The Matrix of Human Breast Tumor Cells Is Mitogenic for Fibroblasts

RICHARD T. KAO, DDS, JACKSON HALL, BS, LINDA ENGEL, MS, and ROBERT STERN, MD

From the Department of Pathology, University of California, San Francisco, California, and Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

The basis of the scirrhous reaction to human breast carcinoma was investigated. When normal human skin fibroblasts were plated on the preformed extracellular matrix of human breast tumor cells, a remarkable series of changes was observed. The matrix of the tumor cells was mitogenic for the fibroblasts. An increased growth rate and a fourfold increase in cell density was observed. There was also a change in cell morphology and in the pattern in which the cells grew, with an apparent loss of contact inhibition. The spindle-shaped fibroblasts became more elliptical and grew in a series of whorls and dense ridges with spaces between them. These observations were made with the use of newborn foreskin fibroblasts and the matrix of an established line of human breast cancer cells, ZR75–1. No such effect was seen when fibroblasts were plated on their own preformed matrix, on the matrixes of other cell types, on various type-specific collagen gels, or on a combination of collagen and fibronectin or when fibroblasts were grown in media conditioned by the ZR75–1 cells. A floating tumor cell matrix added to the cell media also did not provide the mitogenic stimulus. Apparently, fibroblasts required direct contact with the tumor cell matrix for the mitogenic response to occur. *In vivo*, the matrix of breast tumor cells may modulate the growth and the morphology of host stromal cells. Collagen is a major synthetic product of fibroblasts. The stimulation of stromal cells to proliferate by adjacent breast tumor matrix may be the basis of the desmoplastic reaction, the intense fibrotic response associated with human breast cancer. (Am J Pathol 1984, 115:109–116)

MANY HUMAN TUMORS are associated with a marked fibrotic response termed the scirrhous or desmoplastic reaction. The most profound response is elicited by human breast tumors, particularly infiltrating ductal carcinomas. The molecular basis of this reaction is not known, whether the fibrosis tissue is the product of the stromal cells or is synthesized by the malignant epithelial cells themselves. Convincing evidence has been provided to support each of these points of view.[1–4] Alternatively, the fibrotic response may be dependent on a continuous interaction between the two cell types. Since the desmoplastic reaction of breast carcinomas is characteristic only of the human disease, we have utilized an established continuous line of cultured human breast cancer cells, ZR75-1, and human newborn foreskin fibroblasts (NBFs).

It is now realized that the extracellular matrix (ECM) of cells is not only a structural support but is also informational and can modulate phenotypic expression of cells.[5–7] In early studies from this laboratory a four to fivefold increase in collagen synthesis was consistently observed when fibroblasts were plated on a preformed ECM of breast tumor cells. However, when calculated on a per-cell or micrograms of DNA basis, levels of collagen synthesis were only slightly higher in fibroblasts grown on a tumor matrix than in those grown on plastic. The increase in collagen synthesis was due to the much higher cell number in plates of cells grown on the breast cancer matrix. These findings suggested that the ECM of ZR75-1 or some factor associated with the ECM was mitogenic.

In this communication we demonstrate that the

Supported by PHS Grant CA 25179 PTHB awarded by the National Cancer Institute, DHHS, to Dr. Stern. A portion of this work was published previously (Fed Proc 1983, 42:1839).

Accepted for publication November 14, 1983.

Address reprint requests to Dr. Robert Stern, Department of Pathology, University of California, San Francisco, CA 94143.

breast tumor matrix was mitogenic for fibroblasts, that cells grew at a faster rate and at confluence reached a much higher cell number when plated on breast tumor ECM. The pattern of the fibroblast growth was also markedly different. The spindle-shaped cells became more elliptical and grew in whorls and ridges. Contact inhibition appeared to be lost without cells ever reaching true confluence. *In vivo*, breast tumor cells may be mitogenic for the stromal cells which surround them. We suggest that this effect is mediated through the tumor matrix or some factor associated with the matrix by some unknown mechanism. This may be the basis of the fibrotic reaction associated with human breast cancer.

Materials and Methods

Cell Cultures

The properties and growth characteristics of human breast cell line ZR75-1 have been previously described.[8] The NBFs in the third to fifth passage and the HeLa cells were obtained from the UCSF cell culture facility. Pancreatic and colon carcinoma cells were obtained from Dr. Young Kim, Veterans Administration Hospital, San Francisco, and have been described previously.[9,10] The R22 cells, a line of murine smooth muscle cells, were provided by Dr. Peter Jones.[11] All cells were cultured in RPMI 1640 with 4% fetal calf serum. This was defined as fresh media. Cells were grown at 37 C in an atmosphere of 95% air and 5% CO_2. For conditioned media, culture medium was conditioned by cells for 5 days and then diluted with an equal part of fresh RPMI 1640 containing 4% fetal calf serum.

Preparation of Matrixes

The matrixes produced by cells in culture were prepared in 35-mm dishes by treating 1–2-week-old cultures with 25 mM freshly prepared NH_4OH. Treatment was for 1–2 minutes and was monitored with an inverted phase microscope. This treatment removed cells but left the ECM intact.[12] The matrixes were then washed gently with PBS and stored in Hanks' buffer until used. The ZR75-1 and other tumor matrixes were fragile and were utilized immediately.

Fibronectin purified from human plasma was generously provided by Dr. Pearl Toy and Ms. Li-Wen Lien, UCSF.[13] Fibronectin at a concentration of 0.1 mg/ml was incubated on the collagen-coated culture dishes at 37 C for 1 hour.

Artificial matrixes composed of gels of collagen or collagen with fibronectin were prepared by pipetting 30 μg of collagen or collagen and fibronectin in 0.3 ml into 35-mm wells.[14,15] Types I, IV, and V collagen were respectively obtained from bovine skin, bovine anterior lens capsule, and human placenta. These wells were placed in a dish saturated with fresh NH_4OH at room temperature. Gelation of the artificial matrixes occurred in less than 5 minutes. Such matrixes were stored in Hanks' buffer until used.

Growth Kinetics

The growth kinetics were determined by obtaining cell counts every third day. Triplicate cell counts from three different 35-mm dishes were made by light microscopy with the use of a hemocytometer. Viability was determined by the trypan blue exclusion test. Cell viability was always greater than 97%.

Thymidine Incorporation

The measurement of DNA synthesis was determined by the incorporation of tritiated thymidine. Triplicate 35-mm dishes were used. The cells were incubated with 2.5 μCi methyl-^{3}H-thymidine per milliliter of fresh culture medium (20 Ci/mmol, 1.9×10^{-7} M final concentration) for 4 hours. The labeling period was terminated by the addition of 0.10 ml of cold 0.8% bovine serum albumin, which also served as carrier. After three cycles of cold 10% TCA wash, the amount of ^{3}H-thymidine incorporated into TCA precipitable material was determined. Pellets were solublized in 0.20 ml of 88% formic acid and 7 ml of Aquasol (New England Nuclear) was added. The data are reported as counts per minute (cpm) per 10^6 viable cells. Cells were counted in duplicate cultures at the beginning of the labeling period.

Results

Monolayer cultures of ZR75-1 breast tumor cells were established by seeding cell suspensions onto plastic dishes. After the cultures had become confluent, we treated the dishes to obtain the preformed matrix. The ECMs from other tumor cells and from fibroblasts were obtained in a similar fashion. NBF cells were then plated on these matrixes. Triplicate dishes were harvested every third day, and viable cell counts were determined. We observed that the growth rate of fibroblasts was increased. The final cell density was increased by 380% when seeded on the preformed breast tumor ECM, compared with uncoated plates (Figure 1). When fibroblasts were plated on their own preformed matrix, only a slight enhancement of NBF cell growth was present. The pre-

formed matrixes from HeLa cells, however, had no effect.

The ECM functions in many ways, in addition to being a structural support. One proposed role for the ECM *in vivo* is to sequester biologically active growth factors. This would provide persistent localized stimulation for cells.[16] We tested for the presence of such factors in medium conditioned by breast tumor cells. We examined cell densities of fibroblast cultures exposed to conditioned media in the presence and absence of ECM (Table 1). Neither medium conditioned by the breast tumor cells or that by fibroblasts had any mitogenic effect. As shown, increased fibroblastic proliferation was observed only when the cells were plated on ZR75-1 ECM. A modest increase was seen when fibroblasts were grown on the preformed fibroblast ECM. The combination of conditioned medium and ECM produced cell densities comparable to that produced by the ECM alone. No apparent mitogenic effect could be attributed to the conditioned medium.

We next invoked the possibility that the ZR75-1 breast tumor ECM itself contained sequestered growth factors, which had accumulated during the production of the matrix. The preformed breast tumor matrix was washed vigorously to suspend the matrix. The matrix was then introduced to an NBF culture growing on plastic so that it floated in the media. No change in growth rate was observed (Table 2). Thus it appears that fibroblasts required direct contact with the breast tumor cell matrix for the mitogenic response to occur.

Table 1 – Growth of Fibroblasts Under Various Culture Conditions*

Substrate	Culture media	Cell number ($\times 10^6$)	Relative to control
Plastic (control)	FM	1.25 ± 0.07	1.00
Plastic	ZR75-1 cell CM	1.29 ± 0.05	1.03
ZR75-1 ECM	FM	3.74 ± 0.07	2.99
ZR75-1 ECM	ZR75-1 cell CM	3.83 ± 0.02	3.06
Plastic	NBF cell CM	1.21 ± 0.08	0.97
NBF ECM	FM	1.83 ± 0.04	1.46
NBF ECM	NBF cell CM	1.81 ± 0.07	1.48

* Tissue culture plates (35 mm), uncoated or coated with ZR75-1 breast tumor cell ECM, were used to support fibroblast cell growth. The culture medium was either fresh media (FM) as described in Materials and Methods or conditioned media (CM). The latter was prepared by conditioning media by cells for a 5-day period and then adding 50% fresh media. The initial seeding of newborn fibroblasts was at 5×10^5 cells/plate. The data represent the average number of cells in triplicate plates on Day 15.

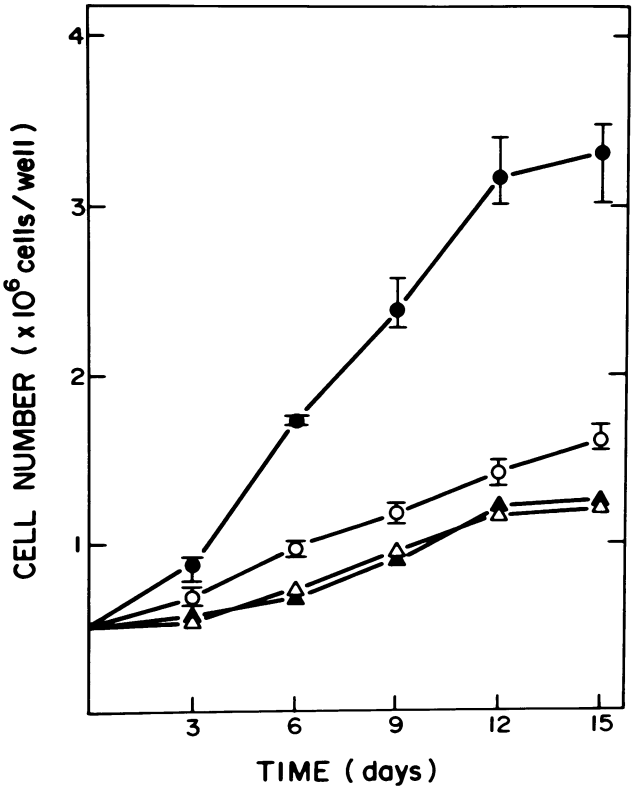

Figure 1 – ZR75-1 breast tumor cell preformed ECM is mitogenic for fibroblast growth. Tissue culture plates (35 mm) were coated with the ECM of various cells. Fibroblasts (5×10^5) were seeded on each plate and cell number was determined in triplicate every 72 hours. ZR75-1 breast tumor cell matrix (●——●); NBF cell matrix (○——○); HeLa cell matrix (△——△); and uncoated plates (▲——▲). For the latter two determinations, the range in triplicate cell counts was too close to indicate by bars.

The permissive growth of mitogen requiring cells on a preformed ECM has been reported previously.[6, 17-19] We examined the ability of various preformed ECMs to stimulate the growth of the human skin fibroblasts. No increases in cell density were observed when fibroblasts were grown on the preformed matrixes of HeLa, bovine corneal endothelium, R-22 smooth muscle, and embryonic chick fibroblast cells (Table 2). There was, however, some increase in cell density when fibroblasts were plated to the preformed ECMs of pancreatic and colon carcinoma cells.

The morphologic characteristics of fibroblasts grown on the breast tumor matrix was markedly different from the characteristics of those grown on other matrixes. Instead of the usual uniform-contact-inhibited monolayer, cells formed multilayered elongated colonies in whorls and ridges resembling isolated bundles and fasciculi. They were irregular, disposed in all directions with spaces between these dense ridges, and spaces between ridges never filled in, even when cultures were left for several weeks (Figure 2A–C). The morphology of the individual cell was also changed. The fibroblasts no longer had the usual elongated spindle shape but took on a more elliptical pattern.

Table 2—Growth of Fibroblasts With Various Matrixes*

Substrate	Cell number ($\times 10^6$)	Relative to control
Plastic (control)	1.22 ± 0.06	1.00
ZR75-1 ECM	3.27 ± 0.05	2.68
HeLa ECM	1.26 ± 0.08	1.03
Pancreatic Ca ECM	1.87 ± 0.09	1.53
Colon Ca ECM	2.01 ± 0.09	1.64
Bovine corneal endothelium ECM	1.42 ± 0.07	1.16
R-22 rat smooth muscle cell ECM	0.79 ± 0.03	0.64
Embryonic chick fibroblast ECM	1.28 ± 0.11	1.05
Plastic with floating ZR75-1 ECM	1.18 ± 0.07	0.97

* Tissue culture plates (35 mm) were coated with preformed matrixes of various cell lines. Newborn fibroblasts were seeded at a concentration 5 × 10^5 cells/plate. The data represents the average number of cells in triplicate plates on Day 15.

These changes were not present when cells were plated on the other matrixes. NBFs grown on plates coated with preformed HeLa cells, NBFs, or Type V collagen matrixes showed morphologic features similar to those of cells grown on uncoated plates (Figures 2D–G). Cells grown on the preformed matrix of bovine corneal endothelium, R-22 smooth muscle, embryonic chick fibroblast, pancreatic cells, and colon carcinoma cells exhibited no morphologic differences from cells grown on plastic (data not shown). Such cells demonstrated the normal spindle shape and retained contact inhibition upon reaching confluence. ZR75-1 tumor cells plated on a preformed matrix of NBF cells did not change in morphologic characteristics or growth pattern, compared with the same cells grown on plastic (Figure 2H and I).

Radiolabeled thymidine incorporation into these various cell preparations was examined on Day 15 of culture. The ZR75-1 breast tumor matrix stimulated thymidine incorporation into fibroblasts over fivefold, compared with fibroblasts grown on plastic (Table 3) during a 4-hour labeling period. Incorporation of label into fibroblasts grown on the preformed HeLa cell matrix was comparable to that of fibroblasts grown on plastic. Some stimulation of radiolabeled thymidine incorporation was observed when fibroblasts were grown on their own preformed matrix.

Collagen is the major component of the ECM and

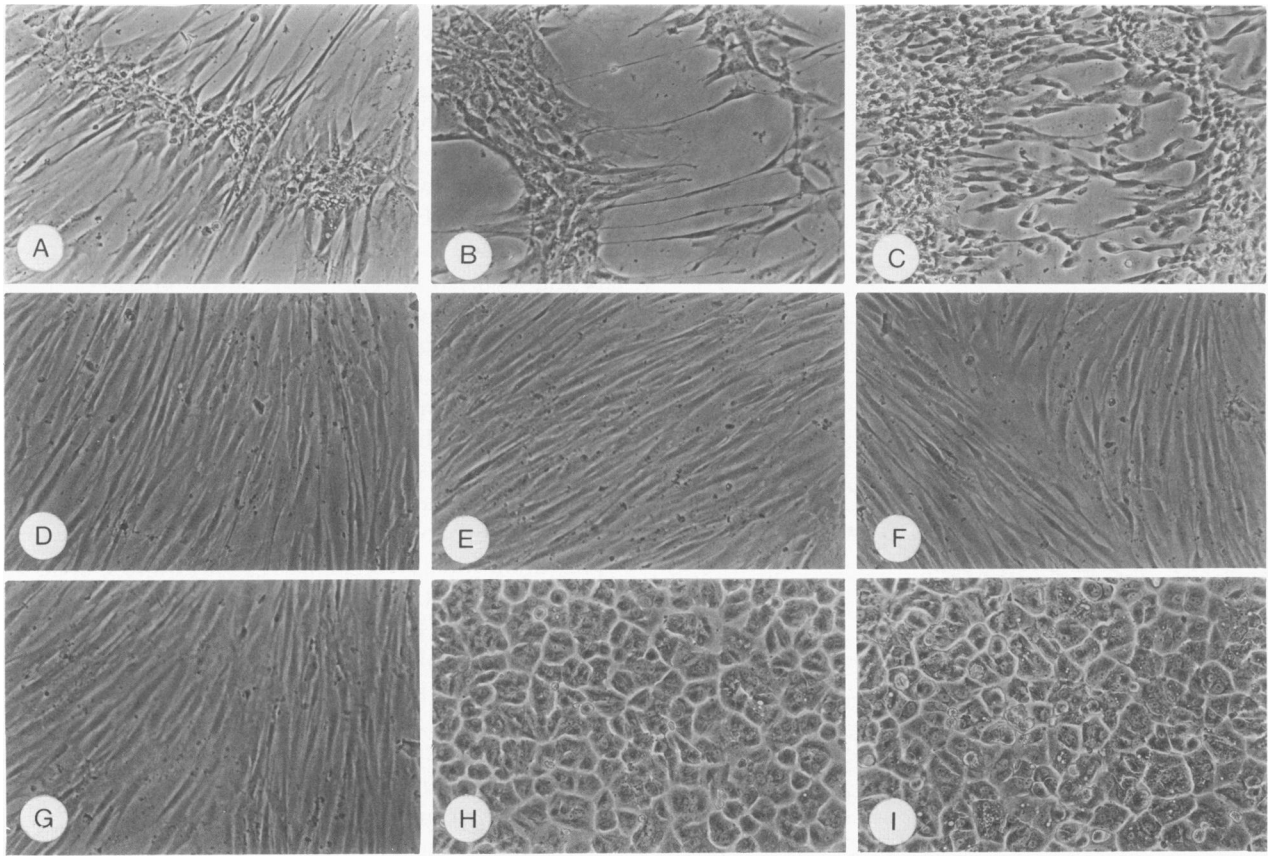

Figure 2—Morphologic appearance of fibroblasts maintained on breast tumor ECM and other substrates. Newborn fibroblasts were seeded on culture dishes coated with ECM or uncoated. Newborn fibroblasts were seeded on ZR75-1 breast tumor cell ECM and photographed on Day 12 (**A**), Day 15 (**B**), or Day 18 (**C**); on HeLa cells matrix (**D**); on the fibroblasts' own preformed matrix (**E**); on Type V collagen matrix (**F**); and on plastic (**G**). Breast tumor cells were seeded on plastic (**H**) and on the fibroblast preformed matrix (**I**).

is an important molecule in cell-matrix interactions.[20] Fibroblasts were grown on various type-specific collagens in an attempt to reproduce the mitogenic effect. Malignant epithelial cells can produce Type IV, basement-membrane-specific collagen. In addition, high levels of Type V collagen are characteristic of the desmoplasia of human malignant breast tumors.[21] Fibroblasts were plated on gels composed of these different type-specific collagens. Types I, IV, and V collagen gels were unable to elicit a mitogenic response in fibroblasts. Cells plated on a composite gel of Type I collagen and fibronectin also failed to stimulate cell growth (Figure 3).

Next, we attempted to identify the nature of the material in the preformed ECM responsible for the mitogenic stimulus to fibroblasts. NBFs were plated on breast tumor ECM that had been subjected to different pretreatments (Table 4). Prewashing the matrix with saline, with alcohol, or with the detergent Triton X-100 did not change the mitogenic effect. Fibroblasts were also grown on tumor cell lysates prepared hypotonicly. This produced no change in the rate of fibroblasts cell proliferation as compared with those grown on plastic. In this experiment, the breast tumor cells had been plated on plastic at high density; and after 1 day, before sufficient time had transpired for breast tumor ECM to accumulate, the cells were lysed. However, the mitogenic property of the breast tumor matrix was diminished after pretreatment with SDS and trypsin.

Discussion

Many human tumors have a dense fibrotic reaction called the desmoplastic or scirrhous response. Breast tumors have the most profound such reaction, followed by prostate, pancreatic, gastric, and colon carcinomas.[22] The basis of the desmoplastic reaction associated with these tumors is not known. Our experimental approach to this problem was to use the ECM produced by cultured breast tumor cells as a substrate for the growth of fibroblasts. We present evidence here that the mechanism for the desmoplastic reaction may be the mitogenic effect of the tumor cell ECM for adjacent host stromal cells.

Table 3—Thymidine Incorporation by NBF Cells on Various Substrates*

Substrate	cpm incorporated/10^6 cells
Plastic	15,248 ± 376
HeLa ECM	18,624 ± 147
NBF ECM	41,758 ± 465
ZR75-1 ECM	84,774 ± 794

* On Day 15, the incorporation of tritiated thymidine was determined. The labeling procedure is as described in Materials and Methods. Cell numbers were determined in triplicate in parallel cultures.

Table 4—Mitogenic Activity of Breast Tumor Cell ECM After Various Treatments*

NBF cell growth	% growth
On plastic	100
On NBF ECM	151
On ZR75-1 ECM	
Untreated	379
PBS treatment	382
0.5% Triton X-00	375
1% SDS	172
95% methanol	398
TPCK-trypsin	182
On ZR75-1 cell lysate layer	109

* Tissue culture plates (35 mm) with breast tumor ECM were treated in various fashions for a period of 1 hour. The ZR75-1 cell lysate layer was prepared by treating 24-hour culture with 0.2 M sodium phosphate buffer (pH 7.4), 0.1 M NaCl. After cell lysis, the dishes were washed gently with PBS. This procedure resulted in a dish coated with residual plasma membrane and cellular organelles. The data represent the average of experiments performed in triplicate. After treatment, the NBF cells (5×10^5) were seeded, and cell counts of triplicate plates on Day 15 are shown in terms of percentage of NBF cells grown on uncoated plates.

Grobstein proposed originally a role for the cell matrix in the control of cell proliferation and morphogenesis.[23] Since then, there have been many examples described in which the proliferative capacity of cells is modulated by the substrate upon which the cells are maintained.[7,17] What is unique in the present situation is that the matrix of one cell type is having a modulating influence on another cell which *in situ* is the adjacent cell population. In this tissue culture situation an interaction has been reproduced which may occur *in vivo* and which may form the basis of an important pathologic process, the scirrhous reaction.

The synthesis and deposition of collagen and glycosaminoglycans by corneal epithelial cells are stimulated when cells are grown on killed lens capsule.[24,25] Here, the lens capsule represents the matrix provided *in situ* by the adjacent stromal tissue. This situation may be analogous to the one described in the present studies, except here it is the epithelial cells that are responding to the matrix provided by the adjacent stromal cells. A general biologic principal can be recognized, that ECM is informational, not only for the cells that produce that matrix, but also for adjacent cell populations.

In the present system, the breast tumor ECM increased the growth rate of fibroblasts nearly fourfold. Associated with this increased growth rate was an apparent loss of contact inhibition. Groups of cells piled up in ridges and whorls with spaces between them. These spaces did not become filled, even after

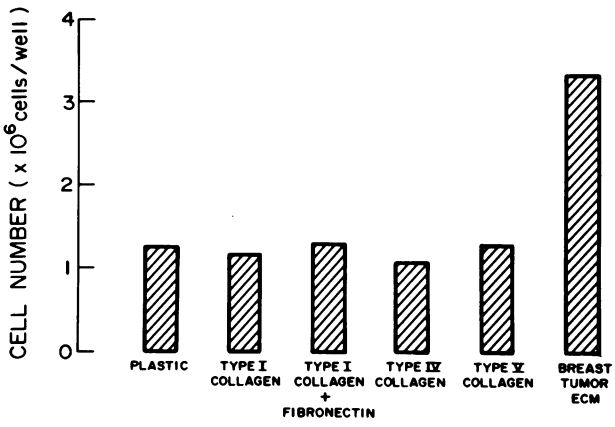

Figure 3—Comparison of newborn fibroblast cell growth on various artificial matrixes. Tissue culture plates (35 mm), coated with various type-specific collagens, uncoated, or with ZR75-1 breast tumor cell ECM were used to support fibroblast cell growth. The initial seeding of newborn fibroblasts was 5 × 10^5 cells/plate. The data represent the average number of cells in triplicate plates on Day 15. Deviations were too close to present by bars in the figure.

several weeks of culture. This was not observed when fibroblasts were plated on their own preformed matrix or the matrixes of other cells. The morphologic features of the cell patterns resembled those of myofibroblast cultures.[26] Transformation to myofibroblasts may be occurring in these cultures, a possibility that is being investigated.

The mitogenic stimulus was not observed when fibroblasts were plated on the preformed matrix of mesenchymal- and endothelial-derived cell lines. The ECM of HeLa cells, a continuous line of tumor cells of epithelial origin, also produced no effect. However, moderate mitogenic properties were observed with cells grown on the preformed matrixes of colon and pancreatic carcinoma cells. The desmoplastic reaction is also associated with both colon and pancreatic carcinomas *in vivo*. But it is much more moderate in these tumors than it is in breast tumors. The mitogenic response their ECM supports *in vitro* correlates with the desmoplastic reaction induced *in vivo*.

The substrate upon which cultured cells are maintained has been shown to be capable of phenotypic modification of cells.[7,18,19,27] When fibroblasts were seeded on the breast tumor ECM, changes in phenotypic characteristics were observed. Instead of the usual elongated spindle shape, an elliptical cell shape was adopted. Clinically the spindle cell population, the presumptive fibroblasts, is not conspicuous by light microscopy in the stroma of breast tumors. A change in shape may account for the scarcity of spindle-shaped cells in scirrhous carcinomas of the breast. No explanation can be offered as to why no such morphologic change was observed for fibroblasts grown on colon and pancreatic carcinoma cell lines. Perhaps the mitogenic nature and the ability to induce morphologic change are two independent properties of breast tumor ECM, only one of which is shared by other scirrhous tumors.

The desmoplasia of human breast carcinoma contains a 10-fold increase in Type V collagen.[21] This increase is attributed to host myofibroblasts that are recruited by the tumor. The origin of such myofibroblasts is not clear. We speculate from the morphologic changes we have observed that resident fibroblasts are transformed to myofibroblasts, and this is the actual origin of such cells *in vivo*.

We have explored the nature of the mitogenic property of breast tumor ECM by two additional approaches. The first of these was to seed fibroblasts or artificial matrixes, including those containing Types IV and V collagen, the predominant collagenous components of naturally occurring breast tumor ECM. No change in phenotype or proliferative capacity could be reproduced with any of the artificial matrixes.

The mitogenic property of the ECM is associated with the matrix, and only by direct contact can the fibroblast growth pattern be enhanced. The floating tumor cell matrix did not provide mitogenic stimulus, nor did breast-cell-conditioned medium. The findings suggest that the mitogen is neither diffused from matrix nor released into conditioned medium by tumor cells. These results do not exclude the possibility that growth factors may be adhering tenaciously to the matrix. Growth factors such as PDGF and FGF have been shown to adhere tenaciously to collagen-coated plates.[16] Such may be the case in our system whereby growth factors produced by the tumor cells are bound to matrix components. Indeed, the ECM may be functioning as a concentrating mechanism for such cellular and circulating growth factors. With cell contact, these growth factors may stimulate fibroblast proliferation. It is not possible from the present experiments to discern whether the mitogenic effect is associated with the matrix itself or with growth factors bound to the matrix.

In another series of experiments, the preformed matrix was perturbed by treatment with various agents. The mitogenic stimulus was lost with SDS or trypsin pretreatment of the ECM. Since growth factors may adhere tenaciously to the ECM, SDS or proteolytic treatment might be expected to remove or degrade such growth factors. Alternatively, some important structural component of the matrix may have been lost after such treatment, a component vital to the mitogenic stimulus. Proteoglycans at the matrix

surface may be such trypsin-sensitive components. Another interpretation is that the three-dimensional integrity of the breast tumor ECM was disrupted.

Another possibility was addressed, that the mitogen was associated with residual plasma membrane cell or organelle contaminants adherent to the matrix. The breast tumor cells were plated at high density. After 1 day, before sufficient time had transpired for ECM to accumulate, the cell layer was lysed, leaving a residual plasma membrane layer. No mitogenic effect was observed. This suggested that it was the ECM of the tumor cells which was mitogenic and not residual cellular contaminants adherent to the plastic plate.

The matrix of cells is composed of a complex of various type-specific collagens, elastin, glycoproteins, many of which are adhesion proteins, and a spectrum of proteoglycans and their attendant glycosaminoglycans in a highly ordered three-dimensional structure.[28] The components of the breast tumor ECM are now being characterized. It contains Type IV basement-membrane-specific collagen, as well as Type I and III interstitial collagens. Lamin and fibronectin have been observed by immunofluorescence microscopy and elastin is also detected (manuscripts in preparation). There are also high concentrations of proteases, including collagenases and elastases elaborated by breast tumor cells onto their surfaces as well as into the tissue culture medium.[29,30] Such degradative activities make characterization of tumor ECM components more difficult than that of other cells and tissues. There are many tumors that have elevated levels of associated proteolytic activity, compared with normal tissues. *In vivo* transformed cells have much higher protease levels when compared with their parental cells.[31] Trypsin and other proteases are known to stimulate division of cells in culture.[32,33] From these observations, another mechanism for the mitogenic activity of the tumor matrix can be suggested. Fibroblasts may be stimulated to divide by proteases imbedded in or on the surface of the tumor ECM.

Another possible model is that a structural component of the ECM is directly responsible for the mitogenic stimulation. Receptors for soluble collagen[34,35] and for other components of the ECM[36,37] have been demonstrated. Some structural moiety within the matrix may thus be responsible for the stimulation, and some as yet unidentified plasma membrane receptor of the stromal cell perhaps perceives that material and relays the signal to divide.

In summary, a preformed matrix deposited by human breast tumor cells was mitogenic for fibroblasts. There are several mechanisms that can be involved. The tumor matrix may be trapping and concentrating growth factors which are mitogenic for the fibroblasts. The matrix may be stimulating cell division by associated proteases. Finally, some structural matrix component may be providing the mitogenic stimulation. Whether this mitogenic effect is an artifact of tissue culture or reflects an actual *in vivo* situation cannot be established from the present experiments.

It has long been appreciated by pathologists that epithelial and connective tissues have mutual influences on one another *in vivo*. The German pathologists of the last century were the first to observe in fixed, stained sections of human breast cancer, mitotic figures in the stromal cells around tumor cell nests. They suggested that the malignant epithelial cells were accelerating the multiplication of fibroblasts.[38] We add here a further refinement, that it may be the ECM or some moiety or activity associated with the ECM of the tumor cells which is providing that mitogenic stimulus.

References

1. Al-Adnani MS, Kirrane JA, McGee JO'D: Inappropriate production of collagen and prolyl hydroxylase by human breast cancer cells *in vivo*. Br J Cancer 1975, 31:653–660
2. Willis RA: Spread of Tumor in the Human Body. London, Butterworths, pp 114–120
3. Trembly G: Ultrastructure of elastosis in scirrhous carcinoma of the breast. Cancer 1976, 37:307–316
4. Azzopardi JG, Laurini RN: Elastosis in breast cancer. Cancer 1974, 33:174–183
5. Tseng SCG, Savion N, Gospodarowicz D: Modulation of collagen synthesis by a growth factor and by the extracellular matrix: Comparison of cellular response to two different stimuli. J Cell Biol 1983, 97:803–809
6. Gospodarowicz D, Delgado D, Vlodavsky I: Permissive effect of the extracellular matrix on cell proliferation *in vitro*. Proc Natl Acad Sci USA 1980, 77:4094–4098
7. Vlodavsky I, Lui GM, Gospodarowicz D: Morphologic appearance, growth behavior and migratory activity of human tumor cells maintained on extracellular matrix versus plastic. Cell 1980, 19:607–616
8. Engel LW, Young NA, Tralka TS, Lippman ME, O'Brien SJ, Joyce MJ: Establishment and characterization of three new continuous cell lines derived from human breast carcinomas. Cancer Res 1978, 38:3352–3364
9. Kim YS, Tsao D, Hicks J, McIntyre L: Surface membrane glycoproteins of cultured human pancreatic cancer cells. Cancer 1981, 47:1590–1596
10. Kim YS, Tsao D, Siddiqui B, Whitehead JS, Arnstein P, Bennett J, Hicks J: Effects of sodium butyrate and dimethylsulfoxide on biochemical properties of human colon cancer cells. Cancer 1980, 45:1185–1192
11. Jones PA, Scott-Burden T, Gevers W: Glycoprotein, elastin, and collagen secretion by rat smooth muscle cells. Proc Natl Acad Sci USA 1979, 76:353–357
12. Jones PA: Construction of an artificial blood vessel wall from cultured endoethelial and smooth muscle cells. Proc Natl Acad Sci USA 1979, 76:1882–1886

13. Ruoslahti E, Hayman EG, Pierschbacher M, Engvall E: Fibronectin: Purification, Immunochemical Properties, and Biological Activities. Methods in Enzymology, Vol 82, Structural and Contractile Proteins. Part A, Extracellular Matrix. Edited by LW Cunningham, DW Frederickson. New York, Academic Press, 1982, pp 803-831
14. David G, Bernfield MR: Collagen reduces glycosaminoglycan degradation by cultured mammary epithelial cells: Possible mechanism for basal lamina formation. Proc Natl Acad Sci USA 1979, 76:786-790
15. Ruoslahti E, Engvall E: Complexing of fibronectin, glycosaminoglycans and collagen. Biochim Biophys Acta 1980, 631:350-358
16. Smith JC, Singh JP, Lillquist JS, Goon DS, Stiles CD: Growth factors adherent to cell substrate are mitogenically active *in situ*. Nature 1982, 296:154-156
17. Wicha MS, Lowrie G, Kohn E, Bagavandoss P, Mahn T: Extracellular matrix promotes mammary epithelial growth and differentiation *in vitro*. Proc Natl Acad Sci USA 1982, 79:3213-3217
18. Gospodarowicz D, Greenburg G, Birdwell CR: Determination of cellular shape by the extracellular matrix and its correlation with the control of cellular growth. Cancer Res 1978, 38:4155-4171
19. Gospodarowicz D, Vlodavsky I, Greenburg G, Johnson LK: Hormone and Cell Culture. Cold Spring Harbor Conferences on Cell Proliferation. Vol 6. Edited by G Sato, R Ross. New York, Cold Spring Harbor Laboratory 1980, pp 561-592
20. Hay ED: Collagen and Embryonic Development, Cell Biology of Extracellular Matrix. Edited by ED Hay. New York, Plenum Press, 1982, pp 379-409
21. Barsky SH, Rao CN, Grotendorst GR, Liotta, LA: Increased content of type V collagen in desmoplasia of human breast carcinoma. Am J Pathol 1982, 108:276-283
22. Robbins SL, Cotran RS: Pathologic Basis of Disease. Philadelphia, W. B. Saunders, 1979
23. Grobstein C: Tissue interaction in morphogenesis of mouse embryonic rudiments *in vitro*, Aspects of Synthesis and Order in Growth. Edited by D Rudnick. Princeton, NJ, Princeton University Press, 1955, pp 233-256
24. Meir S, Hay ED: Control of corneal differentiation by extracellular materials: Collagen as a promoter and stabilizer of epithelial stroma production. Dev Biol 1974, 38:249-270
25. Meier S, Hay ED: Stimulation of extracellular matrix synthesis in the developing cornea by glycosaminoglycans. Proc Natl Acad Sci USA 1974, 71:2310-2313
26. Buck RC: The influence of contact guidance on the orientation of colonies of subcultured vascular smooth muscle cells. In Vitro 1982, 18:783-788
27. Folkman J, Moscona A: Role of cell shape in growth control. Nature 1978, 273:345-349
28. Hay ED: Extracellular matrix. J Cell Biol 1981, 91: 205s-225s28.
29. Kao RT, Wong M, Stern R: Elastin degradation by proteases from cultured human breast cancer cells. Biochem Biophys Res Commun 1982 105:383-389
30. Kao R, Wong M, Stern R: A hormone responsive collagenase of human breast carcinoma cells. J Cell Biochem Suppl 1982, 6:163
31. Goldberg AR: Increased protease levels in transformed cells: A casein overlay assay for the detection of plasminogen activator production. Cell 1974, 2:95-102
32. Sefton BM, Rubin H: Release from density dependent growth inhibition by proteolytic enzymes. Nature 1970, 227:843-845
33. Greene LA, Tomita JT, Varon S: Growth stimulating activities of mouse submaxillary esteropeptidases on chick embryo fibroblasts *in vitro*. Exp Cell Res 1971, 64:387-395
34. Goldberg B: Binding of soluble type I collagen molecules to the fibroblast plasma membrane. Cell 1979, 16:265-275
35. Goldberg BD: Binding of soluble type I collagen to fibroblasts: Effects of thermal activation of ligand, ligand concentrations, pinocytosis, and cytoskeletal modifiers. J Cell Biol 1982, 95:747-751
36. Rao NC, Barsky SH, Terranova VP, Liotta LA: Isolation of a tumor cell laminin receptor. Biochem Biophys Res Commun 1983, 111:804-808
37. Lesot H, Kuhl U, von der Mark K: Isolation of a laminin-binding protein from muscle cell membranes. EMBO J 1983, 2:861-865
38. Hansemann D: Das Krebsstroma und die Grawitz'sche Theorie der Schlummerzellen. Virchows Arch 1893, 133:147-165

Acknowledgments

We thank Mrs. Susan Girod and Ms. Diana Sansoe for their skillful preparation of this manuscript.