Ductal Metaplasia of Human Exocrine Pancreas and Its Association with Carcinoma

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ABSTRACT

Monoclonal antibodies to cell surface markers of human exocrine pancreas were used to establish the cytotypic expression of cells forming "tubular complexes" in pancreases from six adults without carcinoma and in the nontumorous pancreatic parenchyma of 16 pancreases with carcinoma. These cells manifested duct cell determinants. In general, the presence of cells with duct cell surface markers within the acini corresponded to the normal distribution of centroacinar cells in the 30 control human pancreases (from cadaveric donors); however, focal of abnormal acini were seen in these pancreases independent of or intermingled with the "tubular complexes." The acini in these abnormal areas were formed by a core of cells and cell processes that expressed duct cell determinants. They were partially surrounded by acinar cells and showed slight or no lumenal dilation. While the causative agent(s), the cell(s) of origin, and the regression and/or progression of these lesions are yet to be determined, the replacement of acini by the spectrum of lesions composed of cells with duct cell surface marker is suggested to constitute ductal metaplasia.

INTRODUCTION

Pancreatic carcinoma has been the subject of intensive study in the past decade, and several rodent models of pancreatic carcinoma have been advanced in this period (6, 11, 18-23) which have contributed to the understanding of pancreas carcinogenesis in rodents. The apparent species specificity of the carcinogen and the seeming disparity of the target cells in these models have generated serious controversy as to the cell(s) of origin of pancreatic carcinoma. In the azaserine-treated rat model, the earliest morphological alterations were described in acinar cells, leading to the development of nodules of atypical acinar cells (10) and, eventually, to acinar cell carcinoma (11). The early morphological changes in the hamster (6, 7, 9, 13, 14), guinea pig (24, 25), and rat (2, 5) models have been described as degeneration of acinar cells, loss of zymogen granules and apicil cytoplasm, proliferation of cells devoid of zymogen granules, and formation of duct-like structures. In the absence of secretory granules and cytotypic markers, there is a controversy as to whether the proliferating cells represent preexisting centroacinar and ductular cells or "dedifferentiated" acinar cells. Because of its resemblance to the most common malignant pancreatic neoplasm in humans, ductal carcinoma in the hamster induced by β-oxidized derivatives of N,N-propylnitrosamine has been the subject of intensive studies, and the cells of origin in this cancer model have become the center of controversy. Preexisting duct cells and proliferating intralobular ductular cells are considered the exclusive progenitor cells of this tumor by some (1, 13, 14, 17, 19, 20, 26, 29), and the phenotypic alteration of acinar cells leading to the development of duct-like epithelium is suggested to be the origin of carcinoma in this model by others (6, 7, 27, 29).

Duct cell carcinoma of the human pancreas, the most common type, is generally assumed to derive from duct cells, an assumption based primarily on the morphological resemblance of tumor cells to duct cells and the frequent association of atypical, dysplastic duct epithelial lesions and in situ ductal carcinoma with pancreatic carcinoma (3, 4). Acinar cell carcinoma is regarded as rare: its incidence reported to be between 1 and 15% of exocrine carcinoma. Focal pancreatic acinar cell dysplasia, similar to the atypical acinar cells described in carcinogen-treated animals, was reported in a series of 108 autopsy cases (12).

This paper describes the occurrence of early, intermediary, and advanced stages of ductal metaplasia in human exocrine pancreas as identified by the use of monoclonal antibodies to human pancreas cell surface markers.

MATERIALS AND METHODS

Human Pancreas. Pancreases (tail and body) were removed in the operating room from 30 cadaveric donors, ranging in age from one to 52, with no pancreatic disease. The warm ischemia times varied from 2 to 30 min. The pancreases were kept at 0-5°C for up to 4 hr before sectioning. The sections were used as control pancreas.

Human Pancreas with "Tubular Complex." Sections from 6 autopsy cases with no pancreatic tumor but showing tubular complexes were used in this study. These were contributed by the Dartmouth-Hitchcock Medical Center (3 cases), the Epply Institute for Research in Cancer (one case), and Downstate Medical Center (2 cases). The patients' ages ranged between 55 and 81, and all died of non-pancreas-related causes.

Human Duct Cell Carcinoma. Sections were taken from 16 patients with pancreatic cancer; the noncancerous portions of the pancreas were examined as well. These were contributed by the Dartmouth-Hitchcock Medical Center (2 surgical cases), Northwestern University Medical School (one surgical and 2 autopsy cases), the Epply Institute for Research in Cancer (2 autopsy cases), Walter Reed Army Medical Center (3 autopsy cases), and Downstate Medical Center (6 surgical cases).

Monoclonal Antibody. The specificity of AC1 and HP-DU-1 antibodies for the human pancreatic duct cell surface has been reported previously (15, 16). The hybrid producing specific IgG was developed by the fusion of splenocytes from BALB/c mice, immunized with human acinar or ductal cells, and mouse myeloma cells (SP2). The IgG-secreting cells were screened, and AC1- or HP-DU-1-producing cells were cloned. Peritoneal exudates, rich in specific antiaacin or antiductal cell surface IgG from BALB/c mice receiving injections i.p. of specific hybrids, were

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used in this study.

Microscopic Preparation. Tissues were fixed in Bouin’s fluid or buffered formaldehyde (pH 7), embedded in paraffin, and sectioned at 2- to 4-μm thickness. Sections were stained with H & E. Fluorescence Microscopy. For fluorescence microscopy, sections were deparaffinized in xylene, hydrated gradually, and placed in PBS, containing NaCl (9 g/liter) in 0.01 m sodium phosphate (pH 7.2 to 7.4), for 10 min, followed by a 20-min incubation in goat serum diluted with PBS (1:10) and 3 washes with PBS, all at room temperature. The sections were then incubated for 30 min at room temperature in pooled ascitic fluid rich in antiduct (HP-DU-1) or antiacinar (AC1) cell surface IgG diluted with PBS (1:200), given 3 washes in PBS, and incubated in fluorescein-conjugated goat anti-mouse IgG for 30 min at room temperature, washed with PBS, and examined for fluorescence.

Double Staining. Sections were first stained as above using HP-DU-1 antibody and fluorescein-conjugated goat anti-mouse IgG, washed in PBS to remove excess of conjugated antibody. The sections were then incubated in monoclonal antibody to human acinar cell surface (AC1) for 30 min at room temperature, rinsed in PBS, incubated for 30 min in rhodamine-conjugated rabbit anti-mouse IgG, washed with PBS, mounted in glycerol, and examined for green and red fluorescence.

Peroxidase Staining. Deparaffinized sections were incubated in normal goat serum, washed with PBS, incubated with HP-DU-1 diluted with PBS (1:800), and washed again by PBS. The sections were incubated in 0.1% H2O2 in methanol for 20 min, washed in PBS, and incubated in goat anti-mouse horseradish peroxidase-conjugated IgG (New England Nuclear, Boston, MA) (diluted 1:10 with normal goat serum) for 45 min at room temperature. They were then subjected to PBS washes and incubation in diaminobenzidine for 3 min, counterstained with methylène blue, and examined by light microscopy.

Mitotic Count. Random H & E-stained sections from pancreases of 10 cadaveric donors, 6 autopsy cases without pancreatic carcinoma selected because they contained aggregates of duct-like structures, and from the nontumorous pancreases of 16 carcinoma patients were screened using x40 objective. The numbers of mitotic figures per section associated with ductal and acinar cells in normal-appearing pancreas and tubular complexes were counted. The mean ± S.D. of mitotic figures per area of normal and ductal complexes in each group was determined.

RESULTS

Human Pancreas. Sections from 30 cadaveric pancreases stained with HP-DU-1 showed intense surface fluorescence of the cells lining the main duct, the interlobular and intralobular ducts, and the centroacinar cells. Acinar and endocrine cells, connective tissue, and blood vessels showed a total absence of fluorescence. Peroxidase-stained sections showed similar results. Sections stained with both HP-DU-1 and AC1 revealed intense green fluorescence of ductal and centroacinar cells and red fluorescence of acinar cells. Thus, the exocrine pancreas was divided into 2 populations of ductal and acinar cells according to their surface markers.

Pancreatic Carcinoma. Examination of the H & E sections from pancreatic carcinomas (surgical or autopsy specimens) revealed extensive variation in the preservation of tumor cells from case to case and among the sections of a given case. In every case, there were sections with well-preserved areas of tumor separated by foci of tumor cells showing various degrees of preservation and necrosis. Duplicate sections from each case were examined for both peroxidase reactions and immunofluorescence. All sections of pancreatic tumors (9 surgical and 7 autopsy cases) stained with HP-DU-1 antibody showed cell surface fluorescence and peroxidase reaction. The extent of the fluorescence or peroxidase staining correlated well with the degree of preservation and necrosis of tumor cells detected on the H & E-stained adjacent sections. Well-preserved tumor cells showed an intense surface fluorescence and peroxidase reaction with HP-DU-1 antibody only. No definite staining was discernible by either HP-DU-1 or AC1 antibody in poorly preserved or necrotic cells.

Tubular Complexes in Noncancerous Pancreas. Sections from the pancreases of 6 autopsy cases showing foci of tubular complexes were examined. These sections were selected because they contained aggregates of duct-like structures of various sizes and shapes that were identified in routine H & E-stained sections. These structures were lined by cuboidal to columnar epithelium with basally located nuclei and moderate to large amounts of apical cytoplasm (Fig. 1). Sections stained with HP-DU-1 antibody showed an intense surface fluorescence of the epithelial cells lining the duct-like structures (Fig. 2). The fluorescence or peroxidase staining in these sections was not limited to the tubular complexes, ducts, ductules, and centroacinar cells, but also multiple clusters of intensely staining HP-DU-1-positive acinus-like structures were present (Fig. 3). These acinus-like structures, against the negative background of generally nonfluorescing normal acini, conveyed the impression of a lobular distribution. The degree of staining in the HP-DU-1-positive acinus-like structures was variable and was often confined to their lumenal aspects. Careful examination of these acinus-like structures on the adjacent sections stained with H & E revealed normal to slightly dilated acinar lumens (Fig. 4) formed primarily by cells showing basal basophilia and recognizable as acinar cells. Many of these cells were devoid of zymogen granules. There were also some cells with the morphological criteria of centroacinar cells, as distinguished by their lightly staining cytoplasm.

To determine the identity of the cells forming the HP-DU-1-positive acini, adjacent sections were double stained with both HP-DU-1 and AC1 antibodies and examined to detect any acinar and ductular cells. Normal acini were formed by AC1-positive cells with intense red surface fluorescence (rhodamine) and showed only occasional HP-DU-1-positive cells corresponding to the normal distribution of centroacinar cells. In contrast, the HP-DU-1-positive acinus-like structures were partially constituted by AC1-positive cells, showing intense surface and cytoplasmic red fluorescence, interspersed with AC1-negative-staining cells (Fig. 5a). These AC1-negative-staining cells showed HP-DU-1-positive green fluorescence (Fig. 5b) and possessed extensive thin cytoplasmic processes which often covered the lumenal surface of the acinar (AC1-positive) cells. The intensity of fluorescence staining of both HP-DU-1- and AC1-positive cells varied considerably from cell to cell and from section to section. The cells forming acinus-like structures, however, could be identified as having either acinar or ductal antigenic expression. Due to variations in tissue and cell preservation, it was not possible to rule out with certainty the existence of cells without either of these 2 antigenic expressions.

Lesions Associated with Carcinoma. Sections from the nontumorous parenchyma of carcinoma cases were examined in H & E-stained preparations to detect the presence of tubular complexes; they were also examined by immunofluorescence.
and immunoperoxidase techniques to detect cytotypic markers. Multiple foci of tubular complexes and HP-DU-1-positive acinar-like structures per section were noted in each case of pancreatic carcinoma. The fluorescence stainings and the peroxidase reactions, however, were subject to extensive variation according to the degree of tissue preservation.

Mitoses and Intermediary Lesions. Mitotic figures were conspicuous in tubular complexes (Fig. 6), HP-DU-1-positive acini, and intermediary lesions. Intermediary lesions, as distinguished by the presence of a few (Fig. 7) or many (Fig. 8) duct-like structures within the HP-DU-1-positive foci, were more abundant in the nonmalignant portion of pancreases from patients with pancreatic carcinoma but were also seen in pancreas sections from each of the 5 noncancerous autopsy cases. There were 38 mitotic figures in 20 sections from normal pancreases with an average of 1.9 ± 0.7 mitoses per section, all belonging to ductal and ductular lining cells. In contrast, there was an average of 11.7 ± 3.1 mitotic figures per section in the normal parenchyma of cancer-bearing pancreases and in those of the 6 autopsy cases with tubular complexes. The normal duct and ductular epithelial lining in these cases contained an average of 1.6 ± 0.6 mitoses per section. The remaining mitotic figures (an average of 10.1 ± 3.7) belonged to the epithelium of tubular complexes, HP-DU-1-positive acini, and the intermediary lesions.

**DISCUSSION**

The monoclonal antibodies HP-DU-1 and AC1 make it possible to distinguish between human exocrine pancreas cells bearing ductal and those bearing acinar cell surface antigens. In the present study, it is established that human pancreatic lesions resembling those of the rat, guinea pig, and hamster pancreas under experimental conditions (here referred to as tubular complexes or pseudoductular lesions) are composed of cells with ductal antigenic expression. Early acinar lesions often with a lobular distribution, undetectable by routine histological examination, were also demonstrated to consist of cells with enhanced ductal antigenic expression forming the core of each acini in the involved foci. The spectrum of lesions at the early, intermediary, and advanced stages, all composed of cells with ductal antigenic expression that are essentially replacing acinar cells, warrants the term "ductal metaplasia" of the pancreatic acini. Due to its extent and multiplicity, ductal metaplasia is most likely an expression of a more general response pattern to injury and like metaplasia of other organs, e.g., bronchi, stomach, breast, and bladder; its presence in tumor-bearing pancreas may or may not have direct relevance to the cancer.

The data obtained from human pancreases do not allow evaluation as to the degree of reversibility, persistence, or progression of ductal metaplasia toward neoplasia in the human exocrine pancreas. The occurrence of early lesions as a response to cellular or molecular injury by carcinogenic and/or noncarcinogenic environmental chemicals may be transient and potentially reversible. Amplification of the metaplastic process by selection or by differential growth which is no longer environment dependent may, on the other hand, constitute an early neoplastic step. The relatively high mitotic activity associated with ductal metaplasia, as compared to that of normal and nonmetaplastic pancreas, suggests some proliferative activity in the metaplastic epithelium.

The present observations establish the occurrence of a lesion, ductal metaplasia, in the human pancreas and its early detectability by monoclonal antibody. The data are consistent with both views on the cell(s) of origin of the tubular complexes in experimental pancreas carcinogenesis, namely that the duct-like structures (ductal metaplasia) may result from proliferation of either centroacinar and ductular cells (1, 13, 14, 19, 29) or dedifferentiated acinar cells that express ductal cell morphology (2, 6, 7, 24, 25, 27, 28). The absence of cells bearing both ductal and acinar cell determinants in normal pancreases, in early lesions, and in a wide range of intermediary foci refutes the argument for an obvious transition of acinar cells to cells bearing ductal antigen. The loss of acinar cell determinants in cells which may later express HP-DU-1 antigen may, however, be supported by the existence of exocrine cells having neither of the 2 markers.

**REFERENCES**


Figs. 1 to 8. These micrographs are from human pancreas fixed in phosphate-buffered formaldehyde (pH 7.0), embedded in paraffin, cut at 2- to 4-μm thickness, stained with H & E (Figs. 1, 4, 6, 7, and 8), or incubated with murine monoclonal antibody to acinar cells (AC1) and/or to ductal cells (HP-DU-1) and rhodamine-conjugated and/or fluorescein-conjugated anti-mouse IgG (Figs. 2, 3, and 5).

Fig. 1. Section from pancreas of a 64-year-old female cigarette smoker showing duct-like structures ("tubular complexes") of various sizes and shapes lined by cuboidal epithelium. × 180.

Fig. 2. Section adjacent to that shown in Fig. 1 revealing duct-like structures lined by HP-DU-1* cells. × 400.

Fig. 3. Section from pancreas of a 72-year-old cigarette smoker showing clusters of HP-DU-1* cells against a negative background of normal acini. × 180.

Fig. 4. Section adjacent to that of Fig. 3 showing normal acini with slight or no luminal dilatation (arrowheads) formed partially by recognizable acinar cells and also cells with lightly staining cytoplasm. × 400.

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Fig. 5. a, section adjacent to that of Fig. 4 double stained with AC1 and HP-DU-1 examined for red fluorescence showing acini formed by acinar (AC1⁺) cells and a core of AC1⁻ cells (arrowheads). × 400. b, same section as in a examined for green fluorescence. AC1⁺ cells and their processes shown by arrows in a are HP-DU-1⁺. × 400.

Fig. 6. Section from pancreas of an 81-year-old female with type 2 diabetes showing "tubular complex" formed by duct-like structures lined by cuboidal to high columnar epithelium (double arrowhead) and an occasional mitotic figure (arrowhead). × 400.

Fig. 7. Section from pancreas of a 61-year-old female showing a few duct-like structures in an area showing an early lesion formed by HP-DU-1⁺ acini. × 280.

Fig. 8. Section from the same case as Fig. 7 showing a relatively large number of duct-like structures in an early lesion formed by HP-DU-1⁺ acini. × 280.
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