In vitro formation of neuroclusters in microfluidic devices and cell migration as a function of stromal-derived growth factor 1 gradients

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\textbf{ABSTRACT}

Central nervous system (CNS) cells cultured \textit{in vitro} as neuroclusters are useful models of tissue regeneration and disease progression. However, the role of cluster formation and collective migration of these neuroclusters to external stimuli has been largely unstudied \textit{in vitro}. Here, 3 distinct CNS cell types, medulloblastoma (MB), medulloblastoma-derived glial progenitor cells (MGPC), and retinal progenitor cells (RPC), were examined with respect to cluster formation and migration in response to Stromal-Derived Growth Factor (SDF-1). A microfluidic platform was used to distinguish collective migration of neuroclusters from that of individual cells in response to controlled concentration profiles of SDF-1. Cell lines were also compared with respect to expression of CXCR4, the receptor for SDF-1, and the gap junction protein Connexin 43 (Cx43). All cell types spontaneously formed clusters and expressed both CXCR4 and Cx43. RPC clusters exhibited collective chemotactic migration (i.e. movement as clusters) along SDF-1 concentration gradients. MGPCs clusters did not exhibit adhesion-based migration, and migration of MB clusters was inconsistent. This study demonstrates how controlled microenvironments can be used to examine the formation and collective migration of CNS-derived neuroclusters in varied cell populations.

\textbf{Key Words}

central nervous system; chemotaxis; collective migration; medulloblastoma; retinal progenitors; SDF-1; stromal-derived growth factor

\textbf{Introduction}

The assembly of cells into organized 3-dimensional structures is fundamental to morphogenetic events that occur during normal tissue development and regeneration and in tumorigenesis.\textsuperscript{1-7} These morphogenetic processes often involve the coordinated migration of cells as large assemblies rather than as individual cells. Examples of this behavior include cell movements in gastrulation,\textsuperscript{8} vasculogenesis and angiogenesis.\textsuperscript{9} In addition, different types of tumors are known to grow by spreading along defined pathways, e.g. along vascular tracks.\textsuperscript{10}

The ability of many cell types, including progenitors, de-differentiated cells and stem cells,\textsuperscript{11-15} to form aggregates or clusters \textit{in vitro} has made it clear that cells often function differently in 3-dimensional arrays than in traditional monolayer cultures.\textsuperscript{7} In particular, many insights have been gained into the control of cell proliferation, differentiation and maintenance of phenotype in tissue-like clusters. Relatively little is known, however, about how cells migrate as part of organized complexes, i.e., “collective migration” after forming neuroclusters.\textsuperscript{2,5,16-18}

Neuroclusters have been pivotal in the dynamic study of cluster and cell outgrowth, but underutilized to examine the collective migration of the bulk cluster itself. For example, clusters of embryonic stem cells,\textsuperscript{11} mesenchymal stem cells,\textsuperscript{19} and cancer stem cell populations have been widely-used to monitor cell differentiation and matrix production.\textsuperscript{20} However, the directed, collective migration of neural cells is also critical for tissue repair\textsuperscript{21,22} and initiation of metastasis,\textsuperscript{23,24} while cell replacement\textsuperscript{22} and migration-targeted therapies for CNS tissue\textsuperscript{25} can be greatly aided by collective chemotactic migration.

The majority of migration studies have focused on measuring the movements of individual cells rather than cells in clusters, and utilize conventional transmembrane assays.\textsuperscript{26} Our group has developed a microfluidics-based system, called the \textit{$\mu$-Lane}, that enables measurement of cell migration within well-established gradients of chemotactic agents, thereby permitting analysis of cell migration as a function of both gradient and bulk concentration.\textsuperscript{27} In the present study, we utilized a previously developed microfluidic system, the \textit{$\mu$-Lane} as shown in Figure 1, to examine the migration...
of 3 neural-derived cell lines, each of which has the capability to form clusters in vitro: Medulloblastoma (MB), Medulloblastoma-derived Glial Progenitor Cells (MGPC) and Retinal Progenitor Cells (RPC). In vivo, MB tumors infiltrate the microvasculature as both single cells and as cell clusters.\textsuperscript{5,28} In vitro, MB cells are routinely maintained in monolayer cultures, but are also known to form clusters in neurobasal medium (NBM). MGPCs represent tumor stem and progenitor cell populations believed to play significant roles in the collective migration of neuroclusters that contribute to metastasis of CNS tumors.\textsuperscript{29,30} In vitro, MGPCs are normally maintained as poorly adherent clusters. Lastly, RPCs persist in clustered form both in vitro and in vivo,\textsuperscript{31,33} a feature considered crucial for the effective use of these cells in regenerative therapy.\textsuperscript{34,35}

We examined the migration of these 3 populations as clusters and as individual cells in response to signaling from Stromal cell-derived factor 1 (SDF-1), also known as C-X-C motif ligand 12 (CXCL12). SDF-1 has been well established as a chemoattractant for cells of the CNS, including neural progenitors,\textsuperscript{36} oligodendrocytes,\textsuperscript{37} and others.\textsuperscript{38,39} In addition, signaling from SDF-1 gradient fields has been shown to produce a chemotactic effect on cells, singularly, and in combination with other growth factors such as epidermal growth factor (EGF)\textsuperscript{40} and fibroblast growth factor (FGF).\textsuperscript{41} Specifically we examined whether potential differences in cell or cluster migration were related to i) the level of SDF-1 receptor (CXCR4) or ii) the expression of Connexin 43 (Cx43),\textsuperscript{42} a gap junction protein used as an indicator of the degree to which cells are junctionally coupled.

**Results**

Experiments of this study examined the cell clustering and migration of 3 neural cell lines, MB, RPC, and MGPC. The first set of experiments examined clustering behavior of these cells, both average number of clusters and cluster size by area, at different seeding densities over time. All three cell lines exhibited cluster formation. This behavior was seen starting at 1 hr after plating until

![Figure 1. \(\mu\)Lane microchannel device to measure chemotactic cell migration. (A) Device schematic. (B) Gradient profile based on measurements of fluorescent dextran (MW = 10kDa) at steady-state, \(t = 18\) hours. Position \(x = 0\) is assigned to the sink well.](image-url)
the final time point of 48hr, with the latter shown in Figure 2. The average number of clusters present scaled with seeding density for MGPC and RPC, while consistently large numbers of clusters were seen at all seeding densities of MB, as shown in Figure 3. MGPC cells plated at $10^4$ and $10^5$ cells/mL exhibited increased number of clusters over time, while average cluster size remained constant. By contrast, MGPC cell clusters seeded at the highest density of $10^6$ cells/mL remained constant in number but grew in average cluster size over 48hr. RPCs seeded at $10^4$ and $10^5$ cells/mL formed clusters that increased in both size and number over time, while cells seeded at $10^6$ cells/mL showed a decline in cluster number with time and an increase in average cluster size.

Lastly, MB cells displayed uniformly high average numbers of clusters at all seeding densities, and exhibited consistent and moderate decreases in the average numbers of clusters with comparable increases in average cluster size.

The next experiments examined migration of these 3 neuro-cell lines to exogenous SDF-1 signaling using conventional transwell assays. As seen in Figure 4, all cell lines showed low levels of transfilter migration in the absence of SDF-1, and both single cells and cell clusters were observed on the underside of the migration membrane. Single cell migration under control conditions was highest for MGPC. SDF-1 elicited 13-fold and 18-fold increases in the numbers of individual RPC cells and RPC clusters, respectively. In addition exogenous signaling from SDF-1 led to a nearly 25-fold increase in the average number of MGPC clusters over controls. However, the numbers of clusters that migrated in response to SDF-1 was extremely variable with no statistically significant difference from control. Lastly, individual MPGCs did not migrate toward SDF-1, nor did SDF-1 signaling lead to statistically-significant differences in the migration of individual MB cells or clusters over control.

The μLane system was next used to examine the collective and individual migration of cells in response to signaling from defined fields of SDF-1. Microchannel experiments illustrated that all cell types survived and

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**Figure 2.** Cell cluster formation 48 h after plating. Suspensions of MGPCs (Row A) RPCs (Row B), and MB (Row C) at $10^4$ cells/mL (Col 1), $10^5$ cells/mL (Col 2), and $10^6$ cells/mL (Col 3) were plated and photographed at 48 h. Representative single cells and clusters (arrows) are shown for each cell type.
formed clusters within the device at all seeding densities over time in an SDF-1 gradient, as seen in Figure 5. Further, average cluster area largely increased with SDF-1 stimulation for MPGCs and MB, but decreased for RPC cells, as shown in Table 1. The percentage of total cells that were located in clusters also increased with plating density, but decreased under SDF-1 stimulation, also show in Table 1. Additionally, the μLane system enabled real-time imaging and tracking of individual cells and collective migration of neuroclusters within SDF-1 gradient fields. Chemotactic migration was assessed using the parameter length of center of mass, $L_c$, which is defined as the straight line distance traveled by the cell or cluster center of mass.48 Here, positive values indicate net movement toward the SDF-1 source. As shown in Table 2, RPCs were chemoattracted to SDF-1 as both clusters and single cells. Clusters illustrated a greater relative increase in $L_c$ compared to unstimulated controls. MB cells showed mixed results in migration toward SDF-1 with seeding density. A higher seeding density of $10^6$ cells/mL resulted in increased collective cell migration and single

Figure 3. Changes in cluster number and size with time in culture. Cluster number (Column 1) and size by area (Column 2) are shown for suspensions of MGPCs (Row A), RPCs (Row B) and MB (Row C) at cell densities indicated in each panel. Data show mean and standard deviation, n > 4 wells.

Figure 4. Chemotactic migration to SDF-1 assessed by transwell assay. Average number of cells migrated through an 8µm porous membrane toward 100 ng/mL SDF-1. Data show total migrated cell count, n > 3 wells.
cell migration, while measured migration from experiments using lower densities of $10^4$ and $10^5$ cells/mL displayed no statistically-significant difference from controls. Lastly, no attachment of MGPCs was observed within microchannel experiments, thus preventing study of their adhesion-based migration.

In addition plots of mean cell trajectory plots for both single cells and neuroclusters of RPCs and MB can be seen in Figure 6. As shown, clusters and single cells move toward increasing SDF-1 concentration in response to concentration gradients generated by 100 ng/mL of SDF-1 in the source reservoir. No statistically significant differences were observed between single cells and clusters.

The uLane further enabled study of additional parameters to evaluate cell migratory behaviors in gradient fields. First, the time-dependent speed of cell clusters and individual cells was examined during each 24-hour experiment. Speed of MB cells and RPCs and were determined to be continuous and stable, but time and position-independent along positions of the uLane. Further, the growth of motile cell clusters over time was also measured. MB, RPC and MGPC clusters did not exhibit statistically-significant differences in average cluster size over the time course of experiments along all positions of the microchannel.

Additional experiments used immunocytochemistry (ICC) to examine the change in expression of CXCR4, the SDF-1 receptor, within clusters and single cells of the 3 cell types studied upon stimulation with 100 ng/mL SDF-1, as seen in Figure 7. The expression of CXCR4 was observed to be evenly distributed along the cell membrane for MB, RPC and MGPC. ICC results showed no differences between average expression of CXCR4 at different plating densities of the same cell type. However, data did highlight significantly increased CXCR4 expression with SDF-1 stimulation for all cell types at varied densities.

Similarly, ICC was performed to evaluate Cx43 expression for all 3 cell types at different plating densities in clusters and single cells, as shown in Figure 8. Cx43 was present in all cell types, however, despite significant increase in Cx43 with SDF-1 stimulation for whole cell populations, there were mixed results as a function of single cells or clusters stimulated individually, and no statistically-significant differences between neuroclusters and single cell populations or between plating densities.

**Discussion**

This study examined the migration of self-assembled neuroclusters to exogenous signaling from defined SDF-1 concentration fields, and examined the extent to which sensitivity for collective cell migration can be assessed via expression of the cognate CXCR4 receptor and/or the gap junction protein Cx43. Experiments first confirmed that RPCs, MGPCs and MB were able to recapitulate time-dependent neurocluster formation within conventional plates, as reported for *in vivo* neurosphere

| $\rho$ (cells/mL) | MGPC SDF- | MGPC SDF+ | RPC SDF- | RPC SDF+ | MB SDF- | MB SDF+ |
|------------------|-----------|-----------|---------|---------|---------|---------|
| Avg cluster area ($\mu$m$^2$) | 10$^4$ | 2838.2 | 1089.6 | 2330.0 | 798.3 | 3735.8 | 11202.1 |
| % cells in clusters | 10$^4$ | 2776.9 | 6470.0 | 1377.1 | 1426.6 | 3250.6 | 10406.4 |
| | 10$^5$ | 3390.6 | 5225.2 | 6019.4 | 2708.6 | 5787.3 | 18494.7 |
| | 10$^6$ | 567.0 | 40.7 | 82.3 | 3.2 | 35.1 | 34.1 |
| | 10$^7$ | 59.2 | 42.0 | 58.3 | 24.2 | 78.7 | 43.3 |
| | 10$^8$ | 75.4 | 93.1 | 96.8 | 38.0 | 84.0 | 55.3 |
We further replicated this self-assembled clustering within our microfluidic devices, which highlights the ability of these systems to enable study of collective migration with minimal confinement effects.

Chemoattraction of all 3 CNS cell types to exogenous SDF-1 signaling was then confirmed via both conventional transwell assays and microdevices. Migration was consistently observed toward SDF-1 in transwell assays, but with a wide range of results for varied cell types. MGPCs demonstrated large numbers of clusters relative to SDF-1 negative control, while RPCs showed an increase in numbers of motile single cells and MB did not exhibit significant migration. The intrinsic clustering nature of the cell types is thought to be a primary reason for differences the observed. MGPCs exist in clusters under basal culture conditions and likely migrated through the pores as single cells, but quickly clustered on the membrane underside. By contrast, RPCs exist in NBM culture as a mixed population of single cells and clusters, which is representative of the mixed migratory populations seen in the assay. Lastly, as MB exist as single cells in standard culture but form large clusters in NBM, it is likely that little migration was observed because MB clusters were too large to migrate through the membrane pores.

Microfluidic environments were then used to examine collective cell migration, enabling study of cell behavior in response to controlled exogenous SDF-1 signaling.

Table 2. Migration in the μLane microfluidic device. Measured by length of center of mass, Lc. MB and RPCs tested with and without SDF-1 gradient stimulus at 10⁴, 10⁵ and 10⁶ cell/mL. n = 3 channels. *statistical significance (p<0.05).

| ρ (cells/mL) | RPC Single Cells | MB Single Cells |
|--------------|------------------|-----------------|
|              | Lc (μm) SDF(-)  | Lc (μm) SDF(+)%change | Lc (μm) SDF(-) | Lc (μm) SDF(+)%change |
| 10⁴         | 40.4 ± 29.7     | 46.1 ± 50.7     | 14.00%       | 25.6 ± 14.9     | 39.0 ± 21.8     | 52.60%       |
| 10⁵         | 27.0 ± 20.0     | 43.1 ± 29.8     | 59.7%*       | 62.4 ± 5.6      | 44.4 ± 10.7     | -28.8%*      |
| 10⁶         | 40.9 ± 23.3     | 61.5 ± 23.9     | 50.3%*       | 17.9 ± 3.4      | 121.29 ± 55.8   | 576.7%*      |

Figure 6. Spatial plots of single cell and neurocluster trajectory. Representative and mean trajectories for both single cells (Column 1) and clusters (Column 2) along with length of center of mass (Lc) distribution for clusters vs single cells (Column 3) over 24 hr toward an increasing (0–100 ng/mL) SDF-1 gradient in RPCs (Row A) and MB (Row B). Positive y-axis indicates direction of increasing SDF-1 concentration.
and eliminating limitations of pore size presented by the transwell assay. First, while MGPCs self-assembled into neuroclusters within the µLane system, no collective migration was observed because the clusters did not attach onto channel surfaces. Here, we postulate that cell to cell communication leading to assembly of MGPC clusters played a more dominant role over the substrate attachment signaling needed for collective migration of the cluster.50,51 By contrast, MB illustrated mixed results for movement of its neuroclusters in SDF-1 fields. MB showed large increases in migration distance, Lc, at high plating density for both neuroclusters and single cells, but inconsistent migration at low cell densities for both single cells and neuroclusters. Further, cell tracking showed a clear migration pattern as a mean of the entire cell population. We attribute the aberrant behavior at low density to potential effects of mutations accumulated in this long-running cancer cell line over the culture lifetime.52 We note that additional experiments are needed to isolate the effect of SDF-1 on MB collective migration.

Lastly, RPCs exhibited both single and collective chemotaxis along SDF-1 gradients. Interestingly, RPC neuroclusters demonstrated more directed collective migration than individual cells, as evidenced by the larger distances of migration, Lc. Further, real-time images from our microfluidic system demonstrate the collective chemotaxis of entire RPC neuroclusters, highlighting the directionality of bulk cluster movement. Cell tracking illustrates the direct path of both single cell and clusters toward increasing SDF-1 concentration. These data are novel because such previously-unreported behavior indicates that collective RPC migration is chemosensitive to exogenous signaling from the local environment. However, it was unexpected to measure cluster speeds within the channel that did not change as functions of gradients and/or time. Numerous studies have illustrated such concentration dependence effects in multiple cell types, such that our results were unconventional. It is speculated that this may be due to a high sensitivity of the cells to any change in concentration, independent of magnitude, or may be a result of the nature of collective migration itself. A more precise measure of cell motility per gradient would help elucidate the specific effect of position and concentration, beyond simple illustration of collective migration as a mode of chemotaxis.

Potential factors implicated in collective migration were further explored by examining expression of the

Figure 7. Immunocytochemistry of CXCR4 expression. ICC images of CXCR4 staining at 10⁴ (Column 1), 10⁵ (Column 2), and 10⁶ cell/mL (Column 3) stimulated with 100 ng/mL SDF-1 and comparison of relative fluorescence intensity to unstimulated control (Column 4) for MGPCs (Row A), RPCs (Row B) and MB (Row C). Green staining denotes CXCR4 for MGPCs. Red staining denotes Connexin 43 for RPCs and MB. Blue staining denotes cell nuclei. All scale bars 50 µm. *statistical significance (p<0.05).
CXCR4 receptor and gap junction Cx43 upon SDF-1 stimulation. No significant changes in CXCR4 expression with plating density were seen for any of the cell types, though all cell types showed upregulated surface receptor expression with ligand stimulation. This may indicate a relationship between migratory behavior and ligand-receptor availability and upregulation. Cx43 expression was then measured to determine the role of cell-cell communication within neuroclusters when excited by chemoattractants, which may be implicated in the process of collective migration. It was shown that Cx43 is upregulated with SDF-1 stimulation for each cell type as a function of the whole cell population, but no statistically significant differences in Cx43 expression between single cells and cell clusters were measured. However, this result is likely due to limited antibody penetration that prevents accurate measurement of Cx43 within neuroclusters. Further, Cx43 was not shown to localize to cell-cell boundaries as expected, but was uniformly distributed across the cell surface. We postulate that the increased surface expression of Cx43 in RPCs and MB independent of single cell or cell cluster morphologies, indicates the increased ability of cells to self-assemble in clusters and communicate via gap junctions: This consequently may increase their predisposition to collectively migrate. However, the role of Cx43 in neuroclusters requires further study.

In conclusion, CNS-derived cells, well known to exist and respond as clusters in vivo, have been largely unexamined for their collective migratory behavior in vitro. This study has shown the ability to

**Figure 8.** Immunocytochemistry of Connexin 43 expression. ICC images of Cx43 staining for both single cells (Column 1) and clusters (Column 2) stimulated with 100 ng/mL SDF-1 and comparison of relative fluorescence intensity to unstimulated control (Column 3) for MGPCs (Row A), RPCs (Row B) and MB (Row C). Green staining denotes Connexin 43 for MGPCs. Red staining denotes Connexin 43 for RPCs and MB. Blue staining denotes cell nuclei. All scale bars 50 μm. Statistical significance (p<0.05)** or (p<0.1)** between respective single cell or cluster SDF- and SDF+ groups.
culture MGPCs, MB, and RPCs as clusters, both in conventional plates and within microfluidic devices. Neuroclusters presented many of the same behaviors as individual cells, including uniform receptor levels and in some cases, chemotaxis. Surface expression of receptors, as well as other possible co-activated signaling pathways, may be implicated in migration modality, including bulk cluster migration. Preliminary tests illustrate promising SDF-1 gradient-induced migration for RPCs. RPCs compared favorably to MB and MGPC in microchannel chemotaxis tests, possibly indicating a proliferative tendency in the presence of SDF-1 relative to the oncogenic CNS cells studied. Additional data are needed to mechanistically examine the effect of SDF-1 on collective RPC migration, which will greatly aid in development of migration-targeted regeneration therapies in the retina.

Methods and materials

Cell culture and maintenance

Human MGPCs (CHLA-01-MED, #CRL-3021, ATCC) were maintained in neurobasal medium (NBM) consisting of DMEM:F-12 (ATCC #30–2006) supplemented with 10% (v/v) B-27 supplement (Life Technologies #17504–044), 100 μg/mL penicillin-streptomycin (Corning #30–001C1), 50 ng/mL EGF (Life Technologies #PHG0313), and 50 ng/mL FGF (Life tech #PHG0264). MB (human medulloblastoma derived cancer cells, #HTB-186, ATCC) were maintained in EMEM (ATCC #30–2003) supplemented with 10% fetal bovine serum (FBS) (Corning #35–010-CV) and 100 μg/mL (U/mL) penicillin-streptomycin but tested in NBM. Mouse RPCs were initiated43 and maintained in NBM supplemented with B-27 supplement, 100 μg/mL penicillin-streptomycin, 50 ng/mL EGF and 50 ng/mL FGF.

Cluster formation

Cells were trypsinized, resuspended in NBM, seeded in 24-well plates (Corning 353504) at 0.7 mL per well and densities of 104, 105 and 106 cells/mL and cultured. Cell cluster formation was monitored 1 hr, 24 and 48 hr after seeding. Brightfield images of each well were obtained from 5 regions of interest using a standard checkerboard analysis32 and the average number of clusters, diameter of clusters and number of individual cells was determined by manual counting. Analysis of clustering behavior was performed using Image J (NIH) to measure cluster size, growth and distribution. Number of clusters and individual cells was normalized per area. Clusters were defined as groups of cells with total area larger than 300 μm2 or approximately 3 tightly packed cells.

Transwell migration assays

Transwell (Corning 353097, 8um pore size) modified Boyden Chamber assays were used to measure number of cells migrating in response to 100ng/mL of SDF-1. Each cell type was seeded at 105 cells/mL in 0.3 mL aliquots in supplemented NBM in the upper well of the Boyden Chamber System.28 The bottom Boyden Chamber reservoirs were then loaded with 0.7 mL of supplemented NBM either with or without 100ng/mL SDF-1 (Peprotech #250–20A). Cells were incubated for 8 hours at 37°C, after which the transwells were fixed and stained according to manufacturer’s protocols (Seimens Diff-Quik Staining System). Individual cells and clusters on the bottom surfaces of the transwells (defined as in the previous experiment) were counted under brightfield microscopy.

Microfluidic system

The chemotactic migration of RPCs, MB, and MGPCs was evaluated using the μLane microfluidic system developed in our laboratory.26,27, 44,45 In brief, the μLane (Figure 1A) is a 2-tiered polydimethyl siloxane (PDMS) (Sylgard #24236–10) elastomer that is ozone plasma-bonded to a glass microscope slide. The migration channel in the lower tier is 250μm in width, 100μm in height and 13mm in length, and connects 2 reservoir wells that are also connected via a much larger bridge channel in the upper tier. The ligand added to the source well transports through the channel and toward the sink well via uniaxial diffusion and minimal bulk convection, to rapidly develop a steady-state gradient profile. Figure 1B illustrates the steady-state concentration distribution within the μLane system (modeled in MATLAB) of fluorescent dextran (Mol wt 10kDa, Life Technologies #D22911) used to model SDF-1 (Mol wt 7.9 kDa). For these experiments, microchannel surfaces were coated with 20 μg/mL laminin (Corning #354232) in PBS. The coating solution was injected into the channels and allowed to incubate at 37°C for approximately 1 hour. The laminin solution was aspirated to leave a thin coating within the channel, after which fresh NBM was added to fill both reservoirs. The cells were allowed to attach to laminin-coated channel surfaces overnight (12 hours), after which 100 ng/mL SDF-1 was added to the source well. The device was then further incubated at 37°C for 18 hours in order to establish the steady state SDF-1 gradient before
initiating the measurements of cell migration. Analysis of individual cell and cell cluster motility was performed using the cell migration package in ImageJ (NIH Image). Percentage of cells in clusters in the µLane was determined by calculating the ratio of cluster area to total cell area (i.e. individual cells + clusters).

**Expression of CXCR4 and Connexin 43**

Expression of CXCR4 and Cx43 were measured in all cell lines using immunocytochemistry (ICC). Cells were plated in NBM at densities of 10^4, 10^5 and 10^6 cells/mL in confocal well plates (Thermo Scientific #155383) in aliquots of 0.4 mL per well and allowed to adhere for 1 to 5 d depending on cell type. Once 80% of cells had adhered, for the stimulated condition, cells were exposed to 100 ng/mL SDF-1 for 1 hr at 37°C. The cells were rinsed 3 times with sterile PBS (Sigma Aldrich #D8537) and fixed with 10% formalin (Sigma Aldrich #HT501128-4L) for 10 minutes. After fixing, the cells were rinsed 3 times with PBS and permeabilized using a solution of 0.1% BSA (Sigma Aldrich #A7906-50G) and 1% Triton X (Sigma Aldrich #T8787-50ML) in PBS for 10 minutes. After permeabilization, cells were blocked using a 1% BSA in PBS solution for 60 minutes, then rinsed 2 times with blocking solution and exposed to primary antibody (mouse anti-CXCR4, Life Technologies #35-8800; rabbit anti-Connexin 43, Life Technologies #71-0700) for 2 hours at room temperature (20–25°C). After primary antibody incubation, cells were rinsed 3 times with PBS and incubated at room temperature for 30 minutes with the appropriate fluorescent secondary antibody (AlexaFlour conjugated anti-mouse, Life Technologies #A11005, Millipore #AP124JA4; anti-rabbit antibody, Life Technologies #A11037, Millipore #AP132F). After secondary antibody incubation, cells were rinsed 2 times with PBS, stained with Hoescht33342 Nuclear dye (Life Technologies #R37605) for 20 minutes, then rinsed and mounted in glycerol (Invitrogen #15514-011) to prevent drying.

**Imaging**

Time lapse migration experiments were performed using a Nikon Morrell Eclipse TE200-U microscope retrofitted with an incubated stage for environment control as previously described29. Experiments were run for 24 hours with images captured every hour at approximately 1–2 mm intervals along the length of the channel.

Cells were imaged using a Zeiss LSM 710 confocal microscope using a 63x oil immersion objective, at identical laser intensity and signal gain, and analyzed using ImageJ. Relative fluorescence for each cell was defined as the mean grayscale intensity value of the immunostain fluorescent channel, without the DAPI stained nuclei channel, divided by the total cell area. Background fluorescence was measured for each image in non-cell containing area and subtracted from the mean value.

**Statistics**

Determinations of statistical significance were performed using a one-way ANOVA and post hoc bonferroni correction with unpaired student’s t-test of equal variance. Values of p<0.05 were considered statistically significant.

**Abbreviations**

CNS Central Nervous System
MB medulloblastoma
MGPC medulloblastoma-derived glial progenitor cells
RPC retinal progenitor cells
SDF-1 stromal-derived growth factor 1
CXCR4 C-X-C motif receptor 4
Cx43 Connexin 43
NBM neurobasal medium

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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**References**

[1] Askenasy N, Zorina T, Farkas DL, Shalit I. Transplanted hematopoietic cells seed in clusters in recipient bone marrow in vivo. Stem Cells 2002; 20(4):301-10; PMID:12110699; http://dx.doi.org/10.1634/stemcells.20-4-301
[2] Friedl P, Gilmour D. Collective cell migration in morphogenesis, regeneration and cancer. Nat Rev Mol Cell Biol 2009; 10:445-4; http://dx.doi.org/10.1038/nrm2720
[3] Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Witterner BS, Spencer JA, Yu M, Pely A, Engstrom A, Zhu H et al. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Cell 2014; 158 (5):1110-22; http://dx.doi.org/10.1016/j.cell.2014.07.013
[4] Gilbert SF. Developmental Biology. 6th edition. Sunderland (MA): Sinauer Associates; 2000
[5] Galanternik MV, Kramer KL, Pietrowski, T. Heparan sulfate proteoglycans regulate Fgf signaling and cell polarity during collective cell migration. Cell Rep 2015 10, Issue 3, p414-428; http://dx.doi.org/10.1016/j.celrep.2014.12.043
[6] Morrison LC, McClelland R, Aiken C, Bridges M, Liang L, Wang X, Di Curzio D, Del Bigio M, Taylor MD, Werbowetski-Ogilvie TE. Deconstruction of medulloblastoma cellular heterogeneity reveals differences between the most highly invasive and self-renewing phenotypes. Neoplasia 2013; 15:384-98; http://dx.doi.org/10.1093/neo.13148

[7] Zhao X, Zhao Y, Lin Q, Litian Yu L, Liu Z, Lindsay H, Zhao X, Zhao Y, Lin Q, Litian Yu L, Liu Z, Lindsay H, Kogiso M, Rao P, Li X, Lu X. Cytogenetic landscape of paired neurospheres and traditional monolayer cultures in pediatric malignant brain tumors. Neuro Oncol 2015; 17(7):965-977.

[8] Ulrich F, Krieg M, Schötz EM, Link V, Castanon I, Schnabel V, Taubenberger A, Mueller D, Puech PH, Heisenberg CP. Wnt11 function in gastrulation by controlling cell cohesion through Rab5c and E-cadherin. Dev Cell 2015; 9:555-64; PMID:16198297; http://dx.doi.org/10.1016/j.devcel.2005.08.011

[9] Arima S, Nishiyama K, Ko T, Arima Y, Hakoza K, Sugihara K, Koseki H, Uchijima Y, Kurihara Y, Kurihara H. Angiogenic morphogenesis driven by dynamic and heterogeneous collective endothelial cell movement. Development 2011; 138(21):4763-76; http://dx.doi.org/10.1242/dev.068023

[10] Im and Muschel. Brain Metastasis. Metastatic Cancer: Clinical and Biological Perspectives edited by Rahul Jandial. 2013 Landes Bioscience.

[11] Hoehn M, Küstermann E, Blunk J, Wiedermann D, Trapp T, Wecker S, Föcking M, Arnold H, Hescheler J, Bernd K. Fleischmann BK et al. Monitoring of implanted stem cell migration in vivo: A highly resolved in vivo magnetic resonance imaging investigation of experimental stroke in rat. 99:16267-72; http://dx.doi.org/10.1073/pnas.242435499

[12] Khieu DN, Nyabi O, Maerckx C, Sokal E, Najimi M. Adult human liver mesenchymal stem/progenitor cells participate in mouse liver regeneration after hepatectomy. Cell Transplant 2013; 22:1369-80; http://dx.doi.org/10.3727/096368912X659853

[13] Chaddah R, Arnfield M, Runciman S, Clarke L, van der Kooy D. Clonal neural stem cells from human embryonic stem cell colonies. J Neurosci 2012; 32:7771-81; http://dx.doi.org/10.1523/JNEUROSCI.3286.2011.12.02

[14] Guo C, Luo L, Urata Y, Goto S, Huang W, Takamura S, Hayashi F, Doi H, Kitajima Y, Ono Y et al. Sensitivity and dose dependency of radiation-induced injury in hematopoietic stem/progenitor cells in mice. Sci Rep 2015; 5:8055; http://dx.doi.org/10.1038/srep08055

[15] Yamazoe T, Koizumi S, Yamasaki T, Amano S, Tokuyama T, Namba H. Potent tumor tropism of induced pluripotent stem cells and induced pluripotent stem cell-derived neural stem cells in the mouse intracerebral glioma model. Int J Oncol 2015; 46(1):147-52; http://dx.doi.org/10.3892/ijo.2014.2702

[16] Chang WK, Carmona-Fontaine C, Xavier JB. Tumour-stromal interactions generate emergent persistence in collective cancer cell migration. Interface Focus 2013; 3 (4):20130017; http://dx.doi.org/10.1098/rsfs.2013.0017

[17] Theveneau E, Steventon B, Scarpa E, Garcia S, Trepat X, Streit A, Mayor R. Chase-and-run between adjacent cell populations promotes directional collective migration. Nat Cell Biol 2013; 15(7):763-72; http://dx.doi.org/10.1038/ncb2772

[18] Cai D, Chen SC, Prasad M, He L, Wang X, Choesmel-Cadamuro V, Sawyer JK, Danuser G, Montell DJ. Mechanical feedback through E-cadherin promotes direction sensing during collective cell migration. Cell 2014; 157(5):1146-59; http://dx.doi.org/10.1016/j.cell.2014.03.045

[19] Akiyama K, You YO, Yamaza T, Chen C, Tang L, Jin Y, Chen XD, Gronthos S, Shi S. Characterization of bone marrow derived mesenchymal stem cells in suspension. Stem Cell Res Ther 2012; 3(5):40; http://dx.doi.org/10.1186/scrt131

[20] Xu Q, Li B, Yuan L, Dong Z, Zhang H, Wang H, Sun J, Ge S, Jin Y. Combination of platelet-rich plasma within periodontal ligament stem cell sheets enhances cell differentiation and matrix production. J Tissue Eng Regen Med 2014; http://dx.doi.org/10.1002/term.1953

[21] Jeon S, Oh IH. Regeneration of the retina: toward stem cell therapy for degenerative retinal diseases. Sohee Jeon1,2, Il-Hoan Oh. BMB Rep 2015; 48(4):193-9.

[22] Mouhieddine TH, Kobeissy FH, Itani M, Nokkari A, Wang KK. Stem cells in neuroinjury and neurodegenerative disorders: challenges and future neurotherapeutic prospects. Neural Regen Res 2014; 9(9):901-6; PMID:25206908; http://dx.doi.org/10.4103/1673-5374.133129

[23] Cage TA, Louie JD, Liu SR, Alvarez-Buylla A, Gupta N, Hyer J. Distinct patterns of human medulloblastoma dissemination in the developing chick embryo nervous system. Clin Exp Metastasis 2012; 29(4):371-80; http://dx.doi.org/10.1007/s10585-012-9456-6

[24] Korenberg MJ. On predicting medulloblastoma metastasis by gene expression profiling. J Proteome Res. 2004; 3(1):91-6. PMID:14998168; http://dx.doi.org/10.1021/pr034069s

[25] Elhtesham M, Min E, Issar NM, Kasl RA, Khan IS, Thompson RC. The role of the CXCR4 cell surface chemokine receptor in glioma biology. J Neurooncol 2013; 113(2):153-62; http://dx.doi.org/10.1007/s11060-013-1108-4

[26] Able RA Jr, Ngnabuey C, Beck C, Holland EC, Vazquez M. Low concentration microenvironments enhance the migration of neonatal cells of glial lineage. Cell Mol Bioeng 2012; 5(2):128-142; http://dx.doi.org/10.1007/s12195-012-0226-y

[27] Rico J, Singh T, McCueteon S, Vazquez M. Epidermal growth factor as a new therapeutic target for medulloblastoma metastasis. Cell Mol Bioeng 2015; 8:553-65

[28] Jenkins NC, Kalra RR, Dubuc A, Sivakumar W, Pedone Ehtesham M, Min E, Issar NM, Kasl RA, Khan IS, Thompson RC. The role of the CXCR4 cell surface chemokine receptor in glioma biology. J Neurooncol 2013; 113(2):153-62; http://dx.doi.org/10.1007/s11060-013-1108-4

[29] Venkataraman S, Alimova I, Balakrishnan I, Harris P, Birks DK, Griesinger A, Amani V, Cristiano B, Remke M, Taylor MD et al. Inhibition of BRD4 attenuates tumor cell self-renewal and suppresses stem cell signaling in MYC driven medulloblastoma. Oncotarget 2014; 5(9):2355-71; PMID:24796395; http://dx.doi.org/10.18632/oncotarget.1659

[30] Zigmond SH, Hirsch JG. Leukocyte locomotion and chemotaxis. New methods for evaluation, and demonstration of a cell-derived chemotactic factor. J Exp Med 1973; 137(2):387-410; http://dx.doi.org/10.1084/jem.137.2.387

[31] Seiler MJ, Aramant RB. Cell replacement and visual restoration by retinal sheet transplants. Prog Retin Eye Res 2012; 31(6):661-87; PMID:22771454; http://dx.doi.org/10.1016/j.preteyeres.2012.06.003
[32] Karl MO, Reh TA. Regenerative medicine for retinal diseases: activating the endogenous repair mechanisms. Trends Mol Med 2010; 16(4):193-202; PMID:20303826; http://dx.doi.org/10.1016/j.trendsmolmed.2010.02.003

[33] Stern JH, Temple S. Stem cells for retinal replacement therapy. Neurotherapeutics 2011; 8(4):736-43; PMID:21948217; http://dx.doi.org/10.1007/s13311-011-0077-6

[34] Ramsden CM, Powner MB, Carr AJ, Smart MJ, da Cruz L, Stern JH, Temple S. Stem cells for retinal replacement therapy: present and future. Development 2013; 140(12):2576-85; PMID:23715550; http://dx.doi.org/10.1242/dev.092270

[35] Johnson TV, Bull ND, Martin KR. Identification of barriers to retinal engraftment of transplanted stem cells. Invest Ophthalmol Vis Sci 2010; 51(2):960-70; PMID:19850833; http://dx.doi.org/10.1167/iovs.09-3884

[36] Merino JJ, Bellver-Landete V, Oset-Gasque MJ, Beatriz Cubelos B. CXCR4/CXCR7 molecular involvement in neuronal and neural progenitor migration: focus in CNS repair. J Cell Physiol 2015; 230(1):27-42; http://dx.doi.org/10.1002/jcp.24695

[37] Williams JL, Patel JR, Daniels BP, Klein RS. Targeting CXCR7/ACKR3 as a therapeutic strategy to promote remyelination in the adult central nervous system. J Exp Med 2014; 211(5):791-9; http://dx.doi.org/10.1084/jem.20131224

[38] Müller N, Michen S, Tietze S, Töpfer K, Schulte A, Lamiszus K, Schmitz M, Schackert G, Pastan I, Temme A. Engineering NK Cells Modified With an EGFRvIII-specific chimeric antigen receptor to overexpress CXCR4 improves immunotherapy of CXCL12/SDF-1α-secreting Glioblastoma. J Immunother 2015; 38(5):197-210; http://dx.doi.org/10.1097/CJI.0000000000000882

[39] Chen HY, Clayman ES, Ma WF. Phenotypic Knockout of CXCR4 expression by a novel intrakine mutant hSDF-1α/54/KDELInhibits breast cancer metastasis. J Interferon Cytokine Res 2015; 35:771-8

[40] Kim BJ, Hannanta-anan P, Chau M, Kim YS, Swartz MA, Mingming Wu. Cooperative roles of SDF-1α and EGF gradients on tumor cell migration revealed by a robust 3D microfluidic model. PLoS One. 2013; 8(7):e68422; http://dx.doi.org/10.1371/journal.pone.0068422

[41] Peplow PV. Influence of growth factors and cytokines on angiogenic function of endothelial progenitor cells: a review of in vitro human studies. Growth Factors 2014; 32(3-4):83-116; http://dx.doi.org/10.1024/09227010.3109.08977194.2014.904300

[42] Schajnovitz A, Itkin T, D’Uva G, Kalinkovich A, Golan K, Ludin A, Cohen D, Shulman Z, Avigdor A, Nagler A, et al. CXCL12 secretion by bone marrow stromal cells is dependent on cell contact and mediated by connexin-43 and connexin-45 gap junctions. Nat Immunol. 2011; 12(5):391-8; http://dx.doi.org/10.1038/ni.2017

[43] Unachukwu UJ, Sauane M, Vazquez M, Redenti S. Microfluidic generated EGF-gradients induce chemokinesis of transplantable retinal progenitor cells via the JAK/STAT and PI3Kinase signaling pathways. PLoS One 2013; 8(12):e83906; http://dx.doi.org/10.1371/journal.pone.0083906

[44] Kong Q, Able RA Jr, Dudo V, Vazquez M. A microfluidic device to establish concentration gradients using reagent density differences. J Biomech Eng 2010; 132(12):121012; http://dx.doi.org/10.1115/1.4002797

[45] Kong Q, Majeska RJ, Vazquez M. Migration of connective tissue-derived cells is mediated by ultra-low concentration gradient fields of EGF. Exp. Cell Res 2011; 317:1491-502; PMID:21536028; http://dx.doi.org/10.1016/j.yexcr.2011.04.003

[46] Bland JM, Altman DG. Multiple significance tests: The bonferroni method. BMJ 1995; 310(6973):170; PMID:7833759; http://dx.doi.org/10.1136/bmj.310.6973.170

[47] Ropella K. Introduction to statistics for biomedical engineer. Morgan & Claypool Publishers, 2007

[48] Pacey LKK, Stead S, Gleave JA, Tomczyk K, Doering LC. Protocol Exchange 2006; http://dx.doi.org/10.1038/nprot.2006.215

[49] Pepperell EE, Watt SM. A novel application for a 3-dimensional timelapse assay that distinguishes chemotactic from chemokinetic responses of hematopoietic CD133+ stem/progenitor cells. Stem Cell Res 2013; 11(2):707-20; http://dx.doi.org/10.1016/j.scr.2013.04.006

[50] Yanagi Y, Inoue Y, Kawase Y, Uchida S, Tamaki Y, Araie M, Okochi H. Properties of growth and molecular profiles of rat progenitor cells from ciliary epithelium. Exp Eye Res 2006; 82(3):471-8; http://dx.doi.org/10.1016/j.exer.2005.08.005

[51] Schwindt TT, Motta FL, Barnabé GF, Massant CG, Guimarães AO, Calcagnotto ME, Pesquero JB, Mello LE. Effects of FGF-2 and EGF removal on the differentiation of mouse neural precursor cells. An Acad Bras Cienc 2009; 81(3):443-52; http://dx.doi.org/10.1590/S0001-37652009000300009

[52] Jacobsen PF, Jenky DJ, Papadimitriou JM. Establishment of a human medulloblastoma cell line and its heterotransplantation into nude mice. J Neuropathol Exp Neurol 1985; 44:472-85; PMID:2993532; http://dx.doi.org/10.1097/00005072-198509000-00003