Laminin and Fibronectin Promote the Haptotactic Migration of B16 Mouse Melanoma Cells In Vitro

JAMES B. McCARTHY and LEO T. FURCHT
Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota 55455

ABSTRACT The migration of tumor cells through basement membranes and extracellular matrices is an integral component of tumor invasion and metastasis. Laminin and fibronectin are two basement membrane- and extracellular matrix-associated noncollagenous glycoproteins that have been shown to promote both cell adhesion and motility. Purified preparations of laminin and fibronectin stimulated the directed migration of B16 murine metastatic melanoma cells in vitro as assessed in modified Boyden chambers. The stimulation of migration occurred over a concentration range of 1–100 μg/ml of laminin or fibronectin, with a peak response occurring between 12.5 and 25 μg/ml. The maximal response of these cells was 80–120-fold higher than control migration. Affinity-purified antibody preparations specifically abrogated the migration of these cells in response to the respective proteins. Tumor cells in suspension were preincubated in physiologic levels of plasma fibronectin prior to assay to partially mimic what occurs when a metastasizing cell is in the blood stream. This preincubation with plasma fibronectin had no effect on the subsequent migration of cells in response to either laminin or fibronectin. Furthermore, experiments using filters precoated with fibronectin or laminin indicated that these cells could migrate by haptotaxis to these two proteins. We conclude that tumor cell migration in response to such noncollagenous adhesive glycoproteins could be an important aspect in the invasion and metastasis of certain malignant cell types.

Metastasis is a process that is composed of a number of interrelated events that culminate in the successful translocation and growth of tumor cells within the host (1, 2). These events include penetration and entry into the vascular or lymphatic circulation from the primary site of tumor growth, followed by attachment to endothelium or subendothelial components, extravasation, and proliferation of cells at apparently selective sites that are distant from the original tumor site. The similarity between tumor metastasis and inflammation has been alluded to previously (3). Among a number of processes the two have in common is that both involve the recruitment and subsequent migration of cells through basement membrane and interstitial matrices. Numerous factors have been described that stimulate chemotaxis, the directed migration in response to concentration gradients of attractant, of inflammatory cells in vitro (4, 5). Several studies have also reported the existence of specific chemoattractants for tumor cells. These attractants include a proteolytic fragment of the complement fragment C5a (6), collagen and related peptides (7), and factors derived from resorbing bone (8) or tumor tissues (9). Injection of the complement-derived tumor cell attractant (10) as well as a tumor-derived attractant (11) have been reported to influence the formation of experimental metastases at the site of injection. These findings imply a role for tumor chemotactants in the "recruitment" of metastasizing cells to sites distant from the primary tumor mass.

Cell adhesion proteins play an important role in the phenotypic behavior of diverse cell types ranging from normal to highly metastatic cells (12, 13). Fibronectin and laminin are two of the best characterized cell adhesion proteins. Both proteins apparently have discrete regions or domains that serve unique functions, such as binding to specific collagens and proteoglycans, as well as to cell surfaces (12, 13). Initially, it was believed that epithelial cells attach specifically to laminin and that mesenchymal cells utilize fibronectin for adhesion (14). However, recent evidence indicates that both cell types can synthesize and utilize either fibronectin or laminin for attachment (15, 16). One important function of fibronectin involves the ability to direct cell movement. Studies have shown that fibronectin will promote the directed movement in vitro of various cells, including fibroblasts (17) and neural crest cells (18). This activity may be important in embryolog-
Migration of Melanoma Cells

The data in Fig. 1 depict migration of the tumor cells to increasing concentrations of fibronectin and laminin. Fig. 1A shows that the metastatic murine melanomas B16F10 and B16F10 cells migrate in response to increasing concentrations of purified fibronectin. The increase in cellular migration occurs within a range of from 1.5 to 12.5 µg/ml of fibronectin, and plateaus at protein concentrations higher than this. The maximum level of migration to fibronectin was 80-95-fold greater than control migration for both cell types. A highly significant concentration-dependent increase in cell migration of B16F10 and B16F10 to laminin was also observed (Fig. 1B). Maximum migration of these cells occurred in response to 12.5 µg/ml of laminin. The highest level of laminin tested (200 µg/ml) led to lower B16 migration compared with the peak response, a phenomenon that occurs for other cells and chemoattractants. Neither cell type responded to BSA at any concentration tested (1.5-100 µg/ml) (data not shown).

Effect of Antibody on Melanoma Migration

The addition of affinity-purified antibody to fibronectin or laminin (Fig. 2) specifically inhibited melanoma migration in response to the respective attractants. Thus, the migration of B16 melanomas in response to low levels of laminin (3.1 µg/ml) was completely inhibited by the addition of 8 µg/ml affinity-purified antilaminin antibody (Fig. 2A). In contrast, the addition of 25 µg/ml affinity-purified antifibronectin had no effect on laminin-stimulated movement. A similar specific inhibition was observed for antifibronectin when fibronectin was used as the attractant with no effect of antilaminin in these circumstances (Fig. 2B).

Checkerboard Analyses

Checkerboard analyses (34) were performed to study the nature of cell migration in response to laminin and fibronectin (Fig. 3). The assay is constructed by examining the migration of cells in the presence of increasing levels of attractant both above and/or below the filter. A comparison of cell migration levels toward a positive gradient of fibronectin or laminin (Fig. 3, A and B, below the diagonal) with movement observed in a reversed gradient, when more attractant is on the near side of the filter (Fig. 3, A and B, above the diagonal) demonstrated that migration in response to both attractants was directional in nature. Additionally, random migration, or the migration in the absence of an established gradient, was increased in response to both proteins (Fig. 3, A and B, along

---

1 Abbreviation used in this paper: DME, Dulbecco’s modified Eagle’s medium.
Preincubation of Melanoma Cells with Plasma Fibronectin

Since metastasizing cells in the blood stream come into contact with plasma fibronectin, experiments were performed to more closely parallel this circumstance. Suspensions of B16-F10 melanoma cells were adjusted to a final concentration of 5 x 10^5/ml in DME/HEPES containing 2 mg/ml BSA. Cells were preincubated for 1 h at 37°C in the presence or absence of plasma levels (250 µg/ml) of fibronectin and washed twice to remove excess fibronectin. Studies with other chemoattractants have shown that preincubation may desensitize cells to further stimulation immediately after this incubation (35, 36). This preincubation step had no effect on the subsequent migration of cells towards laminin or fibronectin (Table I). This suggested that the fibronectin present in this soluble form did not interact with cells.

Responsiveness of Melanoma Cells on Precoated Filters

Previous work has shown that a rat Schwann cell tumor line migrated over substratum-bound laminin in the absence of additional soluble attractant (23). The migration of cells due to substratum-bound attractant, termed haptotaxis, was more pronounced in the presence of a density gradient of substratum-bound laminin than in the absence of such a gradient (23). It was therefore of interest to determine if metastatic melanoma cells could respond similarly on precoated filters in the absence of additional soluble attractant. The data in Table II illustrate the results of this type of experiment. Low level random migration of these cells is observed on filters precoated on both sides in the absence of additional soluble attractant. Importantly, more cells accumulate on the lower surface when it is the only surface coated with the same concentrations of either fibronectin (a threefold difference) or laminin (a 15-fold difference), compared with "random" migration levels when both sides are uniformly coated with attractant proteins.

Effect of Soluble Attractant on Tumor Cell Migration over Attractant-precoated Filters

Preincubation studies suggested that fluid-phase fibronectin was not interacting with cells to promote migration in this system but instead was stimulating cells by first depositing on the filter surface. The precoating experiments further indicated that substratum-bound fibronectin (and laminin) could promote haptotaxis of these metastatic melanoma cells. To further elucidate the relationship between bound and soluble attractant, we next investigated melanoma migration using...
Fibronectin Concentration (μg/ml) | Preincubation | Challenge
---|---|---
0 | 0.4 | 0.4 | 2.8 | 0.2 | 7.4
6.2 | 36.9 | 28.2 | 4.6 | 7.4 | 1.0 | 10.6
12.5 | 53.2 | 35.6 | 14.0 | 38.1 | 11.2 | 13.0
25 | 44.0 | 34.8 | 27.2 | 19.2 | 27.4 | 16.4
50 | 32.8 | 22.0 | 23.0 | 21.6 | 19.8 | 23.8
100 | 39.2 | 25.8 | 27.3 | 28.6 | 17.0 | 20.7

B16 (F10) Melanoma Checkerboard

Laminin Concentration (μg/ml) | Preincubation | Challenge
---|---|---
0 | 0.4 | 0.4 | 2.8 | 0.2 | 7.4
6.2 | 59.4 | 19.4 | 14.4 | 11.2 | 9.8 | 4.8
12.5 | 69.8 | 30.2 | 20.0 | 14.6 | 12.6 | 3.4
25 | 67.2 | 27.3 | 26.8 | 25.0 | 16.6 | 4.8
50 | 66.6 | 37.2 | 45.0 | 16.0 | 16.0 | 4.0
100 | 40.2 | 34.4 | 34.4 | 25.0 | 12.8 | 4.4

B16 (F10) Melanoma Checkerboard

Table I: Lack of Effect of Preincubation of B16f10 Cells with Plasma Fibronectin on Subsequent Cellular Migration

| Preincubation | Challenge |
|---|---|
| Medium | Fibronectin |
| μg/ml | |
| Laminin | 60 | 29.2 | 31.0 |
| 15 | 72.0 | 79.8 |
| 3.8 | 46.4 | 51.4 |
| 0.9 | 17.6 | 8.4 |
| Fibronectin | 30 | 20.2 | 19.0 |
| 7.5 | 58.2 | 46.2 |
| 1.9 | 33.1 | 19.0 |
| Medium | 0 | 0 |

Table II: Migration of B-16 Melanoma Cells on Precoated Filters

| Protein coated | Surface coated | Migrated cells per HPF |
|---|---|---|
| Medium | Fibronectin |
| μg/ml | |
| Fibronectin | 100 | Both | 11.4 ± 2.9 |
| 100 | Distal | 31.4 ± 4.4 |
| 100 | Distal | 11.4 ± 2.9 |
| 10 | Both | 2.4 ± 1.0 |
| 10 | Distal | 30.0 ± 2.4 |
| No coating | 0.4 ± 0.2 |

The response of metastatic cells to challenge with soluble fibronectin on both sides of the assay was abrogated by the response of these cells to soluble fibronectin in the lower well. We considered the possibility that this inhibition of the migration response to soluble fibronectin was due to the presence of potentially "deactivating" levels of fibronectin on the filter surface, which were created by the necessity of using high levels of fibronectin during coating to saturate the filter. However, this concentration of fibronectin when applied only to the lower surface of the filter, enhanced migration levels significantly compared with control (both sides coated, no soluble fibronectin), indicating that cells could effectively migrate over this density level of bound fibronectin.

The response of metastatic cells to challenge with soluble laminin on laminin-coated filters (Fig. 4B) differed from that of fibronectin in one respect: the addition of soluble laminin to the lower wells in chambers containing coated laminin filters did cause enhanced melanoma migration compared with the migration on coated filters in the absence of such soluble attractant. However, the increased response to soluble laminin on laminin-coated filters was less than that observed when noncoated filters were used. Again, results using filters on which only the lower surface was laminin coated indicated that cells could migrate effectively on this density level of soluble fibronectin in the presence of or absence of additional soluble attractant. High levels of attractant (50 μg/ml of fibronectin and 100 μg/ml of laminin) were used to precoat filters on both the upper and lower surfaces. These concentrations of protein were chosen to saturate filter surfaces with bound attractant prior to use in the assay. The migration of tumor cells in response to additional soluble attractant on such uniformly coated filters was compared with migration levels observed on noncoated filters. The data in Fig. 4 illustrate the result of such experiments.

Melanoma cells were observed to migrate in a concentration-dependent manner, in response to increasing levels of soluble fibronectin using uncoated filters (Fig. 4A). In contrast, precoating the filter with fibronectin on both sides before the assay abrogated the response of these cells to soluble fibronectin in the lower well. We considered the possibility that this inhibition of the migration response to soluble fibronectin was due to the presence of potentially "deactivating" levels of fibronectin on the filter surface, which were created by the necessity of using high levels of fibronectin during coating to saturate the filter. However, this concentration of fibronectin when applied only to the lower surface of the filter, enhanced migration levels significantly compared with control (both sides coated, no soluble fibronectin), indicating that cells could effectively migrate over this density level of bound fibronectin.
DISCUSSION

Highly significant migration of melanoma cells in response to laminin and fibronectin was observed. The response was clearly due to specific protein effects, since the addition of BSA to the lower wells did not stimulate tumor cell migration. Both proteins stimulated maximal migration at concentrations between 12.5 and 25 μg/ml. Migration of tumor cells to laminin or fibronectin could be inhibited by the addition of the appropriate affinity-purified antibody.

Checkerboard analyses were performed to determine whether or not the migration was directional. These results indicate that maximal migration of these cells in response to laminin and fibronectin occurred in response to positive gradients of attractant (directed migration). Significant migration also occurred in response to challenge with increasing levels of attractant added simultaneously on both sides of the filter (increased random migration). Thus, migration of these metastatic melanoma cells to both attractants was due to accelerated random as well as increased directional components, as judged by this method.

The results of previous work in our laboratory on laminin-stimulated movement of a Schwann cell tumor line indicated that migration of these cells occurred primarily in response to substratum-bound attractant. This conclusion was supported by the observation that the filters bound laminin under the assay conditions used, and that filters precoated with laminin could support cell migration in the absence of additional soluble laminin. This type of migration due to substratum-bound laminin was operationally termed haptotaxis (based on Carter, reference 39) to distinguish it from chemotaxis, which involves the directed migration of cells in response to soluble concentration gradients of attractant. The distinction is important, since chemotactic mediators would be predicted to be operative over longer distances and as such may be important in the margination and active recruitment of metastasizing tumor cells at distant sites (10, 11). In contrast, haptotactic migration would be more involved with insolubilized constituents of the matrix or basement membrane directly promoting the invasion of metastatic tumor cells. Haptotaxis would be important for extravasation once the...
tumor cell had come into contact with matrices following endothelial cell retraction or in regions of exposed basement membranes.

The results in this study indicate that metastatic melanoma cells can migrate in a haptotactic manner to both insolubilized fibronectin and laminin. This conclusion is supported by results obtained from measuring migration levels on attractant-precoated filters in the absence of a soluble stimulus. Precoating of only the lower side of the filter with either attractant promoted more cellular migration to the lower surface than that observed using filters coated on both surfaces with the same concentration of attractant. This finding was similar to that observed for the haptotactic migration of Schwannoma cells on laminin-precoated filters (23).

Metastasizing tumor cells in the blood stream would come into contact with high levels of soluble plasma fibronectin. It was therefore important to analyze the effect of preincubation with plasma levels of fibronectin on melanoma cell migration in response to fibronectin and laminin. It was necessary to wash away excess soluble fibronectin prior to the assay, in this case to eliminate the potential influence on cell migration created by binding of this level of excess soluble fibronectin onto the upper filter surface (see below). The results indicated that preincubation of melanoma cells with physiologic levels of plasma fibronectin had no appreciable effect on subsequent laminin- or fibronectin-promoted movement of these cells. This indicates that soluble plasma fibronectin, encountered by hematogenously metastasizing tumor cells, would likely not inhibit laminin-mediated extravasation. However, if fibronectin were deposited on a surface, or occurred naturally in a "solid phase," such as in basement membranes or within connective tissues, then it could effectively mediate tumor cell migration. The observation is consistent with previous reports that have demonstrated that plasma fibronectin in solution does not interact well with vertebrate cells in suspension (37, 38). Furthermore, it is suggestive that fibronectin-mediated melanoma migration in this system may be totally in response to substratum-bound attractant.

The preincubation experiments with plasma fibronectin suggested that cells in suspension did not bind soluble plasma fibronectin. It was not clear if this represented a property of the suspended cell or instead was due to differences (e.g., conformational) between bound and soluble fibronectin (40, 41). Thus, it was of interest to examine migration of melanoma cells on fibronectin-precoated filters in the presence of soluble fibronectin. These results indicated that cells promoted to adhere to a fibronectin-precoated surface did not respond to challenge with increasing levels of soluble fibronectin in the lower well. Furthermore, the high levels of melanoma cell migration, observed on filters coated on the lower surface only, indicated that precoated filter-mediated inhibition of the response to soluble fibronectin was not due to "deactivation" of the tumor cells. We therefore concluded that the interaction and subsequent migration of the tumor cells to fibronectin in this system is a result of deposition of density gradients of attractant on the filter surface, and as such represents a haptotactic response (22). It is quite possible that the haptotactic requirement for this migration is determined by conformational alterations of fibronectin that occur upon binding of the protein to the filter, thus allowing it to interact with the cell surface. This conformational alteration has previously been determined to regulate the cell attachment properties of fibronectin in promoting the cell attachment to collagen-coated (40) as well as to synthetic surfaces (41). These conclusions contrast with those of Seppä et al. (42) in which they indicated that a chymotryptic cell-binding fragment of fibronectin stimulated the chemotactic response of fibroblasts. These differences may indicate a difference in the nature of the migratory response to various cell types to fibronectin, or may reflect an altered configuration of the cell-binding fragment relative to intact fibronectin. Further work using proteolytic fragments in our system is necessary to distinguish between these two possibilities for fibronectin-induced tumor cell movement.

The same type of experiment using laminin-coated filters and soluble attractant (laminin) is more difficult to interpret. Results using filters coated on both sides with attractant indicate that B16 melanoma cells are partially responsive to challenge with soluble laminin in the presence of a high level of bound laminin. This partial responsiveness could be due to additional binding of laminin to the lower filter surface in the presence of excess soluble laminin, contributing to the formation of increased density gradients on the substratum. Alternatively, the effect of soluble laminin on melanoma migration may reflect both binding of soluble laminin to membrane receptors as well as substratum-mediated interactions of bound laminin with the cell surface. Experiments using [3H]laminin indicated that a soluble concentration gradient was established and maintained by these filters for long periods of time. Only 10% of the available radioactive laminin diffused to the upper well after 3 h of incubation even with the highest concentration of laminin tested. Thus, it was concluded that cells are in the presence of a relatively steep (10-fold) concentration gradient of soluble laminin during the assay. At least one receptor for soluble laminin has been described for a number of tumor cell types (28, 29) and recently isolated (29, 43). Interestingly, the work of Brown et al. (44) would indicate that this receptor moiety, which they have termed connectin, can interact with actin filaments and cause bundling of these filaments. This observation allows the speculation that this cell surface receptor for soluble laminin is directly involved with stimulating the motility of the metastatic melanoma cells in the current study. Clearly, further work using different assay systems to assess tumor cell migration in the presence of bound and soluble attractants is necessary to accurately relate the nature of the cellular interactions of the matrix proteins with the subsequent attachment and motility responses of metastatic tumor cells.

It is clear that invasion of tissues by aggressive tumor cells must also be accompanied by the localized dissolution of matrices. Differences in the production and release of proteolytic enzymes that have been reported for cell types of varying metastatic potential are likely important in this regard (45-47). Of particular interest is a recent report indicating that migrating endothelial cells digest substratum-bound types IV and V collagen (48). An interruption of matrix-mediated tumor cell migration and associated enzymatic functions would greatly reduce, if not eliminate, the metastatic potential of a tumor population. A complete understanding of the involvement of extracellular matrix adhesive glycoproteins such as laminin and fibronectin in this regard may provide useful tools to ultimately control cancer metastasis, the major cause of death in cancer patients.

It is a pleasure to acknowledge the high quality of assistance of Ms. Elaine Oberle, Mr. Peter E. Schad, and Mr. Scott Hagen. The authors
also thank Ms. Kara Turnblom, Ms. Linda Kenny, and Ms. Carol Larson for facilitating the preparation of this manuscript.

This work was supported by National Institutes of Health grants CA 29995 and CA 21463 and the Leukemia Task Force. L. T. Furcht is a Stone professor of pathology and a recipient of Research Career Development Award K04-CA 00651 from the National Cancer Institute/National Institutes of Health.

Received for publication 26 August 1983, and in revised form 5 December 1983.

Reference:

1. Mushy, G. R., S. DeMartino, and D. W. Rowe. 1981. Collagen and collage-derived fragments are chemotactic for tumor cells. J. Clin. Invest. 68:1102-1105.

2. Furcht, L. T. 1982. Structure and function of the adhesive glycoprotein fibronectin. In Modern Cell Biology, B. Saito, editor. Alan R. Liss, Inc., New York. 203:176-179.

3. Klahr, K., H. Nishi, A. Ishikura, and H. Hayashi. 1976. Characterization of two different factors chemotactic for cancer cells from tumor tissue. Virchows Arch. B Cell Pathol. 21:119-131.

4. Lea, W. C. E., D. E. Delikatny, F. W. Orr, J. Varani, and P. A. Ward. 1981. Comparison of the chemotactic responsiveness of two fibroblast subpopulations of differing malignancy. Am. J. Pathol. 102:160-167.

5. Schaffman, E., and J. E. Gallin. 1979. Biochemistry of phagocyte chemotaxis. Curr. Top. Cell. Regul. 15:263-361.

6. Snydman, R. R., and E. J. Goedl. 1981. Molecular and cellular mechanism of leukocyte chemotaxis. Science (Wash. D.C.) 213:830-837.

7. Furcht, L. T. 1982. Structure and function of the adhesive glycoprotein fibronectin. In Modern Cell Biology, B. Saito, editor. Alan R. Liss, Inc., New York. 203:176-179.

8. Lea, W. C. E., F. E. Delikatny, F. W. Orr, J. Varani, and P. A. Ward. 1981. The chemotactic response of tumor cells. Am. J. Pathol. 104:69-76.

9. Osaki, T., K. Yoshida, K. Ohshigama, and H. Hayashi. 1971. Studies on the mechanism of invasion in cancer. II. In vivo effects of a factor chemotactic for cancer cells. Int. J. Cancer. 7:93-100.

10. Furcht, L. T. 1982. Structure and function of the adhesive glycoprotein fibronectin. In Modern Cell Biology, B. Saito, editor. Alan R. Liss, Inc., New York. 203:176-179.

11. Lea, W. C. E., F. W. Orr, J. Varani, and P. A. Ward. 1981. The chemotactic response of tumor cells. Am. J. Pathol. 104:69-76.

12. Furcht, L. T. 1982. Structure and function of the adhesive glycoprotein fibronectin. In Modern Cell Biology, B. Saito, editor. Alan R. Liss, Inc., New York. 203:176-179.

13. Yamada, K. M. 1983. Cell surface interactions with extracellular materials. Annu. Rev. Biochem. 52:761-779.

14. Tchividova, V. P., and D. H. Rohrbach, and G. R. Martin. 1980. Role of laminin in the attachment of PAM 212 (epithelial) cells to basement membrane collagen. Cell 22:719-726.

15. Couchman, J. R., M. Hooi, D. A. Rees, and R. Timpl. 1983. Adhesion, growth, and matrix production by fibroblasts on laminin substrates. J. Cell Biol. 96:177-183.

16. Oliver, N., R. F. Newby, L. T. Furcht, and S. Bourgeois. 1983. Regulation of fibronectin biosynthesis by glucocorticoids in human fibroblast cells and normal fibroblasts. Cell 32:287-296.

17. Gaunitz-Muller, V. H., K. Kleinman, G. R. Martin, and E. Schifman. 1980. Role of attachment factors and attractants in fibronectin chemotaxis. J. Lab. Clin. Med. 96:1071-1080.

18. Greenburg, J. H., S. Seppa, H. Seppa, and A. Tyl-Hewitt. 1981. Role of collagen and fibronectin in neural crest cell adhesion and migration. Dev. Biol. 87:259-276.

19. Repeha, L. A., T. J. Fitzgerald, and L. T. Furcht. 1982. Fibroblast involvement in granulation tissue and wound healing in rabbits. J. Histochem. Cytochem. 30:351-358.

20. A. B. Cinelli, K. E. Billingham, and L. Burgess. 1981. Distribution of fibronectin during wound healing in vivo. J. Invest. Dermatol. 76:181-189.

21. Donaldson, D. J., and J. T. Mabon. 1983. Fibrinogen and fibronectin as substrates for epidermal cell migration during wound closure. J. Cell Sci. 62:117-127.

22. Schur, S. L., A. M. Schor, and G. W. Bazett. 1981. The effects of fibronectin on the migration of human foreskin fibroblasts and Syrian hamster melanoma cells into three-dimensional gels of native collagen fibers. J. Cell Sci. 48:361-364.

23. McCarthy, J. B., S. L. Palm, and L. T. Furcht. 1983. Migration of haptoytic of a Schwann cell tumor line to the basement membrane glycoprotein laminin. J. Cell Biol. 72:777-782.

24. Rogers, S. L., P. C. Letoumeven, S. L. Palm, J. B. McCarthy, and L. T. Furcht. 1983. Neurite extension by peripheral and central nervous system neurons in response to substratum-bound fibronectin and laminin. Dev. Biol. 98:212-220.

25. Baron-van Evercooren, A., H. K. Kleinman, S. Ohno, P. Marangos, I. F. Schwartz, and M. Dubois-Dalal. 1982. Nerve growth factor, laminin, and fibronectin promote neurite growth in human fetal sensory ganglion cultures. J. Neurosci. Res. 8:179-193.

26. Terranova, V. P., L. A. Liotta, R. G. Ruso, and G. R. Martin. 1982. Role of laminin in the attachment and metastasis of murine tumor cells. Cancer Res. 42:2265-2269.

27. Varani, S. E. L., E. J. Lovett, J. P. McCoy, S. Shihab, D. E. Maddox, J. J. Goldstein, and M. Wicha. 1983. Differential expression of a laminin like substance by high- and low-metastatic tumor cells. Ann. J. Pathol. 111:27-34.

28. Repeha, L. A., T. J. Fitzgerald, and L. T. Furcht. 1983. Distribution of fibronectin during wound healing in vivo. J. Invest. Dermatol. 76:181-189.