Determinants of Human Astrocytoma Migration

Alf Giese, Monique D. Rief, Melinda A. Loo, and Michael E. Berens

ABSTRACT

A unique characteristic of astrocytic malignancies is their frequent dissemination through the brain. Cellular determinants of migration include adhesion to the substratum, restructuring of the actin cytoskeleton to generate motion, and (in the setting of invasion into tissue) secretion of enzymes for remodeling interstitial space to accommodate forward motion of the migrating cell. In order to better understand these features in the context of local brain invasion by astrocytoma cells, the adhesion and migratory properties of these cells have been investigated in an in vitro monolayer system. Adhesion of 8 different astrocytoma cell lines to different purified human extracellular matrix (ECM) proteins (collagen type IV, cellular fibronectin, laminin, and vitronectin) revealed that there is no "astrocytoma-specific" ECM protein that consistently leads to high cell binding. Similarly, migration of astrocytoma cells was found to be variable and dependent on different ECM proteins. Laminin was frequently the most permissive for adhesion and migration. Adhesion to collagen, fibronectin, and vitronectin was integrin dependent and could be blocked using anti-β1 integrin antibodies; in contrast, attachment to laminin could not be blocked using these antibodies. A comparison of adhesion with migration for each of the cell lines on each of the 4 ECM proteins revealed that poor adhesion was associated with minimal migration and that frequently, high adhesion was correlated with rapid migration. When tested for migration on autologous, cell-derived ECM, none of the cell lines were as migratory as they were on one of the purified ECM proteins, with the exception of SF767 cells. Furthermore, it was found that ECM from SF767 cells promoted the migration of other astrocytoma cells. The results from this study indicate that migration is a constitutive behavior of glioma cells which is dependent on, or modified by, the presence or absence of permissive ligands in the environment.

INTRODUCTION

The local invasive behavior of astrocytic neoplasms into surrounding normal brain accounts for the difficult clinical management and poor outcome of patients with these tumors (1). The ability to successfully permeate the brain is a unique feature of astrocytomas, which stands in contrast to the localized, well delineated lesions formed by other tumors metastatic to the brain (2). The infrequency with which astrocytomas successfully establish extracranial metastases (3) further highlights the unique interaction of astrocytomas with their local environment. The current study was undertaken to characterize astrocytoma cells for determinants of their migratory behavior in a monolayer culture system.

The biological attributes of cell migration and invasion include adhesion to the immediate tissue structure (either extracellular matrix or cell membranes), motility mechanisms to generate locomotion, and the ability to influence the local environment so as to create space into which to move (4). The latter process is generally believed to include selective remodeling of the extracellular matrix by the secretion of proteases (5, 6).

The brain is known to include the basement membrane glycoproteins fibronectin, laminin, and vitronectin, as well as collagen types I, III, and IV (7, 8); however, these structures are largely confined to major blood vessels and to the glial limits externa. Conceptually, these are border structures isolating the brain from the rest of the body and do not account for the patterns of invasion seen in spontaneous human astrocytomas. In this context, the mechanisms used by astrocytoma cells to move through the brain parenchyma remain poorly understood. It is uncertain whether invasive astrocytoma cells utilize constitutive brain ECM for migration or cell-cell interactions or whether they elaborate their own matrix for subsequent invasion. As an initial evaluation of the role of ECM proteins on the migratory behavior of human astrocytoma cells, purified human collagen type IV, laminin, cellular fibronectin, and vitronectin were used as substrates in this study.

Cell adhesion to ECM proteins is mediated by different types of receptors, among which is a class of transmembrane heterodimeric proteins called integrins (9). The assortment of α and β subunits (of which there are 16 and 8 presently described, respectively) (10) leads to differential affinity to different ECM ligands, although there may be some promiscuity for different ligands demonstrated by individual integrins. Further complicating this binding activity is the diversity of integrins which are able to bind to the same ECM ligand (reviewed in Ref. 11). The integrins serve to attach cells to the ECM and, in some instances, also function in conjunction with the cytoskeleton to provide the force for cell movement (12–14). The signal transduction events between adhesion to the ECM and cell migration are being elucidated for selected integrins and are revealing processes consistent with signal transduction pathways driven by other transmembrane receptors (15–18).

This study was undertaken to identify features of the adhesive and motility properties of human astrocytoma cells on defined ECM proteins or astrocytoma-derived ECM. The interrelationship between adhesion and migration was also investigated.

MATERIALS AND METHODS

Cells, Culture, and Extracellular Matrix. Human astrocytoma cell lines [NCE G-22, NCE G-28, NCE G-63, NCE G-112, and NCE G-118 (19, 20); U-251MG (21); and SF767 (22)] and one culture of normal human astrocytes (QG-4, kindly supplied by Joan R. Shapiro) were propagated in monolayer culture in minimal essential medium with 5–10% FCS (HyClone Laboratories, Logan, UT) for tumor lines or Waymouth's MAB 87/3 (GIBCO) supplemented with 20% FCS for the normal astrocytes. Cells were passaged using trypsinization at regular intervals depending on growth characteristics. Human extracellular matrix proteins and their sources were collagen type IV and vitronectin (GIBCO-BRL, Gaithersburg, MD) and laminin and fibronectin (Sigma Chemical Co., St. Louis, MO). Culture dishes or microtiter wells were coated with the ECM proteins by preparing stock concentrations (100 μg/ml) of the proteins and allowing these to coat the surface for 1 h at 37°C, rinsing the wells with PBS, blocking the surface with 1% bovine serum albumin (Sigma) for 30 min at 25°C, and then storing the vessels at 4°C with PBS. Production of astrocytoma-derived ECM was done using cultures of the cells which were allowed to persist at confluence for 10 days. After a thorough rinsing, the cells were lysed from the ECM using treatment with 0.5% Triton X-100, followed by 0.1 M NH4OH and 3 rinses with PBS (23, 24). Protein-coated dishes or

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2 To whom requests for reprints should be addressed, at Neuro-Oncology Laboratory, Barrow Neurological Institute of St. Joseph's Hospital and Medical Center, Phoenix, Arizona 85013-4496.

3 The abbreviations used are: ECM, extracellular matrix; FCS, fetal calf serum; PBS, phosphate-buffered saline; RT-PCR, reverse transcription-polymerase chain reaction.
cells were harvested from monolayer culture, suspended at a concentration of 10^6 cells/ml, and then deposited into microtiter wells (50,000 cells/well). The microtiter plates were incubated for 30 min on ice and then at 37°C for 60 min to allow adhesion. The plates were then subjected to vigorous agitation (350 rpm for 6 min on a horizontal rotator), after which nonattached cells were removed by aspiration and rinsing with PBS. Attached cells were fixed in 1% glutaraldehyde and stained using crystal violet (0.1% in H_2O). Absorbance of stained nuclei was quantified using spectrometry at 540 nm (BioTek Plate Reader; BioTek, Winooski, VT). Replicates from 6 wells were averaged. The number of attached cells is reported as absorption units for each cell line, which was linear over the range of cell numbers studied. The quantitative uptake of stain by the different cell lines, however, was not the same, and similar absorbance values do not reflect the same number of cells for each line.

In experiments using anti-integrin antibodies, the antibody was added to the wells prior to the cells. Anti-β integrin antibody was the hybridoma supernatant AIIB2 (kindly provided by Caroline Damsky, University of California at San Francisco, San Francisco, CA) which is biologically active in blocking the ligand-binding site of β-containing integrins (26).

**RT-PCR Analysis of Integrin Subunits.** Total RNA was isolated from monolayer cultures according to Chomczynski (27) and quantified by measuring absorbance at 260/280 nm. Reverse transcriptase synthesis of complementary DNA was done using a First Strand Synthesis Kit (Stratagene, La Jolla, CA) according to manufacturer's recommendations; aliquots of 2.5 μl were used for PCR. Primer pairs for each of the integrin subunits are shown in Table 1. These were derived from published material (28, 29) or were designed based on available integrin sequences with the assistance of Oligo 4.1 Primer Analysis Software (National BioSciences, Inc., Plymouth, MN). Amplification of DNA [2.5 μl of RT product, 2.5 μl of each primer, 0.5 μl of Taq polymerase (Perkin Elmer/Cetus), and 2.0 μl of nucleotides in 10 μl of 10X buffer, total volume of 100 μl] was allowed to run for 22–38 cycles (1 min at 94°C, 1 min at 60°C, and 2 min at 72°C) (MJ Research, Inc., Watertown, MA); aliquots of 9 μl were collected every 2 cycles. These aliquots were run in 2% agarose gels, stained with ethidium bromide, and photographed with Polaroid 665 film under UV illumination. The film negative was scanned for absorbance using visible light (Beckman DU-70). Absorbance values versus distance along the gel were collected and analyzed for peak area using PeakFit (Jandel Scientific, San Rafael, CA). The quantitative measures are reported as the interpolated number of PCR cycles needed to generate a product of peak area equal to 0.1 absorbance unit/mm. The molecular weights of the products were calculated relative to known size standards run in separate lanes on the same gels.

**Migration Assay.** A recently developed monolayer migration assay (30) was used to investigate the influence of different ECM proteins on astrocytoma movement. Briefly, surfaces of 8-well LabTek chamber slides (Nunc, Inc., Naperville, IL) were coated with ECM proteins or astrocytoma-derived ECM as described above. Custom produced sedimentation cylinders (G&G Glass, Gilbert, AZ) cut from 1-ml glass pipets with an internal diameter of 1 mm were placed into each chamber with 200 μl of culture media. A 1-μl cell suspension containing 2000 cells was added to each cylinder; the slide was kept on ice for 60 min to allow cell sedimentation and then incubated overnight. The cylinders were removed and fresh media supplemented with FCS and autologous astrocytoma-conditioned media were added. Daily serial diameter measurements of the cell population area were made using inverted microscopy (Axiovert; Zeiss, Thornwood, NY) and image analysis (VIDAS; Kontron, Eching, Germany). The diameter of the cell population increased linearly over time (30).

For contingency analysis (χ^2), each ECM protein studied with each cell line was ranked from 1 to 4 based on the extent of cell attachment and on the degree of migration. Substrates to which the cells bound or moved most rapidly were scored as 1; 4 was assigned to the substrate of least adhesion or migration. In instances where results were equivocal for different proteins, the ranking scores were averaged.

**Actin Staining.** Cells deposited within sedimentation cylinders as described above were allowed to migrate for 1–2 days and then fixed for 30 min in 1% paraformaldehyde at 23°C. Actin was stained using bodipy phalloloid (Molecular Probes, Eugene, OR) according to manufacturer's recommendations. Photomicrographs of the actin cytoskeleton were made under 546 nm excitation-580 nm emission fluorescence microscopy (Zeiss Axioplan, rhodamine filter set).

**RESULTS**

**Adhesion of Cells to ECM Proteins.** Human astrocytoma cells and normal cultured astrocytes adhered after a brief incubation to different purified human ECM proteins in a dose-dependent manner (Fig. 1). Relative to plastic "blocked" with bovine serum albumin, each cell line showed a preference for specific adhesion to at least one ECM protein. There was no "astrocytoma-specific" ECM which was consistently most effective for adhesion. Laminin was frequently the optimal adhesive substrate (5 of 7 cell lines); however, vitronectin and fibronectin were each optimal in separate instances (G-22 and G-118, respectively).

The best studied integrins which mediate specific adhesion of nonhematopoietic cells to ECM proteins are those containing a β subunit (11). When anti-β antibodies (AIIB2) were tested for their effect on SF767 astrocytoma adhesion to different ECM proteins, it

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**Table 1 Primer sequences for RT-PCR analysis of integrins**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Oligonucleotide</th>
<th>Annealing temperature</th>
<th>Size of fragment (base pairs)</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integrin β1</td>
<td>5' AAT GGG AAC AAC GAG GTC ATG GTT 3' 5'TTG TGG GAT TTG CAC GGG CAG TAC 3'</td>
<td>60°C</td>
<td>300</td>
<td>Bates et al., 1991 (28)</td>
</tr>
<tr>
<td>Integrin β3</td>
<td>5'TGC TCA TGG TTC CCG CCC TGC 3' 5'TGA TCT GAG GAT GAC TGA TTA TCA 3'</td>
<td>60</td>
<td>200</td>
<td>Bates et al., 1991 (28)</td>
</tr>
<tr>
<td>Integrin β4</td>
<td>5'GAC ACC AGG GGC ACC TAC AC 3' 5'CCT CCT GCC CTC TCT TA 3'</td>
<td>60</td>
<td>1132</td>
<td></td>
</tr>
<tr>
<td>Integrin α2</td>
<td>5'TGG GTG GCA AAC AGA CAA GGG 3' 5'GTA GTG CTG CTT GGT CAT C 3'</td>
<td>60</td>
<td>541</td>
<td>Milam et al., 1991 (29)</td>
</tr>
<tr>
<td>Integrin α3</td>
<td>5'TGG GCA GAT GGA TGT GGA TGA GAA 3' 5'GAT GAT GGG GGG GGG GTT GTC 3'</td>
<td>60</td>
<td>406</td>
<td></td>
</tr>
<tr>
<td>Integrin α5</td>
<td>5'GCC CTC CAC TGT ACA GCT G 3' 5'CAG CAA GTC ATC CAG CCC G 3'</td>
<td>60</td>
<td>564</td>
<td>Milam et al., 1991 (29)</td>
</tr>
<tr>
<td>Integrin α6</td>
<td>5'GAG CAC CAA GGA CCT TGG G 3' 5'GGG TAC ACT TCA AGA CCA GC 3'</td>
<td>60</td>
<td>619</td>
<td>Milam et al., 1991 (29)</td>
</tr>
</tbody>
</table>

* Gene sequences were retrieved from the Rockefeller Gene Bank and primer sequences were designed using Oligo 4.1.
DETERMINANTS OF ASTROCYTOMA MIGRATION

Fig. 1. Effect of different ECM protein coatings on the adhesion of human astrocytoma cells. Collagen type IV (ColIV), fibronectin (FN), laminin (Lam) and vitronectin (Vit) were incubated in microtiter plates at concentrations of 1, 10, and 100 μg/ml to coat the surface. Astrocytoma cells were deposited and incubated for 60 min to allow specific attachment. Nonspecifically attached cells were removed by shaking, aspirating, and rinsing. Cell number was determined by crystal violet spectroscopy of stained nuclei. Columns, mean of triplicate determinations; bars, SD; BSA, bovine serum albumin.

Fig. 2. Effect of anti-β1 integrin antibody on astrocytoma cell attachment to purified ECM proteins. Antibody was added to microtiter wells prior to adding the cells for the adhesion assay. Columns, mean of triplicate determinations; bars, SD.

Fig. 3. Expression of integrin subunits in astrocytoma cells determined by RT-PCR. Interpolated PCR cycle numbers needed to produce a product of 0.1 absorbance unit/mm peak area are shown for the integrin subunits from the different astrocytoma cell lines. The absence of bars indicates that a product did not appear after 38 cycles.
was found that attachment to collagen was inhibited in a dose-dependent manner (Fig. 2) but that only minimal effects on laminin adhesion were demonstrated. Effects on the attachment of the other cell lines to collagen and laminin by treatment with anti-β₁ antibodies was similar (data not shown). Attachment of SF767 to fibronectin and the limited adhesion to vitronectin were also blocked by anti-β₁ antibodies; adhesion to laminin was not affected by either anti-β₃ or anti-β₄ antibodies (data not shown).

RT-PCR Analysis of Integrin Expression. In order to determine the relative levels of expression of the integrin subunits by the different astrocytoma cells, RT-PCR was done (Fig. 3). The values represent the number of PCR cycles needed to generate a product of 0.1 absorbance unit/mm peak area. For each cell line, β₁ dominated the integrin subunit expressed; however, all the cultured astrocytic cells also produced at least one other β chain (β₂, β₄, or both). The levels of α subunits expressed by the cells showed a wide variation. In 5 of 7 cell lines, α₅ was the most prevalent α subunit; α₅ was never the most abundant. While this does not mean that the proteins were expressed at levels analogous to the mRNA or that the translated integrin proteins were functionally active, these results indicate that astrocytoma cells in culture exhibit varying profiles of integrin α and β subunit transcription.

Migratory Response of Astrocytoma Cells to ECM Proteins. Astrocytoma cells deposited onto different purified ECM proteins manifest different behaviors relative to their lateral movement and pattern of dissemination. Generally, two different morphological patterns of cell dispersion were seen; these are presented using data from

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Fig. 4. Human astrocytoma cells on different ECM proteins. G-28 cells (a-d) and G-112 (e-h) were deposited in sedimentation cylinders onto ECM-coated slides and fixed after 3 days of migration. Cells were plated on laminin (a and e), collagen type IV (b and f), fibronectin (c and g), or vitronectin (d and h). G-28 cells expanded as a contiguous front of advancing cells, whereas G-112 demonstrated features of single cell outward migration.

Fig. 5. Migration behavior of human astrocytoma cells on different ECM proteins. The increase in area (measured in pixels) occupied by the migrating astrocytoma cell population is plotted as a function of time. △, laminin; ○, collagen type IV; ■, fibronectin; ▼, vitronectin. Points, means of triplicate determinations; bars, SD.
DETERMINANTS OF ASTROCYTOMA MIGRATION

On all substrates, G-28 cells expanded as a contiguous front of advancing cells; however, on collagen G-28 cells showed the fastest migration rate as well as a larger number of solitary migrating cells (Fig. 4b). G-112 dispersed on laminin and collagen in a manner reminiscent of a starburst; these cells moved only minimally on vitronectin, and the population appeared to retract onto itself when plated on fibronectin.

The quantitative migratory behavior of the different cell lines showed even greater differences. Migration was quantified as the incremental increase in area occupied by the cell population beyond that area measured on the day the sedimentation cylinders were removed. This incremental expansion of the advancing rim of migrating cells follows a linear relationship over time on every substrate for each cell line, regardless of the degree of permissiveness for migration (Fig. 5). G-22 cells were essentially nonmigratory on collagen and expanded on the other three ECM proteins, reaching the greatest distance on laminin. G-28 was a relatively fast moving cell line on all

Fig. 6. Association between adhesion and migration of astrocytoma cells on different ECM proteins. Each ECM protein for each cell line was ranked from 1 to 4 according to its permissiveness for adhesion and migration (1 represents most adhesion or greatest migration). ▲, laminin; ◆, collagen type IV; ▲, fibronectin; ▼, vitronectin. . . , separation of data into 4 quadrants for χ² analysis. Contingency was tested using χ² analysis of the distribution of the data pairs; P < 0.01.

Fig. 7. Polymerized actin cytoskeleton in human astrocytoma cells on different ECM proteins. G-22 (a and b), G-28 (c and d), and G-112 (e and f) cells were seeded onto permissive (left) or nonpermissive (right) ECM proteins. These were laminin (a and f), collagen (b and c), and fibronectin (d and e). Astrocytoma cells on permissive substrates contained polymerized actin at the plasma membrane that modeled filopodia (a and e) and lamellipodia (c and e), whereas nonpermissive substrates induced heavy actin bundles that were largely confined to cytoplasmic structures (b and f) or immature lamellipodia (d).
the substrates but showed the fastest rate of dispersion on collagen. G-112 cells displayed a wide range of migratory responses to the different ECM proteins, with laminin being the most permissive and vitronectin being almost completely nonpermissive for movement; collagen was more permissive than fibronectin but both of these were less effective than laminin.

The migratory behavior of astrocytoma cells on different ECM proteins demonstrated that these cells respond to their insoluble environment with consistent and stable rates of migration. The speed of migration on the different ECM proteins can be ranked according to permissiveness, ranging from 1 to 4, with a score of 1 indicating a highly migratory response. Similar scores were assigned for the adhesive behavior of the astrocytoma cells on the different ECM proteins. It was of interest to determine whether the pattern of adhesion to the different ECM proteins after 60 min was related to the eventual migratory behavior observed over 2 days. Paired adhesion and migration data from each cell line on each ECM protein were plotted (Fig. 6). The ranked data could be divided into 4 quadrants based on high/low attachment (ordinate) and high/low migration (abscissa). Statistically, this analysis indicates that migration of astrocytoma cells is contingent on adhesion (P < 0.01, χ²). Substrates to which the cells adhered poorly were relatively nonpermissive for migration; ECM proteins to which the cells showed a rapid attachment also were relatively more supportive of migration.

**Cytoskeletal Changes Associated with Migration.** The influence of different ECM proteins on the actin cytoskeleton was studied. Cells were allowed to migrate in monolayer culture on surfaces coated with the different ECM proteins and then stained for polymerized actin (Fig. 7). Cells on all substrates contained similar cytoplasmic actin bundles. Astrocytoma cells migrating on permissive substrates showed intense staining of actin filaments that either condensed parallel to the lateral aspects of the cell membrane or projected into structures reminiscent of broad lamellipodia and advancing filopodia. Cells on substrates nonpermissive or less permissive for migration showed polymerized cytoplasmic actin bundles similar to cells on permissive substrates; however, there were no filopodia or mature lamellipodia. A frequent observation in nonmigrating cells was the strong staining for actin at the plasma membrane that was interpreted to be immature lamellipodia (Fig. 7d). These structures appeared as closely grouped, overlapping bubbles of actin-containing membrane, in contrast to more dispersed, maturing lamellipodia in migrating cells. The actin-containing plasma membrane in nonmigrating cells appeared to be lamellipodia, which failed to develop into a “leading edge”. Nonmigrating cells also showed less space between adjacent cells than migrating cells.

**Astrocytoma-derived ECM: Adhesion and Migration.** In order to determine whether astrocytoma cells produce their own extracellular matrix conducive to migration, the influence of autologous ECM on cell migration was studied. Cells were allowed to grow at confluence for 10 days. Cells were then lysed and the residual ECM was rinsed in PBS. Autologous cells were studied for adhesion in ECM-coated microtiter wells and for migration using sedimentation cylinders in 8-well chamber slides onto which autologous ECM had been deposited. The rapid adherence of astrocytoma cells to autologous ECM occurred to an extent similar to or greater than that to laminin (Fig. 8). However, autologous ECM was found to be less permissive for migration than laminin (Fig. 9), with the exception of SF767 cells, which migrated more rapidly on their own ECM than on laminin. When other astrocytoma cells were tested on SF767 ECM, adhesion and migration were also supported. These results suggest that the movement of astrocytoma cells in monolayer culture is almost exclusively determined by the nature of the surface features present on the matrix independent of modifications made by the tumor cells.

**DISCUSSION**

Our results indicate that human astrocytoma cells show preferential adhesion and migration on specific extracellular matrix proteins and that the preferred substrate varies according to cell line. Some cell lines, such as U251-MG, G-112, G-118, and QG-4, showed a single preference for high levels of rapid adhesion; others demonstrated more promiscuous adhesion to several ECM proteins. Overall, laminin was the most effective substrate for the adhesion and migration of astrocytoma cells.

Cell adhesion to collagen, fibronectin, and vitronectin was blocked partially or fully by incubation of the cells with anti-β1 antibodies, implicating β1-containing integrins in adhesion. Since β1 integrin dimerizes with a wide assortment of α subunits (11), leading to receptors recognizing many ECM ligands, the key role of β1-containing integrins in astrocytoma adhesion is not unexpected.

It was of interest that adhesion of the astrocytoma cells to laminin could not be blocked by anti-β1 antibodies. Laminin is a complex molecule relative to cell adhesion and has been described to contain several domains that serve as ligands for integrins (31). The majority of integrins which recognize different proteolytic cleavage fragments of laminin utilize the β1 chain combined with different α subunits (32); αβ1, αβ2 have also been described as laminin receptors (Refs. 33 and 34, respectively). Because the different astrocytoma cell
lines also contained mRNA for either β3 or β4 subunits, further studies are in progress to determine whether integrin heterodimers with these subunits mediate adhesion to laminin (preliminary studies indicate that they do not). Sobel (35) has described a nonintegrin receptor for laminin. It is unknown whether human astrocytes or astrocytoma cells express this M, 67,000 protein.

The inability to predict substrate adhesion from the levels of integrin subunit mRNA suggests that translational regulation and mature protein activation are likely to be central features in the manifestation of functional integrin dependent behavior of astrocytoma cells (11). Furthermore, the interactions of integrins with cytoskeletal elements (36) and other integrin-mediated signal transduction mechanisms, such as phosphorylation (37–39), may override attempts at simplistic correlations between levels of expression and function (40).

Migration of astrocytoma cells in monolayer culture on defined ECM proteins follows a linear expansion over time (up to 3 days). All the cell lines migrated on at least one substrate, and several were nonmigratory on some substrates. This suggests that the cellular determinants of migration are relatively unchanged and are not induced or modified over several days. There were some concerns that astrocytoma cells customized their environment through the modification of an extracellular matrix that would impair our ability to determine effects from individual ECM proteins deposited exogenously. The results in Figs. 8 and 9 demonstrate that with only one exception, autologous cell-derived ECM is a poor substrate for astrocytoma migration, despite the finding that these same matrices were effective adhesive substrates for autologous cells. The one instance in which autologous ECM was a superior migratory substrate (SF767), also proved it to be a substrate on which other astrocytoma cells freely moved. This diverges from the correlation between adhesion and migration seen on purified ECM proteins. It is likely that the concert of ligands represented on cell-derived ECM activates a diversity of integrins on adhering and migrating cells, which would be different than those activated by matrices of single, purified proteins. These results also suggest that integrins which mediate adhesion are not necessarily the same integrins which mediate migration. The data do, however, allow the conclusion that astrocytoma cells in culture are opportunistic for migration and do not generally synthesize their own matrix for migration.

Local environmental factors (i.e., the composition of the matrix or the repertoire of cell surface ligands) determine the migratory behavior of transplanted mouse astrocytes rather than the developmental stage of the astrocytes (fetal versus adult) (41) or the anatomical region from which the transplanted cells are harvested (42). Astrocytes derived from different brain regions do not contain homing information that targets their eventual migratory destination but rather move according to local environmental cues (43). Our results suggest that local factors (ECM, cell ligands, or local soluble factors) in particular regions of the brain may also dictate the patterns and routes of astrocytoma invasion.

The appearance of the polymerized actin cytoskeleton in cells on permissive and nonpermissive substrates provides a lead for mechanisms by which the ECM modifies motility behavior of astrocytoma cells. Actin remodeling is a critical mechanical determinant of cell migration and is mediated by events at the cell membrane (44, 45). The actin cytoskeleton in the migrating astrocytoma cells in our study showed features of filopodia extension and maturation of lamellipodia. The latter structures could become rather large relative to the overall cell area and yet this was highly indicative of cell migration. Nonmigrating cells were noticeably deficient in the generation of filopodia and mature lamellipodia. Frequently, the lamellipodia that did develop in nonmigrating cells resembled overlapping bubbles of condensed actin in the membrane. These we interpret to be immature lamellipodia that were unable to advance over the substrate.

White matter tracts are the predominant route of migration for transplanted normal astrocytes (46) and are the preferred avenues for dissemination of malignant astrocytes (47). There may be ligands within this anatomical structure, either as an accessible matrix for astrocytoma cells or as cell membrane epitopes, which are highly permissive for migration and invasion. Our results strongly indicate that migration is a constitutive behavior of human astrocytoma cells.
which is dependent on, or modified by, the presence or absence of permissive ligands in the environment.

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