Cell Differentiation in Pancreatic Cancer*

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ABSTRACT

Cell differentiation in cancer of the exocrine pancreas is currently under intensive study. In this report, new developments are critically reviewed on the use of transplantable pancreatic carcinoma and the hybridoma technique to define cell membrane changes during pancreatic cancer growth. It is concluded that two categories of plasmalemma structure hold promise as differentiation markers specific for pancreatic cancer: (1) membrane receptors for cholinergic and peptide secretagogues and (2) membrane glycoproteins as detected by monoclonal antibodies. Assay of secretagogue receptors and membrane glycoprotein antigens will be central to elucidation of mechanisms of pancreatic carcinoma cell differentiation (? stem cell differentiation or retrodifferentiation) and, hopefully, will provide tumor-specific or tissue-specific markers for the laboratory diagnosis and monitoring of pancreatic cancer.

Introduction

Cancer of the pancreas now ranks fifth in the United States among causes of death owing to cancer, behind only lung, bowel, breast, and prostate cancer.11 Internationally, pancreatic cancer accounts for 3 to 6 percent of all cancer deaths in most countries, and the mortality rate continues to increase with time. It can be expected that essentially every patient among the approximately 24,000 new cases of pancreatic carcinoma presently diagnosed in the United States each year will die of that disease. Pancreatic carcinoma clearly represents a major challenge in the laboratory diagnosis and monitoring of cancer.

A number of tumor-associated antigens has been utilized as diagnostic markers for pancreatic cancer. Most notable among these is carcinoembryonic antigen (CEA), first demonstrated by Gold and Freedman in human colonic carcinoma.4 Serum and pancreatic juice levels of CEA have been extensively studied in pancreatic cancer patients. Unfortunately, CEA has been found insufficiently specific for early diagnosis of pancreatic carcinoma, and neither specific nor sensitive enough for use as a single diagnostic tool in monitoring the disease of individual patients.5 Other
pancreatic oncofetal and tumor-associated antigens have been identified, but none demonstrate the specificity required of a meaningful diagnostic test.

A major limitation in the development of a sensitive and specific marker for pancreatic cancer has been absence of information on mechanisms of differentiation in pancreatic carcinoma. Several transplantable pancreatic cancers have recently been developed in rodent species, which provide experimental models for elucidating the nature of cellular differentiation in pancreatic cancer. In addition, the hybridoma technique is being utilized in current investigations on membrane differentiation in cancer of the human pancreas. This review will describe our present understanding of differentiation in transplantable pancreatic carcinoma, examine the production of monoclonal antibodies in human pancreatic carcinoma, and discuss how progress in these two areas might contribute to improved techniques of laboratory diagnosis of pancreatic cancer. Comprehensive reviews of cytological, radiological, and ultrasound techniques for the diagnosis of pancreatic carcinoma can be found in the published proceedings of the 1980 International Meeting on Pancreatic Cancer.

Transplantable Pancreatic Carcinoma

In 1977, Reddy and Rao reported successful transplantation into weanling rats of a pancreatic acinar cell carcinoma, which developed in a male Fischer 344 rat fed 0.1 percent nafenopin for 20 months. The nafenopin-induced pancreatic acinar carcinoma can be dissociated into single cells by combined treatment with collagenase and ethylenediamine tetraacetic acid (EDTA), and patterns of cytodifferentiation have been established in the tumor by electron microscopic examination of the dissociated cells. The most common type of cell in the carcinoma is a well-differentiated cell containing abundant rough endoplasmic reticulum, prominent Golgi apparatus, and numerous zymogen granules. Progressively less differentiated cells are also present, including undifferentiated cells completely lacking phenotypic features of zymogen maturation. The spectrum of undifferentiated to well-differentiated carcinoma cells makes the nafenopin-induced tumor an excellent model for study of cellular differentiation in pancreatic cancer.

Use of Lectins to Characterize Cell-Surface Glycoconjugate Patterns

The maturational sequence of acinar cell membrane glycoprotein/glycolipid patterns has been established in embryonic rat pancreas by Maylie-Pfenninger and Jamieson utilizing a battery of lectins capable of interacting with major membrane saccharides. Undifferentiated epithelial cells of the day 15 embryonic pancreas demonstrate a restricted range of membrane receptors for lectins and, in particular, are devoid of receptors for concanavalin A (con A). During days 15 to 19 of embryogenesis, pancreatic acinar cells develop a full complement of zymogen granules, and concomitantly acquire membrane receptors for con A. Thus, in normal differentiating pancreatic acinar cells, acquisition of membrane binding sites for con A is tightly coupled to zymogen maturation. To determine whether or not con A binding correlates in similar fashion with zymogen differentiation in pancreatic carcinoma, con A binding to acinar carcinoma cells was examined both by electron microscopy utilizing the peroxidase method of membrane labeling and by radioreceptor assay using 125I-labeled con A. Heavy membrane labeling owing to specific con A binding was detected by the peroxidase method on both undifferentiated carcinoma cells lacking
zymogen maturation and differentiated carcinoma cells containing zymogen granules.\textsuperscript{26} Radioreceptor assay revealed an identical number of con A receptors on acinar carcinoma and fully-differentiated normal pancreatic acinar cells, although only one-third of pancreatic acinar carcinoma cells demonstrate mature zymogen maturation.\textsuperscript{22,26}

In recent work, well-differentiated acinar carcinoma cells containing zymogen granules have been separated from undifferentiated carcinoma cells lacking zymogen granules by isopyknic Percoll gradient centrifugation,\textsuperscript{1} and have directly compared the con A-binding properties of differentiated and undifferentiated carcinoma cells by radioreceptor assay (figure 1). Equivalent con A binding was noted for the granule-containing and granule-deficient carcinoma cells. It is concluded, therefore, that unlike embryonic pancreatic acinar cells, the presence of con A receptors in membranes is not a differentiation marker for carcinoma cells containing zymogen granules.

**Secretagogue Responsiveness and Acinar Cell Differentiation**

Secretion of protein by pancreatic acinar cells is an integrated response involving membrane structures of the cell periphery. For the peptide secretagogue cholecystokinin (CCK) and cholinergic agents such as carbamylcholine, initial binding of secretagogue to specific plasma membrane receptors results in displacement into the cytosol of acinar cells of Ca\textsuperscript{2+} from membrane-bound stores.\textsuperscript{28} The increased cytosolic Ca\textsuperscript{2+} levels activate protein secretion by acinar cells. It has recently been demonstrated by us that both the C-terminal octapeptide of CCK (CCK-OP) and carbamylcholine stimulate protein secretion in the nafenopin-induced acinar carcinoma, but only at one-fifth to one-half the rate observed in normal pancreatic lobules.\textsuperscript{24,25,27} This modest secretory response is consistent with presence in the carcinoma of undifferentiated cells lacking a secretory apparatus (no Golgi complex, no zymogen granules), which would not be expected to secrete protein in response to CCK-OP or carbamylcholine.

To compare directly the secretagogue responsiveness of differentiated and undifferentiated acinar carcinoma cells, carcinoma cells containing zymogen granules were separated from cells lacking granules on a Percoll gradient, and actions of carbamylcholine on Ca\textsuperscript{2+} efflux in the two cell populations were measured. Stimulation of Ca\textsuperscript{2+} efflux in the two cell populations were measured.
linergic secretagogues or CCK which reflects elevated cytosolic Ca\(^{2+}\) levels. As reported in table I, both granule-containing and granule-deficient cells demonstrated brisk stimulation of Ca\(^{2+}\) efflux in response to carbamylcholine. Similar results were obtained with the secretagogue CCK-OP (data not shown). The occurrence of post-receptor intracellular Ca\(^{2+}\) responses and protein secretion indicates that pancreatic cancer cells are capable of functional as well as cytomorphological acinar differentiation.

A significant qualitative difference has been detected in the responsiveness of normal and neoplastic cells toward the Ca\(^{2+}\) ionophore A23187 and the cyclic nucleotide N\(^6\)O\(^2\)-dibutyryl cyclic AMP (db-cAMP). Protein secretion in embryonic pancreas can be stimulated by A23187 and db-cAMP within a day or so after acinar cells achieve morphological zymogen maturation, and before the acinar cells become responsive to carbamylcholine and CCK. Unexpectedly, neither A23187 nor db-cAMP stimulated protein secretion in the nafenopin-induced pancreatic carcinoma. Since A23187 stimulates normal acinar cell secretion by increasing the influx of extracellular Ca\(^{2+}\), failure of A23187 to stimulate tumor secretion suggests insensitivity of even highly differentiated carcinoma cells to extracellular Ca\(^{2+}\). Likewise, inability of db-cAMP to stimulate acinar carcinoma secretion indicates defective differentiation of cyclic AMP-dependent protein kinases.

### TABLE I

<table>
<thead>
<tr>
<th>Cell Preparation*</th>
<th>Stimulation Index† (Mean ± SD)</th>
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<tbody>
<tr>
<td>Granule-containing</td>
<td>1.560 ± 0.214</td>
</tr>
<tr>
<td>Granule-deficient</td>
<td>1.604 ± 0.110</td>
</tr>
<tr>
<td>Unfractionated</td>
<td>1.527 ± 0.131</td>
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*Granule-containing and granule-deficient preparations were obtained by Percoll gradient centrifugation as described in the legend to figure 1. The unfractionated preparation consisted of dissociated carcinoma cells not fractionated on the Percoll gradient.

†Carcinoma cells were labeled to quasi-steady state levels of \(^{45}\)Ca\(^{2+}\) and were then incubated in buffer without added \(^{45}\)Ca\(^{2+}\) for six minutes. Stimulation index = \(\text{pm \(^{45}\)Ca\(^{2+} \text{ per 10}^6 \text{ cells present in buffer after incubation with } 10^{-3}\text{M carbamylcholine per pm \(^{45}\)Ca\(^{2+} \text{ per 10}^6 \text{ cells present in buffer after incubation without carbamylcholine. Values reported here are based on results obtained in three separate experiments.}

**Mechanisms of Cellular Differentiation in Transplantable Pancreatic Carcinoma**

Two different mechanisms of cellular differentiation can be proposed for the growth of pancreatic acinar carcinoma. First, a stem cell mechanism of neoplastic acinar cell differentiation can be suggested, in which stem cells lacking phenotypic features of zymogen maturation give rise to carcinoma cells of intermediate and mature zymogen differentiation. Evidence has been obtained with other types of transplantable tumor, including teratocarcinoma, squamous cell carcinoma, and mammary adenocarcinoma, that neoplastic stem cells proliferate along normal maturational pathways to yield partially and highly differentiated progeny. However, lectin binding and secretory responsiveness to A23187 and db-cAMP, both expressions of acinar cell differentiation in embryonic pancreas, diverge detectably from normal patterns in pancreatic acinar carcinoma. If a stem cell mechanism operates in pancreatic acinar carcinoma, then membrane and secretory phenotypes of differentiating carcinoma cells differ significantly from normal differentiating acinar cells.

A second mechanism, retrodifferentiation, can be proposed in which well-differentiated carcinoma cells lose phenotypic features of zymogen maturation, resulting in partially and poorly differentiated cells. Indeed, both mitotic activity and deoxyribonucleic acid (DNA)
synthesis have been detected in acinar carcinoma cells containing mature zymogen granules\textsuperscript{22} and, therefore, granule-containing carcinoma cells could theoretically serve as a proliferative pool for generation of carcinoma cells lacking zymogen maturation. Retrodifferentiation of zymogen granule-containing acinar cells would result in less mature “duct-like” cells without zymogen granules but possessing plasma membrane receptors for con A,\textsuperscript{12} phenotypic features observed for the population of undifferentiated cells in pancreatic acinar carcinoma.

The most common type of carcinoma in the human pancreas is designated ductal adenocarcinoma, constituting up to 89 percent of all reported cases,\textsuperscript{6} and it is generally assumed that ductal epithelial cells are the cells of origin for most human pancreatic cancers. However, the evidence for ductal origin is purely morphological, being based on: (1) presence of duct-like structures and mucin (intracellular and/or extracellular); (2) absence of zymogen granules by electron microscopy; and (3) presence of ductal carcinoma \textit{in situ} in about one-quarter of patients with ductal adenocarcinoma.\textsuperscript{6} None of these morphological criteria unequivocally defines pathways of cellular differentiation in human pancreatic cancer. It is well to remember that acinar and ductal epithelial cells develop from common progenitor cells in normal pancreas.\textsuperscript{13} Also, careful studies of sequential changes induced in normal pancreas by carcinogen treatment strongly suggest that ductal structures can arise from modulation of fully differentiated acinar cells.\textsuperscript{6} Thus, mechanisms of cell differentiation in pancreatic acinar carcinoma have potential relevance to ductal adenocarcinoma of the pancreas. Experimental acinar carcinoma is clearly important as a tumor model for the relatively small number of patients with pancreatic acinar carcinoma (1 to 15 percent of all pancreatic cancer cases).\textsuperscript{6}

Using the nafenopin-induced acinar carcinoma, as well as other transplantable acinar and ductal tumors (human and experimental),\textsuperscript{6} it should be possible to define whether pancreatic carcinoma grows by proliferation of stem cells along maturational pathways divergent from those of normal pancreas, or by retrodifferentiation of acinar cells. Regardless of which mechanism proves correct in future work, it can be concluded on the basis of presently available data that differentiation in pancreatic acinar carcinoma does not mimic normal embryonic differentiation. It is suggested, therefore, that the search for accurate laboratory tests of pancreatic cancer should focus on tumor rather than embryonic membrane markers. As will be noted, a membrane antigen has recently been described in human ductal adenocarcinoma which is absent in normal fetal pancreas.\textsuperscript{14}

Finally, the secretagogue responsiveness of Percoll fractionated carcinoma cells (table I) suggests that undifferentiated granule-deficient pancreatic carcinoma cells as well as differentiated granule-containing carcinoma cells contain plasma membrane receptors for secretagogues. Secretagogue receptors may, therefore, provide markers of membrane differentiation in pancreatic cancers which lack morphological features of acinar cytodifferentiation. Cholecystokinin is a trophic hormone as well as secretagogue and stimulates the growth of normal pancreatic acinar cells.\textsuperscript{7} It will be important to determine if CCK regulates growth of malignant pancreatic cells and also to assess secretagogue receptor content of pancreatic tumors in individual patients.

Monoclonal Antibodies in Pancreatic Carcinoma

The hybridoma technique has gained wide acceptance for the production of ho-
mogeneous antibodies specific for a single antigenic site. In this technique, spleen cells from mice immunized with antigen are fused with a continuously proliferating mouse myeloma cell line, and somatic cell-hybrids secreting antibodies monospecific for the antigen are recovered by growth in selective medium and cloning. The hybridoma technology has been successfully used to identify tumor-specific antigens in malignant melanoma and differentiation antigens in acute lymphocytic leukemia. In very recent work, monoclonal antibodies have been elicited which are reactive against human pancreatic adenocarcinoma cells and normal pancreatic acinar cells.

Human Pancreatic Carcinoma Antigens

Metzgar and his co-workers have recently elicited and characterized five murine monoclonal antibodies, which they designate DU-PAN-1,2,3,4, and 5, to the human pancreatic adenocarcinoma cell line HPAF. Each of the five monoclonal antibodies was tested against different pancreatic carcinoma cell lines, pancreatic and other types of tumors, and a variety of normal and fetal human tissues. One of the monoclonal antibodies, DU-PAN-1, strongly reacted with human pancreatic carcinoma cells, did not react with adult or fetal normal pancreas, and showed very limited reactivity with non-pancreatic tumors. In this preliminary work, therefore, the DU-PAN-1 antigen meets the criteria to be considered a tumor-specific antigen for human pancreatic carcinoma.

The DU-PAN-2 and 3 antigens were detected on pancreatic carcinoma and normal ductal epithelial cells (but not acinar cells) and were absent on normal cells from other types of adult tissue. Perhaps, therefore, DU-PAN-2 and 3 represent tissue-specific differentiation antigens in pancreatic carcinoma. In addition, the DU-PAN-1 and 2 antibodies failed to react with all HPAF pancreatic carcinoma cells, which were used to elicit production of the antibodies in BALB/c mice. By indirect immunofluorescence, DU-PAN-1 antibodies reacted with 70 percent and DU-PAN-2 antibodies only 10 percent of HPAF cells. This restricted staining pattern indicates that DU-PAN-1 and 2 are differentiation antigens whose expression is dependent on the maturational stage of human pancreatic carcinoma cells.

Human Pancreatic Acinar Cell Antigens

A monoclonal antibody reactive towards normal human pancreatic acinar cells, but unreactive with ductal, centroacinar, islet, and interstitial cells, has been obtained by Parsa using collagenase-dissociated adult human pancreas to immunize BALB/c mice. Of 14 tumors obtained by in vitro treatment of human pancreatic explants with methyl-nitrosourea and histologically consistent with ductal carcinoma, one contained cells reactive with the anti-acinar antibody. Interestingly, intensity of cell surface staining in this tumor by indirect immunofluorescence varied considerably from cell to cell, again suggesting that the antigen detected by the anti-acinar antibody is a differentiation antigen.

Of the five monoclonal pancreatic cancer antibodies isolated by Metzgar and colleagues, only DU-PAN-4 revealed some reactivity with normal pancreatic acinar cells. However, DU-PAN-4 antigen is broad in its distribution, and detectable on cells in many nonpancreatic tumors, as well as fetal and adult prostate, breast, and salivary gland, and blood vessels of many organs. The DU-PAN-4 antigen is thus neither tissue-specific nor tumor-specific for the pancreas.

Summary

1. Transplantable tumors of exocrine pancreas are now available which dem-
onstrate morphological and secretory maturation, and hence serve as excellent models to elucidate the mode of cellular differentiation (stem cell differentiation) versus retrodifferentiation in pancreatic cancer.

2. Evidence has been obtained from lectin-binding and secretion measurement that pathways of cellular differentiation in pancreatic carcinoma diverge significantly from normal maturational pathways operative in embryonic pancreas. It is suggested, therefore, that the search for diagnostic markers in pancreatic cancer focus on tumor-specific rather than embryonic membrane properties.

3. Preliminary data indicate that plasma membrane receptors for secretagogues and glycoprotein antigens detected by the hybridoma technique are expressed as differentiation markers in membranes of pancreatic carcinoma cells.

4. The use of pancreatic secretagogues and monoclonal antibodies offers promising new directions for the identification of specific and sensitive differentiation markers in pancreatic cancer. Work with these markers should elucidate mechanisms of ductal and acinar cell differentiation in pancreatic cancer and provide additional criteria for the evaluation of pancreatic cancer in individual patients.

References


