

Laboratory Investigation

Glioma-produced extracellular matrix influences brain tumor tropism of human neural stem cells

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Summary

A major obstacle in the treatment of gliomas is the invasive capacity of the tumor cells. Previous studies have demonstrated the capability of neural stem cells (NSCs) to target these disseminated tumor cells and to serve as therapeutic delivery vehicles. Less is known about the factors involved in brain tumor tropism of NSCs and their interactions within the tumor environment. As gliomas progress and invade, an extensive modulation of the extracellular matrix (ECM) occurs. Tumor-ECM derived from six glioblastoma cell lines, ECM produced by normal human astrocytes and purified ECM compounds known to be upregulated in the glioma environment were analyzed for their effects on NSCs motility *in vitro*. We found that tumor-produced ECM was highly permissive for NSC migration. Laminin was the most permissive substrate for human NSC migration, and tenascin-C the strongest inducer of a directed human NSC migration (haptotaxis). A positive correlation between the degree of adhesion and migration of NSCs on different ECM compounds exists, as for glioma cells. Our *in vitro* data suggest that the ECM of malignant gliomas is a modulator of NSC migration. ECM proteins preferentially expressed in areas of glioma cell invasion may provide a permissive environment for NSC tropism to disseminated tumor cells.

Abbreviations: Astro-ECM – Astrocyte-derived ECM; ECM – Extracellular matrix; G-ECM – Glioma-derived ECM; HB1.F3 h-NSCs – HB1.F3 human neural stem cells; NSC – Neural stem cell

Introduction

Gliomas are among the most common type of human primary brain tumor; malignant gliomas represent 22–27% of all brain tumors [1]. Despite many technological advances in neuroimaging, neurosurgery, chemotherapy and radiation therapy, the average survival after diagnosis remains only 12–18 months [2]. The major reason for this dismal prognosis is based on the highly infiltrative nature of glioma cells. The glioma cells that have migrated from the core mass generate tumor microsatellites in the normal brain parenchyma. These microsatellites are not accessible by surgery and are the seed for the recurrent tumor growth [3].

The eradication of invading glioma cell microsatellites, before they give rise to a recurrent tumor, may be a valuable therapeutic strategy for glioma treatment, which could prolong survival time [4]. Genetically modified neural stem cells (NSCs) are an ideal tool to deliver therapeutics to distant glioma microsatellites [5,6] and disseminated brain metastasis [7]. Experimental

in vivo studies have demonstrated that NSCs are capable of migrating long distances within the brain, and specifically target and enrich in the tumor mass. It has been shown that NSCs “track down” single glioma cells migrating away from the main tumor mass [5]. The administration of NSCs engineered to express various therapeutical molecules, increased survival time in glioma animal models [6,8,9]. However, little is known about the signals and factors influencing the tumor tropism of NSCs and their interactions within the tumor environment. Answers to these key questions are essential for clinical realization of stem cell technology in human cancer.

It has been speculated that soluble factors which are overexpressed by tumor cells may be an important signal for the long-range attraction of NSCs from distant sites. The first experimental evidence indicates that tumor-upregulated VEGF [10] and SDF-1 [11] are serving as soluble chemotactic factors for inducing glioma tropism of NSCs. However, the observation that even microsatellites and invading glioma cells distant from the main tumor mass are targeted by NSCs suggests that additional local signals for NSC guidance exist. The

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migration of glioma cells during the invasion process is associated with a complex and continuous remodeling of the pre-existing normal extracellular matrix of the brain parenchyma (reviewed in Ref. [12]). *In vitro* and *in vivo* studies revealed that in contrast to the pre-existing normal ECM, the ECM of gliomas and their migration pathways consist mainly of tenascin, fibronectin, laminin, vitronectin and different types of collagen [12–16]. These ECM proteins are known to play an important role in controlling and facilitating the motility of glioma cells. Hence, we hypothesize that the tumor ECM may have a significant influence on the migration and tropism of NSCs. Invading glioma cells may leave a trail through the normal brain parenchyma, serving as a permissive and attractive guidance signal for tumor-targeting NSCs.

Here, we demonstrate that the tumor-produced ECM of different human glioma cell lines is a permissive substrate for the *in vitro* migration of human NSCs. Purified ECM proteins (of those which are known to be upregulated in the tumor ECM of malignant gliomas) are highly stimulatory for human NSC migration, especially laminin and tenascin which are preferentially expressed by invading glioma cells *in vivo*. Our *in vitro* data suggest that the ECM of malignant gliomas is a modulator of NSC migration and may potentially serve as an additional local guidance signal for NSC tropism to disseminated tumor cells.

Material and methods

Cell culture

The human neural stem cell line HB1.F3 (HB1.F3-hNSCs) used was provided by Dr Seung Kim [17,18] and maintained as a monolayer culture in Dulbecco's modified eagle medium (DMEM) (Life Technologies, Grand Island, NY, USA) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.25 µg/ml fungizone, and 10% heat inactivated fetal bovine serum (Life Technologies). One rat glioma cell line (CNS-1 provided by WF Hickey), five human glioblastoma cell lines (U251, U343, U87 from ATCC; D566 from Darryl Bigner and SJ-GBM [19]) and human astrocytes derived from normal brain tissue adjacent to a surgical specimen of a resected arterial venous malformation were used in these studies. They were cultured in DMEM supplemented with 2 mM L-glutamine, 2 mM sodium pyruvate, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.25 µg/ml fungizone, and 10% FBS. All cells were propagated in tissue culture flasks in 5% CO₂/95% air at 37 °C in a humidified incubator and routinely passaged using trypsinization.

ECM coating

The following purified human ECM compounds were used for the monolayer migration-, adhesion- and haptotaxis assay: collagen I (Cohesion, Palo Alto, CA), tenascin-C and vitronectin (Chemicon International, Temecula, CA), fibronectin, hyaluronic acid and lami-

nin (Sigma, St Louis, MO). Furthermore, normal and growth factor reduced Matrigel (Becton Dickinson Labware, Bedford, MA) were used. Teflon ten-well printed microscope slides were used (Creative Scientific Methods Inc., Phoenix, AZ) for the monolayer migration assay, and 96-well plates (Corning Inc., Acton, MA) for the adhesion assay. They were coated with each of the ECM compounds at a concentration of 100 µg/ml diluted in PBS and incubated at 4 °C overnight. The following day the surface of the wells was blocked with PBS containing 0.1% bovine serum albumin (BSA) (Sigma) for 30 min at room temperature and then rinsed one time with DMEM.

Preparation of glioblastoma cell line derived ECM

Tumor-ECM derived from cultured glioblastoma cell lines and non-tumoral ECM derived from normal human astrocytes was prepared as described previously [13]. Briefly, the cell lines were allowed to grow to confluence for 3 days, then lysed from the ECM using 20 mM of NH₄OH and carefully rinsed with PBS. Teflon ten-well coated slides and 96-well plates coated with cell – derived ECM, were used for adhesion and migration studies on the same day of the preparation.

Cellular monolayer migration assay

The permissive characteristics of different ECM compounds on HB1.F3-hNSCs migration were quantified using a modified method previously described by Kaczarek [20]. Briefly, Teflon ten-well printed microscope slides were pretreated with 3-aminopropyltriethoxysilane (AES) (Pierce Biotechnology Inc., Rockford, IL) and 0.1% glutaraldehyde to optimize protein and cell adhesion, then, the slides were passively coated with ECM proteins, hyaluronic acid, Matrigel or G-ECM as described above. After pipetting 50 µl of culture media in each well a 10-cylinder cell sedimentation manifold (CSM) (Creative Scientific Methods Inc.) was carefully placed over the slide. A 1.5 µl cell suspension containing 3500 cells/µl of HB1.F3-hNSCs was added to each cylinder of the CSM. The slide was placed at 4 °C for 45 min to allow rapid sedimentation of the cells and then transferred to a cell culture incubator for 6 h. The circular area occupied by attached cells in each well was then photographed using an inverted microscope (Nikon Eclipse TE300) connected to a Spot RT Slider digital camera (Diagnostic Instruments Inc., Houston, TX) and daily serial diameter measurements of the cell population area were made using the imaging software IP – Lab for Windows (Scanalytics, Inc., Fairfax, VA) with a calibrated 1 mm scale. A migration value was quantified as the increase in the radius of the circle occupied by the cell population beyond that measured on the time zero (the moment when the CSM was removed). The average of the radius increase of four replicates was calculated. The measurements were conducted every 24 h for 72 h after the removal of the CSM. All values were expressed as mean ± standard deviation (SD) of quadruplicate determination.

Cell substratum adhesion assay

HB1.F3 h-NSCs were tested for substrate-adhesion using a modified method of Kueng et al. [21]. Briefly, HB1.F3 h-NSCs were harvested from monolayer cultures and deposited into the wells of a 96-well plate (3×10^4 cell/well dispersed in $100 \mu\text{l}$ of culture medium) passively coated by absorption with ECM purified components ($50 \mu\text{l}/\text{well}$) or G-ECM. The 96-well plate was placed at 4°C for 45 min to allow rapid sedimentation of the cells and then incubated for 60 min in 5% $\text{CO}_2/95\%$ air at 37°C in a humidified atmosphere incubator. Subsequently the plate was subjected to vigorous agitation (220 rpm for 5 min) on a horizontal rotor. Non-attached cells were removed by aspiration and the wells were rinsed with PBS. Attached cells were fixed with 1% glutaraldehyde (30 min) and then stained with a 0.1% solution of crystal violet (30 min). The excess dye was removed by extensive washing with deionized water and the plate was air-dried for 10 min prior to dye solubilization with a solution of 10% acetic acid (60 min). The optical density on the dye extracts was measured directly in the plate using a spectrophotometer (Lab-system Multiscan MCC 433, Fisher Scientific Inc., Pittsburgh, PA) at 590 nm. Replicates from four wells were averaged. Blanks used were derived from ECM-coated cell-free wells on the same 96-well plate where the growth experiments were performed. A linear relationship between cell number and absorbance at 590 nm was observed for the HB1.F3 h-NSCs on all the ECM substrates used.

Haptotaxis assay

Haptotaxis which is defined as a directed dose-dependent migration of cells induced by gradients of substratum-bound substances was tested by using a modified Boyden Chamber assay [7]. Purified ECM proteins were added to the lower wells of a 96-well modified Boyden chamber in quadruplicate (Neuroprobe, Cabin John, MD) at a concentration of $100 \mu\text{g}/\text{ml}$ in PBS. The wells were covered with an $8\text{-}\mu\text{m}$ pore size Nucleopore filter and coating of the ECM proteins to the underside of the filter was allowed by incubation of the Boyden Chamber at 37°C for 1 h. After incubation the chamber was disassembled and immediately washed extensively with distilled water. The filter was rinsed three times with PBS containing 0.1% BSA followed by a final rinse with DMEM/0.1% BSA. For assessing the haptotactic effects of the ECM proteins, the lower wells were filled with DMEM/0.1% BSA and covered with the underside of the ECM protein coated filter.

HB1.F3 h-NSCs were then suspended at 2.5×10^4 cells in $50 \mu\text{l}$ of serum free DMEM/0.1% BSA and seeded into the upper wells. After incubation for 6 h at 37°C , non-migrating cells were scraped off the upper side of the filter and filters were stained with Diff Quick (Dade, Switzerland). Nuclei of adherent cells on the underside of the filter were counted in five high power fields using a $20\times$ objective with a calibrated ocular grid.

Migrated cell numbers from quadruplicate determination were expressed as the mean \pm standard error. The control migration was assessed in response to serum free DMEM containing 0.1% bovine serum albumin only, and reflects the basal migration rate of the cells in this assay.

Statistical analysis

Linear regression analysis was done for each substrate, displayed in Figures 1 and 2, using MS Excel (Microsoft, Inc. Seattle, WA). Significant differences between the migratory rates of NSCs on purified ECM substrates were determined by using one way analysis of variance (ANOVA) followed by pairwise multiple comparisons using the Tukey test, shown in Figure 3. For the migratory rates of NSCs on cell-produced ECM and for the haptotaxis assay, statistical analysis was performed using Kruskal–Wallis one way analysis of variance on ranks followed by pairwise multiple comparisons using Dunnett's method (Figures 3 and 4). The Pearson product moment correlation coefficient was used to investigate the interrelationship between the migratory rate and the adhesion properties of HB1.F3-hNSCs on different ECM environments, shown in Figure 5. The level of statistical significance was set at $P \leq 0.05$ in all experiments.

Results

Human glioblastoma-derived ECM is permissive for NSCs migration

To assess whether human glioma cell line-produced ECM is stimulating NSC migration, we used the cellular migration monolayer assay. This is designed to quantify net changes in the size and distribution of the migrating cell population. The incremental expansion of the advancing rim of migrating cells followed a linear relationship over time in all ECM substrates studied (Figures 1 and 2). No significant migration of HB1.F3-hNSCs was observed on the surface coated with the control protein (BSA), and on the control ECM derived from normal human astrocytes (non-tumoral). The human astrocyte cultures used for the experiments displayed 100% GFAP immunoreactivity (data not shown). The migration rate (r), calculated by regression analysis of the radius increase of the cell population, on the non-specific substrate BSA was 0.18 mm/day and on the non-tumoral ECM 0.21 mm/day. There was no statistical significant difference between these control substrates ($P > 0.05$). The rate of HB1.F3-hNSC migration on all tested glioma-produced ECM was significantly higher compared to the migration on BSA ($P < 0.001$) and Astro-ECM ($P < 0.005$) (Figures 1 and 3a). Only the migration rate of the murine CNS-1-ECM did not reach statistical significance (0.27 mm/day). The highest migratory rate was observed in the U251 produced ECM (0.42 mm/day). HB1.F3-hNSC migrated significantly less in all other glioma derived

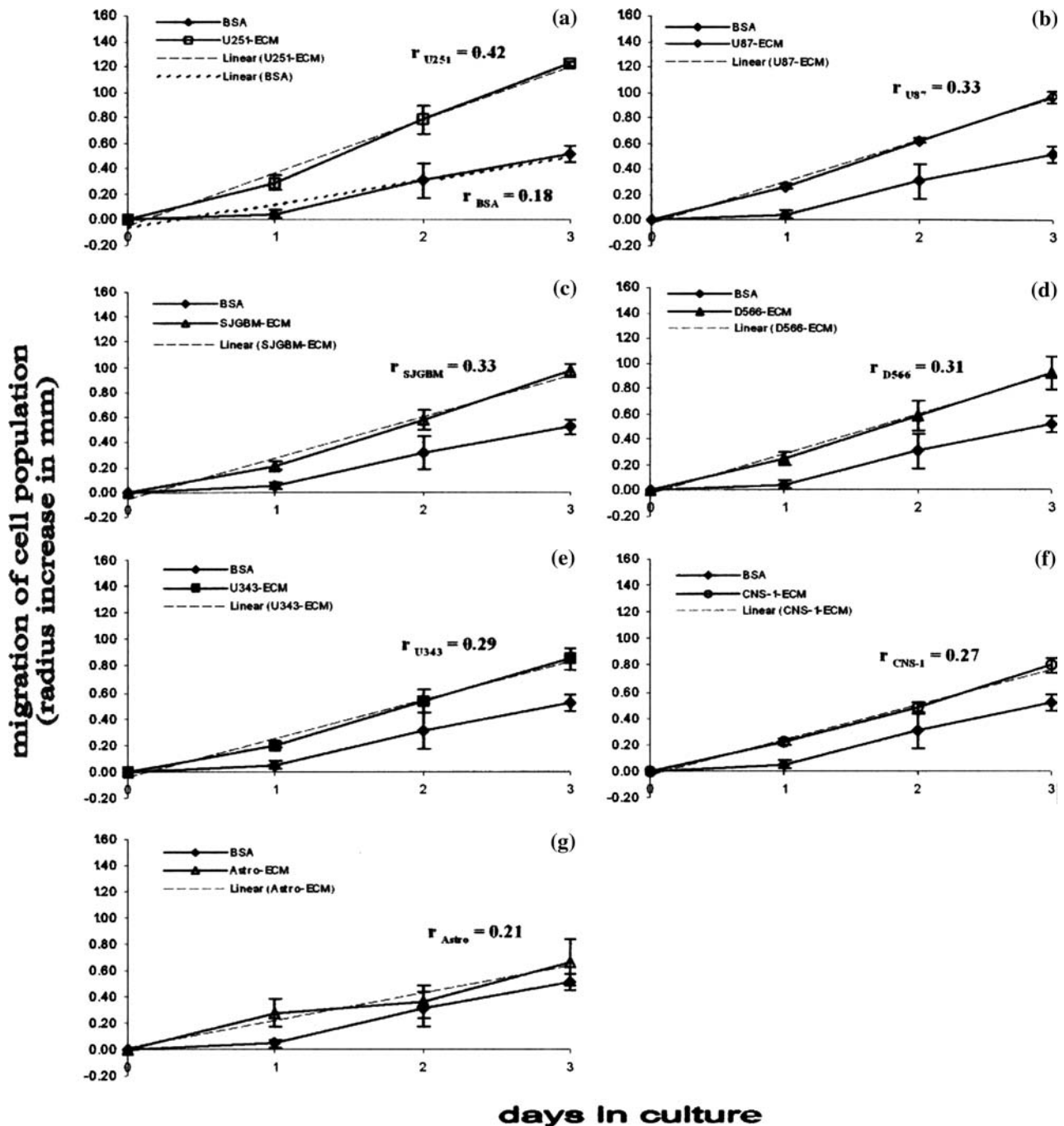


Figure 1. The motility of HBL.F3-hNSCs on the ECM produced by different glioma cell lines was assessed in the monolayer migration assay over time. Tissue culture surfaces were coated with BSA to quantify migration on a non-specific substrate and by deposition of a non-tumoral ECM produced by normal human astrocytes in comparison to tumor cell produced ECM. Migration on (a) U251-derived ECM; (b) U87-derived ECM; (c) SJGBM-derived ECM; (d) D566-derived ECM; (e) U343-derived ECM; (f) CNS-1-derived ECM; (g) Human astrocytes-derived ECM. The migration rate was calculated by regression analysis of the increase of the radius of cell population migration over time. Values shown are means \pm standard deviation of radius increase of quadruplicate determination.

ECM (0.27–0.33 mm/day) and there were no significant differences among these ($P < 0.05$).

Human NSCs migration on purified ECM substrates

We next wanted to investigate if human NSC migration is stimulated by specific ECM proteins which are known to be expressed in malignant gliomas invasion pathways. The migration rates on the purified ECM substrates ranged from 0.27 to 0.51 mm/day, which were all

significantly higher compared to the non-specific migration on BSA (Figures 2 and 3b). The results show that the substrates promoting the highest migration rates were laminin ($r = 0.51$ mm/day) followed by fibronectin ($r = 0.44$ mm/day) and tenascin ($r = 0.41$ mm/day). Laminin was significantly higher than all the other ECM proteins examined. Although fibronectin and tenascin do not have significantly different rates, both substrates were significantly lower than laminin. Tenascin was not significantly higher than vitronectin ($r = 0.39$ mm/day)

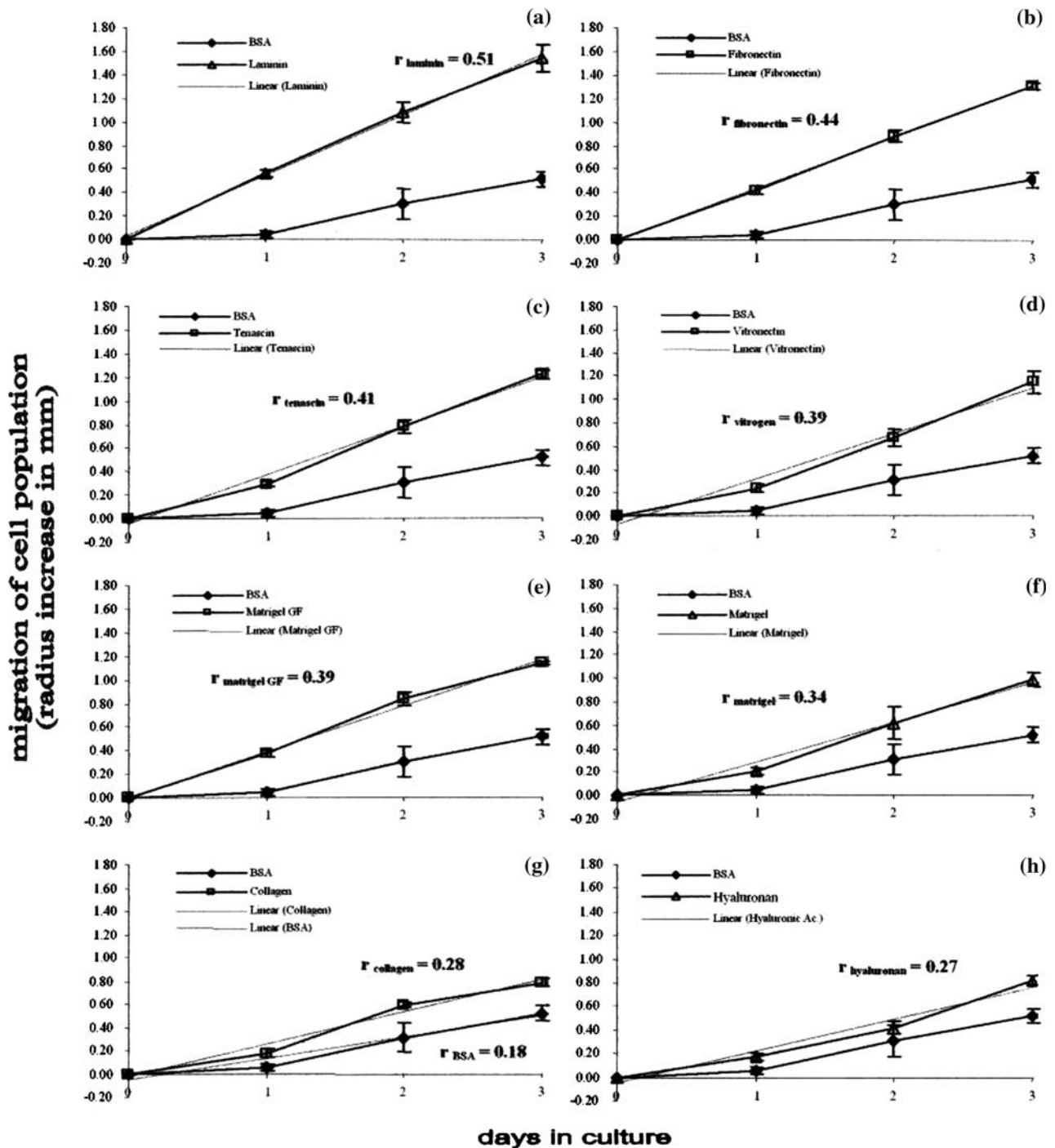


Figure 2. Migration of HB1.F3-hNSCs on different ECM compounds in the monolayer assay over time. Tissue culture surfaces were passively coated with BSA to quantify migration on a non-specific substrate and with different purified ECM compounds to test substrate induction of migration. The migration of HB1.F3-hNSCs on each of the ECM purified compounds was compared with the migration on the non-specific substrate, BSA. (a) Laminin; (b) fibronectin; (c) tenascin-C; (d) vitronectin; (e) matrigel; (f) growth factor reduced matrigel; (g) collagen I; (h) hyaluronic acid. The migration rate was calculated by regression analysis of the increase of the radius of cell population migration over time.

and matrigel with growth factors ($r=0.39$ mm/day). Significantly, lower migration rates were observed on growth factor reduced matrigel ($r=0.34$ mm/day) than on matrigel with growth factors. This is of importance because matrigel with growth factors reflects the composition of the blood vessel basement membrane, indicating the influence of vessel factors on NSC motility. The lowest migration rates were observed with collagen I (0.28 mm/day) and hyaluronic acid (0.27 mm/day), a main component of the grey and white matter of the

normal brain parenchyma. The level of statistical significance was set at $P < 0.05$ in all comparisons.

Purified human ECM proteins induce haptotaxis of human NSCs

Since the ECM of gliomas and their migration pathways consist of a high percentage of tenascin, fibronectin, laminin, vitronectin and collagen, we

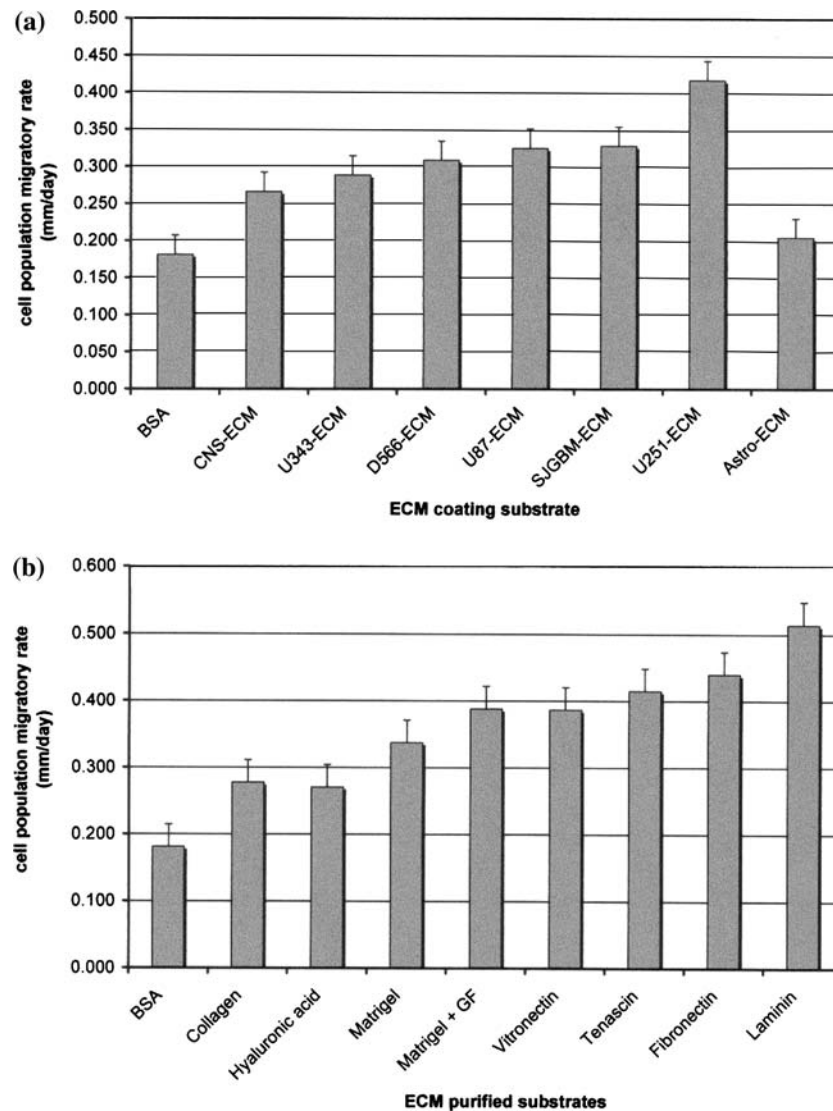


Figure 3. Effects of different ECM substrates on the migration rate of HB1.F3-hNSCs in the monolayer migration assay. Migration rates were determined on BSA coated surfaces to quantify non-specific motility and on different ECM compounds: (a) glioma cell lines produced ECM and (b) ECM purified proteins.

investigated the effect of these substratum-bound proteins on directed migration of human NSCs. Tenascin-C bound to the underside of a Boyden Chamber filter stimulated the highest haptotactic effect (100-fold of the control) followed by fibronectin (71-fold) and vitronectin (65-fold) (Figure 4). Laminin and collagen I-induced haptotaxis, higher compared to BSA or the control with no coated-protein, reflecting the basal migration rate of the HB1.F3-hNSCs in this assay ($P < 0.05$).

The interrelationship between migration and adhesion of human NSCs on different ECM substrates

Previous *in vitro* studies have demonstrated a relationship between the degree of glioma cell migration and their adhesion capacity [13,20]. In order to investigate if a similar relation exists for NSCs, we first examined the adhesion of HB1.F3-hNSCs on different ECM compounds (Figure 5). The highest number of adhering cells after 60 min of incubation was observed on laminin, for

which the specific optical density (s-O.D.) reading was 0.500 at 590 nm of wavelength. A large number of HB1.F3-hNSCs also adhered to vitronectin (s-O.D. = 0.395), fibronectin (s-O.D. = 0.401), and matrigel (s-O.D. = 0.409). Only a small number of HB1.F3-hNSCs adhered to hyaluronic acid (s-O.D. = 0.153) and BSA (s-O.D. = 0.103). Glioblastoma-produced ECM induced a moderate adhesion of HB1.F3-hNSCs with the highest adhesion degree observed on the U251-ECM (s-O.D. = 0.271) and the lowest on the CNS-ECM (s-O.D. = 0.170). The interrelationship between the migration rate and adhesion properties of the HB1.F3-hNSCs on the different ECM substrates was next investigated by plotting the migratory rate of HB1.F3-hNSCs on the different ECM substrates against the adhesion degree on the same ECM substrates (Figure 5). A significant positive correlation was found (Pearson product moment correlation, $r_p = 0.77$; $P < 0.0001$). ECM substrates to which the cells rapidly adhered were more permissive for migration of HB1.F3-hNSCs in the monolayer migration assay.

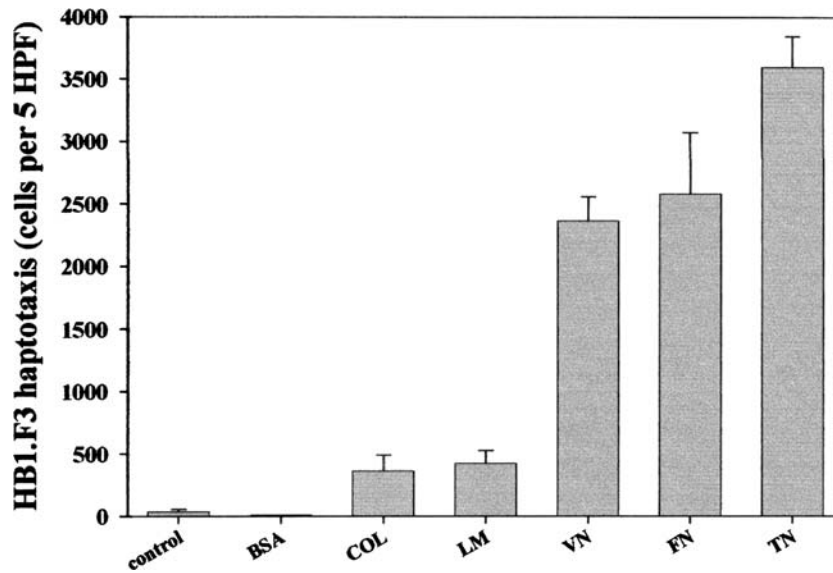


Figure 4. Effects of purified human ECM compounds on the migration of HB1.F3-hNSCs in a haptotaxis assay. ECM proteins coated to the underside of a Boyden Chamber filter induced a directed migration of human NSCs. Minor migration was observed in the control wells containing no ECM (36 ± 32 cells) or BSA (7 ± 3 cells). Bars are means \pm standard error of quadruplicate determination.

Discussion

Cell migration is an important multi-step process that contributes to organism development, tissue repair and regeneration. It also drives disease progression in cancer and inflammation [22]. Each step of the cell migration process depends on extracellular factors that act on the cell itself through intracellular signalling cascades and molecular pathways. The ECM is one of these important extracellular factors that modulate many crucial cell processes such as survival, proliferation, differentiation and migration [23]. It has been shown that NSCs have exceptional migratory abilities within the central nervous system (CNS) under physiological and pathological condition; little is known about how the ECM, as an extracellular factor, influences their migratory behavior.

The ECM is a network of different molecules that is produced by the cells. Previous studies have demonstrated that glioma cells facilitate their migration by producing their own ECM [24]. Furthermore, glioma cells modulate the normal surrounding brain parenchyma in which they invade by inducing the expression of additional ECM molecules [25]. Tenascin, fibronectin, laminin, vitronectin and different types of collagen are upregulated within the glioma stroma and at the advancing edge of the tumor within the brain parenchyma [12,26]. These ECM components appear to be highly expressed during embryogenesis and tumor growth, but are downregulated in the adult normal brain ECM [12,27] or restricted to specified anatomical areas which are assumed to be reservoirs of neural stem cells in the adult [28]. In this study, we demonstrate

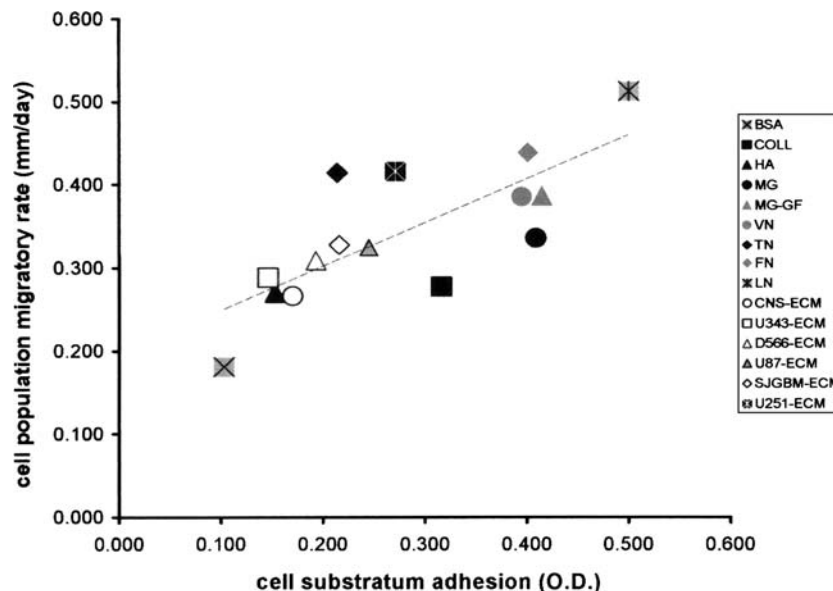


Figure 5. Interrelationship between migration and adhesion of human NSCs on different ECM substrates. Adhesion of HB1.F3-hNSCs to different ECM substrates was plotted against migration rates on the surfaces coated with the respective ECM substrates. Pearson test showed a positive correlation between migration and adhesion in the monolayer assay (Pearson product moment correlation, $r_p = 0.77$; $P < 0.0001$).

in vitro that the ECM of malignant glioma facilitates NSC migration, and that certain ECM proteins known to be upregulated during malignant glioma progression have the ability to influence NSC migration.

Laminin is an important factor in glioma cell migration and invasion [26,29]. It has been found to be deposited in the tumor parenchyma and tumor invasion front, and is also highly expressed on tumor blood vessels which are a major pathway of glioma cell invasion [30]. Angiogenic active blood vessels seem to provide a vascular niche for NSC survival [31] and are necessary for active neurogenesis [32]. We found that laminin is the most permissive substrate for allowing human NSC migration. This is in line with previous findings, showing that laminin was highly permissive for the outgrowth of NSCs from neurospheres [33].

Tenascin is another ECM molecule predominantly expressed in the tumor environment and its important role for glioma invasion has led to the development of the first human clinical trials using anti-tenascin [34]. *In vivo*, tenascin localizes to the invading edge of gliomas and surrounds single invading tumor cells [35]. In our study, tenascin-C was a highly permissive substrate for HB1.F3-hNSCs migration and furthermore, in contrast to laminin, displayed the most potent haptotactic characteristics for human NSCs. Given the fact that tenascin-C is highly expressed in invading gliomas and its essential role for cellular migration during brain development [36], the deposition of tenascin-C and laminin by invading glioma cells in the well organized normal brain parenchyma may serve as a trail for tumor targeting NSCs.

Other ECM proteins like fibronectin and vitronectin also support NSC motility and induce the directed migration of HB1.F3-hNSCs. Fibronectin and vitronectin have been shown to be important factors for cellular migration during normal brain development [29,37,38]. Vitronectin is upregulated in the glioma ECM where it was expressed mainly in the main tumor mass. The lowest level of human NSC migration was seen with collagen I and hyaluronic acid. Hyaluronic acid is a major component of the ECM in the CNS [12]. This glycosaminoglycan, in contrast to other proteoglycans does not contain a protein core and it is attached via specific receptors to many other ECM components. When attached to other ECM proteins, hyaluronic acid has been shown to facilitate glioma cell migration [39,40]. The need for hyaluronic acid to be attached to other ECM proteins in order to influence migration, may explain our findings. Laminin, fibronectin, tenascin-C and collagen I are localized within the basement membrane of existing and newly formed blood vessels [12]. These basement membrane proteins, including the reconstituted basement membrane matrigel, allowed the migration of HB1.F3-hNSCs in the monolayer migration assay. In this study, we demonstrate that all ECM components previously reported to be permissive substrates for glioma cell migration had similar effects on human NSC migration.

Chemotactic mediators play an important role for NSC attraction from distant sites. NSCs migrate toward gliomas because they are tracking a chemotactic gradi-

ent established by tumor cells [11]. Our finding that the ECM plays a crucial role in NSC migration toward tumor cells reinforces the idea that cell migration is a complex process where different factors interact in a fine tuned game in space and time. This is further supported by the fact that HB1.F3-hNSC migratory rate on normal matrigel was significantly higher than on growth factor reduced matrigel. The ECM not only contains the intrinsic molecules that directly affect cell migration, but is also a site of accumulation of soluble molecules that regulate cell movement. Apart from forming the structure that cells use for their movement that is made possible by the interaction of ECM components and cell surface receptors, ECM may contribute to the formation of gradients of attractants, inhibitors or repellents by binding and modulating the activities of these factors [16,41].

It seems unlikely that single invading glioma cells are able to establish a chemotactic gradient which can concur with that of the main tumor mass. Therefore, additional signals for the local guidance of NSCs may be present. Our report suggests that the glioma-produced and modulated ECM stimulates NSC migration *in vitro*. These data support the hypothesis that glioma modulated ECM may potentially function as an additional local guidance signal for neural stem cell migration in concert with soluble factors. An increasing knowledge about relevant factors for stem cell survival, attraction and their interaction within the tumor environment is necessary for the development of rational therapeutic strategies using stem cell technology.

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