Neoplastic Disorganization of Pancreatic Epithelial Cell–Cell Relations

Role of Basement Membrane

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The authors have analyzed the structural relations of a nonmetastatic rat pancreatic acinar carcinoma and contrasted them with those of normal exocrine pancreas in order to better define the role of basement membrane (BM) in early stages of neoplastic disorganization. These studies showed that normal acinar cells rested on continuous BM (containing laminin, heparan sulfate proteoglycan, and Type IV and V collagens) and displayed a polarized distribution of intracellular organelles, cytoskeletal assemblies (concentration of actin within terminal web), and distinct membrane domains (apical leucine aminopeptidase). In contrast, the parenchyma of the pancreatic acinar carcinoma was free of all BM components except for a discontinuous array of laminin. In these regions, acinar tumor cells appeared randomly oriented, displayed actin in uniform cortical distributions, and lost membrane polarity. However, when tumor cells contacted mesenchymally derived connective tissue along tumor capsule and vascular adventitia, they accumulated intact BM and reoriented in a manner reminiscent of normal pancreas. Tumor cell reorganization was observed in the absence of formation of full junctional complexes or normally polarized membrane domains, although leucine aminopeptidase appeared to be excluded from regions of tumor cell surfaces that were in direct contact with BM. The loss of normal epithelial cell–cell arrangements that is the hallmark of early stages of tumor formation could therefore result from failure to match increases in cell number with commensurate BM extension. (Am J Pathol 1985, 121:248–260)

TUMOR CELL penetration through basement membrane (BM) with associated invasion into neighboring connective tissue is commonly used as a criterion for designating a carcinoma as malignant. In the past, BM was viewed as a host connective tissue barrier to malignant invasion, and its disruption was seen as an end result of neoplastic disorganization. However, more recent studies show that BM is produced and maintained by normal epithelia[1,2] and that its metabolism can be altered as a result of neoplastic transformation.[3–5] BM serves as an inductive interface and mediates morphogenetic interactions between neighboring epithelial and mesenchymal tissues during normal development.[1,2,6] Its presence is also required for the maintenance of tissue morphology throughout adult life.[7] Furthermore, loss of BM integrity[1] or interference with its deposition[8,9] during morphogenesis results in disorganization of epithelial form.

We have previously published a preliminary description of a transplantable tumor of rat exocrine pancreas that reorganizes and mimics normal polarized epithelial form when in contact with BM along its vasculature *in vivo*.[5] On the basis of this preliminary observation, we suggested that loss of BM may be involved in early stages of neoplastic disorganization of normal tissue architecture. The rat pancreatic epithelial tumor is comprised of cytologically differentiated acinar cells that have been previously characterized biochemically.[10–14] In the present investigation, we localized different components of the extracellular matrix (lami-

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nin, fibronectin, heparan sulfate proteoglycan, and Type I, III, IV, and V collagens), cell surface (leucine aminopeptidase), and cytoskeleton (F-actin) in both the pancreatic acinar carcinoma and normal exocrine pancreas in order to better characterize the relation between BM and neoplastic disorganization. These studies demonstrated a direct correlation between absence of intact BM and disorganization of normal cell–cell relations, intermixing of specialized membrane domains, and loss of cytoskeletal polarity within the parenchyma of the acinar cell tumor. In addition, tumor cells accumulated intact BM and reoriented along their interface with connective tissue capsule as well as along vascular adventitia. We have recently found that contact with exogenous BM is also sufficient to spatially organize and reorient pancreatic acinar tumor cells *in vitro*.[15] Thus, neoplastic disorganization of normal epithelial cell–cell relations may be the result of failure to maintain normal coordination between cell proliferation and BM extension.

Materials and Methods

Experimental Systems

The pancreatic acinar cell tumor was grown by sequential passage in male F344 Sprague–Dawley rats (Harlan–Sprague Dawley, Madison, Wis) as previously described.[5] All tumors used in this study were between the 18th and 39th passage and 1 to 5 mm in diameter. Although the pancreatic acinar carcinoma was originally discovered with associated metastatic foci in nafenopin-treated rats,[10] in all subsequent passages it has not been metastatic.

Light and Electron Microscopy

Normal and tumor tissue was fixed by perfusion with 1% glutaraldehyde/3% formaldehyde in phosphate-buffered saline (PBS) (pH 7.4). Fixed specimens were osmicated, stained *en bloc* with uranyl acetate, and embedded in Epon–Araldite for transmission electron microscopy. Thick sections (0.5 μ) were stained with 1% toluidine blue in 1% sodium borate. Thin sections (80 nm) were stained with uranyl acetate and lead citrate and photographed on a Siemens 101 electron microscope.

Fluorescence Microscopy

Tissue was fixed by perfusion with 0.1% glutaraldehyde/3% formaldehyde in PBS. Tissue specimens were removed, immersed in fixative for 1 hour and transferred into 2.3 M sucrose in 0.1 M sodium monophosphate buffer (pH 7.4). Semithin (0.5 μ) frozen sections of sucrose-impregnated tissue were cut at −70 C on a Sorvall MT-2B ultramicrotome with a cryotomy attachment. Methods for immunostaining and preparation of our affinity-purified rabbit antibodies to laminin, fibronectin, and Type I, III, IV, and V collagens have been previously described.[16–19] Antiserum to heparan sulfate proteoglycan and affinity-purified antibodies against leucine aminopeptidase were produced in rabbits and generously provided by Dr. G. Martin (National Institute of Dental Research)[20] and Dr. D. Louvard (Pasteur Institute),[21] respectively. Rhodaminated-aminophalloin was a gift of Dr. Th. Weiland (Max Planck Institute).[22] Rhodamine-conjugated goat immunoglobulins (Cappel, Malvern, Pa) were used for detection of the primary rabbit antibodies. Control ex-

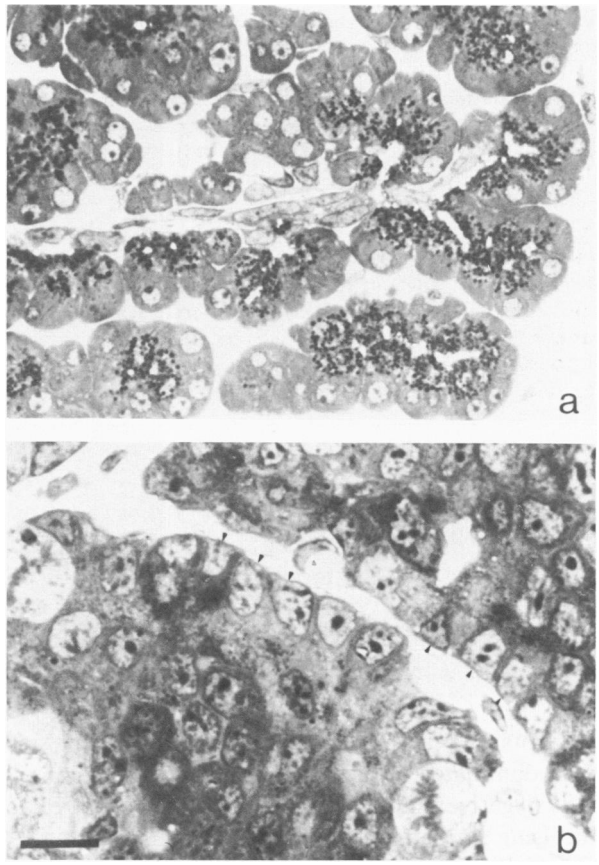

Figure 1—Light micrographs of normal pancreas and pancreatic acinar carcinoma. **a**—Individual epithelial cells were organized into acini within normal exocrine pancreas. **b**—In the parenchyma of the pancreatic tumor, individually anisotropic tumor cells were randomly oriented one next to another. However, acinar tumor cells palisaded in a regular fashion whenever they contacted connective tissue capsule or vascular adventitia along the tumor periphery. *Arrowheads* appear along the connective tissue interface with their tips abutting on basal surfaces of reoriented cells within two adjacent acinar tumor cell nests. Note mitotic figures along the tumor periphery and that there was an increase in cell and nuclear size of the tumor relative to normal pancreas. Both micrographs are at the same magnification. (Toluidine blue, bar = 20 μ; ×565)

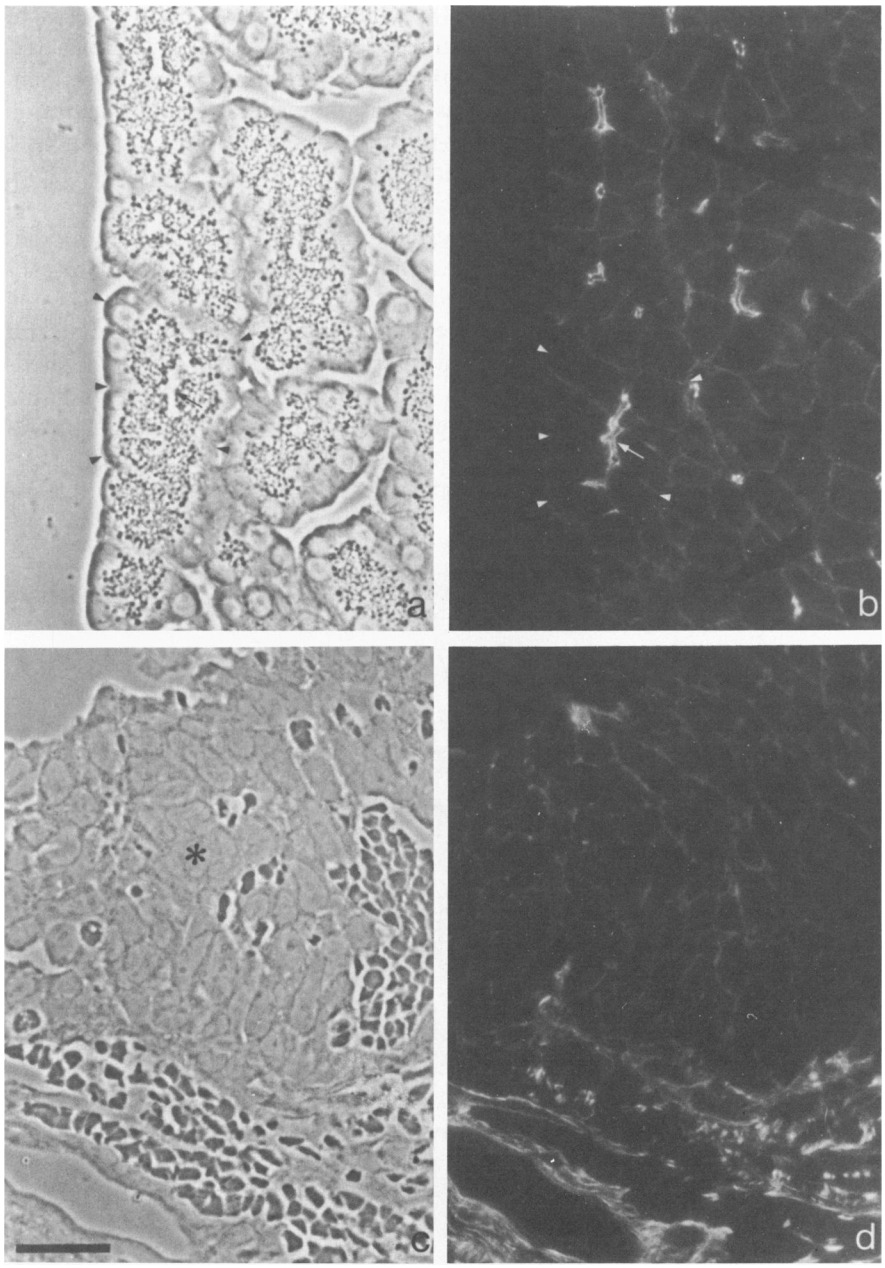

Figure 2—Phase-contrast (**a** and **c**) and corresponding fluorescence micrographs (**b** and **d**) of semithin tissue sections stained for F-actin. **a** and **b**—Normal pancreas. *Arrowheads* surround a single pancreatic acinus, and a *small arrow* points to its centroacinar lumen, which was outlined by intense fluorescent staining. Less intense linear staining also delineated the basolateral surfaces of individual acinar cells. **c** and **d**—Acinar cell tumor. For orientation, an *asterisk* appears over a cell within the disorganized tumor parenchyma in the phase-contrast view. F-actin staining appeared evenly surrounding individual acinar tumor cells. Capsular connective tissue cells also exhibited bright staining as seen across the bottom third of the view. Clusters of erythrocytes appeared both in parenchymatous and capsular regions. (Bar = 20 μ; ×625)

periments with preimmune rabbit serum in the first step or rhodaminated immunoglobulin alone were consistently negative. All images were recorded on Tri-X Pan film using similar times of exposure and printing. A Zeiss photomicroscope II equipped with phase contrast optics and an epifluorescence illuminator containing appropriate filters for rhodamine were utilized for these studies.

Results

Normal Pancreatic Epithelium

In normal exocrine pancreas, individual epithelial cells were consistently oriented one next to another and integrated into an acinar configuration (Figure 1a). Each acinar cell was typically polarized: its nucleus was located within the basal portion of the cell, and the Golgi complex and zymogen granules filled the apical region. Apical cell surfaces were highly specialized, lined by microvilli and limited by typical junctional complexes as previously described.[23] Fluorescently labeled phallotoxin also localized F-actin (a major cytoskeletal component) in a polarized pattern within normal pancreatic acinar cells (Figure 2a and b). Actin appeared throughout the entire acinar cell cortex but was preferentially concentrated beneath apical cell surfaces, where a

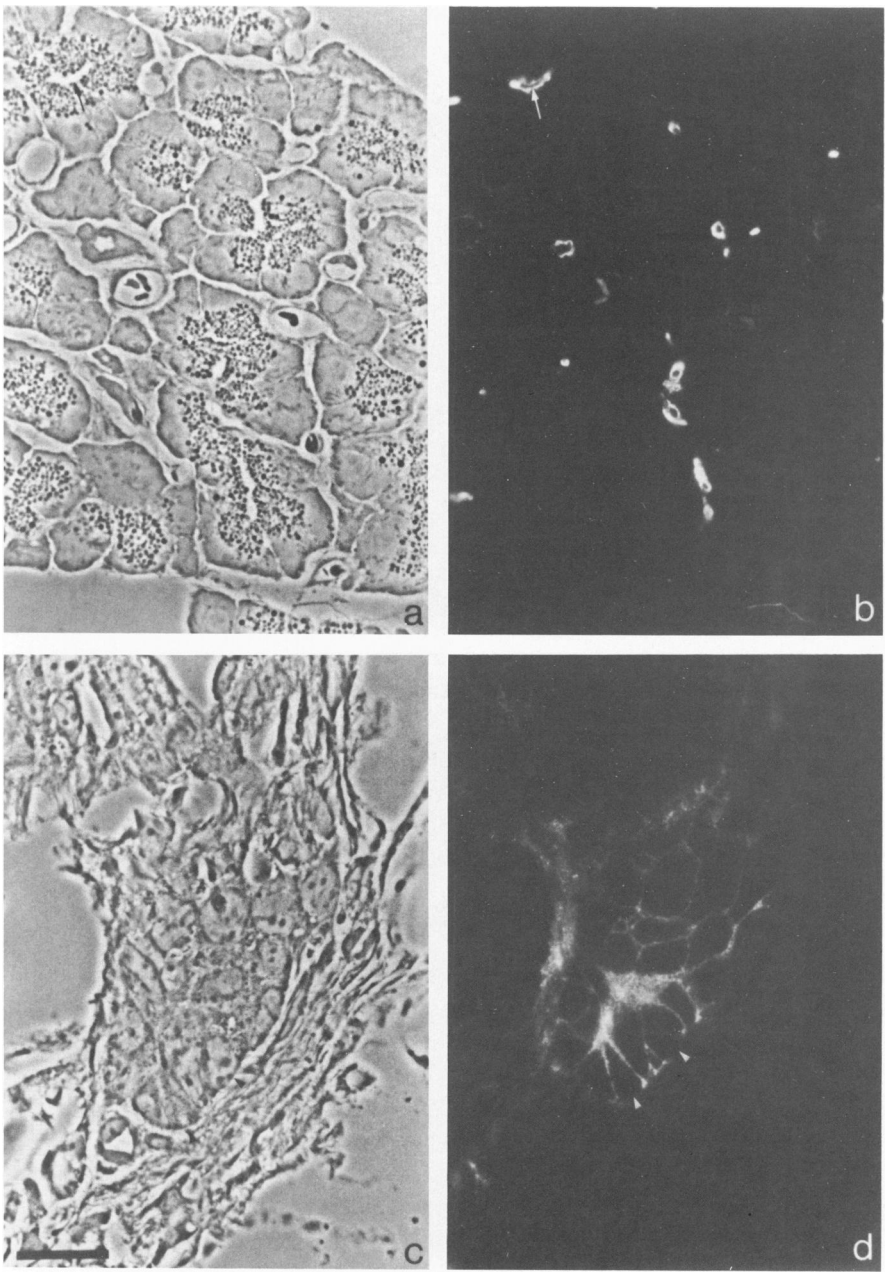

Figure 3—Phase-contrast (**a** and **c**) and corresponding fluorescence micrographs (**b** and **d**) of semithin tissue sections stained for leucine aminopeptidase. **a** and **b**—Normal pancreas. Staining clearly delineated every acinar lumen. A *small arrow* indicates one such lumen. **c** and **d**—Staining appeared abnormally surrounding entire cell surfaces within the central regions of this small nest of tumor cells. *Arrowheads* point to the basal surfaces of tumor cells, which were free of staining along their interface with surrounding connective tissue, although nonbasal surfaces of the same cells stained intensely. (Bar = 20 μ; ×625)

filamentous terminal web can be observed by electron microscopy. Normal pancreatic acinar cell membrane polarity was confirmed by the localization of leucine aminopeptidase, an intrinsic membrane constituent, exclusively within apical surfaces of acinar cells and along pancreatic ducts (Figure 3a and b).

The high resolution of semithin frozen sections enabled us to clearly identify the different BM components that comprised normal pancreatic BM. Continuous linear staining patterns of laminin (Figure 4a and b) and Type V collagen (Figure 5a and b) as well as Type IV collagen and heparan sulfate proteoglycan (not shown) were observed surrounding all acini and neighboring vascular structures, whereas acinar cells were themselves free of any intracellular staining. These linear patterns correspond directly to regions in which basal lamina is seen by electron microscopy. Interestingly, the intensity of Type V collagen staining appeared to be brighter within vascular BM. On the other hand, the distributions of interstitial collagens were clearly different from those of BM components. Staining of Type III collagen (Figure 6c and d) and Type I collagen (not shown) appeared in diffuse patterns which filled the interspaces between acini and vessels. In exocrine pancreas, fibronectin appeared primarily in a discontinuous, wispy pattern that was more characteristic of

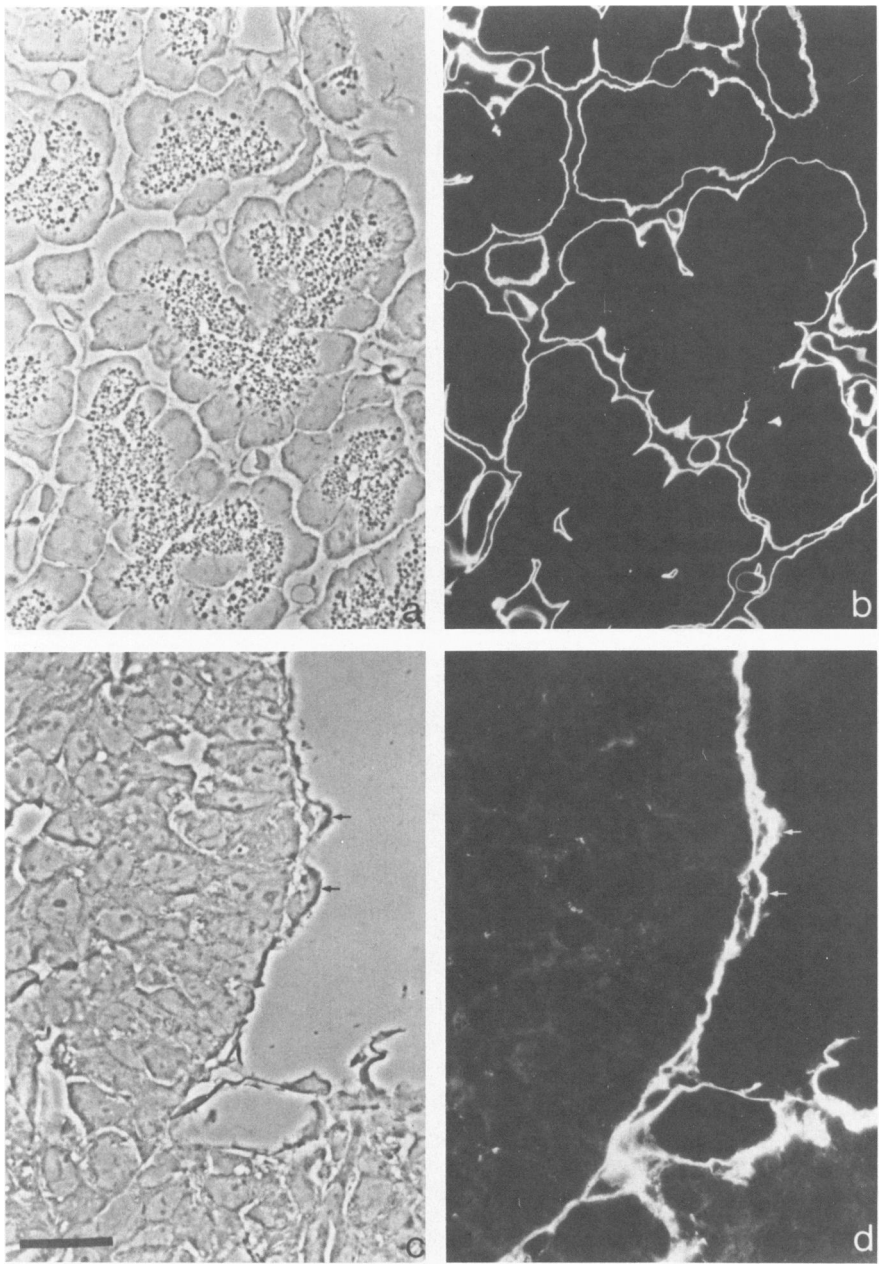

Figure 4—Phase-contrast (**a** and **c**) and corresponding fluorescence micrographs (**b** and **d**) of semithin tissue sectoins stained for laminin. **a** and **b**—Normal pancreas. Acinar cells were free of staining, although a linear staining pattern clearly delineated all acinar and vascular BM. **c** and **d**— Acinar cell tumor. A bright linear pattern of staining outlined the tumor-connective tissue interface, although only low levels of staining in amorphous and punctate patterns were observed within the tumor parenchyma. Note the bright staining surrounding two isolated connective tissue cells at the tumor periphery (*small arrows*). (Bar = 20 μ; × 625)

interstitial collagens than of other BM components (Figure 7a and b).

Pancreatic Acinar Cell Tumor

Neoplastic Disorganization of Normal Epithelial Relations

The pancreatic acinar carcinoma grew as a constantly expanding encapsulated mass. In contrast to normal pancreas, the tumor was characterized by a highly disorganized parenchyma with no evidence of obvious cell orientation (Figure 1b). Mitotic figures have been observed throughout the tumor, although they most frequently appeared along the tumor periphery near the connective tissue interface. Each individual acinar tumor cell was cytologically differentiated and anisotropic, in that it displayed a normal polarized orientation of nucleus, Golgi complex, and zymogen granules from one pole of the cell to the other (Figure 8). Yet the apex of one acinar tumor cell was often abnormally juxtaposed to the basal and/or apical pole of a neighbor. These cells did not display complete junctional complexes on their surfaces, although occasional desmosomes and rare small gap junctions have been observed.

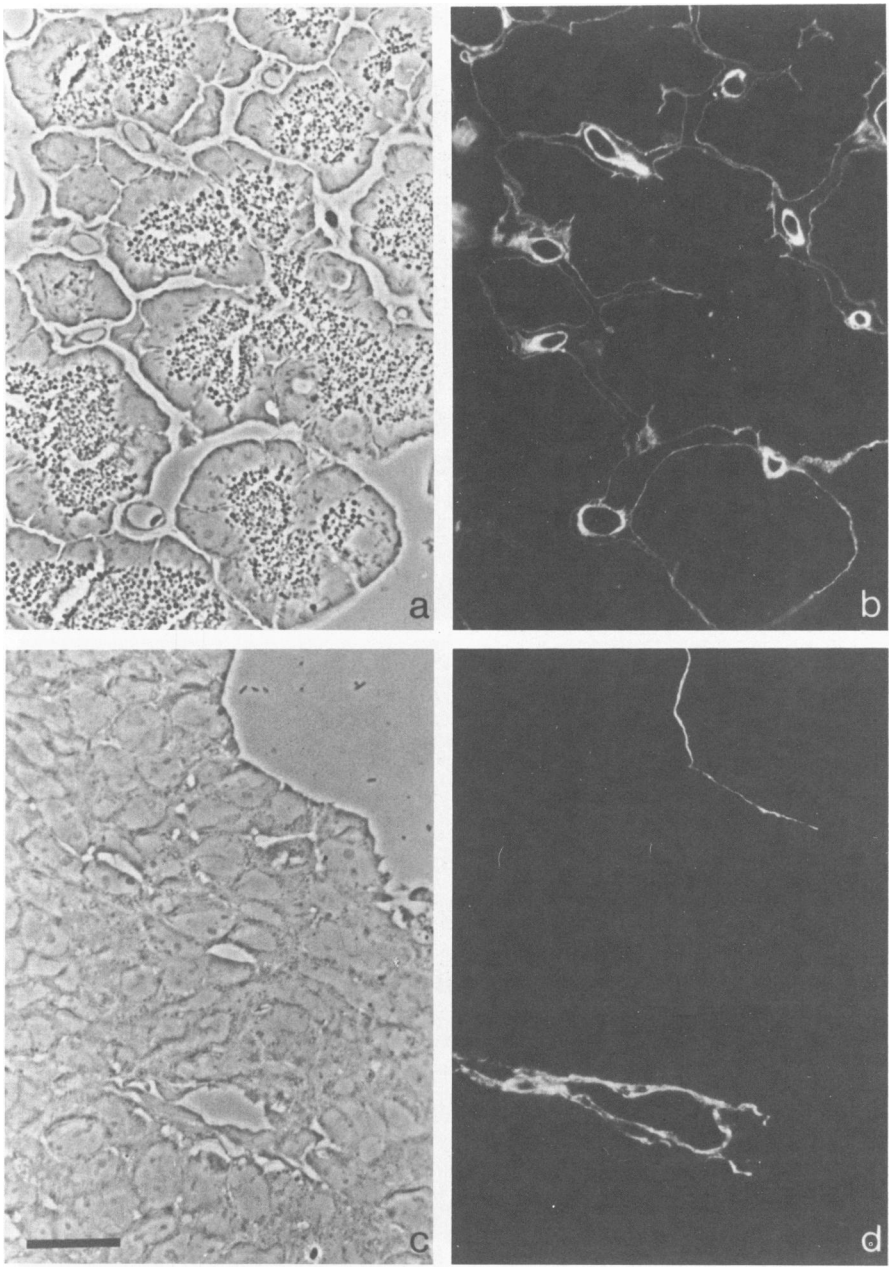

Figure 5—Phase-contrast (**a** and **c**) and corresponding fluorescence micrographs (**b** and **d**) of semithin tissue sections stained for Type V collagen. **a** and **b**—Normal pancreas. Staining delineated all acinar forms, although vascular BM stained more brightly. **c** and **d**—Acinar cell tumor. Staining appeared only in a linear form along the tumor periphery and surrounding a tumor vessel at the bottom of the view. (Bar = 20 μ; ×625)

As previously described, growth and cytodifferentiation are not incompatible within this epithelial tumor, because mitotic figures were seen in cells which contained zymogen granules.[12,13]

Cytoskeletal actin was not observed in polarized patterns within disorganized acinar tumor cells. Rather, actin staining appeared to evenly outline the cortical regions of individual cells (Figure 2c and d). This staining pattern confirmed the absence of organized acinar structures or normal cytoskeletal polarization. Disorganized acinar tumor cells also did not possess organized junctional complexes, apical microvilli, or distinct membrane domains. For instance, leucine aminopeptidase appeared in abnormal continuous patterns delineating the entire circumference of every cell within the tumor parenchyma (Figure 3c and d).

Epithelial Tumor Cell Reorganization

While tumor cells commonly grew piled one atop another within the tumor parenchyma, they palisaded in a regular fashion along the tumor periphery (Figure 1b). Electron microscopy verified that pancreatic acinar tumor cells displayed normal epithelial cell–cell relationships along their interface with mesenchymally derived

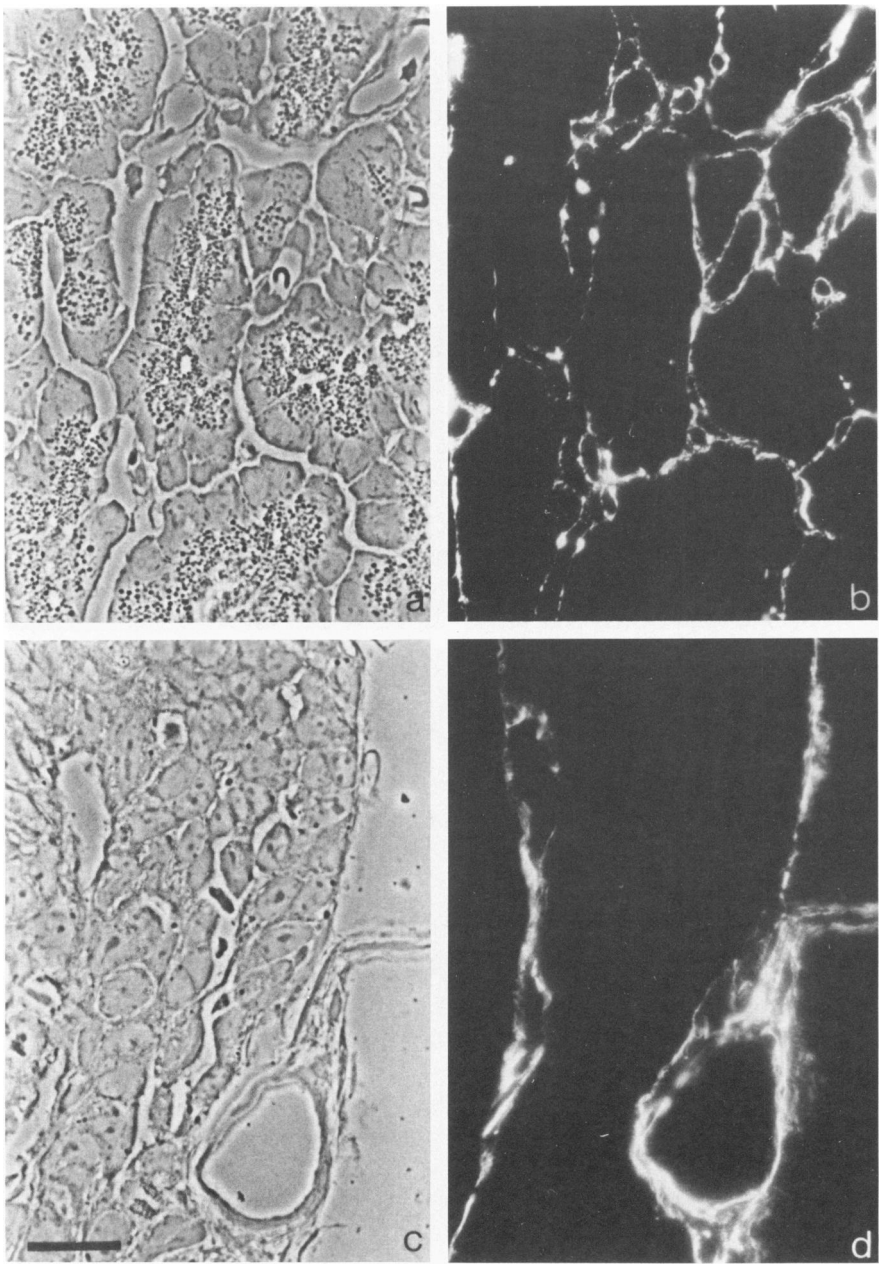

Figure 6—Phase-contrast (**a** and **c**) and corresponding fluorescence micrographs (**b** and **d**) of semithin tissue sections stained for Type III collagen. **a** and **b**—Normal pancreas. Staining appeared in a diffuse pattern, filling the interspaces between acini and vessels. **c** and **d**—Acinar cell tumor. Staining was seen along the tumor periphery and in a diffuse pattern within the connective tissue interspace, although it was absent from the tumor parenchyma. (Bar = 20 μ; ×625)

connective tissue within the tumor capsule (Figure 9). Nuclei were located within the basal portions of reoriented tumor cells near the connective tissue interface, while Golgi complexes and zymogen granules appeared in supranuclear and apical regions of the cytoplasm, respectively. Reorganized tumor cells did not display full junctional complexes in these regions, although apical microvilli and associated membrane specializations reminiscent of adhering zonules have occasionally been observed. Tumor cells reorient in a similar manner along their boundary with vascular adventitia.[5]

Discrete regions of increased actin staining were not readily apparent in the apices of reoriented cells, although this was difficult to assess because the tumor periphery was limited by closely apposed connective tissue cells, which stained brightly for actin (Figure 2c and d). Normal apical and basolateral membrane domains also did not reform in regions of tumor cell reorganization. However, leucine aminopeptidase was observed

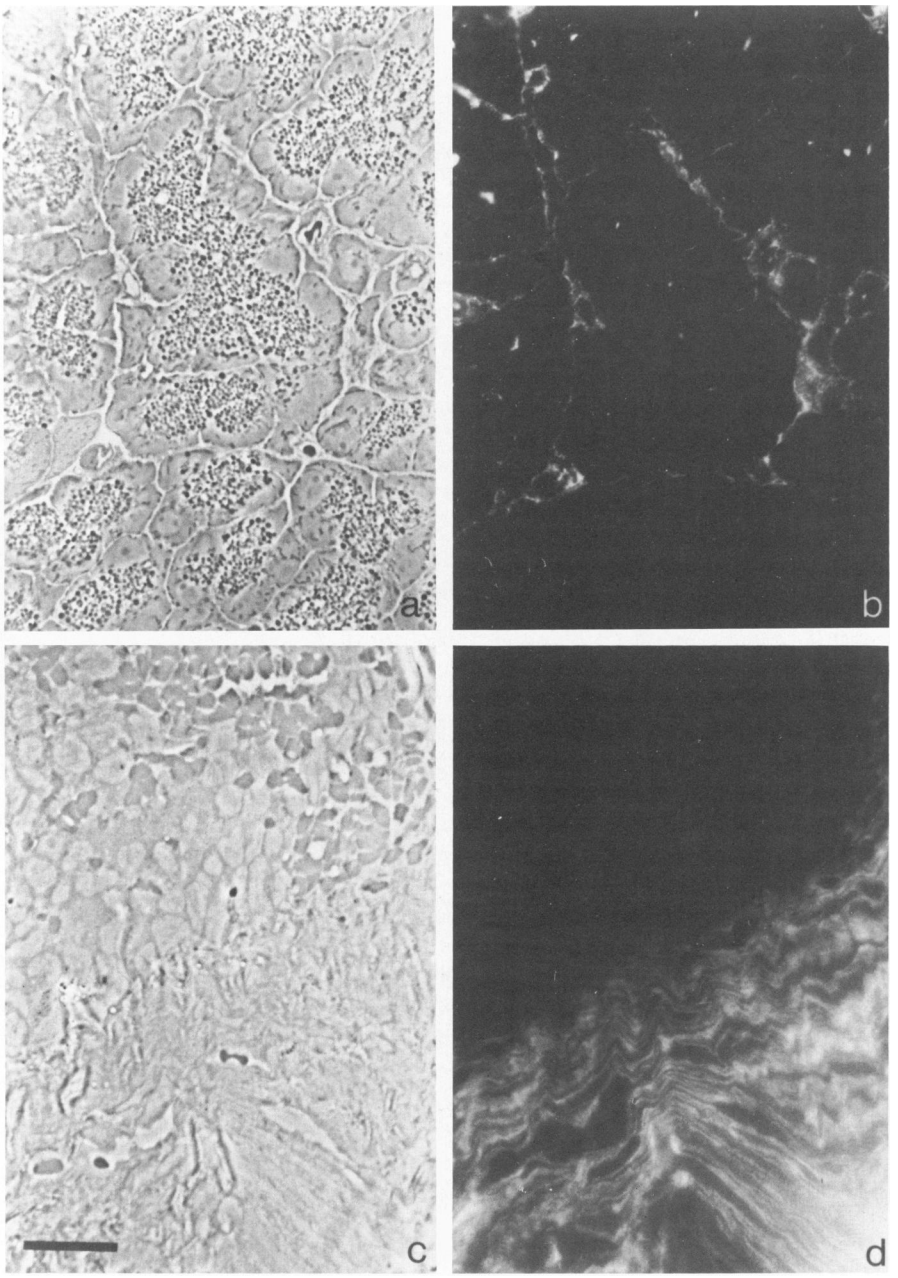

Figure 7—Phase-contrast (**a** and **c**) and corresponding fluorescence micrographs (**b** and **d**) of semithin tissue sections stained for fibronectin. **a** and **b**—Normal pancreas. Staining appeared in a predominantly discontinuous pattern within stroma that separated acinar and vascular structures. **c** and **d**—Acinar cell tumor. Staining was absent from the tumor parenchyma but appeared in a striated pattern within the tumor capsule. (Bar = 20 μ; ×625)

in a partially polarized distribution; that is, it was excluded from the basal surfaces of some reoriented tumor cells along the tumor periphery (Figure 3c and d).

Neoplastic Disorganization Correlates With Loss of BM

In the tumor parenchyma, randomly oriented cells grew free of contact with morphologic basal lamina (Figure 8). This was confirmed by immunolocalization studies, which showed that the tumor parenchyma was free of the normal linear array of laminin (Figure 4c and d), Type V collagen (Figure 5c and d), Type IV collagen, and heparan sulfate proteoglycan (not shown). The only BM component that could be identified within disorganized regions of the tumor was laminin, which appeared within cells and in a discontinuous or punctate intracellular pattern (Figure 4c and d). On the other hand, linear patterns of staining were observed for all normal pancreatic BM components (ie, laminin, heparan sulfate proteoglycan, and Type IV and V collagen)

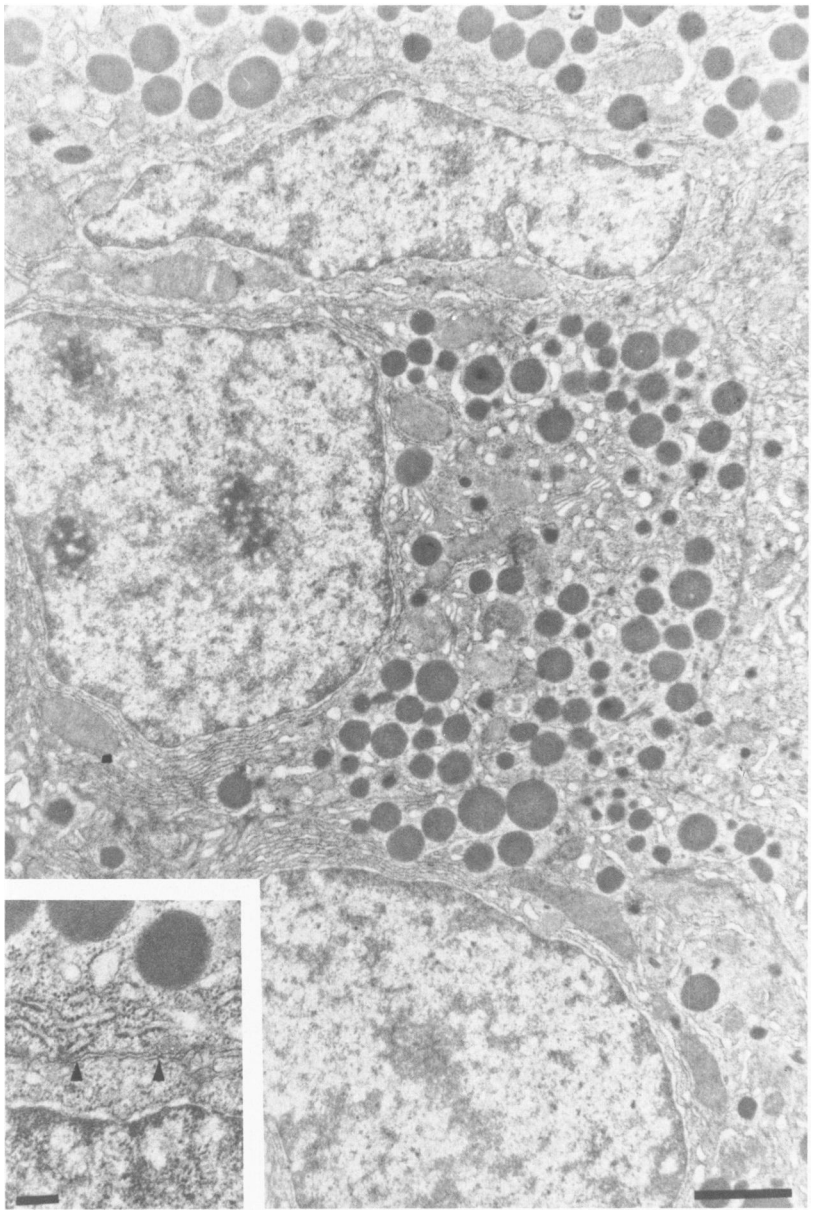

Figure 8—Electron micrograph of the acinar cell tumor parenchyma showing that adjacent tumor cells were out of register, although each individual cell was anisotropic with its nucleus, Golgi complex, and zymogen granules distributed in series along an axis stretching from one pole of the cell to the other. (Bar = 2.0 μ; ×6500) **Inset** demonstrates absence of basal lamina between apposed plasma membranes in this field (*arrowheads*). (Bar = 0.2 μ; ×28,000)

in regions of tumor cell reorganization along the tumor periphery (Figure 4c and d and Figure 5c and d). Similarly, basal lamina with characteristic morphologic features appeared closely apposed to the basal surfaces of reoriented tumor cells along the connective tissue interface (Figure 9). While tumor basal lamina was usually continuous, occasional small breaks and focal contacts between epithelial tumor cells and neighboring fibroblasts were observed.

Type III collagen (Figure 6c and d), fibronectin (Figure 7c and d), and Type I collagen (not shown) were not found within the parenchyma of this epithelial tumor and were limited to surrounding connective tissue. This stromal staining pattern correlated with the presence of bundles of collagen fibrils detected in these regions by electron microscopy (Figure 9). Staining for BM components was also occasionally observed surrounding isolated connective tissue cells (Figure 4c and d).

Discussion

It is becoming increasingly clear that cancer is not solely the result of a single pathologic gene product but rather is an aberration of normal cellular organization.[24-27] In embryogenesis, tissue architecture is con-

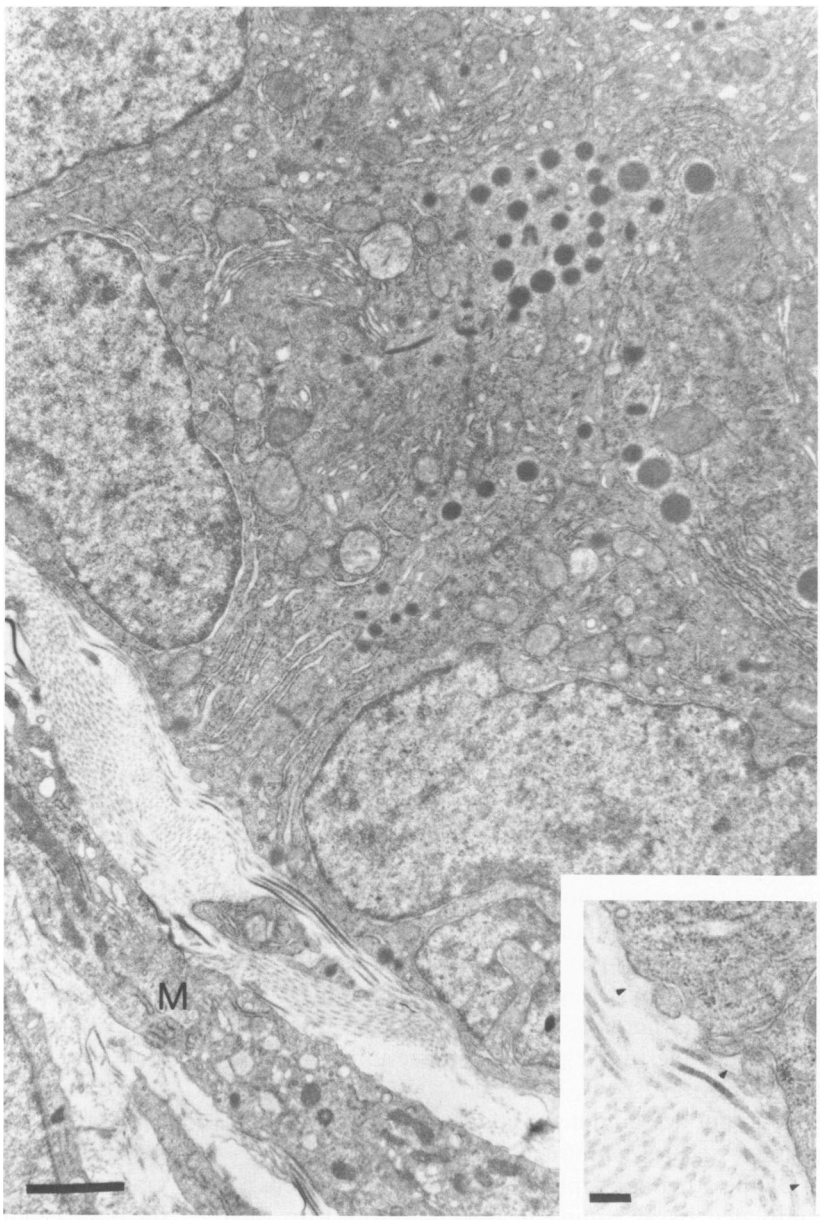

Figure 9—Electron micrograph of tumor-connective tissue capsule interface. Acinar tumor cells palisaded and reoriented their intracellular organelles in areas of contact with the connective tissue capsule (*M*, mesenchymally derived connective tissue cell). (Bar = 2.0 μ; ×6500) **Inset**—Tips of *arrowheads* abut on basal lamina that physically separated reoriented tumor cells from collagen fibrils within neighboring connective tissue. (Bar = 0.2 μ; ×28,000)

structed in an epigenetic fashion through a combination of cell proliferation, differentiation, and progressive remodeling of increasingly complex tissue structures. Maintenance of tissue function throughout adult life is then assured through preservation of characteristic tissue form (ie, patterns of intercellular connections and structural supports) over many cell generations. Cancer represents a failure to sustain this hierarchical scheme of architectural organization. A more thorough understanding of the mechanism by which cells are integrated and maintained within organized tissue structures should therefore provide greater insight into the carcinogenic process.

Because the pancreatic acinar carcinoma grew in organized and disorganized forms within the same tumor, it provided an excellent opportunity to investigate the role of BM in both epithelial organization and neoplastic disorganization. While individual acinar tumor cells were cytodifferentiated and anisotropic, they grew randomly oriented one next to another. Normal acinar cell cytoskeletal polarity (ie, concentration of actin in the region of the apical terminal web) was lost during neoplastic transformation, and normally polarized membrane components (eg, leucine aminopeptidase) appeared over entire acinar tumor cell surfaces. Destabilization of normal epithelial architecture corre-

lated directly with lack of organized BM within the tumor parenchyma and resulted in loss of normal pancreatic function (eg, abnormally high levels of digestives enzymes appear in serum[11]) even though secretory proteins continued to be processed in a normally polarized fashion within each individual tumor cell.[14]

Loss of distinct membrane domains within acinar tumor cells was consistent with the observation that tight junctions were not seen by transmission electron microscopy. Freeze-fracture electron-microscopic studies of the pancreatic acinar carcinoma also demonstrate short, fragmented tight junction strands, rare small gap junctions, and a random distribution of intramembranous particles in the plasma membranes of these cells.[13] Tight junctions physically limit lateral diffusion of integral membrane components in a variety of epithelia.[28,29] The absence of normal junctional complexes may also be related to failure of tumor cells to maintain discrete actin-containing terminal web structures and apical microvilli, because terminal web components anchor to both microvillar filaments[30] and junctional elements.[31]

Tumor cells grew free of BM and piled one atop another within the tumor parenchyma. The absence of BM in these regions was probably not due to increased levels of BM degradation by acinar tumor cells (ie, while maintaining normal synthetic levels), because the pancreatic acinar carcinoma was not metastatic, and dissociated tumor cells do not invade through BM when maintained on human amnion *in vitro*.[15] Invasion through BM has been shown to correlate with both production of BM degrading enzymes and invasive potential.[4] Rather, it is more likely that the absence of BM within the tumor parenchyma resulted from decreased BM synthesis, because isolated acinar tumor cells do not accumulate basal lamina or the full complement of BM components *in vitro*.[15]

On the other hand, pancreatic acinar tumor cells accumulated basal lamina in tight apposition to their basal surfaces and reoriented along their interface with mesenchymally derived connective tissue in the tumor capsule. Linear distributions of laminin, heparan sulfate proteoglycan, and Type IV and V collagen were only seen in these organized areas. When surrounding the vasculature, tumor cell basal lamina was also physically separated from endothelial basal lamina by stroma comprised of adventitial cells and collagen fibrils.[5] The fibrillar stroma in both capsular and vascular regions contained at least fibronectin and Types I and III collagen.

Tumor cells reorganized along the connective tissue capsule as well as vascular adventitia suggesting that tumor organization was related to contact with mesenchymally derived tissue and was not due to some vessel-specific quality such as nutrient availability. Tumor-associated BM was probably produced by the tumor cells as a result of an epithelial–mesenchymal interaction. Normal embryonic pancreatic epithelia only deposit BM when in contact with live mesenchymal tissue.[32,33] and coculture of dissociated acinar tumor cells with embryonic pancreatic mesenchyme also results in accumulation of basal lamina along basal tumor cell surfaces *in vitro* (preliminary observation). Fibroblast products can similarly stimulate BM production by other epithelia.[34,35] In addition, it is possible that some of the components of the tumor BM were contributed by mesenchymal cells, because desmoplastic stroma has been shown to secrete small amounts of Type V collagen.[36]

Pancreatic epithelial tumor cells palisaded and consistently reoriented their intracellular organelles (cell base toward connective tissue) whenever they contacted BM along their periphery. Tumor cell reorganization occurred in the absence of reformation of full junctional complexes or normally polarized membrane domains and thus was not secondary to establishment of membrane polarity. While we could not visualize clear cytoskeletal reorganization of a terminal web within repolarized tumor cells *in vivo*, contact with BM induces assembly of actin into fibrous bundles within these cells *in vitro*.[15] BM may have also subtly affected adjacent tumor cell membrane topography, because leucine aminopeptidase appeared to be excluded from regions of reoriented tumor cell plasmalemma that were directly involved in cell anchorage. This was not due to an inability to resolve antigens within a single membrane bilayer, because basolateral membrane proteins could be seen in these regions in parallel sections (not shown). Extracellular BM components also have specific effects on the localization and lateral mobility of muscle fiber cell membrane constituents,[37,38] and an integral membrane component is similarly excluded from the basal cell membranes of isolated MDCK cells during attachment *in vitro*.[39]

Pancreatic epithelial tumor cell reorganization was analogous to epithelial organization during embryogenesis. In normal development, *de novo* formation of a polarized epithelium immediately follows a complex series of events which include production of a punctate intercellular pattern of laminin, subsequent deposition of other BM components, and finally their organization into a planar array.[40,41] Embryonic epithelium usually gains the ability to produce BM through induction by adjacent mesenchyme.[6,33] Mesenchyme in turn directs the generation of specific tissue pattern[42,43] by means of alterations in BM turnover and associated epithelial cell proliferation.[1,44,45] Similarly, while pancreatic acinar tumor cells grew in a disorganized form in as-

sociation with a discontinuous array of laminin, they accumulated BM and reorganized in a regular fashion when in contact with mesenchymal tissue. The presence of mitotic figures, occasional BM discontinuities, and focal epithelial–mesenchymal contacts along the expanding tumor boundary (ie, continually extending BM) were also reminiscent of regions of most rapid cell growth, BM turnover, and tissue remodeling during normal histogenesis.[46,47] Interestingly, the secretory protein profile of pancreatic acinar tumor cells also resembles that of embryonic pancreas.[48]

In summary, neoplastic disorganization of pancreatic epithelium may be viewed as an aberration of normal epithelial–mesenchymal interactions. While mesenchymally derived connective tissue supported local deposition of BM and histodifferentiation of acinar tumor cells, it acted in a deregulated fashion (for in depth discussion see Ingber and Jamieson[49]). Sustained pancreatic acinar cell growth in the absence of commensurate boundary extension apparently resulted in a piling up of epithelial cells, which lost normal proximity to mesenchymal tissue and were unable to organize BM. Contact with BM is sufficient to induce acinar tumor cell reorganization *in vitro*,[15,50] and so BM which accumulated as a result of epithelial-mesenchymal interactions was probably the trigger for acinar tumor reorganization *in vivo*. It is also important to note that the pancreatic tumor is not exclusive in its ability to reorganize in an epigenetic fashion, because many carcinomas similarly histodifferentiate and produce BM when mixed with embryonic tissues.[27,51,52] BM may also serve in a general role as a spatial organizer of normal polarized epithelia.[1,40,41,53] Thus, neoplastic disorganization of normal epithelial cell–cell relations could result from failure to maintain intact BM beneath every daughter cell in an actively growing transformed cell population. Malignant invasion (ie, BM disruption) may then represent an end point in a spectrum of progressive deregulation of BM turnover and related schemes of architectural organization.

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