forming microvilli on luminal surfaces with intercellular tight junctional complexes. The cytoplasm contained polysomes but not RER. One specimen from regrafted DSL-6B/C1 cells generally showed less evidence of glandular differentiation, forming relatively few lumens. The cells were generally small and formed few lumens. Fewer than 10% of the cells contained a few dense membrane-bound granules of variable size.

**Immunocytochemical Studies**

The results obtained by using several antibodies to stain selected specimens from cycle 1 suggested that when acinar cells from the transplanted tumor were passaged in vitro, a subpopulation of these cells “dedifferentiated,” losing acinar cell phenotype, and began to acquire a ductal phenotype. Alternatively, the possibility existed that DSL-6 contained rare ductal cells that were selectively expanded in culture and subsequently produced ductal tumors when reimplanted into rats. To address this question, the “cycle” experiment was repeated, and MAbs that distinguished acinar and ductal cells were employed to characterize subcutaneous tumors and representative stages in the derivation of cell lines. In general, results from IIF and immunoperoxidase studies (Table 1) show a

![Figure 8. In vivo growth of AR42J cells as a subcutaneous tumor following regrafting of the cultured cells. A few rudimentary acini are present, but the tumor generally grows as a solid tumor without formation of glands or ducts. The tumor contains no fibrous tissue. H&E, x240.](image-url)
trend for loss of acinar cell markers and acquisition of ductal markers in the cell lines and regrafted tumors. The parent tumor line, DSL-6, was composed of cells that expressed OC.2 but lacked detectable levels of OC.1. Although a subpopulation of these OC.2+ cells (<10%) weakly expressed OV-6, CK-7, and CK-19 in vitro (Table 1, Figure 11), a phenotypic characterization of DSL-6B/C1 demonstrated that cell lines derived from DSL-6 in culture were composed primarily of OC.2+ cells that lacked the ductal specific markers OV-6, CK-7, and CK-19. However, when DSL-6B/C1 was regrafted into syngenic hosts, the resulting tumors (eg, DSL-6B/T1) were classified as adenosquamous tumors that contained areas of ductal metaplasia that expressed ductal specific markers (Figure 12). The squamous areas of the regrafted tumor were positive for CK-14. The stability of the ductal phenotype was confirmed by the observation that these tumor cells continued to express OC.2, OV-6, CK-7, and

Table 1. Indirect immunofluorescence analysis of normal and neoplastic pancreatic cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>OC.1</th>
<th>OC.2</th>
<th>OV-6</th>
<th>CK-7</th>
<th>CK-19</th>
<th>CK-14</th>
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<tr>
<td>Acinar cells</td>
<td>+++</td>
<td>+++</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ductal cells</td>
<td>–</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>DSL-6</td>
<td>–</td>
<td>+++</td>
<td>(R)*</td>
<td>(R)</td>
<td>(R)</td>
<td>ND</td>
</tr>
<tr>
<td>DSL-6/C1</td>
<td>–</td>
<td>+++</td>
<td>(R)</td>
<td>(R)</td>
<td>(R)</td>
<td>ND</td>
</tr>
<tr>
<td>DSL-6/T1</td>
<td>–</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>ND</td>
</tr>
<tr>
<td>DSL-6/C2</td>
<td>–</td>
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<td>+</td>
<td>++</td>
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<td>+++</td>
<td>+</td>
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</table>

*(R) indicates the presence of rare positive cells. ND, not done.
Figure 11. Double labeling IF analysis of DSL-6 and DSL-6G2. Acetone-fixed frozen sections of the DSL-6 pancreatic transplantable tumor were reacted with OY-6 (A) and OC-2 (B), and positive cells were visualized using isotype-specific secondary antibodies. Micrographs were photographed using Ektachrome 200 film, and negatives were printed on Kodak Pautwade paper. Using this technique, reactive areas appear as dark staining cells. The tumor strongly expresses OC-2. Arrows indicate rare OC-2 positive cells which weakly express the ductal marker designated OY-6 (×100). C and D, acetone-fixed cultures of DSL-6G2 cells reacted with OY-6 (C) and OC-2 (D). In contrast to cultures of OC-2+ DSL-6/C1 cells (not shown), this secondary cell line also coexpresses the ductal marker designated OY-6 (×100).

CK-19 (Table 1, Figure 11) when reintroduced into culture (eg, DSL-6B/C2). It should be noted that all tumors and cultured cell lines lacked detectable levels of OC.1, an observation consistent with the loss of this acinar cell marker by early eosinophilic preneoplastic lesions and primary tumors (R. A. Faris, unpublished observations).

**CCK Receptor Levels**

Although DSL-6 exhibits both high- and low-affinity receptors for CCK, DSL-6A/C1 does not bind CCK with high affinity (Table 2). AR42J cells, utilized as a positive control in this experiment, did exhibit both high- and low-affinity receptors for CCK.

**Discussion**

Similar but slightly divergent results were obtained in experimental cycles 1 and 2 (Figure 1). In both experiments, the cell lines derived from the transplantable acinar cell carcinoma lost most markers of acinar cell differentiation and acquired new phenotypic characteristics of ductal differentiation over the course of about 1 year. Regrafted tumor in cycle 1 mimics the appearance of human ductal adenocarcinoma and in cycle 2 mimics an adenosquamous carcinoma, which in humans has been classed as a variant of ductal carcinoma. Regrafting of the cell lines elicits a desmoplastic host response. Desmoplasia characteristically was present in the ductlike areas but not in the acinar components of primary carcinomas that developed in azaserine-treated rats.

Regrafting of the established cell lines into rats did not cause a reappearance of the acinar cell phenotype. When such tumors were returned to cell culture they did not produce amylase *in vitro* but instead expressed the ductal phenotype morphologically. To this time, we have not observed a reversal of the ductal phenotype with reexpression of the acinar phenotype either *in vitro* or *in vivo*.

The best characterized cell line derived previously from an azaserine-induced carcinoma is
Figure 12. Double labeling IF analysis of DSL-6BT1. Acetone-fixed frozen sections of the DSL-6BT1 tumor were reacted simultaneously with OC-2 (A, C, and E) and CK-7 (B), OV-6 (D) or CK-14 (F). Positive cells were visualized with isotype specific secondary antibodies. Micrographs were photographed on Ilfordchrome 200 film and printed on Kodak Panatomic paper. With this technique, reactive areas appear as dark staining cells. Glandular epithelia within the adenosquamous carcinoma strongly express OC-2, CK-7, and OV-6. In contrast, squamous-like epithelial cells appear to lose both OC-2 (C and E) and OV-6 (D) and acquire CK-14 (E). Arrows indicate areas of squamous epithelium. A, B, E, F, × 100. C and D, x 200.

Table 2. CCK receptor levels in DSL-6A and AR42J cell lines

<table>
<thead>
<tr>
<th>Specimen</th>
<th>High-affinity</th>
<th>Low-affinity receptor</th>
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<tbody>
<tr>
<td></td>
<td>$K_d$ (nmol/L)</td>
<td>$B_{max}$</td>
</tr>
<tr>
<td>DSL-6</td>
<td>0.33 ± 0.04</td>
<td>122 ± 23</td>
</tr>
<tr>
<td>DSL-6A/C1</td>
<td>Not detected</td>
<td></td>
</tr>
<tr>
<td>AR42J</td>
<td>0.30 ± 0.05</td>
<td>85 ± 11</td>
</tr>
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AR42J. It has maintained acinar cell differentiation, including the capacity to make and secrete amylase in culture for years, and maintains an acinar phenotype when grown in vivo. AR42J exhibits high- and low-affinity receptors for CCK. When AR42J was regrafted into Lewis rats, the phenotype of the resulting tumor was a poorly differentiated acinar cell carcinoma. The in vivo growth of AR42J did not yield either ductlike or adenosquamous phenotypes. Thus, there is a distinct difference between AR42J and the cell lines derived from the DSL-6 tumor in regard to the tendency to change phenotype. Cells from a transplanted azaserine-induced carcinoma (DSL-1) formed ductlike structures when they were cultivated on seminiferous tubules, but in contrast, a transplantable pancreatic acinar cell carcinoma derived by Reddy from a F-344 rat maintained an acinar cell phenotype when cultured under similar conditions. These observations suggest that individual tumors vary in regard to the stability of phenotype.

Two mechanisms could explain the derivation of neoplastic ductal cell lines from the DSL-6 tumor in these experiments. It is generally accepted that pancreatic tumors arise from azaserine-altered acinar cells in this rodent model. The most likely possibility is that neoplastic acinar cells may lose acinar cell differentiation and begin to express ductal markers, i.e., undergo metaplasia. The second possible mechanism is that overgrowth of a minor and undetected ductal component coincides with complete loss of neoplastic acinar cells in culture. Although we found that DSL-6 tumors contained a rare population of cells expressing ductal markers, these tumors lacked a histologically identifiable ductal component. Moreover, immunocytochemical characterization of DSL-6/C1 demonstrated that ductlike cells were not selectively propagated in culture. In the conceptual context of the clonal origin of the tumors, the DSL-6 cell lines employed in the present study would be considered to be of acinar cell origin under either mechanism. This view is supported by the finding that transplantable tumors continue to express amylase when passaged in vivo. Although the possibility exists that the metaplastic process may begin in vivo, our findings demonstrate that acquisition of the ductal phenotype is accelerated by adapting the tumor cells to culture, a manipulation that resulted in the acquisition of ductal phenotype when these cells were regrafted.

Our findings suggest that loss of the differentiated phenotype of neoplastic acinar cells in culture allows these cells to differentiate along a ductal pathway when regrafted into a syngeneic host. Since acquisition of the ductal phenotype does not initially occur in culture, our findings further suggest that progression of these less differentiated cells along a ductal pathway is determined, in part, by the in vivo microenvironment of the subcutaneous transplantation site. This observation is in contrast to the findings of De Lisle and Logsdon, who found that normal adult mouse acinar cells dedifferentiate in culture to a more ductlike cell that expresses duct-specific antigens.

The studies described here provide additional evidence to support the view that some pancreatic carcinomas with a ductal phenotype might be derived by transformation and dedifferentiation or metaplasia of acinar cells. Other studies that offer support for this hypothesis have been done primarily in rats, but a similar result is reported for guinea pigs. Carcinomas that develop in the DMBA-induced model of pancreatic cancer in rats are considered to be of acinar cell origin, although their histological phenotype is ductal. A similar situation pertains in methylnitrosourea-treated guinea pigs in which a sequence of formation of pseudoductules from acinar cells preceded the development of ductlike carcinomas. Ela-1-myc transgenic mice that were created using a pseudogene with an elastase 1 promoter/enhancer linked to myc have developed tumors with both acinar and ductal elements. The model is based on the expectation that myc will be overexpressed specifically in acinar cells. This is supported by prior studies in the ELSV transgenic mouse model based on the elastase 1-simian virus 40 T antigen pseudogene in which the vast majority of exocrine tumors retain some degree of acinar cell phenotype.

The implications of these studies for humans are uncertain. It is commonly assumed that ductal carcinomas arise as a result of transformation and neoplastic progression of duct cells. The finding of severe atypia of carcinoma in situ grade in the pancreatic ductal epithelium supports the validity of this pathway. Foci of atypical (hyperplastic or dysplastic) acinar cells resembling those induced by azaserine in rats have been described in the human pancreas, but at this time there is no firm evidence to link such foci with the genesis of ductal carcinomas. Ductal complexes in lobular tissue of human pancreas have been considered to arise by metaplasia of acinar cells and to provide a pathway for the origin of tumors of ductal phenotype from acinar cells. The observations in animal models reported or reviewed here suggest that the matter of histogenesis of human carcinomas with a
ductal phenotype should remain an open question that requires further evaluation.

References