Structural Characterization of a Rat Acinar Cell Tumor

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ABSTRACT A transplantable acinar cell tumor of the rat pancreas has been examined by light and electron microscopy. The tumor cells, though highly cytodifferentiated and characterized by the presence of abundant rough-surfaced endoplasmic reticulum, elements of the Golgi complex, and zymogen granules, undergo mitosis in a manner similar to that seen in the developing pancreas. Cells in the parenchyma of the tumor grow as disarrayed cords and sheets, are randomly oriented with respect to each other, and do not form acinar structures. However, when in contact with the adventitial surface of blood vessels, the tumor cells palisade and form a polarized layer of cells with their zymogen granule-rich poles oriented away from the vessel lumen. Only in this area of the tumor is a basal lamina present that underlies the basal plasmalemma of the reoriented epithelial cells. Freeze-fracture electron microscopy of tumor cells in the parenchyma shows extensive disruption of tight junctions whose sealing strands are randomly distributed over the entire plasmalemma. Gap junctions are infrequent and when present are often enclosed by tight-junctional strands. Intramembrane particles are randomly distributed over the cell surface. Both the absence of basal lamina and derangement of the junctional complexes may account in part for the altered morphogenesis of this tumor.

The pancreatic acinar cell has for many years been used in the study of the biosynthesis, packaging, and discharge of secretory proteins. Though many of the steps leading to the discharge of digestive enzymes and proenzymes have been identified (1, 2), the details of controls involved in the release mechanism are still obscure.

The role of tissue organization and its influence upon cell function has been under consideration in our laboratory (3–5). One approach to this problem has been to examine the developing rat pancreas in an attempt to correlate the concomitant events of histogenesis and cytodifferentiation with secretagogue response (6). In this paper we characterize morphologically a pancreatic acinar cell tumor, first described by Reddy and Rao (7, 8), that shows a high level of cytodifferentiation without organization into acinar structures. This system, therefore, provides an opportunity to study the relationship between epithelial organization and the stimulated release of secretory proteins. We report here on the morphologic features of the tumor obtained from passages 14 through 20 using light and electron microscopy and freeze-fracture techniques. With this morphologic baseline, we describe in the subsequent papers of this series the functional and biochemical properties of the tumor cells. Portions of this study have been published in abstract form (9).

MATERIALS AND METHODS

Tumor Transplantation

Fischer 344 Sprague-Dawley rats bearing tumors in the tenth passage were the generous gift of Drs. J. K. Reddy and M. S. Rao of Northwestern University. The tumors were passaged in weanling Sprague-Dawley Fischer 344 rats (Harlan-Sprague Dawley, Madison, WI) and the results presented here were obtained from passages 14 through 20. For transplantation, rats were anesthetized with Metofane (Pittman-Moore, Washington Crossing, NJ), and 1- to 2-mm pieces of tumor were implanted under sterile conditions subcutaneously in the inguinal region. For intraperitoneal or intrapancreatic transplantation, tumor fragments were suspended in Dulbecco's modified minimal essential medium (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) and passed through a 21- or 26-gauge needle to produce small clumps of cells before inoculation.

Techniques for Light and Electron Microscopy

Tumor fragments for routine light microscopy were fixed with 10% formaldehyde in 0.1 M Na cacodylate (pH 7.4). Paraffin embedding was carried out by standard procedures. Sections 4–5 μm in thickness were stained with hematoxylin-eosin, PAS, or Jones silver-methenamine.

Tissue for embedment in Epon-Araldite was fixed in 2% glutaraldehyde in 0.1

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M Na cacodylate (pH 7.4) and then postfixed in 1% osmium tetroxide in the same buffer followed by in-block staining with 0.5% magnesium uranyl acetate in 0.9% NaCl. Thin sections were doubly stained with uranyl acetate and lead citrate.

Freeze-fracturing of tumor tissue was carried out as follows. Pieces of freshly excised tumor parenchyma were minced in 2% glutaraldehyde in 0.1 M Na cacodylate (pH 7.4) and transferred to fresh fixative for a total of 30 min. The tissue was rinsed with 0.1 M Na cacodylate and slowly infiltrated with glycerol by addition of an equal volume of 60% glycerol over 30 min. Baker's yeast was also infiltrated with 30% glycerol, and a small pellet of the yeast was placed on a gold support disk to provide a matrix into which pieces of tumor were embedded. The material on the disks was frozen in liquid Freon 12 cooled to liquid N_2 temperature and fractured at −110°C in a Balzers BAF-300 apparatus equipped with platinum and carbon guns (Balzers, Hudson, NH). The replicas were cleaned in chlorine bleach followed by chromic acid and examined in a Philips 301 electron microscope.

Immunocytochemistry

Antibodies to normal rat pancreatic secretory proteins were raised as follows. Pancreatic lobules were prepared from the glands of normal Fischer 344 rats and stimulated in vitro with 10^{-5} M carbamylcholine according to Scheele (10) except that BSA and SBTI were omitted from the medium that consisted of fortified KRH (11). Secretion released into the medium was subjected to high-speed centrifugation (100,000 g_{avg} for 30 min) in order to remove particulate matter. The secretory proteins thus obtained were mixed 1:1 with complete Freund's adjuvant and injected subcutaneously into the back and hind legs of New Zealand white rabbits. The procedure was repeated three times at two-week intervals prior to collection of antiserum.

The polyspecific antiserum formed precipitin lines against rat secretory proteins and soluble extracts of pancreatic homogenates but did not cross-react with rat serum proteins. IgG from rabbit serum was purified by $(NH_4)_2SO_4$ precipitation followed by DEAE-Sephadex (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, NJ) chromatography (12).

For immunocytochemical localization of secretory proteins, normal Fischer 344 rat pancreas or fragments of tumor tissue were fixed for 3 h at room temperature in 4% formaldehyde/0.1% glutaraldehyde in 0.1 M Na cacodylate, pH 7.4. Osmication was omitted and the tissues were dehydrated and embedded in Epon. 1-μm thick sections were cut on a Sorvall-MT2B microtome (DuPont Instruments, DuPont Co., Newtown, CT) and mounted on glass slides. Appropriate dilutions (50–100-fold) of antisecretory protein antibodies or preimmune serum were applied to etched sections (13) preconditioned with 1% BSA in PBS. First-step antibodies were detected using rhodamine-conjugated goat anti-rabbit IgG (Cappel Laboratories, Inc., Cochranville, PA). Sections were viewed under epifluorescent illumination in a Zeiss Photomicroscope II (Carl Zeiss Inc., New York, NY). Micrographs were taken on Kodak Tri-X film.

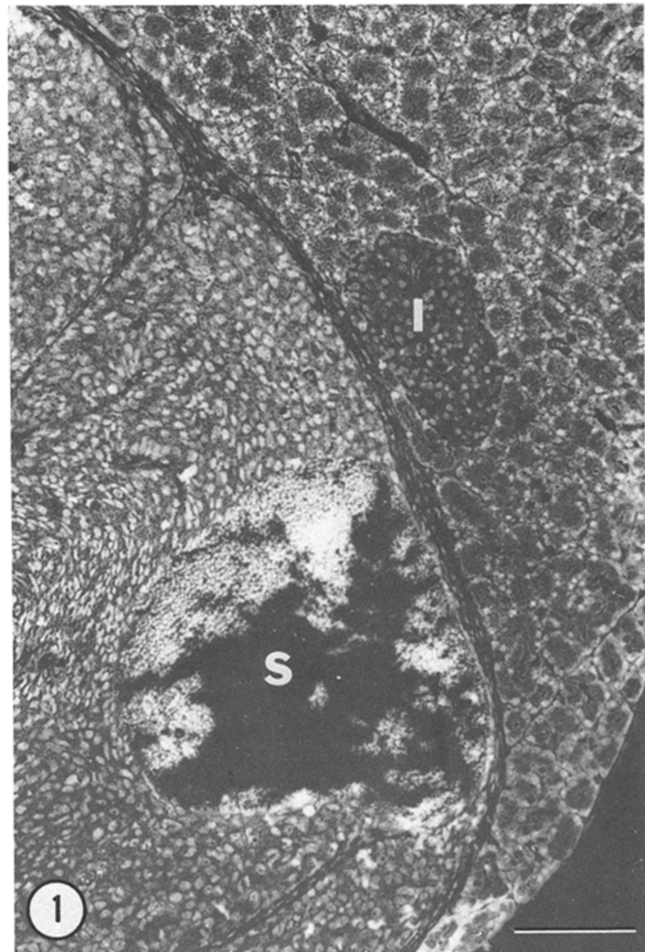


FIGURE 1 Low-magnification view of pancreatic tumor and adjacent normal pancreas from a pancreas inoculated with tumor fragments. The lower left part of the field shows sheets and cords of acinar tumor cells separated from normal pancreas and from an islet of Langerhans (*I*) by a connective tissue capsule. A large sinusoid containing red blood cells is indicated (*S*). Paraffin section stained with hematoxylin and eosin. Bar, 100 μm. × 160.

RESULTS

Tumor Appearance

Acinar cell tumors were propagated by serial transplantation in the peritoneal cavity or subcutaneous tissue, or after direct injection into the pancreas. Regardless of the site of tumor inoculation, the neoplasm always appeared as an encapsulated, soft gray-white mass with extensive vascularization.

The tumor parenchyma consisted of cords of small cuboidal or columnar cells with prominent nuclei (Fig. 1) and frequent mitotic figures regardless of the number of passages, size of tumor, or site of growth. Tissues embedded in paraffin and stained with PAS showed an absence of mucus-containing cells, which are characteristic of ductal carcinomas of the human pancreas (14).

Whereas the cells in the parenchyma of the tumor were randomly oriented, those located adjacent to capillaries and small blood vessels formed a palisaded layer consisting of tumor cells whose nuclei were adjacent to the blood vessel wall and whose secretory granule-rich fields were oriented oppositely (Fig. 2). Because the granule fields were in register around the blood vessel, the tumor cells appeared to be organized in the form of an epithelial sheet (Fig. 2*b* and 3). Concomitantly, we have observed by transmission electron microscopy that the polarized tumor cells adjacent to the vasculature rest upon a basal lamina, whereas no morphologic basal lamina is seen in the tumor parenchyma (Fig. 3). Furthermore, histochemical stains such as PAS and silver methenamine applied to sections of paraffin-embedded tumor confirmed the presence of basement membrane only around the blood vessels (data not shown). This observation has been extended by immunocytochemical localization of basal lamina components, such as fibronectin (3), laminin, and type IV collagen, in the areas surrounding tumor vasculature, but not around the cells in the tumor parenchyma as reported elsewhere (15).

Intracellular Structures

As already observed at the light microscopic level (Fig. 2), the majority of the cells in both the parenchyma and perivascular portions of the tumor possessed secretory granules, which indicates a high level of cytodifferentiation. By electron microscopy, the cells of the tumor contained numerous parallel profiles of rough-surfaced endoplasmic reticulum (RER) that occupy the majority of the cytoplasm. In contrast to their distribution on the RER in normal rat acinar cells, attached ribosomes were arranged in groups frequently separated by areas of ribosome-free membrane similar to the situation in

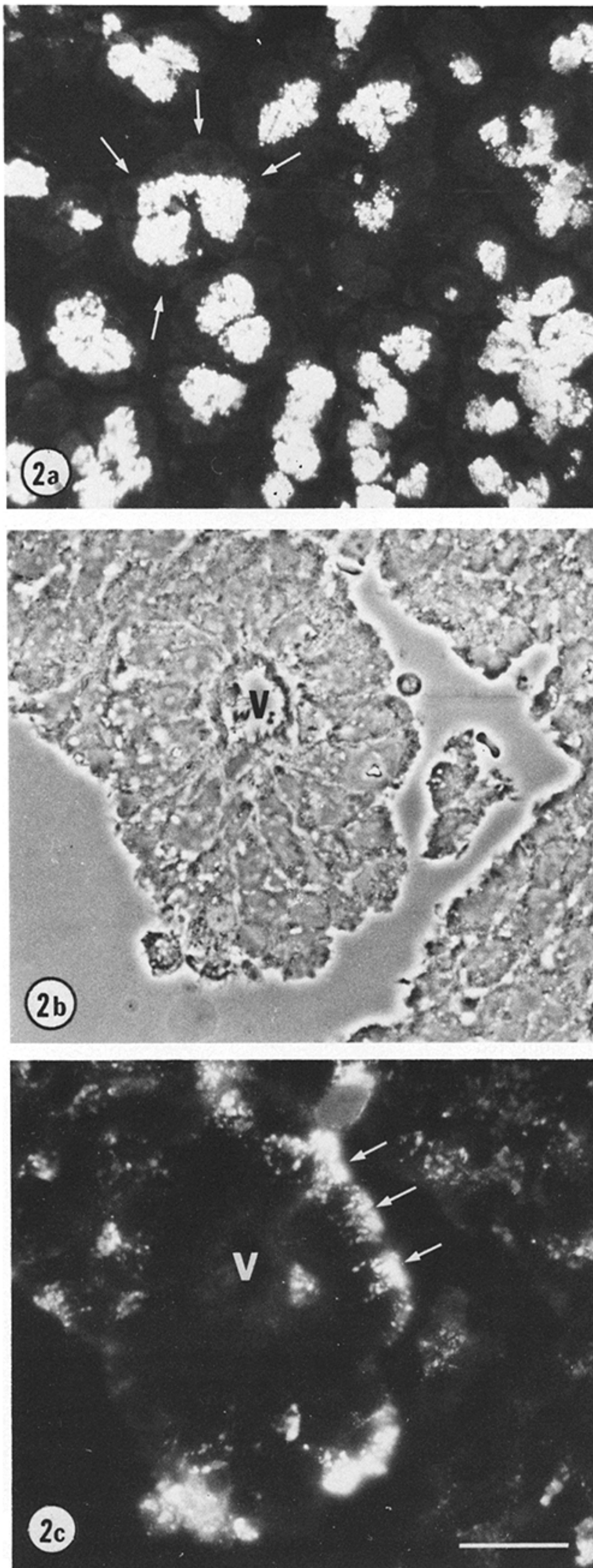


FIGURE 2 Immunofluorescent localization of secretory proteins on 1-μm sections of Epon-embedded and etched sections of normal rat pancreas (*a*) and of the acinar cell tumor (*c*). The corresponding phase-contrast view of (*c*) is shown in (*b*). *V* is a blood vessel in the tumor surrounded by polarized tumor epithelial cells whose granule-rich fields are oriented abluminally (arrows). Application to sections of preimmune serum, or of rhodaminated goat anti-rabbit IgG alone, consistently produced negative images which are not shown. Bar, 2 μm. × 850.

fetal liver (16). The tumor cells also contained well-developed Golgi complexes, whose vacuoles were distended with proteinaceous material. Numerous coated vesicles were seen in the region of the Golgi complex and extended into the secretory granule field (Fig. 4).

In general, the secretory granules were located as a group within a defined cytoplasmic zone that was separated from the nucleus by the Golgi complex. In this sense, the cells of tumor parenchyma exhibited cellular polarity even though they were not regularly organized into acini. As noted above (Figs. 2 and 3), the acinar cells in juxtaposition to the vasculature clearly showed polarized distribution of secretory granules on the abluminal side of blood vessels.

The majority of the cells in the parenchyma of the tumor contained typical secretory granules which, although usually spherical, occasionally displayed aberrant shapes (Fig. 4). The spherical granules, usually 1–1.5 μm in diameter, on occasion were as small as 0.5 μm and, in contrast with their appearance in normal acinar cells, varied in size within individual cells as well as between adjacent cells. All granules contained a densely packed content, surrounded by a typical bilayer; no endocrine type granules were seen. The tumor cells undergoing cell division (Fig. 3*a*) also contained secretory granules, which appeared to be approximately equally distributed at the two division poles of the cell.

All secretory granules in tumor cells as well as those in normal pancreatic acinar cells reacted positively with polyspecific antisecretory protein antibodies (Fig. 2*a* and *c*), indicating that the acinar tumor cells contain secretory proteins. Hansen and co-workers (17) have also reported the presence of secretory proteins in acinar tumor cell granules, but based their conclusion on the use of antibodies raised against bovine and porcine secretory proteins.

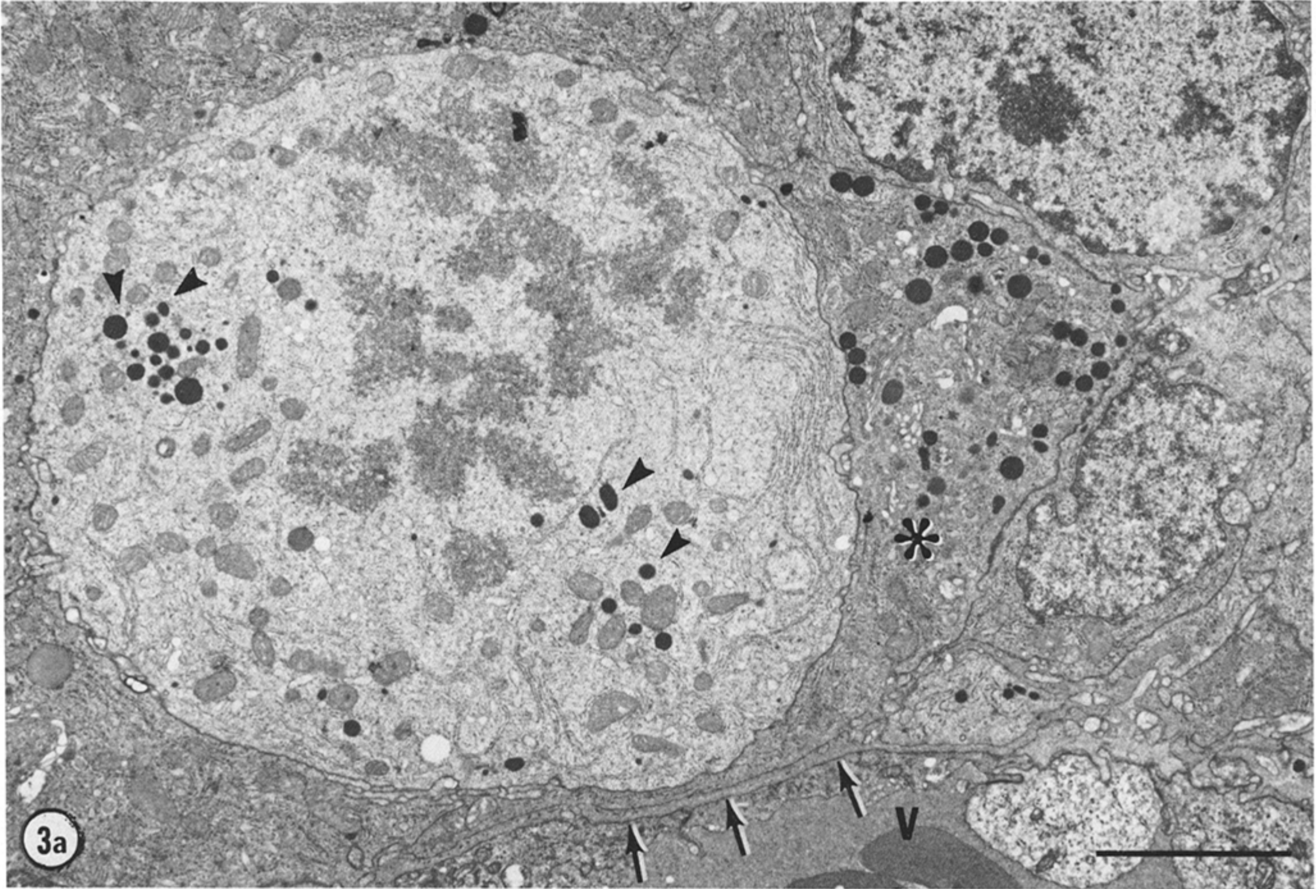
The nuclei of the acinar tumor cells were irregularly shaped with deep invaginations (Figs. 3*b* and 4) in contrast to the oval-shaped nuclei of normal acinar cells. Nucleoli were large and possessed a distinct granular appearance. Heterochromatin was prominent and distributed mainly along the nuclear membrane.

The other organelles of the cell were not unusual in their morphological features.

Plasmalemma and Junctional Complexes

Electron microscopy of the tumor parenchyma showed that the plasmalemma of the tumor cells possessed extensive convolutions of their cell surface and that these convolutions frequently interdigitated more extensively than was normally seen in the lateral intercellular zones between normal acinar cells. Occasional desmosomes were noted between the tumor cells although recognizable adhering zonules, tight junctions, and gap junctions were apparently absent between cells. To better examine the nature and distribution of junctional complexes in the tumor parenchyma, we employed freeze-fracture electron microscopy.

As shown in Fig. 5, tumor cells, identified by the presence of secretory granules, possessed broken and incomplete tight-junctional strands that were randomly disposed over the cell surface and did not form complete sealing belts. Gap junctional particles, when identifiable, were usually enclosed within cir-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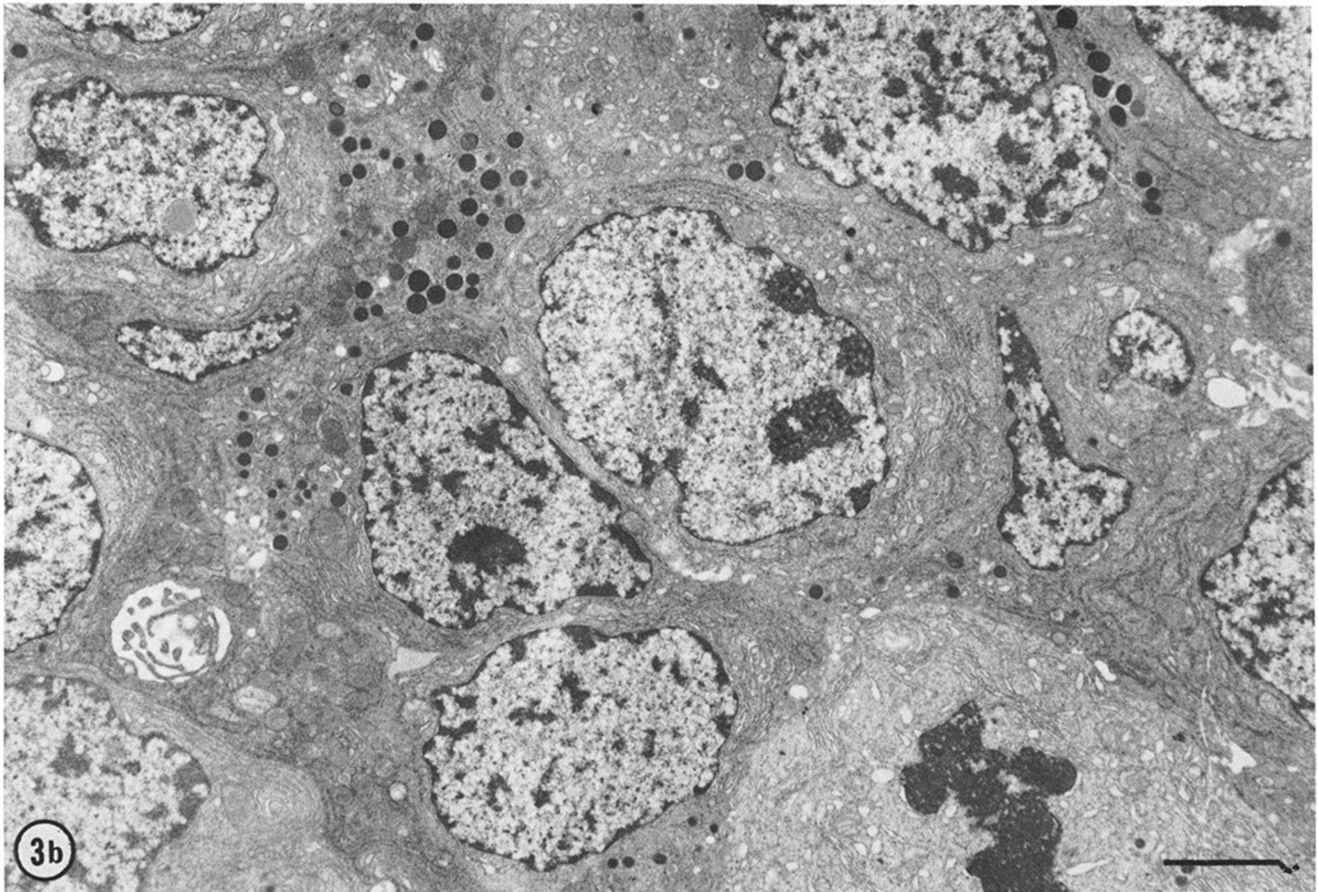
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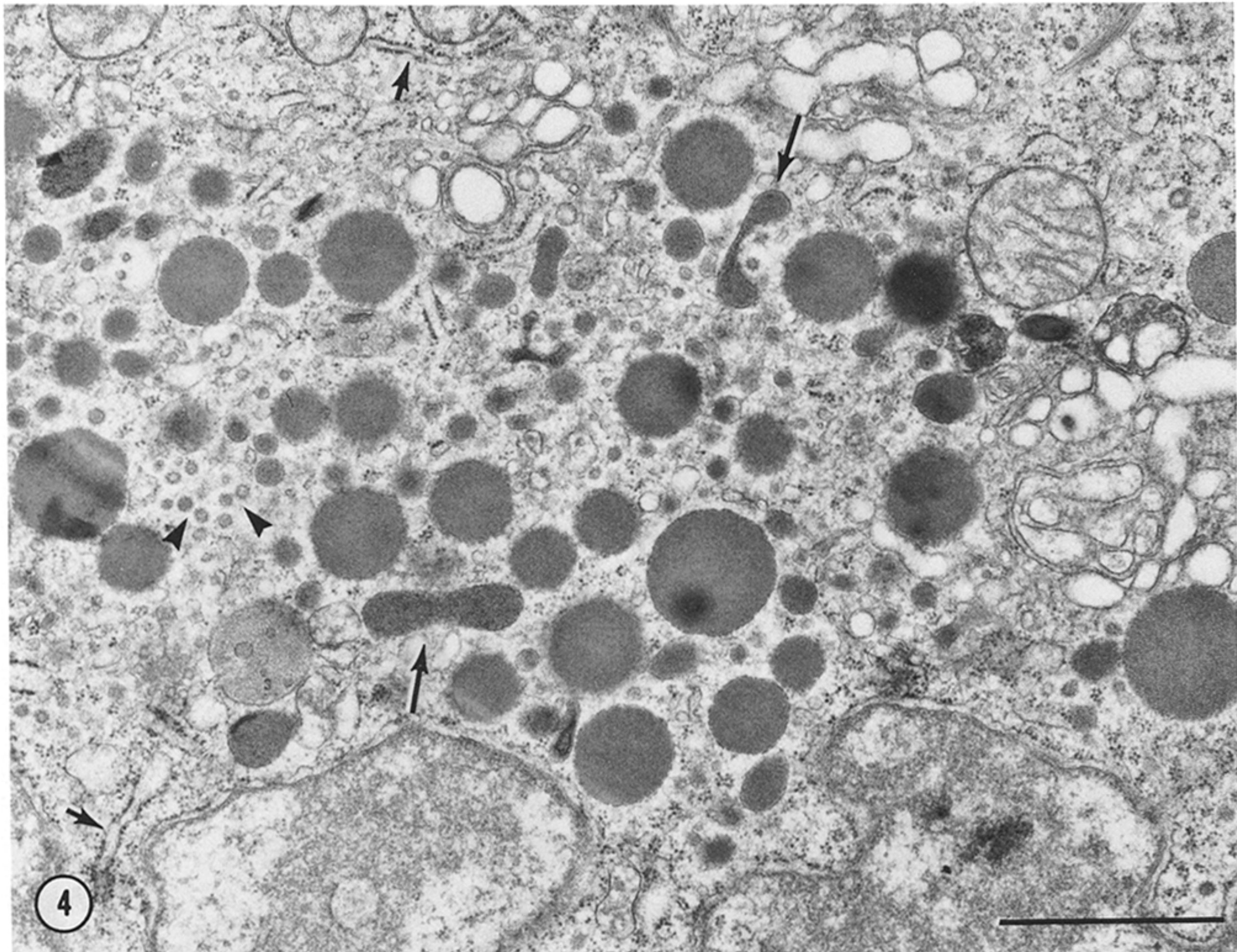

FIGURE 4 Electron micrograph of the juxtanuclear region of an acinar tumor cell. Note that whereas the majority of secretory granules is spherical, many are discoidal and irregularly shaped (long arrows). Numerous coated vesicles are seen in this field (arrowheads). RER profiles showing clusters of ribosomes separated by ribosome-free membrane segments are indicated by short arrows. Portions of an irregularly shaped, deeply indented nucleus are seen in the bottom of the figure. Bar, 1 μm. × 31,000.

cular arrangements of tight-junction strands (Fig. 5, inset).

Intramembrane particles were randomly distributed over the entire cell surface except that they were absent from small circular zones which may correspond to pinocytotic pits. Similar observations have been reported by Pauli and Reddy (18).

DISCUSSION

In this paper we describe the morphological features of an acinar cell tumor that was induced in rats by dietary treatment with nafenopin (7). This tumor has now undergone more than 20 passages in rats without obvious changes in morphology and growth properties except that the gross metastatic lesions found with the primary tumor (7, 8) are no longer seen.

The rat acinar cell tumor not only serves as a model for the study of some of the properties of human pancreatic acinar cell neoplasms (14, 19), but also represents an aberration of development in which cytodifferentiation appears to proceed without histogenesis. The tumor offers, therefore, an opportunity to examine the relationship between cytodifferentiation and histogenesis and the cellular processing and hormonal discharge of secretory proteins.

Our morphologic findings indicate that whereas cells of the parenchyma of the tumor do not organize into acinar structures and are randomly oriented with respect to each other, those tumor cells that abut on blood vessels undergo polarization and form oriented epithelial layers in which the nuclei of the cells face the vessel wall and the secretory granule-rich areas orient in the opposite direction. Although the mechanisms involved in the reorientation and polarization of tumor cells are yet to be fully defined, it is possible that basal lamina

FIGURE 3 Low magnification electron micrographs of pancreatic tumor cells adjacent to a blood vessel (*a*) or located in the tumor parenchyma (*b*). In (*a*), a blood vessel (*V*) is separated from overlying tumor cells by basal lamina (arrows). Note the presence of secretory granules (arrowheads) in a lightly stained tumor cell in metaphase. The cell indicated by the asterisk shows secretory granules located in the cytoplasmic pole opposite the vascular lumen. In (*b*), the apolar arrangement of parenchymal tumor cells containing secretory granules is seen, and a dividing tumor cell is present in the lower part of the figure. Note the irregularly shaped nuclei and prominent nucleoli in the tumor cells. Bars, 4 μm. *a*, × 6,500; *b*, × 4,400.

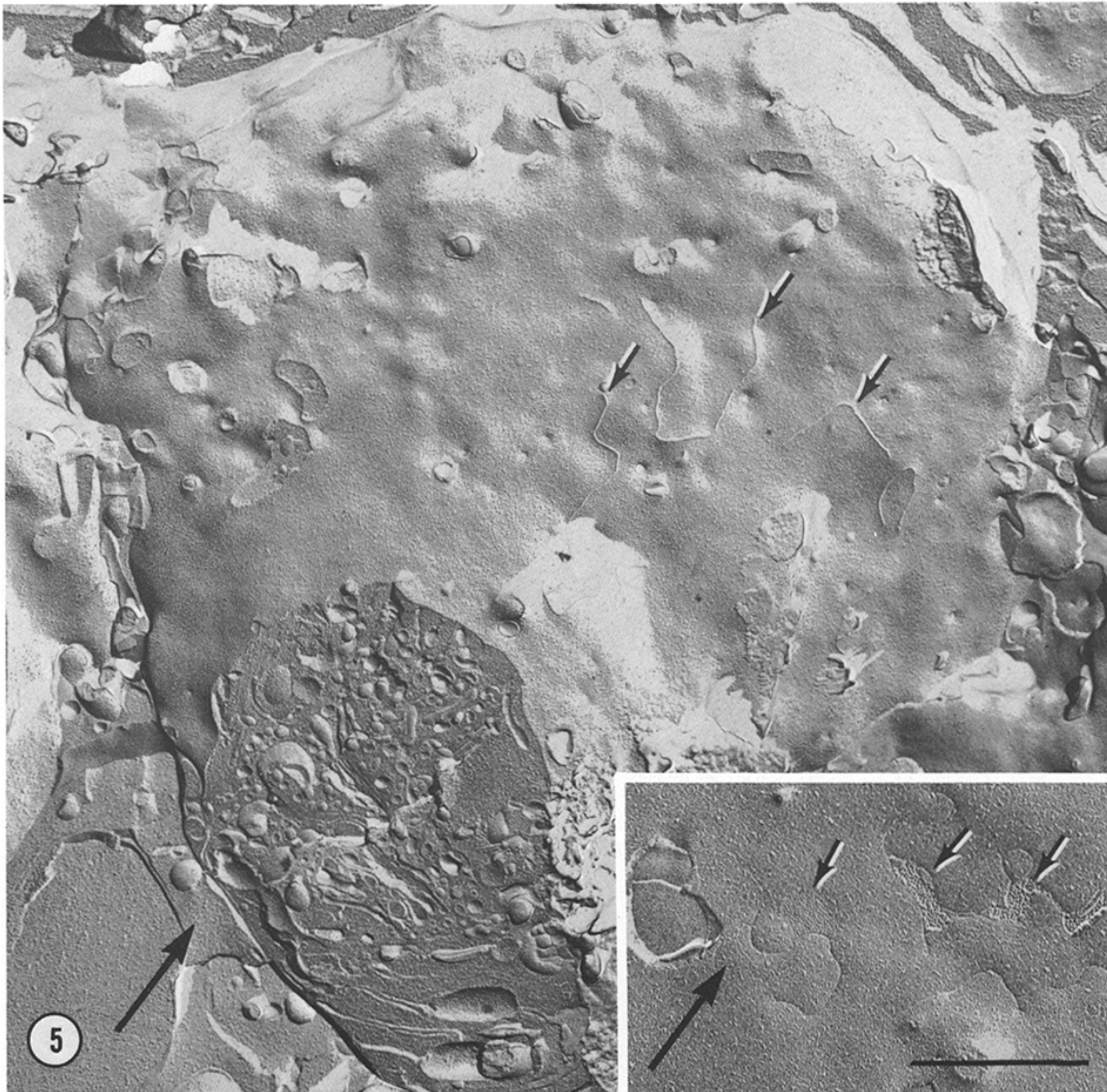

FIGURE 5 Freeze-fracture view of an acinar tumor cell. Note the individual tight-junction strands randomly distributed on the P-face of the plasmalemma (arrows). Intramembrane particles are randomly distributed over the plasmalemma. The insert shows the E-face of the tumor cell plasmalemma in which gap-junction particles (arrows) appear in close association with tight-junction strands. Large arrows indicate direction of shadowing. Bar, 1 μm. × 26,000.

components such as laminin, type IV collagen, or fibronectin (3, 15) may serve as organizing sites to align acinar tumor cells although we cannot completely rule out the possibility that nutritional factors from the circulation may be involved in the epithelial-like organization of the neoplastic cells.

The acinar cells of the tumor are characterized by abundant profiles of the RER that are typical of cells committed to the rapid production of exportable proteins (1, 2). Indeed, the studies of Warren and Reddy (20) as well as those from our laboratory (9) have shown that the acinar tumor cells are capable of synthesizing, packaging, and discharging secretory proteins. In comparison to adult pancreatic acinar cells, however, the RER profiles are less densely packed in the cytoplasm and the distribution of ribosomes along the RER membranes is reminiscent of that seen in cells rapidly producing large amounts of cell membranes (e.g., fetal liver) (16).

Although the majority of acinar tumor cells contains recognizable secretory granules, many of which are similar in size and shape to those of normal acinar cells, we frequently see cells with granules of abnormal sizes and shapes. It is of interest to note that some of the cells have few, if any, secretory granules. Reddy et al. (21) have proposed that this population of cell results from "retrodifferentiation," whereby the population of new cells developing within the tumor possesses a less differentiated state than their progenitors. It is of importance to note in this context that many of the cells in the tumor that are undergoing mitosis contain recognizable secretory granules. If the rate of cell division exceeded the ability of the daughter cells to assemble organelles involved in secretory protein processing, then by a simple dilution process one could account for a population of secretory granule-poor cells.

A striking feature of this neoplastic acinar cell is the disruption of tight-junctional elements, which results in their distribution as short broken strands and incomplete zonulae occlu-

dentes randomly distributed over the plasmalemma. Gap junctions are also few in number and when present are surrounded abnormally by tight-junctional strands. A similar derangement of junctional complexes has also been observed in a canine acinar cell carcinoma (22). Loewenstein (23) has proposed that a correlation may exist between the presence of aberrant gap junctions and the absence of cell communication in neoplastic cells. The cause of the aberration of junctional complexes is currently unknown although one possibility, based upon the observations of other investigators (24, 25), is that cells undergoing rapid proliferation show incomplete assembly of junctional complexes. Alternatively, because disruption of junctional complexes between acinar cells can be induced in the normal pancreas by exposure to Ca^{2+} chelators (26-28) or proteolytic enzymes (11), it is possible that local alterations in Ca^{2+} metabolism or protease release by the tumor cells may lead to similar alterations.

In summary, we have described a pancreatic acinar cell tumor whose cells show changes in their surfaces as indicated both by disrupted junctional complexes and by absence of cell-cell polarity. Within the tumor cells we have observed granules that are abnormal in their shape, size, and location. How these alterations may influence cell function, particularly with respect to hormone-receptor interaction and hormonally induced discharge of secretory proteins, is the subject of the following paper.

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