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Cell-Substrate Interactions Feedback to Direct Cell Migration along or against Morphological Polarization

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Abstract

In response to external stimuli, cells polarize morphologically into teardrop shapes prior to moving in the direction of their blunt leading edge through lamellipodia extension and retraction of the rear tip. This textbook description of cell migration implies that the initial polarization sets the direction of cell migration. Using microfabrication techniques to control cell morphologies and the direction of migration without gradients, we demonstrate that after polarization, lamelipodia extension and attachment can feedback to change and even reverse the initial morphological polarization. Cells do indeed migrate faster in the direction of their morphologically polarization. However, feedback from subsequent lamellipodia extension and attachment can be so powerful as to induce cells to reverse and migrate against their initial polarization, albeit at a slower speed. Constitutively active mutants of RhoA show that RhoA stimulates cell motility when cells are guided either along or against their initial polarization. Cdc42 activation and inhibition, which results in loss of directional motility during chemotaxis, only reduces the speed of migration without altering the directionality of migration on the micropatterns. These results reveal significant differences between substrate directed cell migration and that induced by chemotactic gradients.

Introduction

Directional cell motility plays a central role in embryonic development [1], tissue morphogenesis[2], wound healing[3] and cancer metastasis[2]. Studies have unraveled the mechanism of directional cell movement during chemotaxis initiated by gradients of chemoattractant including chemokines, growth factors and cytokines[4]. Gradients of these soluble attractants trigger an increase in phosphatidylinositol phosphates (PIPs)[5, 6] that promote RhoGTPase activation[7–9], and activate Cdc42 that promote lamellipodia protrusion at the leading edge of migrating cells. Cell migration under these conditions is generally accepted to follow a sequential four-step polarize-extend-attach-retract model. In response to chemoattractant gradients, mammalian cells first polarize into "front" forward moving and "rear" retracting sections that are defined by distinct signaling activity[4, 10]. The immediately visible indicator of

polarization is the morphological transformation of cells into a prototypical teardrop shape, defined by a broad leading edge and a retracting tip. This is accompanied internally by cytoskeletal reorganization and relocation of the microtubule-organizing centre (MTOC) and Golgi apparatus toward the front of the nucleus[10]. Directed protein targeting from the Golgi apparatus has been proposed to maintain the distinct protein composition in the front [11].

The second step of cell migration, directional protrusion of lamellipodia at the leading edge, is driven by actin polymerization[10, 12] and Cdc42/PIPs activation of WASP, which induces protrusions[13]. Chemokines and growth-factor receptors also activate PI3K and PI45K to generate PIPs[9, 14] and engage Rac, Cdc42, and Rho [15, 16]. In the third attachment step, integrins cluster and bind with ECM[17] recruiting α-actinin, focal adhesion kinase, and actin binding proteins (vinculin, paxillin and more α-actinin) to form focal contacts. The assembly of focal contacts is regulated by various "inside-out" signaling pathways that involve active PI3K, protein kinase C, and Rho GTPases[18–20]. In the final step of rear retraction, Rho[21] regulates actin-myosin induced contraction[4, 21, 22] to enable forward cell movement.

The RhoGTPases, Rac, Rho, or Cdc42, are key players in modulating cell migration and cytoskeletal dynamics in all four steps[23–27]. Cdc42 regulates the cell polarity by influencing the location of lamellipodia protrusion and by orienting the microtubule-organizing centre (MTOC), microtubules and Golgi apparatus to the front of the nucleus[12]. Rac activation promotes and maintains lamellipodia extension[28]. Rho activation at the rear of the cell increases actomyosin based contractility[29] and promotes disassembly of adhesions and retraction[30, 31].

Cells interact simultaneously with soluble signalling molecules and their substrate. While significant progress has been made in identifying the molecular components and signalling pathways involved in cell migration during chemotaxis[32–38], how cells explore and respond to non-uniformities and patches in adhesiveness of their surrounding ECM, which are present in-vivo[23, 39, 40], remain poorly understood. Much of the difficulty in probing the role of cell-ECM interactions comes from the fact that ECM environments necessary to drive directional migration typically triggers simultaneous changes in cell morphology. For example, gradients in substrate properties, e.g., cell adhesiveness, that are necessary to induce directional cell migration, simultaneously alter cell shape and spreading area. To isolate the effects of cell morphology, we devised a gradient-free method for directing cell migration using microfabricated adhesive islands to intermittently control their size and shape.

Micropatterned cells have been observed to extend lamellipodia, filipodia, and microspikes most aggressively at sharp corners of their constraining adhesive islands[41]. Cells confined to teardrop shaped islands extend lamellipodia predominantly from the sharp tip, but upon release, migrate preferentially from their blunt ends, consistent with the initial morphological polarization induced by the teardrop shape[42]. Using microarrays of closely spaced teardrop-shaped adhesive islands to amplify the natural directional persistence (MANDIP) of cells, we have demonstrated that 3T3 fibroblasts and human microvascular endothelial cells can be directed to migrate continuously along preset paths without chemoattractants, external fields, or mechanical manipulation[43, 44]. Cells, confined intermittently within the teardrop islands, extend lamellipodia from both ends in directions parallel to the major axis of the teardrops. The asymmetric arrangement of teardrop islands dictate whether lamellipodia extended from the blunt end or narrow tip can attach to a neighboring island and initiate an inter-island hopping step. Thus, while the teardrop shape islands polarize the cells morphologically, the direction of cell migration is imposed upon the cells by the arrangement of islands.

The ability to guide cells to migrate along and against their morphological polarization enables us to investigate if and how substrate guided directional migration differs from the sequential four-step polarize-extend-attach-retract model for chemotaxis. During chemotaxis, gradients in the binding of soluble chemoattractants lead to local activation of RhoGTPases,

which regulate actin cytoskeleton organization and contractility, induces directional migration. However, in contrast to soluble chemoattractants whose binding cannot impart physical forces to cells, the binding of cell integrins to the ECM result in focal adhesion complexes that transmit both physical and chemical cues. Here we will examine if substrated directed cell movement requires the activation of specific RhoGTPases that are central to chemotaxis.

Results

MANDIP micropatterns for independent control of morphological polarization and attachment of protrusions

We use MANDIP micropatterns A and B (Fig 1) to direct cell migration either along (Micropattern A) or against (Micropattern B) their morphological polarization (Table 1). The teardrop islands of micropatterns A and B are identical and mimic the natural shape of motile cells, with a wide leading edge that tapers to a narrow rear end. The area of each teardrop (1000 μm^2) was chosen based on the previous study that show the migration tendency is higher when cell spreading is restricted[45]. The spacing between islands (3.5 μm) were designed to temporarily define the cell morphology while allowing membrane protrusions to reach and attach to adjacent islands[43]. On micropattern A, lamellipodia extended along the elongated cell body from the blunt end span the gap to attach to the adjacent island enforces cyclical clockwise hopping of fibroblasts around the four island array[43].

The seemingly minor rearrangement of the teardrop islands to form micropattern B forces the cell movement against the morphological polarization. As cells adopt the shape of the teardrop islands, they are polarized morphologically to migrate from their blunt end. However, lamellipodia extended from the blunt end rarely attach perpendicularly to a neighboring island due to the absence of permissive adhesion sites directly in front of their blunt-end. In contrast, lamellipodia extended from the tip, can attaches readily to the blunt of a neighboring island. As a result, tip-to-blunt, counter-clockwise migration along the teardrops dominates (>90%, Table 1). This suggests that in addition to the generally accepted notion of cell polarization as

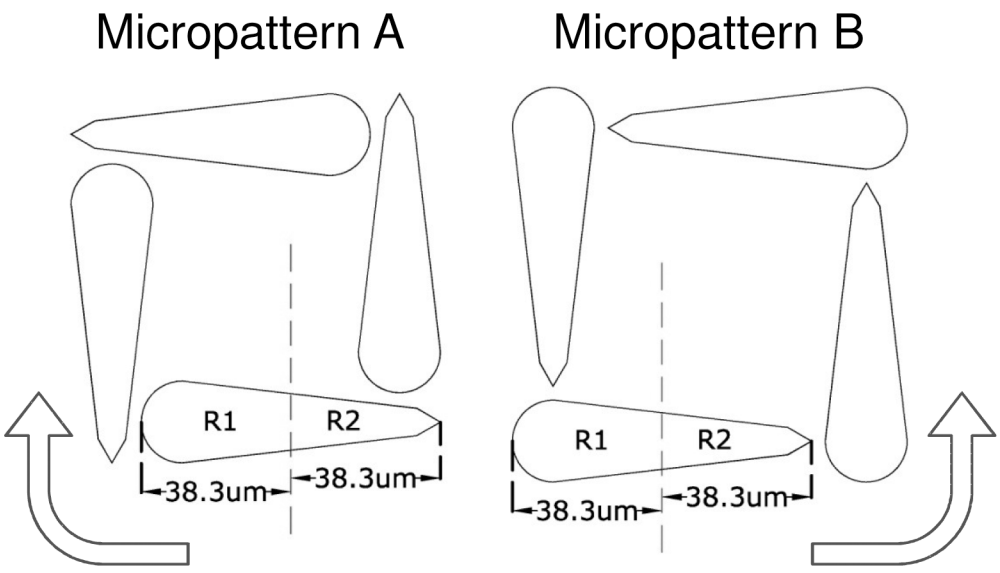

Fig 1. Micropatterns for directing cell migration along (Micropattern A) or against (Micropattern B) the morphological polarization induced by the teardrops. As cells traverse across the teardrop islands, the location of the cell nucleus is categorized to be in the blunt (R1) or tip (R2) halves of the teardrops.

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Table 1. Migration direction and average duration between inter-island hops for wild type fibroblasts and various mutants.

Cell Type	Microarray	#CW	#CCW	Average time* (hrs/hop) *	p-value
WT	A	82	1	2.7 ± 0.6	
WT	B	2	91	4.2 ± 0.6	
CA-Rac	A	58	3	3.0 ± 1.0	4.0E-02
CA-Rac	B	4	53	5.0 ± 1.8	1.9E-03
CA-Rho	A	78	5	1.4 ± 0.8	2.4E-23
CA-Rho	B	6	68	2.3 ± 0.7	5.4E-40
CA-Cdc42	A	81	4	4.1 ± 1.0	1.1E-20
CA-Cdc42	B	7	73	5.9 ± 1.3	9.0E-19
DN-Cdc42	A	66	4	7.4 ± 2.1	7.5E-30
DN-Cdc42	B	9	63	8.0 ± 2.4	2.0E-21

*Average time required for single hop (both CW and CCW) is represented as Mean ± SD, p-values are calculated using Welch's t-test for non-equal sample sizes and variances under the null hypothesis that the average time between hops is equal to the wild type on the same microarray. DN Rac and DN Rho cells did not move in microarray A and B within the time frame used for observing all listed cells. Migration behaviors for the wild type is from Ref [43].

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being the first step of directional migration, there exists a feedback mechanism through which lamellipodia attachment redirects morphological polarization.

Golgi apparatus reorient toward the direction of cell movement

Migrating fibroblasts have been shown to respond to external cues such as chemotactic gradients, wounding, and electric fields by reorienting their Golgi apparatus toward the direction of migration[46]. The reorientation of Golgi toward the cell's leading edge supplies membranes and secreted products to support the formation of protrusions[47]. With the Golgi apparatus typically located between the nucleus and the leading edge of motile cells, cell polarity is deduced from the relative position of the Golgi apparatus and the cell nucleus.

Here, we investigate if and how cells on micropattern B reorient their Golgi as they reverse the polarity imposed by the teardrop shape, so as to migrate in the direction of successful lamellipodia attachment from their rear tip. To do this quantitatively as the cells migrate around the micropatterns, the location of the nucleus was identified to be either in the blunt half (R1) or tip half (R2) of the teardrop shapes. When the nucleus is located in the tip half (R2), the cell is translocating and spanning between the two neighboring islands and the nucleus typically do not reside completely within the tip but suspended between the two neighboring islands (Fig 2A left panel). When the nucleus is located in the blunt half (R1), the cell just completed the migration and the nuclear typically reside within the adhesive island (Fig 2A right panel). With the elliptically shaped nucleus translocating alternately between R1 and R2, the peripheral region around the nucleus was subdivided into front (r1), side (r2), and rear (r3) regions with the front (r1) side oriented towards blunt end of the tear drops (Fig 2A).

For cells migrating on micropattern A, the Golgi apparatus is localized predominantly in front of nuclei (r1) irrespective of whether the cell is transvering between the two adhesive islands (nucleus in R2; Fig 2A left panel) or has completed the migration (nucelus in R1, Fig 2A right panel). This is consistent with the observation that cells on continuous tear drop arrays with the blunt end pointing toward the tip of the neighboring island show preferential Golgi localization toward the blunt ends as in isolated tear drop patterns[42]. Intermittently confined cells adopt the leading edge at their blunt end from which successfully attachment of lamellipodia and migration is biased further by the presence of an adjacent island directly in front of the blunt end.

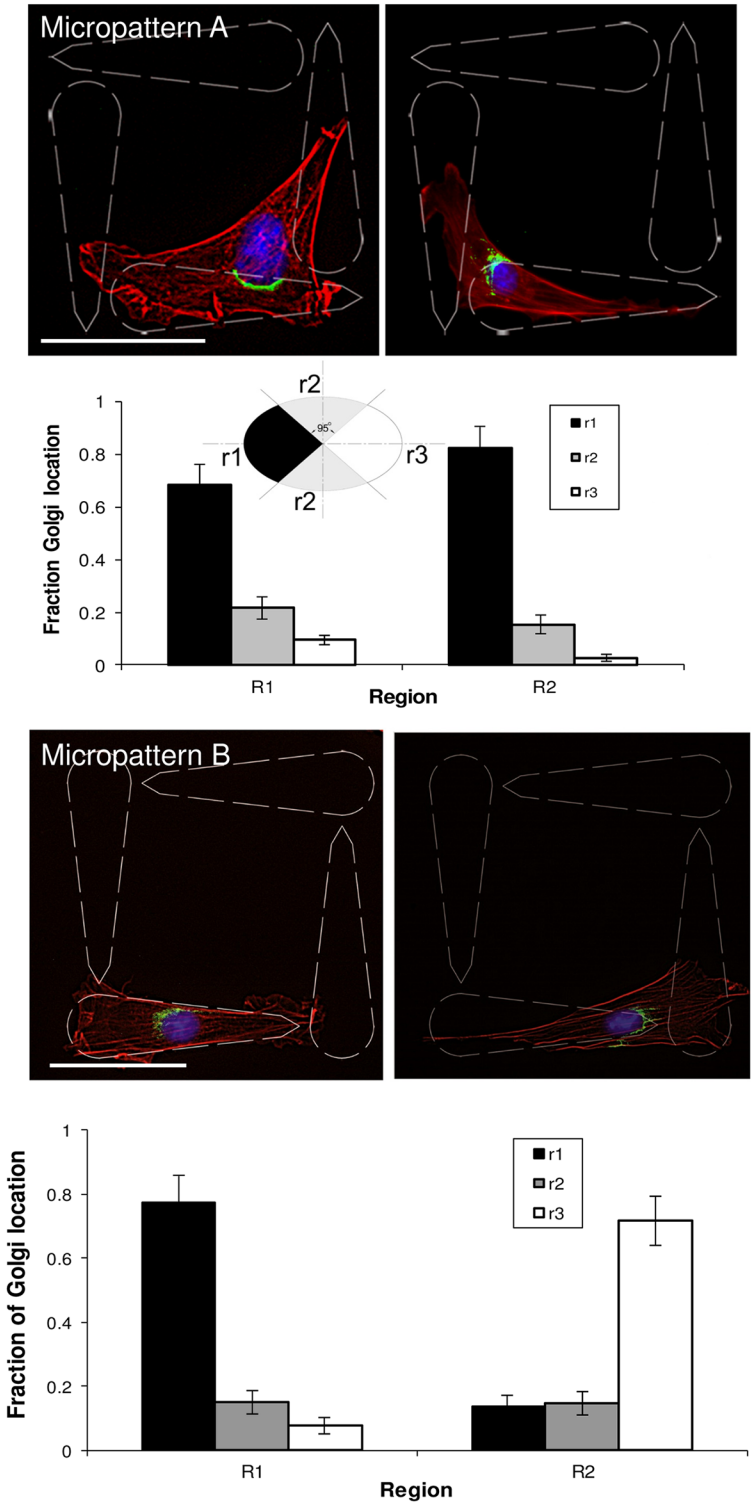

Fig 2. Orientation of Golgi apparatus in cells migrating on micropatterns A and B. Cells are stained for actin filaments (red), Golgi apparatus (green), and nuclei (blue). The polarization of the Golgi apparatus around the nuclear periphery is categoried to be toward the blunt (r1), side (r2), or tip (r3). (A) Cells migrating along the direction their morphological polarization (Micropattern A), polarize their Golgi predominantly towards the blunt end of the islands (r1) regardless of wherther the cell is transvering across the two adhesive islands (nucleus in R2; Fig 2A left panel) or completed the migration (nucleus in R1; Fig 2A right panel). (B)

On micropattern B, the Golgi is polarized towards the blunt end when the nucleus is in the blunt half (R1; Fig 2B left panel) following the morphological polarization induced by the teardrop islands. Feedback from exploratory lamellipodia extension from the rear tip cyclically reverts the morphology to migrate against the polarization of the island indicated by the predominant polarization of the Golgi towards the tip end (r3) when the nucleus is also in the tip end (R2; Fig 2B right panel). At least 30 cells from 5 independent experiments were analyzed for each case. Scale bar: 50 μm.

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In contrast, cells migrating on micropattern B, reverse their polarity cyclically as they traverse across the islands (Fig 2B). Initially cell adopts the shape of a teardrop island, with its nuclei in the blunt R1 region, orients its Golgi predominantly in front of the nucleus (r1) towards the blunt end of the teardrop (Fig 2B left panel). As lamellipodia extended from the tip attaches onto a neighboring island, the nucleus shifts towards the R2 tip region, and the Golgi apparatus relocates towards the tip of the island (r3) revealing a clear reversal of polarization (Fig 2B right panel). The cell adopts the shape of its new host, reorients its Golgi towards r1, and the process repeats. This cyclical reorientation of Golgi apparatus for cells migrating on micropattern B indicate that cells are initially polarized based on the adhesive island shape but upon exploring their surroundings can repeatedly reverse their polarity so as to migrate from their tip. Accompanying this reversal in polarity, is a major cytoskeletal rearrangement to transform what was once a receding tip into a blunt leading edge that is attached to the blunt end of the adjacent teardrop island. The reorientation of Golgi and rearrangement of cytoskeleton takes time, and cells consistently require more time to move between islands on micropattern B compared to micropattern A (Table 1).

Activation of Rac or Rho alters migration speed but not directionality

We investigated the role of RhoGTPases on migration of fibroblasts in directions along or against their morphological polarization using mutants with constitutively activated (CA) and dominant negative (DN) Rac1, RhoA, and Cdc42. Rac1 activation at the blunt leading edge of cells promotes and maintains lamellipodia protrusions[48–50]. However, it is unclear if cells with global activation of Rac1 also promotes lamellipodia extension from the tip that would enhance feedback-induced morphological polarization and migration from the tip. Global activation of intracellular signals that mediate lamellipodia extensions (Rac1) or regulate cell polarity (Cdc42) has been shown to reduce directional persistence and promote random migration [51–53]. From this, we initially expected that the ability of MANDIP microarrays to direct cell movements will be substantially diminished with CA-Rac and CA-Cdc42 fibroblasts. Surprisingly, constitutively active Rac1 fibroblasts migrate on micropatterns A and B with overwhelming directionality, with 95% of observed interisland traversals in the clockwise direction for microarray A and 93% in the counterclockwise direction for microarray B (Fig 3). The non-directional increase in lamellipodia activity associated with activated Rac1 pathways did not alter the directionality of migration on micropatterns A and B. The average time required for interisland traversal with CA-Rac fibroblasts was observed to be 2.95 ± 0.97 h, which is slightly more than wild type cells. This decrease in speed for constitutively activated Rac1 cells may be due to a reduction in net traction force caused by increased non-directional lamellipodia formation/extension around the cells. The period between interisland traversal on micropattern B is slower than on micropattern A to accommodate the cytoskeletal reorganization necessary for tip-to-blunt migration. Cells expressing dominate negative Rac showed no movement on MANDIP micropatterns confirming that Rac is functionally required for cell movement.

Rho activation at the rear of cells increases actomyosin based contractility[54, 55] and promotes adhesion dissassembly and retraction[30, 56]. Overexpression of Rho increases contractile tension, which results in formation of numerous actin bundles and associated mature focal

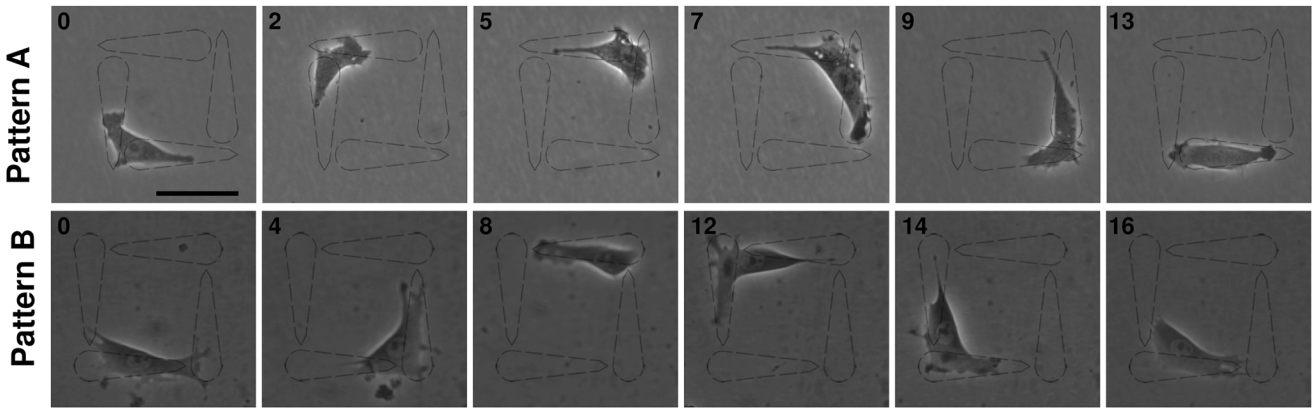

Fig 3. Time-lapse phase contrast images (in hours) showing the continuous directional migration of fibroblasts with constitutively activated Rac1 along micropatterns A and B. Scale bar: 50 μm.

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contacts[57–60], and decreases the rate of cytoskeletal reorganization[61]. Constitutively activated CA-RhoA fibroblasts maintained the directionality as wild-type fibroblasts, clockwise along micropattern A and counter-clockwise on micropattern B (Fig 4). CA-RhoA fibroblasts, with increased contractile tension, migrated at nearly double the speed of wild type fibroblasts (Table 1), implying that a key step limiting the speed of cell migration on these micropatterns is the disassembly of adhesions and retraction of the rear. No movement was observed for dominant negative RhoA cells on MANDIP micropatterns indicating that RhoA is required for cell movement.

DN-Cdc42 cells can move continuously and directionally

CA-Cdc42 cells aggressively extend actin-based membrane protrusions at their edges. These actin protrusions form filopodia that spread beyond the adhesive island and form attachments on neighboring island. As cells migrate from one island to another new filopodia attach and old ones detach (Fig 5). These fingerlike protrusions of parallel bundled actin filaments are key physical means through which cells explore their local environment[38, 62]. It is thus remarkable that dominant negative DN-Cdc42 fibroblasts, migrate in the same direction as their

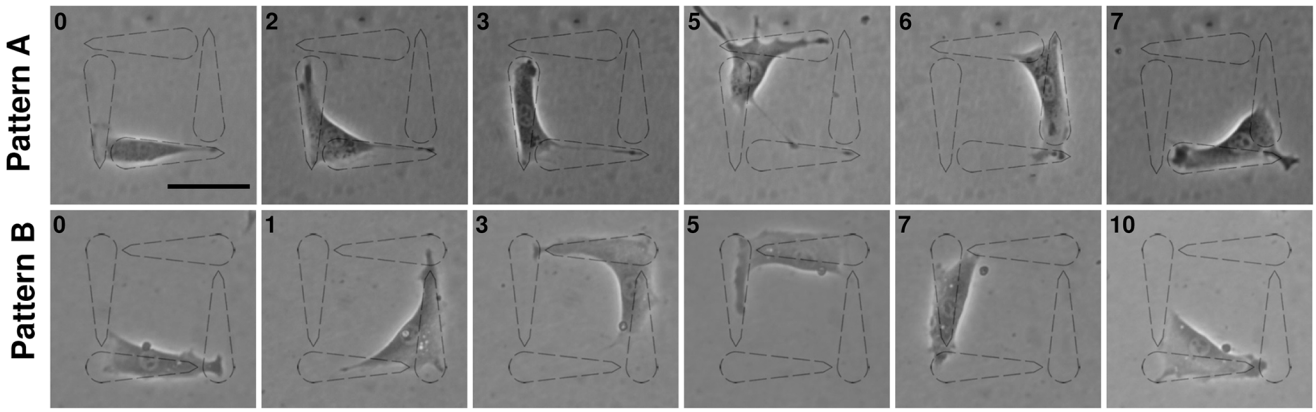

Fig 4. Time-lapse phase contrast images (in hours) showing the continuous directional migration of fibroblasts with constitutively activated RhoA along micropatterns A and B. Scale bar: 50 μm.

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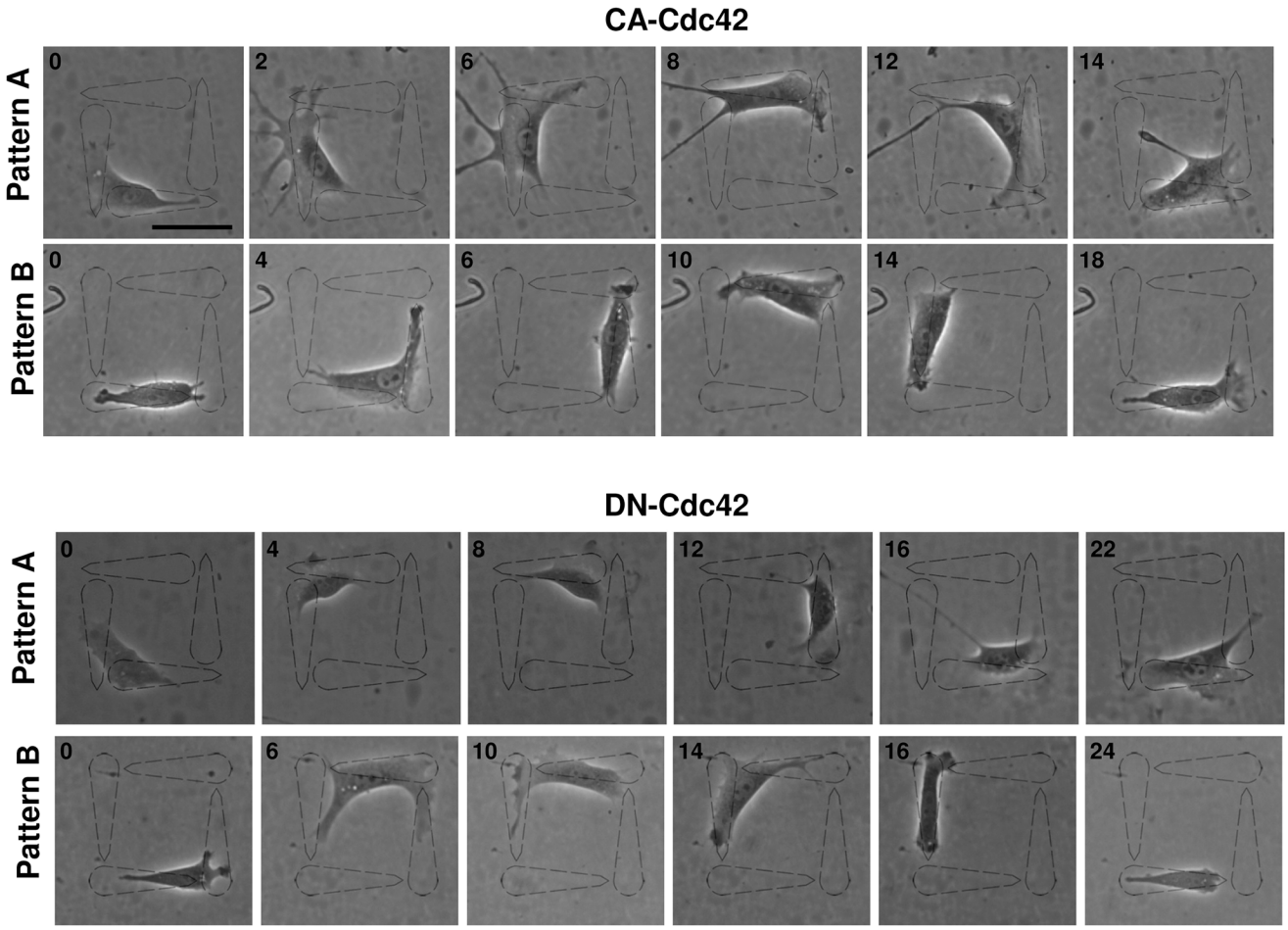

Fig 5. Time-lapse phase contrast images (in hours) showing the continuous directional migration of fibroblasts with constitutively activated Cdc42 or dominant negative Cdc42 along micropatterns A and B. Scale bar: 50 μm.

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constitutatively active CA-Cdc42 counterparts, preferentially in the clockwise direction on microarray A and in the counterclockwise direction on microarray B.

CA-Cdc42 or DN-Cdc42 cells migrate on non-patterned substrates with reduced persistence[63]. The speed of migration on both micropatterns for both CA-Cdc42 and DN-Cdc42 fibroblasts are slower (Table 1) consistent with the observation during chemotaxsis cells move slower with activation or suppression of Cdc42. The overall reduced speed of Cdc42 activated cells is likely due to non-directional filopodia activity at the edges the cell. The average time between island traversals is even longer for DN-Cdc42 compared to CA-Cdc42. This is consistent with *in vitro* wound closure studies[26] where DN Cdc 42 fibroblasts reduced wound closure rates by ~50%.

Table 1 summarizes the results for for CA-Rac1, RhoA, Cdc42 and DNCdc42. With the exception of DN-Rac1 and DN-RhoA which do not migrate, all of the constitutively active and dominant negative RhoGTPase mutants examined here, migrate preferentially in the same direction as wild-type 3T3 fibroblasts, clockwise on micropattern A and counter clockwise on microarray B. Despite drastic changes in Rac1, RhoA and Cdc42 expression, cell migration on these micropatterns remain overwhelmingly directional and all cells retained the ability to migrate against the morphological polarization imposed by the teardrop shapes (micropattern B).

Discussion

In chemotaxis, the first step of cell migration—morphological polarization is thought to direct the subsequent steps of lamellipodia extension and attachment. Here we show that for substrate-directed cell migration, lamellipodia extension and attachment can feedback to change the morphological polarization dictated by the initial shape of the cell. This feedback arising from the continuous exploration of the cell's local environment through membrane protrusions is likely a key mechanism for directed cell movement on non-homogeneous substrates. Unproductive protrusions that fail to attach are retracted, while protrusions that attach successfully to the substrate can reorient the morphology leading to positive feedback. The concept that cell substrate interactions can influence directional cell movement has been proposed by others, but to our knowledge, the idea that cells can move consistently against their initial polarization through substrate interactions and Golgi apparatus reorientation has neither been proposed nor observed. Here, we offer experimental evidence that such a feedback mechanism not only exists but is strong enough that lamellipodia attachment can reverse morphological polarization.

Dominant negative Rac mutants exhibit inhibited chemotaxis for several cell types[64], and substrate-directed cell migration also requires the activation of Rac1. In chemotaxis, Rac1 has been shown to promote the speed of cell migration[65], but directional migration requires localized Rac activation[30]. Here we observed that even with global Rac1 activation, substrate-guided migration can be overwhelmingly directional with only minor reduction in speed for CA Rac1 cells migrating against or along their initial polarization.

RhoA, which regulates contractility, stimulates cell motility in chemotaxis. On our substrate-directed studies, RhoA activation has a very significant effect, with CA RhoA cells migrating almost twice as fast along the initial polarization direction. Even when migrating against their initial polarization (micropattern B), CA RhoA cells migrate at a higher speed than CA Rac1 cells highlighting the significant role of cell contractility during substrate-directed movement. In all cases, the p-values all reject the null hypothesis that the mutants have the same average hop time as the wild type fibroblast.

The most striking difference we found between substrate-guided and chemotactic migration is the role of Cdc42. Cdc42 is generally accepted to be a key regulator of cell polarity during chemotaxis[65–68]. Cdc42 is necessary to establish cell polarity[63], and while dominant negative Cdc42 cells are motile, they fail to respond to chemotactic gradients and migrate randomly [66]. In contrast, when guided by the substrate MANDIP micropatterns here, dominant negative Cdc42 cells migrate with overwhelming directionality. This implies that cell-substrate interactions can define cell polarity even without Cdc42.

It has been shown that signal cross talk between different RhoGTPases and clonal variability can occur when using dominant negative or constitutively active mutants[69]. For example, the dominant negative mutant of Cdc42 functions by sequestering the Rho guanine nucleotide exchange factors (GEF). There are more than 70 GEFs, and among them many are known to regulate multiple GTPases[63]. Thus, dominant negative mutant can influence multiple GEFs and the activities of multiple RhoGTPases. The microarrays for directing cell migration along or against the morphological polarization described here can provide insights on the feedback mechanisms of cell substrate interactions using a wide range of cells including dominant mutant expression approach or cells produced by gene targeting. Combining the knowledge gained from other specific knockout cells in physiologically relevant systems using tissue specific gene targeting[70] will advance our understanding of tissue specific cell substrate interactions and polarity. This approach for probing cell substrate interactions can be extended further by comparing side by side the spatial and temporal activation of signaling molecules,

e.g., the RhoGTPases can be monitored by GFP Raichu probes (Ras and interacting protein chimeric unit)[71] and Golgi can be monitored real time[45].

Altogether, the results presented here brings to light a new layer of complexity on the role of physical cell-substrate interactions in reshaping polarized cell morphology and directional migration even when Cdc42 is inhibited. These findings highlight the importance of understanding the interplay between physical and biochemical cues that guide cell migration during during tissue morphogeneis, wound healing, and cancer metastasis.

Materials and Methods

Materials

Tissue culture dishes were purchased from Fisher Scientific (Catalog No. 430166) and used as received. Polydimethylsiloxane (PDMS; Sylgard 184) was obtained from Dow Corning (Midland, MI). Alexa 488-phalloidin, 4',6-diamidino-2-phenylindole (DAPI), were purchased form Molecular Probes (Eugene, OR). Phosphate buffer saline was purchased from Sigma (St. Louis, MO). Opti-MEM media was purchased from invitrogen, Serum Supreme, were purchased from Cambrex biosciences (Walkersville, MD).

Preparation of poly(OEGMA-co-MA)

Random copolymers of OEGMA and MA (Scientific Polymer Products, NY) were prepared by free radical polymerization of 10 wt% methanolic solutions of the two monomers (80:20 OEGMA to MA mass ratio) at 60°C following a procedure described previously[72]. Polymerizations were initiated with 1 wt% (with respect to monomer) of 2,2'-azobis(2-amidinopropane) dihydrochloride (Wako, VA) and allowed to react for 16 hours.

Preparation of patterned tissue culture dishes

Micropatterns of different geometric shapes, e.g., squares, circles, ellipses and rectangles were fabricated on silicon wafers using standard photolithographic techniques. From this silicon master, complementary polydimethylsiloxane (PDMS) replicas were prepared and used as stamps in subsequent microcontact printing steps[73] to form patterns of poly (OEGMA-co-MA) copolymer directly on cell culture dishes. Patterned dishes were sterilized under UV for 12 hours before cells were plated. The microarrays on the tissue culture dishes were not pretreated with extracellular matrix proteins before cell seeding.

DNA construct, cell culture, and time-lapse microscopy

Rac1, RhoA and Cdc42 constitutively active mutants (Rac1L61, RhoAL63, and Cdc42L63) and dominantly negative mutants (Rac1N17, RhoAN19, and Cdc42N17) were gifts from Prof. Yi Zheng (Cincinnati Childrens Hospital) and were generated as described previously[74–76]. Briefly, the mutants are generated by site-directed mutagenesis based on oligonucleotide-mediated polymerase chain reaction[77]. The retroviral construct expressing mutant were generated by ligating the corresponding cDNA fragments into the BamH1 and EcoR1 sites of the bicistronice vector[78]. GTPases act as binary molecular switches that cycles between an inactive GDP bound state and an active GTP bound state in response to environmental stimuli[24, 27, 79]. The CA cells were modified and selected with mutations that cause the specific GTPases to constitutively accumulate in the active GTP bound active state while DN cells in GDP-bound inactive state. The proteins were not overexpressed. For example, CA-Rac mutants are generally more powerful than endogenous Rac-GTP but cannot be compared directly with endogenous Rac that are cycling between GTP and GDP bound state.

Constitutively active and dominant negative mutants of Rac1, RhoA and Cdc42 (NIH 3T3 fibroblasts) were cultured in Opti-MEM media (w/o phenol red) supplemented with 10% fetal bovine serum and 25mM hepes (Invitrogen). All mutants except CA-Rac1 were grown by selection with G418 (350 μg/ml). Cell cultures were thermostated using a stage-mounted heater equipped with a temperature controller. Patterned cells (500 cells/mL) were supplemented with media containing human PDGF (5 ng/mL) and HEPES buffer (N-(2-hydroxyethyl)-piperazine-N′-2-ethanesulfonic acid; 50 mM) 30 min prior to time-lapse imaging. Phase-contrast images were recorded each hour for the time lapse figures.

Analysis of Cell "Hopping" Direction and Speed

Only single cells confined within single islands were considered for analysis. Cells were considered to have "hopped" when their nuclei and 90% of their cell area have relocated from one island to another. The 99% confidence intervals of the average speed of motile cells were calculated based on the standard deviation of data collected from at least 100 hopping events and a minimum population of 30 motile cells.

Immunostaining

Cells were fixed with 3.7% paraformaldehyde for 10 minutes, washed in phosphate buffered saline, and then permeabilized with 0.2% Triton X100 for 5 minutes. Samples were then rinsed with PBS and incubated with Alexa 594-phalloidin, Alexa 488 conjugated anti-golgin-97 (human) and DAPI to stain for F-actin, golgi apparatus and nuclei respectively. Images of the patterned cells were acquired using a Nikon TE-2000 inverted microscope with Metamorph software (Ver 6.0r4, Universal Imaging, Westchester, PA)

Analysis for Golgi reorientation

The polarization of Golgi patterned on microarray patterns A and B (Fig 2) was quantified by calculating the projected area of Golgi in select regions around the nucleus. We divided the teardrop shape into two equal 38.3μm length sections, and identified cells as having their nuclei either in the blunt R1 or rear tip R2 region to represent when cell is on the island or transvering between the islands. Cells with nuclei span across both R1 and R2 regions were not included in the analysis. An elliptical template (Fig 2A) was aligned to cell nuclei according to the axis of its semi-major axis, semi-minor axis, and centroid. The peripheral region around the nucleus was divided into front (r1), side (r2), and rear (r3) sectors. The fractional localization of Golgi apparatus in these regions was quantified by measuring the projected area of Golgi apparatus in each region and normalizing against the total area of Golgi.

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Author Contributions

Conceived and designed the experiments: GK CH CC. Performed the experiments: GK. Analyzed the data: GK CC. Contributed reagents/materials/analysis tools: CC. Wrote the paper: GK CH CC.

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