Proc. Natl. Acad. Sci. USA
Vol. 78, No. 6, pp. 3901–3905, June 1981
Medical Sciences

Role of basal lamina in neoplastic disorganization of tissue architecture

(basement membrane/epithelial cell polarity/cell shape/immunofluorescence)

DONALD E. INGBER*, JOSEPH A. MADRI†, AND JAMES D. JAMIESON*

*Section of Cell Biology and †Department of Pathology, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510

Communicated by Paul Greengard, March 13, 1981

ABSTRACT **We have studied a transplantable carcinoma of the rat pancreas [Reddy, J. K. & Rao, M. S. (1977) *Science* 198, 78–80] that is composed of cytologically differentiated acinar cells that have lost their epithelial orientation and do not form acini. Light microscopy shows, however, consistent palisading, reorientation, and polarization of these cells in areas of contact with the vasculature. Electron microscopy reveals a normal basal lamina (BL) along the basal portions of repolarized tumor cells that is physically separate from the endothelial BL. We used indirect immunofluorescence to examine the distribution of BL constituents, laminin (Lm) and type IV collagen (type IV), within the different microenvironments of this tumor. In normal pancreas, Lm and type IV are distributed linearly, outlining acini and blood vessels. In the tumor parenchyma, type IV is not detected, whereas Lm appears in a punctate distribution outlining cells. Reorientation of tumor cells is observed only along linearly deposited Lm and type IV bordering vessels. These data indicate that this nonmetastatic tumor has lost the ability to produce or maintain a complete BL within its disorganized parenchyma, while its cells retain the capacity to produce and reorganize along linear BL when in contact with vascular adventitia. We suggest that failure to maintain a complete BL may be involved in the neoplastic disorganization of normal tissue architecture as well as in the breakdown of boundaries during the development of invasive carcinomata.**

Cells in a epithelium are highly organized and commonly exhibit polarized form as well as function. The cells always sit on top of a continuous basement membrane‡ or basal lamina (BL) and are physically separated from the underlying connective tissue. A normal adult epithelium is a dynamic structure (1), and its orderly renewal (e.g., wound healing) requires the continued presence of BL as an extracellular scaffolding or template that maintains the original architectural form and assures for accurate regeneration of preexisting structures (2).

An epithelium may escape its normally tight growth constraints and produce a less ordered arrangement or piling up of atypical epithelial cells and so result in a disorganization of epithelial form termed dysplasia. As the epithelium becomes further disorganized, it reaches the arbitrary point at which it is termed neoplastic. A histologic specimen of this tissue would be designated as carcinoma *in situ*, a premalignant condition, as long as there is no morphologic evidence of invasion through the underlying BL. Once physical penetration occurs, the neoplasm becomes invasive and is malignant, because it is now free to metastasize.

Classically, the BL has been viewed as a host barrier through which a malignant tumor must gain the ability to invade. In fact, BL is normally a specialized product of the overlying epithelial cells (3–5), which also plays a central role as a stabilizer of epithelial form and orientation in embryogenesis (3) and is maintained throughout adult life. Thus, it is possible that neoplastic disorganization of epithelial architecture as well as malignant invasion may result either from loss of maintenance of this epithelial scaffolding or through the acquisition of some new transformed cell product that compromises its structural integrity.

In order to investigate the role of BL in the maintenance of organized tissue structures as well as neoplastic disorganization, we have studied a transplantable carcinoma of the rat exocrine pancreas as a model system. This tumor is composed of cytologically differentiated acinar cells that have lost their normal epithelial organization. The tumor was discovered, with associated metastatic foci, in nafenopin-treated rats in the laboratory of Reddy and Rao (6) and was kindly provided to us for study. As the BL is a complex of different collagenous and noncollagenous macromolecules, the distribution of two ubiquitous BL constituents, the glycoprotein laminin (Lm) (7) and type IV collagen (type IV) (8), was studied in both the tumor and normal pancreas, using indirect immunofluorescence. A preliminary account of these studies has appeared in abstract form (9).

MATERIALS AND METHODS

Experimental System. Weanling male F344 Sprague–Dawley rats (Harlan–Sprague–Dawley, Madison, WI) were inoculated subcutaneously or intraperitoneally with a mechanically prepared suspension of the pancreatic acinar carcinoma in isotonic saline. All tumors used in this study were between the 18th and 23rd passage, 1–4 cm in diameter, and displayed consistent growth characteristics and morphology. In our laboratory, the tumor grows as a nonmetastatic carcinoma. Normal pancreas was obtained from either tumor-bearing animals or non-tumor-bearing animals; the distribution of Lm and type IV was identical in both.

Light Microscopy. Tissue specimens were fixed in 10% formaldehyde in phosphate-buffered saline (P_i/NaCl) and embedded in paraffin, and sections were stained with hematoxylin and eosin.

Electron Microscopy. Tumor tissue was fixed by perfusion through the left ventricle with 1% glutaraldehyde/3% (wt/vol) formaldehyde in P_i/NaCl, treated with osmium tetroxide, stained *en bloc* with uranyl acetate, and embedded in Epon/Araldite. Thin sections were stained with uranyl acetate and lead citrate (10) and were photographed on a Siemens 101 electron microscope.

Fluorescence Microscopy. Tissue was processed according to the method of Beyer *et al.* (11) except that specimens were

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: BL, basal lamina; Lm, laminin; type IV, type IV collagen; P_i/NaCl, phosphate-buffered saline.

‡ "Basement membrane" is a light-microscopic term that at the level of electron microscopy refers to a basal lamina plus adjacent connective tissue matrix.

quenched after fixation with $NaBH_4$ at 1 mg/ml in P_i/NaCl for 40 min and placed either into 2 M sucrose/P_i/NaCl or in Tissue Tek embedding medium. Tissue in sucrose was handled according to the method of Tokuyasu (12) and semithin sections (0.5–1.0 μm) were cut at −90°C on a Sorvall MT-2B ultramicrotome with a cryotomy attachment. Sections (2–4 μm) of the tissue embedded in Tissue Tek were cut at −30°C on a Damon/IEC cryostat.

Methods for preparation of our antibodies to Lm and type IV in rabbits, their characterization, and localization within BL have been published (13, 14). A fluorescein-conjugated sheep anti-rabbit immunoglobulin was used for detection of the primary antibodies (13). Control experiments with preimmune serum in the first step or fluorescein-conjugated immunoglobulin alone were consistently negative. Localizations were done on specimens from seven separate animals on seven different occasions and all findings were confirmed on cryostat sections of frozen unfixed material.

We used a Zeiss photomicroscope II equipped with phase-contrast optics and an epifluorescence illuminator containing appropriate filters for fluorescein isothiocyanate. Images were recorded on Ektachrome 400 film.

RESULTS

Light microscopy of normal rat pancreas displays the organization of exocrine cells into acini (Fig. 1*a*). Each acinar cell is typically polarized, with its nucleus being located within the basal portion of the cell while the zymogen granules fill the apical region. These cells are consistently oriented within each acinus with their bases at the periphery and apices towards the center. Thus, examination of each acinus reveals a central area filled with zymogen granules surrounding a centroacinar lumen as well as a peripheral arrangement of the basally located nuclei along the outer margin of the acinus.

On the other hand, the acinar cell tumor is characterized by a highly disorganized parenchyma with no evidence of obvious cell orientation (Fig. 1*b*). However, consistent palisading and reorientation of tumor cells can be seen in areas of direct contact with the vasculature.

Electron microscopy best displays this arrangement of cells within the tumor. Epithelial cell repolarization can clearly be seen in tumor cells that line up along the abluminal side of a tumor vessel because their nuclei are consistently located in their basal portions while the zymogen granules fill the apical regions (Fig. 2*a*). Cells in the parenchyma of the tumor, however, are highly disorganized in that the apex of one acinar tumor cell is often abnormally juxtaposed with the basal or apical portion of a neighbor. No centroacinar lumina or ductular structures have been observed. While BL is not seen between the cells within the tumor parenchyma (see also figure 4 in ref. 15), a BL with characteristic morphology is closely apposed to the basal portions of the tumor cells in areas of cell repolarization adjacent to vascular adventitia (Fig. 2*b*). The tumor BL and that of the vessel are physically separated from each other by connective tissue matrix, and both basal laminae display occasional discontinuities.

Fig. 3*a* is a phase-contrast micrograph of a semithin section of normal rat pancreas that displays acini as well as neighboring capillaries filled with erythrocytes. The distribution of type IV within this same section is characterized by linear staining outlining all acini and blood vessels (Fig. 3*b*). A phase-contrast view of the tumor (Fig. 3*c*) once again shows a disorganized

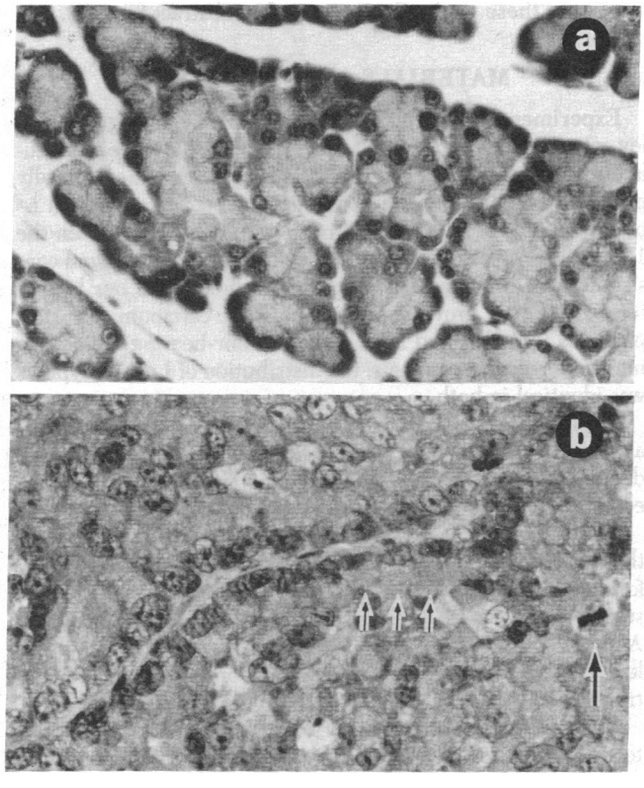

FIG. 1. Light micrographs of normal pancreas (*a*) and acinar cell tumor (*b*). (Hematoxylin and eosin; ×400.) Tips of triple arrows abut on apical poles of three neighboring reoriented tumor cells that are palisading along the adjacent vessel; larger arrow indicates a mitotic figure within the disorganized tumor parenchyma.

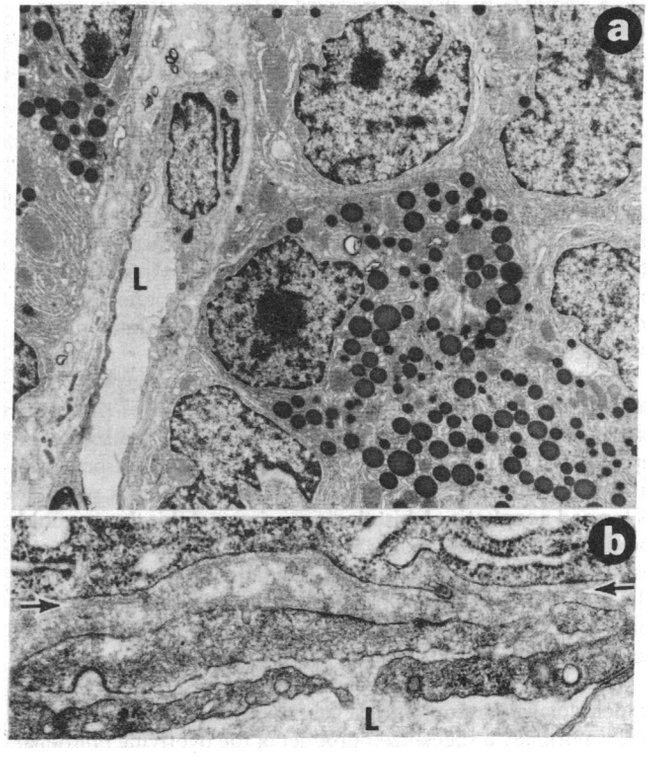

FIG. 2. (*a*) Electron micrograph of tumor containing a small blood vessel. (Uranyl acetate and lead citrate; ×5750.) (*b*) Higher magnification of the epithelial tumor–vascular interface (L, lumen of vessel; apposed arrows indicate BL underlying the basal portions of reoriented tumor cells). (×36,000.)

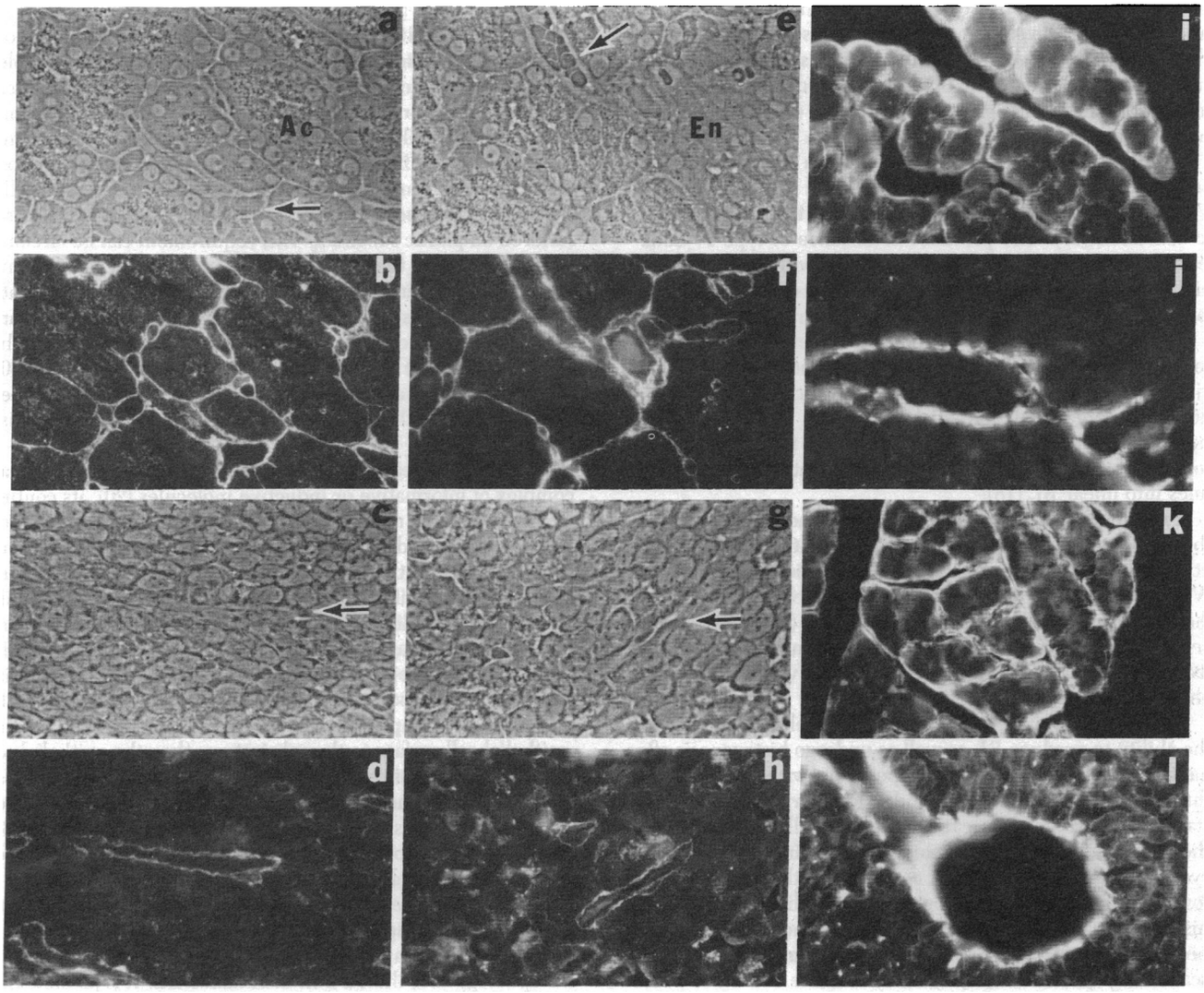

FIG. 3. Phase-contrast (*a, c, e,* and *g*) and corresponding immunofluorescence micrographs (*b, d, f,* and *h*) of semithin sections of pancreas and tumor (Ac, acinus; En, endocrine islet; arrows indicate small vessels). (*a* and *b*) Pancreas stained for type IV. (×415.) (*c* and *d*) Section of tumor stained for type IV. (×415.) (*e* and *f*) Pancreas stained for Lm. (×415.) (*g* and *h*) Tumor stained for Lm. (×415.) Immunofluorescence micrographs of standard cryostat sections of pancreas and tumor: (*i*) Pancreas stained for type IV. (×265.) (*j*) Tumor stained for type IV. (×265.) (*k*) Pancreas stained for Lm. (×265.) (*l*) Tumor stained for Lm. (×265.)

parenchyma free of acinar structures. A tumor vessel stretches horizontally across the center of the view. Under fluorescence microscopy (Fig. 3*d*) a vascular pattern of type IV staining can be seen to dominate as the tumor parenchyma is free of any obvious organized staining. This confirms the absence of any ductular or acinar forms.

Lm staining in normal pancreas (Fig. 3*f*) correlates exactly with that of type IV and once again clearly delineates all acini and vessels. Examination of the right portion of the phase-contrast view (Fig. 3*e*) reveals an endocrine islet that is characterized by linear Lm staining surrounding its capillary network. The distribution of Lm staining within a semithin section of tumor (Fig. 3*h*) is similar to that of type IV. Lm appears only in an organized linear form lining the vasculature, as can be seen in the phase-contrast view (Fig. 3*g*), and is not evident within the surrounding parenchyma.

Because semithin sections present only a very thin slice of tissue, it was possible that our antigens were indeed present within the tumor parenchyma but in a disorganized form and were unresolvable by this technique due to presentation of an insufficient mass of antigen. To examine this possibility, thicker 2- to 4-μm conventional cryostat sections were stained for Lm and type IV as described for semithin sections.

Staining of the thicker sections of normal pancreas is once again identical for Lm and type IV (Fig. 3 *i* and *k*) and acinar forms are clearly delineated. Fig. 3*j* shows that the distribution of type IV within the tumor is identical to that seen by the semithin method with only the vascular pattern of staining evident. On the other hand, in these thicker sections Lm appears in a punctate distribution clearly outlining the tumor cells within the parenchyma as well as in a linear form along the vasculature (Fig. 3*l*).

DISCUSSION

While the pancreatic tumor does not commonly grow as an invasive carcinoma, it is an excellent model system for study of the role of BL in the organization of epithelial architecture as well as its neoplastic disorganization. Our data indicate that this rat pancreatic acinar cell tumor has lost the ability to produce or maintain a complete and organized BL within its parenchyma, and this correlates directly with loss of epithelial cell orientation. We believe that the absence of type IV staining is real

and is not due to tumor-associated changes in antigenicity. Our antibodies were developed against antigens produced by another tumor (EHS sarcoma), and antibodies produced in this manner have been shown to bind to both acid-soluble and pepsin digestion fragments of type IV collagens (unpublished data and ref. 16).

Recently a degradative enzyme has been isolated from the media of cultured metastatic tumor cells that is specific for type IV and is normally produced in a latent form that requires tryptic activation (17). The presence or absence of type IV collagenase activity or of its potential activators within the different microenvironments of this pancreatic acinar cell tumor may in part explain local differences in type IV distribution while Lm is retained. Furthermore, the punctate intercellular distribution of Lm seen in the tumor parenchyma is not unlike that seen within certain embryonic cell populations prior to their orientation into organized epithelial layers (18, 19). In the early mouse embryo, this punctate pattern of Lm is followed by deposition of type IV and subsequent organization of both molecules into linear BL (18).

It is important to emphasize that the tumor also retains the ability to organize when in direct contact with vascular adventitia. In these areas, a normal linear distribution of Lm and type IV is seen and morphologic BL appears in close apposition to the basal portions of repolarized tumor cells (Fig. 2*b*). The BL lies at the interface of the tumor cells with mesenchymally derived connective tissue that surrounds the vasculature and is physically separate from endothelial BL.

In embryological development, an epithelium usually gains the ability to produce BL as well as undergo histogenesis after having remained in direct apposition with mesenchyme throughout its characteristic induction period (20). For instance, embryonic pancreatic epithelial cells separated from their mesenchyme prior to completion of induction undergo cytodifferentiation into acinar cells, but in the absence of mitosis, production of morphologic BL, epithelial polarization, or histogenesis (21). While histogenesis depends on interactions between adjacent cellular societies, it appears that it is the BL scaffolding that serves to physically stabilize the tissue's characteristic form. Maintenance of organized morphology in salivary gland rudiments has been shown to be dependent upon the continued presence of its BL (3). The accumulation of BL by epithelia may in part be mediated through extracellular mesenchymal products such as fibrillar collagen (5, 22), although in certain systems production of new BL requires the added presence of live mesenchymally derived fibroblasts (23).

Thus, the cells of this pancreatic acinar cell tumor may be on the fulcrum of net synthesis or net breakdown of their own BL. They may be able to interact with mesenchymally derived connective tissue and, in a manner remniscient of the embryonic state, organize and concurrently lay down a BL of their own, stabilizing this epithelial reorientation. Resultant cell polarization might redirect potential degradative activity away from the tumor cell base, further promoting accumulation of BL at the tumor margin. This hypothesis is supported by the observation that these tumor cells also organize in areas of contact with the connective tissue capsule as well as along vascular adventitia (data not shown). This finding suggests that tumor organization is not due to some vessel-specific quality such as nutrient availability and may, in part, explain the observation that this rapidly growing neoplasm does not appear to be metastatic. It is interesting to note that examination of various epithelial tumors generally reveals a direct correlation between invasive properties and the absence of BL as well as a correlation of noninvasion with the presence of continuous BL (24–26). A high correlation has also been shown between the enzymatic degradation of type IV and metastatic potential in a variety of tumor systems (27).

The lack of epithelial organization seen within the tumor parenchyma may in part be due to the tumor cells being able to survive and proliferate free of normal contact with either morphologic BL or specific BL components. Normal rat mammary epithelial cell viability appears to be dependent upon contact with an intact BL (28), and the attachment and proliferation of some other normal epithelia (29) and connective tissue cells (30) requires *de novo* collagen deposition when the cells are cultured on plastic substrata. This requirement can be circumvented by plating the epithelial cells on a layer of type IV but not type I collagen (29), and Lm appears to mediate this attachment in at least one epithelial cell line (31). Growth of various tumorigenic cell lines is, however, independent of substratum anchorage and deposition of extracellular collagen (30, 32), suggesting a correlation between loss of substratum dependence, growth autonomy, and subsequent tissue disorganization.

In addition, because the BL may function as an extracellular complex of informative or inductive molecules (20), its continued maintenance may be mandatory for normal growth regulation of organized tissues. A factor has been extracted from embryonic tissue that can replace the requirement of mesenchyme for the proliferation and cytodifferentiation of embryonic pancreatic epithelia (33). Artificial orientation of this mesenchymal factor on agarose beads leads to cell binding, cytodifferentiation, and epithelial polarization (34). If these potential mitogens were produced by mesenchyme *in vivo*, inserted into an acinar BL complex, and neutralized upon adhesion of the epithelial cell surface to the substratum, then the epithelial society should remain stable in number and form. Any process that resulted in the production or release of similar cell-associated mitogens, such as dissolution or loosening of the BL macromolecular complex, could result in the autonomous proliferation of epithelial cells.

Finally, because cell shape is tightly coupled to cell growth in normal anchorage-dependent cells (35), efficient control of cell growth within an epithelium may require a stable tissue morphology and, thus, a well-maintained BL. It is possible that a tissue's three-dimensional physical form may itself serve to regulate cell shape and orientation through transmission of the forces of tension and compression characteristic for a given architectural configuration. An epithelial structure can be regarded as a tensile or tensegrity system, that is, an architectural unit of the highest efficiency, which consists of *discontinuous* compression-resistant members (e.g., microtubules, cytoskeletal microfilaments, fibrillar collagen) interconnected directly or indirectly by a *continuous* series of tension elements (e.g., plasmalemma, contractile microfilaments, BL) (36–38). As dynamic tensile structures, cells alter their shape until an equilibrium configuration is attained that most efficiently and evenly distributes the load, given the characteristic architectural distribution of anchors within the substratum. Dissolution of BL, which frees cell anchors from their normal spatial orientation, could result in a loss of control of cell shape and thus deregulation of cell growth.

In any case, loss of type IV from the parenchyma of this pancreatic acinar cell tumor may play some role in the release of other BL constituents such as Lm from a normally organized BL as well as in the associated development of growth autonomy and loss of epithelial orientation. In other tumor systems that display both uncontrolled proliferation and invasive qualities, failure to maintain BL with additional loss of type IV at the tumor margins may be involved in the breakdown of tissue boundaries during the progression from a normal epithelium to an invasive carcinoma.

We acknowledge the technical assistance of Ms. Amy Hsu and Ms. Rosalie Manzi and the excellent assistance of Ms. Marybeth Hicks in preparing the manuscript. This work was supported by U.S. Public Health Service Grant AM-17389 (to J.D.J.), National Cancer Institute Contract NO1-CB-84255-37 (to J.A.M.), and Medical Scientist Training Program Grant GM-07205 (to D.E.I.).

1. Walker, F. (1972) *J. Pathol.* **107,** 119–121.
2. Vracko, R. (1974) *Am. J. Pathol.* **77,** 314–346.
3. Banerjee, S. D., Cohn, R. H. & Bernfield, M. R. (1977) *J. Cell Biol.* **73,** 445–463.
4. Dodson, J. W. & Hay, E. D. (1971) *Exp. Cell Res.* **65,** 215–220.
5. Emerman, J. T. & Pitelka, D. R. (1977) *In Vitro* **13,** 316–328.
6. Reddy, J. K. & Rao, M. S. (1977) *Science* **198,** 78–80.
7. Timpl, R., Rohde, H., Robey, P. G., Rennard, S. I., Foidart, J.-M. & Martin, G. R. (1979) *J. Biol. Chem.* **254,** 9933–9937.
8. Timpl, R., Martin, G. R., Bruckner, P., Wick, G. & Wiedeman, H. (1978) *Eur. J. Biochem.* **84,** 43–52.
9. Ingber, D. E., Madri, J. A. & Jamieson, J. D. (1980) *J. Cell Biol.* **87,** 127a (abstr.).
10. Reynolds, E. S. (1963) *J. Cell Biol.* **17,** 208–212.
11. Beyer, E. C., Tokuyasu, K. T. & Barondes, S. H. (1979) *J. Cell Biol.* **82,** 565–571.
12. Tokuyasu, K. T. (1973) *J. Cell Biol.* **57,** 551–565.
13. Roll, F. J., Madri, J. A., Albert, J. & Furthmayr, H. (1980) *J. Cell Biol.* **85,** 597–616.
14. Madri, J. A., Roll, F. J., Furthmayr, H. & Foidart, J.-M. (1980) *J. Cell Biol.* **86,** 682–687.
15. Jamieson, J. D., Ingber, D. E., Muresan, V., Hull, B. E., Sarras, M. P., Jr., Maylie-Pfenninger, M.-F. & Iwanij, V. (1981) *Cancer* **47,** 66–75.
16. Timpl, R., Glanville, R. W., Wick, G. & Martin, G. R. (1979) *Immunology* **38,** 109–116.
17. Liotta, L. A., Tryggvason, K., Garbisa, S., Robey, P. G. & Abe, S., *Biochemistry,* in press.
18. Leivo, I., Vaheri, A., Timpl, R. & Wartiovaara, J. (1980) *Dev. Biol.* **76,** 100–114.
19. Ekblom, P., Alitalo, K., Vaheri, A., Timpl, R. & Saxen, L. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 485–489.
20. Grobstein, C. (1967) *Natl. Cancer Inst. Monogr.* **26,** 279–294.
21. Spooner, B. S., Cohen, H. I. & Faubion, J. (1977) *Dev. Biol.* **61,** 119–130.
22. David, G. & Bernfield, M. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 786–790.
23. Dodson, J. W. & Hay, E. D. (1974) *J. Exp. Zool.* **189,** 51–72.
24. Luibel, F. J., Sanders, E. & Ashworth, C.T. (1960) *Cancer Res.* **20,** 357–361.
25. Ozzello, L. (1959) *Am. J. Pathol.* **35,** 887–895.
26. Rubio, C. A. & Biberfeld, P. (1979) *Virchows Arch. A* **381,** 205–209.
27. Liotta, L. A., Tryggvason, K., Garbisa, S., Hart, I., Foltz, C. M. & Shafie, S. (1980) *Nature (London)* **284,** 67–68.
28. Wicha, M. S., Liotta, L. A., Vonderhaar, B. K. & Kidwell, W. R. (1980) *Dev. Biol.* **80,** 253–266.
29. Wicha, M. S., Liotta, L. A., Garbisa, G. & Kidwell, W. R. (1979) *Exp. Cell Res.* **124,** 181–190.
30. Liotta, L. A., Vembu, D., Kleinman, H., Martin, G. R. & Boone, C. W. (1978) *Nature (London)* **272,** 622–624.
31. Terranova, V. P., Rohrbach, D. H. & Martin, G. R. (1980) *Cell* **22,** 719–726.
32. Vembu, D., Liotta, L. A., Paranjpe, M. & Boone, C. W. (1979) *Exp. Cell Res.* **124,** 247–252.
33. Ronzio, R. & Rutter, W. (1973) *Dev. Biol.* **30,** 307–320.
34. Levine, S., Pictet, R. & Rutter, W. J. (1973) *Nature (London) New Biol.* **246,** 49–52.
35. Folkman, J. & Moscona, A. (1978) *Nature (London)* **273,** 345–349.
36. Fuller, R. B. (1975) *Synergetics* (Macmillan, New York), p. 372.
37. Otto, F. (1973) in *Tensile Structures,* ed. Otto, F. (M.I.T. Press, Cambridge, MA), pp. 10, 148.
38. Kenner, H. (1976) *Geodesic Math* (Univ. California Press, Berkeley, CA), pp. 3–7.