8 Exposing Cell-Itary Confinement: Understanding the Mechanisms of Confined Single Cell Migration

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Abstract

Cells in vivo migrate in a complex microenvironment and are subjected to varying degrees of physical confinement provided by neighboring cells, tissues, and extracellular matrix. The molecular machinery that cells utilize to migrate through confining pores or microtracks shares both similarities and differences with that used in unconfined 2D migration. Depending on the exact properties of the local microenvironment and cell contractile state, cells can adopt distinct phenotypes and employ a wide array of mechanisms to migrate efficiently in confined spaces. Remarkably, these various migration modes are also interconvertible and interconnected, highlighting the plasticity and inherent complexity underlying confined cell migration. In this book chapter, an overview of the different molecular mechanisms utilized by cells to migrate in confinement is presented, with special emphasis on the extrinsic environmental and intrinsic molecular determinants that control the transformation from one mechanism to the other.

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8.1 Introduction

Cell migration is an integral process for diverse normal physiological and homeostatic functions, including embryogenesis, tissue morphogenesis, wound healing, and immune response, as well as pathological processes, such as chronic inflammatory diseases and cancer metastasis [1]. There is thus a dire and important need to understand the biochemical and physicomechanical driving forces underlying cell motility, as it can provide critical insights to inform the development of novel and effective therapeutic strategies to ensure proper physiological cellular functions or abate diseases. However, cell migration is an intricate and well-orchestrated biological phenomenon that is modulated by multiple intrinsic (i.e., cell type, actomyosin contractility, integrin-mediated adhesion, cellular and nuclear deformability, etc.) and extrinsic factors (extracellular matrix (ECM) composition and stiffness, porosity, adhesiveness, elastic behavior, etc.) [2, 3]. Much

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of what we currently know about the mechanisms of cell migration stems from in vitro experiments performed on 2D planar surfaces. Although 2D migration is relevant to certain physiological processes like wound healing and neutrophil trafficking on inflamed endothelium, 2D in vitro migration models fail to recapitulate the complex topographical cues presented by the tissue microenvironment that cells experience in vivo [4].

Cells in vivo are typically embedded in and migrate within 3D dense fibrillar ECM with narrow pores. Many of times, the pores present in the ECM network are smaller than the average cell diameter, ranging from 1 to 20 μm [5]. In such instances, cells have to either rely on matrix metalloproteinase (MMP)-dependent pericellular proteolysis to degrade surrounding ECM to generate tracks large enough for cells to migrate into [6] or MMP-independent alternative modes of migration where cells rearrange cytoskeleton and increase actomyosin contractility to facilitate cellular and nuclear deformation and translocation through tight pores [7–9]. In addition to tracks generated de novo by migrating cells with MMP, there also exist preformed 3D longitudinal ECM-free channels that provide paths of least resistance in which cells can exploit to migrate efficiently. These in vivo 3D longitudinal channels can manifest themselves in many forms and are widely prevalent in the human body. Many of these 3D channels form between the connective tissue and the basement membrane of nerve, muscle, and epithelium [2] and in fibrillar interstitial tissues between adjacent bundles of collagen fibers [10]. Microtracks are also present along and within blood [11, 12] and lymphatic vessels [13], as well as in white matter tracks and perivascular spaces within the brain [14]. Additionally, follower cancer cells can also migrate in 3D longitudinal tracks remodeled by leader fibroblasts or surrounding stromal cells [15, 16]. These 3D channels vary considerably in cross-sectional area, ranging from 10 to 1000 μm^2 [5]. As such, cells in vivo have to navigate through tight spaces, be it pores in ECM or tunnellike tracks, and experience different degrees of physical confinement. Numerous recent studies have provided mounting evidence highlighting the differences between unconfined 2D migration and confined migration in terms of cellular morphology, intracellular signaling, and molecular mechanisms [17]. Indeed, many of the hallmarks of conventional 2D migration model are found to be dispensable in confined microenvironments, suggesting a specific and critical role that physical confinement plays in modulating cellular responses.

In this chapter, we focus on the behaviors and mechanisms by which single cells migrate in confinement. Specifically, confined single cell migration is defined as the phenomenon in which a single cell (not tethered or attached to neighboring cells) migrates in an environment where at least one of the three dimensions is about or below cell size; in such a case, the cell has to form additional non-basal contact with the surrounding matrix and deform its cytoplasm and/or nucleus in order to move forward. Recent advances in bioengineering and microfabrication techniques have enabled us to engineer in vitro models to study confined single cell migration at precisely controlled experimental conditions mimicking aspects of the in vivo microenvironment. These models include polydimethylsiloxane (PDMS) microfluidic devices, microcontact printed patterns of prescribed geometries, micro-/nanogroove substrates, vertical confinement devices, and 3D patterned hydrogels. A detailed description of the various techniques and systems to simulate physiologically relevant confined conditions can be found in a recent comprehensive review [18]. Nevertheless, these experimental models are vital, as they have provided us with a rapid and high-throughput platform to study the mechanisms of confined single cell migration, which are discussed below.

8.2 Conventional Paradigm of 2D Cell Motility Cycle

Most of our existing understanding of cell migration originates from initial observations showing how metazoan cells adhere and crawl on 2D flat surfaces [19, 20]. Since then, a plethora of studies have been carried out to decipher the various

steps involved in this highly orchestrated process termed as cell motility cycle. The detailed step-by-step mechanisms of 2D cell motility cycle have been extensively reviewed elsewhere [20–23], but they can be briefly summarized into four sequential steps, namely, protrusion, adhesion, contraction, and retraction. At first, a stationary cell receives motogenic signals, either biochemically with growth factors [24, 25] or cytokines [26] or physicomechanically via physical confinement, differential substrate rigidity [27], or electrical current [28], and becomes polarized, developing distinct leading and trailing edges. This polarized cell state is achieved primarily by internal polarization of microtubule and secretory apparatus [29] that direct the vesicular transport of lipids (e.g., phosphatidylinositol (3,4,5)-trisphosphate, PIP_3) [30] and proteins (e.g., small Rho GTPases such as Rac1 and Cdc42) [31, 32]. Accumulation of these polarized signals at the leading edge facilitates Arp2/3-dependent polymerization of branched actin filaments (F-actin), initiating the formation of wide, fanlike membrane protrusion known as lamellipodia [33]. Adhesion molecules such as integrins present on the lamellipodial protrusions then bind to matrix ligand, forming new small nascent adhesions underneath the leading edge [34]. RhoA and formin family of actin nucleators such as mDia1 and mDia2 subsequently assemble actin stress fibers to connect with adhesions sites [35–37]. Actomyosin contraction of the stress fibers pulls and exerts tension on nascent adhesions, enlarging and maturing them into focal adhesions (FAs) [38]. At the same time, actomyosin contractility also enhances the contractile tension between the leading and trailing edge of the cells. The overall increase in cellular contractility, coupled with localized increase in myosin II activity toward the back of the cells, signals the disassembly of rear adhesions, releasing the rear of the cells from the 2D surfaces in a process known as trailing edge retraction, consequently leading to directed cell movement [39]. As the cycle progresses, retrograde F-actin flow helps to push membrane and lipids rearward and position the nucleus toward the back of the cells, resetting the cells to respond to the next round of motogenic signal [40].

While it is widely believed that most epithelium-derived cells migrate in similar cyclic manner on 2D environments [1], the story becomes increasingly more complicated and less predictable as cells transition to the more physiologically relevant 3D environments where they are now confined within dense fibrillar matrix or preexisting migration tracks. Some of the hallmarks of the conventional 2D cell motility cycles, such as substrate adhesions and actomyosin contractility, are sometimes even dispensable in cells migrating in complex in vivo 3D environment. In fact, numerous studies conducted over the past decade have demonstrated that cells are extremely plastic and are able to adopt a multitude of different migration mechanisms in response to their surrounding environment to enable efficient locomotion. A schematic of the various confined cell migration mechanisms and their key characteristics can be found in Fig. 8.1.

8.3 Pseudopodial-Based Mesenchymal Confined Migration

8.3.1 Comparison to 2D Mesenchymal Migration

In 3D artificial hydrogel networks in vitro and ECM tissues in vivo, cells can migrate with an elongated morphology with protrusions driven by actin polymerization, which we broadly term as pseudopodia and includes actin-rich structures such as lamellipodia, filopodia, and invadopodia, similar to classical 2D mesenchymal migration [9, 41, 42]. This mode of migration is also evident in preformed tunnel-liked conduits in vivo, in collagen/polyacrylamide-based patterned microtracks [27, 43, 44], and in PDMS microfluidic microchannel devices [45–47]. Cells cultured on 1D lines created by microphotopatterning or microcontact printing, in which cells are laterally confined due to limitation of adhesion sites, also exhibit similar elongated morphology as they do on oriented 3D fibrillar ECM in vivo, where a strong correlation between migration speed and movement persistence is noted [41, 48].

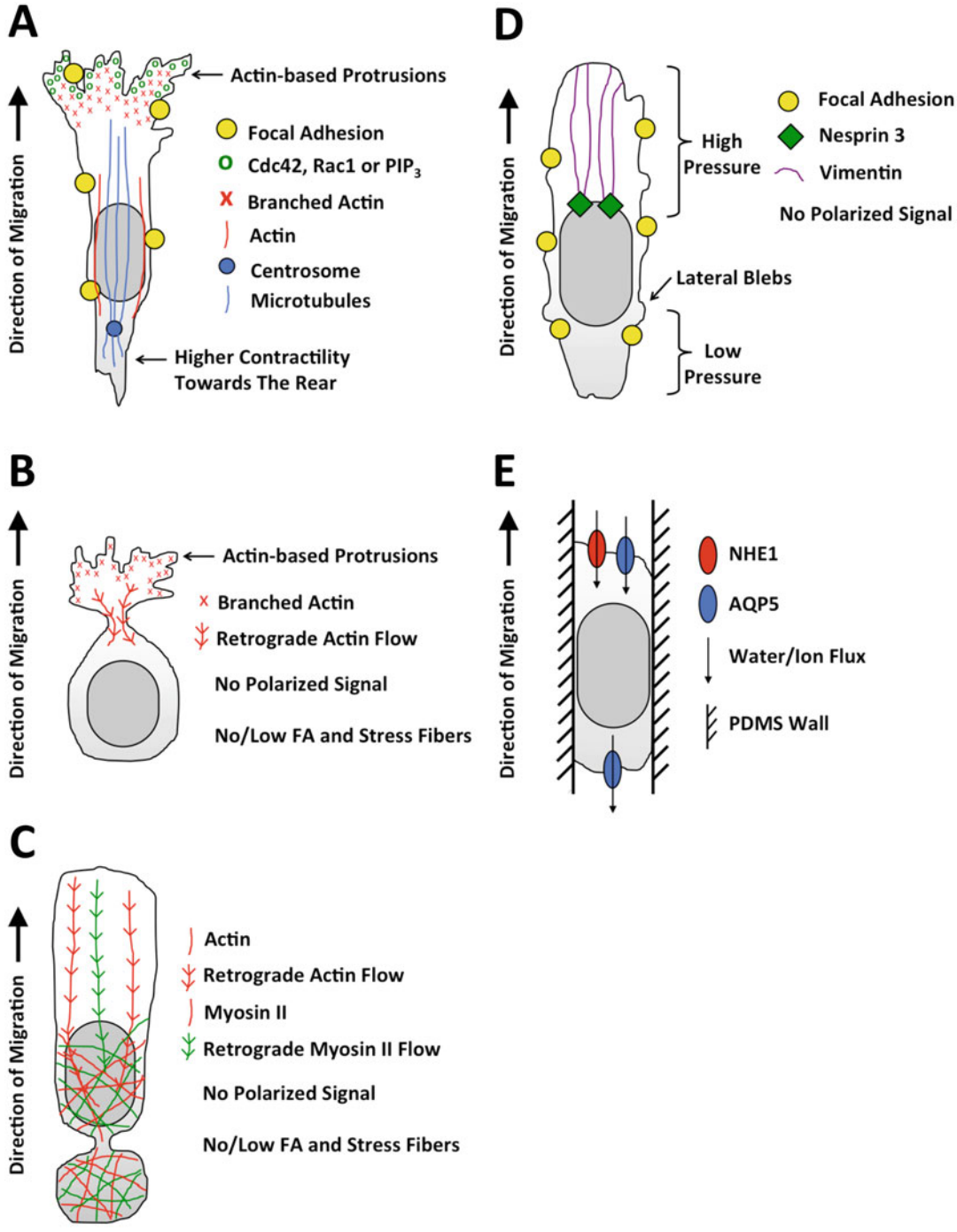

Fig. 8.1 Schematics of the various confined cell migration modes. (**a**) Pseudopodial cell migrates with an elongated morphology and actin-based protrusions initiated by polarized Cdc42, Rac1, and PIP_3 localized at the cell's leading edge. Focal adhesions are distributed in a diffused pattern along the elongated cell. Actomyosin contractility is concentrated toward the trailing edge to aid in rear retraction. Actin is organized around the cell cortex and at the cell's leading and trailing edges. Centrosome is located behind the nucleus while microtubules are concentrated anterior to the nucleus as parallel bundles. (**b**) A1 blebbing cell has a round cell body with small actin-based protrusions at the leading edge. Fast retrograde actin flow is localized at the protruding leading edge. A2 blebbing cells, focal adhesions, and actin stress fibers. (**c**) A2 blebbing

During confined 1D or 3D mesenchymal migration, cells employ similar polarized signals of Rho GTPases and PIP_3 to form actin-based pseudopodia protrusions at the leading edge like they do on 2D surfaces, form adhesions with the substrate via integrins, and activate actomyosin contractility to subsequently detach cell rear [42, 49]. Though both 2D and 1D or 3D confined mesenchymal migration appear to be rather analogous, there still exist some fundamental differences between the two in terms of cytoskeletal and adhesion dynamics, dependence on actomyosin contractility and force generation. First, 1D or 3D confinement induces drastic cytoskeletal remodeling accompanied by fewer F-actin stress fibers [45, 50]. In confined cells, actin is primarily localized at the cortex and/or concentrated on the leading edge as actin-rich wedge-like slab [43, 51]. Second, the role of adhesion is reduced (but not necessarily eliminated) in confinement compared to 2D migration, with FAs demonstrating a smaller size and more diffuse cytoplasmic distribution rather than distinct localization around the cell periphery underneath the pseudopod on 2D surfaces [45, 50]. Third, while actomyosin contractility is indispensable for 2D migration, under specific conditions, for example, within rigid PDMS-based confined microchannels, cells are able to migrate efficiently even if actomyosin contractility is disrupted [45, 52]. However, the role of actomyosin contractility in confinement can also be cell-line dependent and in certain instances is key for efficient confined migration [46, 53]. Fourth, the traction forces exerted by cells in confinement (either in microchannels or on 1D printed lines) are significantly lower than those on 2D flat surfaces and are typically directed toward microchannel walls instead of to the center of the cell on 2D surfaces [50, 54]. In fact, phosphorylated myosin light chain (pMLC)-dependent traction generation is not required for migration in microtracks [43]. All these salient differences suggest that cells are able to modulate intracellular signaling, thereby optimizing their mobility in response to varying degrees of confinement. In order to fully understand the mechanisms of confined mesenchymal migration, we have to dissect each of these factors individually and methodically (Tables 8.1 and 8.2).

8.3.2 Molecular Determinants of Confined Mesenchymal Migration

Pericellular proteolysis is essential for maintaining the mesenchymal phenotypes of tumor cells in 3D matrices. In 3D fibrillar collagen gels, HT1080 and MDA-MD-231 cells display a mesenchymal morphology during migration through proteolytically generated tubelike tracks with β1 integrin co-clustering with MT1-MMP at interaction sites with collagen fibers; MT1-MMP is a membrane-associated surface protease whose activity is needed for focalized ECM degradation [9]. This mode of mesenchymal 3D migration can also be observed in vivo for HT1080 cells migrating in the mouse dermis as imaged with intravital multiphoton microscopy. Interestingly, inhibition of collagenolysis with MMP inhibitors converts mesenchymally migrating cells into a more spherical amoeboidal phenotype (discussed in Sect. 8.4) that is phenotypically and mechanistically distinct from pseudopodial

Fig. 8.1 (continued) cell has an elongated ellipsoidal morphology with a rear uropod and a rounded leading edge. Actin and myosin II are concentrated around the cell cortex and the uropods and demonstrate fast and global retrograde flow toward the cell rear. A2 blebbing cells, focal adhesions, and actin stress fibers. (**d**) Lobopodial cell possess blunt cylindrical protrusions and small lateral blebs around the cell body. Focal adhesions are required for lobopodial migration. Lobopodial cell is separated into a high-pressure compartment anterior to the nucleus and a low-pressure compartment posterior to the nucleus. The nucleus is connected to the anterior cell membrane via a vimentin and nesprin3. Polarized signals are absent in lobopodial cell. High cellular contractility and a linearly elastic matrix are necessary for cell to migrate using lobopodia. (**e**) Osmotic engine is activated when cells are being confined into a pill shape within rigid channels. Ion and water channels such as NHE1 and AQP5 are polarized to the cell leading edge to facilitate water and ion flux that serve to propel the cells forward. Focal adhesion, contractility, and actin polymerization are dispensable in cells migrating using the osmotic engine

Table 8.1 Comparison between unconfined 2D and confined 1D or 3D mesenchymal migration

	2D	1D or 3D
Occurrence		
In vitro	· Flat 2D substrates · Wide PDMS channels	· Microcontact printed 1D line · 3D hydrogel network · Hydrogel patterned microtracks · Narrow PDMS channels
In vivo	· Wound healing · Neutrophil trafficking on inflamed endothelium	· Along oriented ECM fibers · Within dense fibrillar tissues · Preformed ECM-free tunnels
Structural and phenotypic properties		
Actin	· Organized and elongated stress fibers	· Suppression of stress fibers · Actin organized in cortex or concentrated on the leading/trailing edge
Microtubules	· Centrosome in front of nucleus · Nearly isotropic microtubule polymerization from MTOC	· Centrosome behind the nucleus · Stabilized microtubules as parallel bundles in front of nucleus · Alpha tubulin and microtubule growth toward leading edge
Focal adhesion	· Large distinct mature focal adhesions around cell periphery	· Smaller in size · Diffuse and homogenous distribution of focal adhesion proteins
Nuclear shape	· Rounded	· Elongated
Traction force	· Larger · Directed to the cell center	· Significantly lower · Directed toward channel wall
Roles of different molecular determinants		
MMPs	· Not critical	· Essential in 3D ECM to generate migration tracks · Not required if tracks are already preformed
Matrix adhesion	· Migration stops when adhesion is blocked	· Migration persists even when adhesion is blocked, especially in stiff PDMS-based channels
Actomyosin contractility	· Indispensable	· Effect is cell-type dependent · Can be dispensable for cells in rigid microchannels
Microtubule	· Required for signal polarization	· Needed to maintain persistence and directionality

migration without negatively affecting migration speed. In the presence of preexisting microtracks, either generated in vitro with laser ablation or micromolding in 3D hydrogel matrices or in vivo by surrounding or leader cancer-associated stromal cells, cells can still assume a mesenchymal migratory phenotype even when MMP functions are compromised or absent [43, 44]. ECM-free microtracks enable rapid and persistent migration of noninvasive MCF10a breast epithelial cells and MMP-depleted MDA-MD-231, which are unable to invade otherwise in 3D collagen matrices [44]. Microtracks provide a clear unimpeded path of low resistance for migrating cells, reducing the requirement for cell-matrix mechanocoupling, traction force generation, and matrix remodeling required

Table 8.2 Summary of confined single cell migration mechanisms

	Matrix adhesion	Actomyosin contractility	MMPs	Physical microenvironment	Polarization	Other structural requirements	Note
Pseudopodial	· Required	· Cell-type dependent · Can occur with both low and high contractilities	· Needed in 3D ECM · Inhibition leads to amoeboidal phenotype · Dispensable if there are preexisting tracks	· Adhesive matrix · Nonlinearly elastic matrix · ECM-free tracks/channels	· Polarized PIP_3, Rac1, and Cdc42 at the cell leading edge	· Microtubule needed for directional persistence and uniaxial morphology	
A1 blebbing	· Low adhesion	· Low contractility · Activation of contractility converts A1 to A2	· Not relevant	· Vertical confinement	· Unknown	· Localized actin retrograde flow at small leading edge protrusions	· Faster than pseudopodial
A2 blebbing	· Low adhesion	· High contractility · Inhibition of contractility converts A2 to A1	· Not relevant	· Vertical confinement	· Colocalization of contractile machinery and ERM protein family · Stochastic contractility driven by cortical network instabilities	· Global actin and myosin II retrograde flow toward cell rear	· Faster than pseudopodial and A1 blebbing
Lopobodial	· Required	· High contractility needed · Inhibition of contractility converts lobopodial to pseudopodial	· MMP inhibition activates lopobodial mode in fibrosarcoma cells	· Adhesive matrix · 3D linearly elastic matrix (dermal tissue explants or cell-derived matrix)	· Non-polarized PIP_3, Rac1, Cdc42, cortactin, VASP, and F-actin	· Myosin II-vimentin-nesprin3 nucleus-membrane connection · Independent of Rac1, Cdc42, and mDia1	· Observed in only fibroblasts and fibrosarcomas · High intracellular pressure separated by nucleus
Osmotic engine	· Dispensable	· Dispensable	· Not relevant	· Full confinement by rigid PDMS	· Polarized aquaporins, ion channels, and pumps at the cell leading edge	· Overexpression of aquaporins, ion channels, and pumps	· Actin polymerization is dispensable for movement once polarization is achieved but needed for repolarization post osmotic shock

for efficient migration, thereby lowering the mechanistic threshold for local tissue invasion.

Matrix adhesion is needed for pseudopodial migration on 2D, but its role in confinement is markedly diminished. On 2D surfaces or in wide microchannels emulating a 2D microenvironment, FAs (as visualized with phospho-paxillin and phospho-FAK) are localized alongside at the periphery of pseudopod protrusions as distinct complexes. In contrast, FAs are significantly reduced in size in cells migrating inside narrow microchannels (<20 μm) and display a uniform distribution along the cell's migratory axis [45]. On 1D lines, similar long linear localization of adhesion components such as α5 integrin, β1 integrin, FAK, vinculin, and paxillin are also observed spanning the entire length of the cell axis [41]. As a result of the diminished role of substrate adhesion in confinement, blocking β1 integrin has little or no appreciable effect on migration speed in narrow channels or cell-scale collagen microtracks despite completely abrogating planar 2D migration or reducing speed in 3D collagen matrices [43, 45]. 1D migration speed is also resistant to varying ECM ligand densities, as migration speed exhibits a saturating relationship as ligand density increases rather than a classical biphasic phenomenon observed on 2D surfaces [41]. It is however worth noting that while β1 integrin is not required for the maintenance of migration speed in microtracks, they are needed to promote the elongated morphology of the migrating cells. Instead of a stable elongated morphology with pseudopodial protrusion, β1-depleted cells undergo rapid dynamic oscillation between elongated (mesenchymal) and spherical (amoeboidal) morphologies [43].

The effect of actomyosin modulation on confined pseudopodial migration is more variable and dependent on the cell type and matrix dimensions. Inhibiting myosin II activity with blebbistatin impairs migration of fibroblasts and human epithelial keratinocytes on 1D lines and in 3D ECM [41]. In 3D collagen gels, ROCK inhibition with Y27632 significantly diminishes human foreskin fibroblast lamellipodial-based migration, as for other epithelial cells [55]. However, blebbistatin treatment on human foreskin fibroblasts migrating on top of 2D cell-derived matrix is unaffected, consistent with effect of blebbistatin on 2D fibroblast migration [56]. On the other hand, confined migration of various cancer cells, such as MDA-MD-231 breast cancer cells and S180 murine sarcoma cells, is resistant to inhibition of actomyosin contractility. While inhibiting the Rho/ROCK/myosin II signaling cascade with CT04/Y27632/blebbistatin-ML7 suppresses migration on 2D unconfined substrates, these pharmacological interventions have no appreciable effect on confined cell migration through 3 μm narrow microchannels [45, 47]. Along these lines, modulation of actomyosin contractility via the use of blebbistatin (inactivation) or calyculin A (activation) does not alter the traction forces exerted by NIH-3T3 or HOS human osteosarcoma migrating in narrow channels [54].

Microtubules play a key role in regulating the velocity, directionality, and persistence of cell migration in confinement. On 1D microprinted lines, the centrosome (pericentrin) is located behind of nucleus (vs. in front of nucleus on 2D) [41, 57] while stabilized microtubules (i.e., detyrosinated glu-tubulin) are localized as polarized parallel bundle arrays anterior to nucleus, extending into lamellipodia [41, 43]. Confinement induces alpha-tubulin localization and microtubule growth toward the leading edge, as opposed to the rather isotropic microtubule polymerization from microtubule-organizing center on 2D surfaces. Interfering with microtubule dynamics with either Taxol (which prevents depolymerization) or colchicine (which promotes disassembly) significantly decreases cell velocity and directionality in 3 μm narrow channels, indicating a critical role of microtubule in establishing migratory persistence in confinement [45]. Similarly, inhibiting microtubule polymerization with nocodazole or microtubule depolymerization with Taxol causes rounded cell morphology with uncontrolled protrusions in all directions, decreases motile fraction, and reduces migration speed along 1D lines, microtracks, and in 3D ECM, suggesting that microtubules is important in maintaining uniaxial

morphology and alignment in 1D and 3D migration [41, 43].

8.4 Bleb-Based Amoeboidal Migration

8.4.1 General Morphological and Molecular Features of Amoeboidal Migration

When actomyosin contractility is elevated and/or cellular adhesions are diminished, cells typically transform from an elongated, spindle-like mesenchymal morphology that is dependent on adhesion and actin-based pseudopodial protrusion into a rounded amoeboidal morphology resembling that of *Dictyostelium* amoeba and migrating leukocytes. This process is termed as mesenchymal-to-amoeboid transition (MAT) and has been observed both in vitro and in vivo in mouse xenograft models [8, 58]. Amoeboidal migration is associated with rounded cell morphology with spherical membrane protrusions that are devoid of filamentous actin known as blebs, limited and diffuse distribution of cellular adhesion (e.g., β1, paxillin), and higher actomyosin contractility [8]. Moreover, amoeboid cells also exert lower traction forces and exhibit higher cortical tension than mesenchymally migrating cells. Traction forces exerted by Walker 256 carcinosarcoma when they are undergoing non-adherent blebbing motion are several orders of magnitude lower than those exerted during integrin-based FA-dependent mesenchymal motility [59]. Furthermore, the forces are directed outward from the cell body to expand rather than contract the substrate in order to generate sufficient friction to drive migration. Lateral expansion of cells are also able to generate enough traction by extending interdigitating with the surrounding discontinuous confined matrices to provide traction in the absence of adhesion [58].

Cell mechanics represents one of the key determinants of MAT. Whether or not a cell prefers to form blebs or lamellipodia depends on a delicate balance between actin protrusivity, as controlled primarily Rac1 and Arp2/3 complex, and cellular contractility, as dictated by the RhoA/ROCK/myosin signaling axis. The transition between these two phenotypes can be achieved even locally at the cell leading edge without any global change in cell shapes, polarity, and adhesion [59]. Activating Rac1, which recruits and activates downstream Arp2/3 to facilitate nucleation of actin filaments, switches blebs to lamellipodia, increases cell cross-sectional area, and decreases cortical tension. This lamellipodia-promoting role of Rac1 is intimately linked with Arp2/3 activity, whose inhibition via the pharmacological agent CK666 or via siRNA decreases lamellipodial formation in Walker 256 carcinosarcoma [59]. Conversely, Rho/ROCK/myosin signaling promotes rounded bleb-associated mode of motility. Inhibiting actomyosin contractility with RhoA inhibitor C3 transferase, ROCK inhibitor Y27632, or myosin II inhibitor blebbistatin decreases bleb formation while promoting lamellipodia protrusion in Walker 256 carcinosarcoma [59] as well as A375m2 melanoma and LS174T colon carcinoma cells [8]. On the other hand, increasing cell contractility via the use of constitutively active ROCK or overexpression of Rho enhances cell blebbing.

Cells prefer to switch to an amoeboidal mode of migration when cell-ECM adhesion is diminished or eliminated. This can be achieved by either downregulating integrins or decreasing substrate adhesiveness [60, 61]. Typically, amoeboidal migration occurs without FAs and can proceed efficiently even if components of the adhesion machinery such as integrin $\alpha_1\beta_2$ or talin are knocked down or adhesion is completely prevented in nonadhesive PDMS microchannels or in the presence of EDTA which chelates divalent ions needed to establish integrin binding [60, 61]. This is in stark contrast to elongated mesenchymal migration where motility ceases when adhesion is eliminated. The effect of migration phenotypes exerted by changing substrate adhesiveness is also rapid and reversible, as cells (suspension subline of Walker 256 carcinosarcoma) plated on micropatterned surface with al-

ternating adhesive and nonadhesive areas form lamellipodia immediately upon contacting adhesive region which then quickly disappear and resumed blebbing when they move on to nonadhesive region [59]. In HT1080, MAT is associated with decreased surface coverage of $\alpha_2\beta_1$ integrin heterodimers, diminished integrin-mediated adhesion, and downstream signaling via p-FAK [61]. Consequently, inhibition of calpain2 and Src kinase, which participate in FA turnover, suppressed mesenchymal invasion drastically but exerted little or no effect on amoeboid migration where the role of adhesion is already diminished. Interestingly, Rho/ROCK inhibition is able to restore integrin function and calpain2 sensitivity and reverses MAT, indicating that Rho/ROCK signaling also contributes to integrin modulation in addition to enhancing actomyosin contractility to promote amoeboid migration.

Besides altering cellular contractility and adhesion, inhibiting MMP can also induce MAT. HT1080 and MDA-MB-231 transform from an elongated mesenchymal into a spherical amoeboidal morphology that still move at the same speed upon MMP inhibition in vitro in 3D collagen gels as well as in vivo [9]. Similar phenotypic conversion is also observed for BE and WM266.4 melanoma cells during invasion through 3D matrigels [8]. This protease-independent amoeboidal migration occurs without any matrix remodeling and generation of any migration tracks, suggesting that the cells have to now squeeze through the tight collagen fiber network in order to maintain efficient migration. Indeed, during MAT, the cells lose their β1 integrin clusters and surface localization of MT1-MMP and develop diffuse cortical actin rims and narrow region of constriction rings to aid in deforming the cells through narrow pores.

It is noteworthy that MMP inhibition is certainly not a prerequisite for amoeboidal migration. To the contrary, a paradoxical elevated secretion of MMPs, specifically MMP9, was observed in melanoma cells that are already prone to migrate amoeboidally as compared to their elongated mesenchymal counterparts [62]. MMP9 promotes amoeboidal migration through activating actomyosin contractility by binding to CD44 receptor in a non-catalytic, paracrine, and autocrine manner. In turn, the increase in actomyosin contractility activates ROCK/JAK/STAT3 cascade, forming a positive feedback loop that upregulates MMP9 gene expression. Indeed, MMP9 expression was shown to increase over the course of melanoma progression and is highly enriched in invasive lesion front, which incidentally also display more rounded amoeboidal morphology positive for p-STAT3.

The roles of MMP on MAT are hence variable and cell-line dependent. Nevertheless, the ability for the tumor cells to sustain efficient 3D motility via a protease-independent mechanism and the non-catalytic role of MMPs in promoting amoeboidal migration could explain the many failures of MMP inhibitors in human clinical trials despite demonstrating promising potentials in halting migration in vitro and in vivo [63, 64].

8.4.2 Bleb-Based Migration in Physical Confinement

Fascinatingly, physical confinement triggers MAT. Using a sandwich system consisting of two surfaces of tunable surface adhesion characteristics, normal human dermal fibroblasts have been shown to retract and adopt a more compact phenotype with fewer lamellipodia but more elongated pseudopodia when confined to a low ceiling of 3–5 μm [65]. Under high confinement (i.e., 3 μm) and low adhesion, most normal human dermal fibroblasts become immobile with very rounded morphology characterized by continuous uncoordinated blebbing activity. A portion of these confined cells, however, display a round cell body with small leading edge local protrusion and are able to move with an amoeboidal mode of migration, termed as A1 blebbing mode. This subpopulation of cells exhibiting the A1 blebbing mode migrates faster than the remaining spread cells that display a partial mesenchymal morphology when being vertically confined [65]. Similarly, a suspension subline of Walker 256 carcinosarcoma that typically

form non-adherent blebs migrate limitedly on 2D surface, but efficiently when being confined vertically between glass and agarose and within 3D gels with directional persistence [59, 60]. Confinement in this case is essential for cell motility as it enables force transmission in the absence or near absence of adhesions to substrate.

Indeed, computational modeling suggests that cell matrix adhesion is dispensable for cell migration in discontinuous confined environments where blebbing predominates [58]. On an unconfined 2D surface, cells migrate with an elongated morphology with actin-driven protrusion, and highest velocity is predicted at intermediate cell-ECM adhesion. This biphasic migration speed behavior to substrate adhesiveness has been verified experimentally with multiple cell lines on 2D platforms [46]. Conversely, blebbing mode of migration mechanism dominates and maximum cell velocity scale inversely with adhesion on discontinuous confined environment, such as those represented by dense fiber mesh network. The modeling prediction is verified in vitro where β_1 integrin or talin depletion reduces migration on 2D surfaces but increases amoeboidal migration speed in confined environments.

Under high confinement and low adhesion, numerous cell types, including normal or transformed cells of either epithelial or mesenchymal origins, are able to adopt an additional mode of stable bleb-based migration, termed as A2 blebbing, characterized by an elongated ellipsoidal morphology with a large rear uropod and a smooth rounded leading edge, reminiscent of migrating neutrophils [65]. Cells displaying the A2 blebbing morphology typically migrate faster than their A1 blebbing counterparts. The proportions of cells that display the A2 blebbing morphology for each cell type though vary considerably across the group depending on their basal cellular contractility. In general, cell lines that display higher intrinsic cortical contractility also have a higher proportion of cells that migrates via the A2 mode. Similar fast and directionally persistently A2 mode of bleb-based migration is also evident in zebrafish embryonic progenitor cells both in vitro under vertical confinement between two planar glass slides though these cells are immobile on 2D surfaces and in vivo during early development, for instance, at sites of local wounding site where there exists higher actomyosin contractility [66].

Mechanistically, A1 and A2 blebbing differ in their requirement for actomyosin contractility. Increasing contractility via calyculin A treatment or knocking down MYPT1, the PP1 partner targeting myosin II, results in an increased frequency of A2 blebbing cells. The converse is accordingly true upon cell treatment with the ROCK inhibitor Y27632 or myosin II inhibitor blebbistatin where more cells exhibit the A1 rather than A2 mode of migration [65]. Additionally, treating zebrafish embryonic progenitor cells with serum or lysophosphatidic acid, a serum phospholipid capable of activating cortical contractility via the Rho/ROCK pathway, also transforms cells reversibly into the A2 stable bleb morphology, thereby providing further evidence that A2 blebbing depends on high myosin-based contractility [66].

The organization and role of actin are also different between the A1 and A2 blebbing migration modes although they both lack FAs and organized actin stress fibers. In A1 cells, fast retrograde flow of actin is localized at the small protruding leading edge. In A2 cells, however, actin and myosin II are absent from the cell front but instead concentrated around the cell cortex where the uropods are. Both actin and myosin II exhibit fast and global cortical retrograde flow around the central region of the A2 cells, with little to no flow toward the rear, suggesting that the uropod is a dragged passive body [65]. Similar rearward gradient of contractility, cortical actomyosin enrichment, and retrograde flow are also evident in non-adherent blebbing Walker 256 carcinosarcoma [60]. Relaxing cortical contractility at the rear of the cells but not the front by cortex ablation decreases migration velocity. Via computational modeling, it was revealed that rearward contractility gradient is able to drive adhesion-independent amoeboidal migration via two complementary mechanisms. First, frictional forces from counteracting retrograde cortical flow generate propulsive force.

Second, when the friction becomes sufficiently large enough to hold cell body in place, rearward contractility of myosin results in leading edge expansion, leading to net cell movement. Interestingly, the model predicts that cell migration velocity correlates not with amplitude of stress exerted by the cells but rather velocity of the actomyosin flow, highlighting the importance of cortical actomyosin flow in facilitating amoeboidal A2 migration. In summary, cells could fall into two different contractility regimes when they are undergoing MAT following vertical confinement. Under a high contractility regime, global cortical actin retrograde flow results in myosin-dependent mechanical instability of cortex, leading to formation of A2 stable blebs. When contractility is inhibited, the cortex becomes more stable, allowing for more protrusive activity, ultimately leading to an A1 blebbing phenotype.

8.4.3 Establishing Polarity in Blebbing Cells

Amoeboid migration is responsive to chemotractant cues and is not a form of random motility [8]. In mesenchymal cells, specific spatial localization of Rac1, Cdc42, and PIP_3 is needed to establish polarization and direction of migration, but such differential spatial enrichment is absent in amoeboid cells [42]. So then how are amoeboidal cells able to achieve similar polarization? Localization of ezrin/moesin/radixin (ERM) protein family, which are linkers between the plasma membrane and actin cytoskeleton, appears to be involved in this process. Asymmetry contractility is positively related with asymmetry in cortex-membrane linkage [58]. ROCK is able to phosphorylate ERM, and ezrin localization is also dependent on Rho activity. In fact, colocalization of contractile machinery (pMLC) and pERM promotes blebbing and favors migration in confinement [67]. This is achieved by the STRIPAK components, MST3/4 kinases that locally coordinate phosphorylation of ERM and inhibit dephosphorylation of MLC, leading to increased phosphorylation and cortical colocalization of MLC and ERM, resulting in enhanced cortex-membrane linkage and more frequent membrane blebbing. Indeed, increasing actomyosin-membrane linkage with MST3/4 overexpression is associated with increased in vivo metastasis from mammary fat pad to lymph node. Colocalization of actomyosin contractile function and ERM proteins promotes more efficient pulling of contractile cytoskeleton on the plasma membrane, exerting more force on the plasma membrane instead of being coupled to integrins via FAs, thereby producing more blebbing.

A recent study on zebrafish embryonic progenitor cells, however, suggests that polarization in A2 migrating cells is initiated by stochastic contractility that is driven by cortical network instabilities and subsequently maintained by a positive cortical feedback loop [66]. Specifically, addition of lysophosphatidic acid causes rapid redistribution of myosin II to the cell cortex, upregulating cortical contractility and increasing bleb expansion. Interestingly, similar increases in myosin II accumulation, bleb formation, and cortical contractility are also observed in serum-free confined condition, indicating that confinement in itself is able to trigger an increase in cell contractility independent of external biochemical cues, possibly via a yet to be discovered mechanism involving cell and/or nuclear deformation. Nevertheless, these local fluctuations in cortical contractility at the cell periphery disrupt cell symmetry, leading to initial polarization. Polarization is then further enhanced and stabilized by a positive feedback between continuous cortical actin and myosin flow toward cell rear and formation of cortical contractility gradient that reinforces the flow, resulting in the formation and maintenance of stable blebs. Unlike conventional 2D migrating cells where polarization hinges on PIP_3 which is impaired with PI3K inhibition, polarization in A2 blebbing mode is resistant to PI3K inhibition. Instead it is dependent on proper actin turnover as inhibition of actin turnover by latrunculin A or jasplakinolide resulted in disappearance of stable blebs [66].

8.4.4 Mechanotransduction Pathway to Optimize Contractility in Confinement

Cells are able to identify, integrate, and respond to external environmental cues and physical stimuli in a process known as mechanotransduction. However, the exact mechanotransduction mechanisms by which cells sense physical confinement and translate this signal into elevated cortical contractility are still underexplored. Prior work suggested that the existence of an intricate cross talk between Rac1 and RhoA/myosin II signaling [46] serves to optimize actomyosin contractility in order to facilitate efficient migration in confined microchannels. Specifically, Rac1 activity is enhanced in cells migrating on 2D surfaces or inside wide microchannels ($\geq$ 20 μm) to facilitate the formation of lamellipodia protrusions. Conversely, RhoA/myosin II signaling is amplified when cells are migrating inside narrow microchannels ($\leq$ 10 μm), resulting in higher actomyosin contractility and a migration mode with amoeboidal characteristics [46]. These distinct signaling strategies employed by cells in response to physical confinement are modulated by mechanosensors, which can be broadly classified into three major classes: stretch-activated ion channels [68], cytoskeletal and nuclear elements, [69] and integrins [70].

We recently discovered that the membrane-bound stretch-activated cation channel PIEZO1 is responsible for the intracellular calcium increase observed as cells transition from an unconfined 2D environment into confined microchannels [53]. In particular, elevated membrane tension induced by physical confinement activates PIEZO1, leading to increased intracellular calcium levels, which in turn suppresses protein kinase A (PKA) via a phosphodiesterase type 1 (PDE-1)-dependent pathway. Interestingly, confinement-induced inhibition of PKA activity is only negated when both PIEZO1 and myosin II are blocked (but not when either one is individually inhibited), implying that myosin II can also sense physical confinement and suppress PKA directly and independently of PIEZO1. Indeed, external physical forces have been reported to induce assembly of myosin II bipolar filaments and actomyosin bundles [71, 72]. Moreover, myosin II has also been implicated in sensing surface topographical cues in fibroblasts [73] and tumor cells [47]. In relation to mechanosensing of physical confinement, it has been hypothesized that myosin II decreases PKA activity indirectly via downregulation of Rac1 activity, due to the negative cross talk between Rac and Rho/myosin that subsequently reduces recruitment of A-kinase anchoring proteins (AKAPs) to the cell leading edge that is capable of activating PKA. Together, these two independent yet interconnected mechanosensing mechanisms serve to suppress PKA and amplify actomyosin contractility in confinement. Of note, components of adhesion complexes, such as α4 and α5 integrins, do not appear to be essential for cell to sense physical confinement. Rather, they primarily serve to amplify the differential response of contractility increase induced by confinement.

8.5 Lobopodial Migration in Linearly Elastic Matrices

Cells are able to sense the mechanical and rheological properties of ECM and adopt distinct migration mechanisms in different 3D microenvironments. While most migration studies using 3D matrices, such as polyacrylamide or collagen gels, have focused on the ability of the cells to respond to substrate stiffness and pore sizes, limited attention has been devoted to the elastic behavior of the matrix material like strain stiffening [49]. Strain stiffening refers to the ability of a material to resist deformation and handle applied stress. In general, materials can be classified broadly as nonlinearly elastic where they undergo strain stiffening (i.e., the stiffness of the material increases with increasing force application) and linearly elastic where strain stiffening is not observed (i.e., the stiffness of the material is independent of the magnitude of force applied to it).

Fibroblasts are able to recognize the differences in the elastic behaviors of 3D matrices and

migrate via two distinct mechanisms [49]. In 3D collagen gels, which are nonlinearly elastic and softer, fibroblasts migrate via the classical flat lamellipodial protrusions, similarly to how they would migrate on unconfined planar 2D surfaces. In highly cross-linked, stiffer, and linearly elastic materials such as dermal tissue explant or cell-derived matrix (CDM), however, fibroblasts switch to a diametrically opposed morphology, where blunt cylindrical protrusions termed as the lobopodia and small lateral blebs are observed [42]. Notably, the lobopodial mode of migration only occurs when the cells are being confined within the 3D mesh-like structure of CDM but not on top of 2D CDM, indicating that lobopodia-based migration is a unique mechanism that cells can use inside linearly elastic matrices. Unlike lamellipodia where PIP_3, Rac1, and Cdc42 are polarized to the leading edge of the cells, lobopodia are devoid of these polarized signals as well as of other lamellipodial markers such as cortactin, VASP, and F-actin. Instead, the lobopodial protrusions are mainly driven by high intracellular pressure that is highly dependent on RhoA/ROCK/myosin contractility. Fibroblasts continue to migrate using lobopodia after depletion of Rac1, Cdc42, or formin mDia1 with slight variation in velocity in certain instances. In contrast, inhibiting contractility by knocking down RhoA or inhibiting ROCK causes the fibroblasts to switch from a lobopodial to lamellipodial mode without affecting migration velocity. Interestingly, while myosin inhibition also results in the same lobopodial-to-lamellipodial transition, cell migration was significantly impaired, presumably due to inefficient nuclear migration.

Indeed, further studies revealed that the nucleus play a pivotal role in pressurizing the anterior cytoplasm at the cell leading edge by acting as a piston to generate lobopodia [74]. There exists a high intracellular hydrostatic pressure differential between the front and back, as separated by the nucleus, of a lobopodially migrating cells in 3D linearly elastic matrices. The nucleus is being connected to the anterior cell membrane via a myosin II-vimentin-nesprin3 complex and is being pulled forward coordinately as cells traverse through the confined pores of linearly elastic matrices [75]. Knocking down nesprin3 reverses the lobopodial phenotype back to lamellipodia, equalizes intracellular pressure and reduces the velocity of migrating fibroblasts independent of affecting Rho-mediated contractility, indicating the importance structural role of nesprin3 as a nucleoskeleton-cytoskeleton linker in lobopodial-based migration. Unlike the critical role of microtubules in ensuring directionality and polarization of migrating cells displaying a lamellipodial morphology, microtubules do not seem to be involved in promoting the coordinated nucleus movement observed in lobopodial cells. The effect of microtubule inhibition on the velocity and persistence of lobopodial cells, however, remains to be further investigated. Despite the differences in morphology, polarized signals, and motility mechanism, lamellipodia- and lobopodia-based migrations do share a similar requirement for adhesions. Both types of protrusion possess paxillin- and vinculin-based FAs. Blocking integrins also significantly impair lobopodial migration speed and directionality in fibroblasts.

The discovery of this non-polarized, contractility-dependent, and intracellular pressure-driven lobopodial-based migration in normal fibroblasts naturally begs the question: can other cell types such as cancer cells also use a lobopodia-based mode of migration in 3D linearly elastic matrices? While initial studies suggested that HT1080 fibrosarcoma cells do not undergo lobopodial migration but instead migrate via either an amoeboidal (i.e., large blebs with no adhesions) or mesenchymal (lamellipodia with actin stress fibers and adhesions) mode, recent work shows that fibrosarcoma cells (i.e., HT1080 and SW684) are able to activate lobopodia upon protease inhibition in 3D CDM [7]. In general, MMPs are needed for matrix degradation and generation of migration tracks through which cells move using primarily a pseudopodial mode of migration. Upon inhibition of protease activity, cells switch to a bleb-based amoeboidal migration mechanism [8, 9]. It is worth noting that these observations were made using nonlinearly elastic materials such as collagen

gels. In linearly elastic 3D CDM, however, MMP inhibition triggers the activation of nuclear piston mechanism in fibrosarcoma cells without switching to an amoeboid phenotype, possibly as a result of difficulty of efficient nuclear and cell translocation through low porosity confined 3D microenvironments. Similar to fibroblasts, lobopodial migration in tumor cells still depends on integrin adhesion, actomyosin contractility, and nesprin3-vimentin connection.

While it is intriguing that fibroblasts and fibrosarcomas are able to migrate with a lobopodial mode that is completely distinct from the conventional lamellipodial one, it is still unknown how the cells are able to sense the differences of the elastic behaviors of the surrounding 3D microenvironment and trigger the switch of migration mode. Furthermore, it is still unclear how MMP inhibition triggers the switch from lamellipodial to lobopodial migration in fibrosarcomas. More studies are also warranted to determine if the lobopodial migration mode is also applicable in other cancer cell types that are not fibroblast-like and also to elucidate the in vivo functional significance of lobopodial migration.

8.6 The Osmotic Engine Model

Up to this point, all of the confined migration mechanisms that we have discussed so far require intact actin and myosin contractility functions. For instance, actin polymerization is critical for the formation of lamellipodial protrusions; Rho/ROCK/myosin contractility is needed for nucleus to pressurize lobopodial cells; actomyosin contractility and retrograde actin flows are essential to generate blebs and maintain amoeboidal migration. Actin polymerization and myosin contractility are indispensable for cell migration on 2D and 3D microenvironments.

It was fascinating to observe that several tumor cell lines, such as S180 sarcoma and MDA-MB-231 breast carcinoma, are able to migrate through stiff, narrow ($W = 3$ μm and $H = 10$ μm) PDMS-based microchannels even when actin polymerization is completely disrupted by high doses of latrunculin A [45]. Also, efficient migration through narrow channels occurs upon inhibition of β1 integrin function or actomyosin contractility [45]. We proposed the "osmotic engine model" of confined cell migration, which depends on the fluxes of water and ions in and out of the cells through the cell membrane [52]. In this model, cells expand by taking up water at their leading edge and shrink by expelling water at the trailing edge, thereby leading to cell locomotion. Mathematical modeling predicts that the velocity of cell motility is independent of parameters that are influenced by actin polymerization or actomyosin contractility but instead depends on the number and localization of water channels, ion channels, and pumps along the longitudinal cell axis [52]. Indeed, the Na^+/H^+ exchanger-1, NHE-1, is polarized at the cell leading edge during confined migration. Knocking down NHE-1 or aquaporin-5 markedly suppress confined migration [52].

The osmotic engine model operates based on the principles of cell volume regulation as a result of differential osmotic and hydrostatic pressure across the cell membrane of leading and trailing edges. Therefore, any perturbation to the osmolarity of the fluid at either the cell leading or trailing edge has an immediate and pronounced effect on the flow of ions or water across the cell membrane, thereby affecting migration directionality and velocity. Indeed, application of a hypotonic osmotic shock to the cell leading edge or a hypertonic osmotic shock to the trailing edge reverses the direction of cell migration in narrow channels. It is worth noting that though actin is dispensable in maintaining directionally persistent confined migration in these cells once the initial polarization of aquaporins and ion transporters has been established after channel entry, actin is pivotal for the cells to respond to osmotic shock and reverse direction by facilitating NHE1 repolarization [52]. This is in contrast to the role of microtubule in confined migration, where microtubule disruption with nocodazole drastically impairs the persistence and velocity of cells pre-shocked, but only has minor effect post osmotic shock without affecting NHE1 repolarization.

The osmotic engine model relies on the polarization of key molecules, such as

aquaporins, ion channels, and pumps, aided by the actin cytoskeleton and the geometry of confined channels, which induce cells into a longitudinal pill-shaped morphology. Moreover, mathematical modeling predicts that the water permeation mechanism is key to migration inside stiff, narrow microchannels in which cells experience high hydraulic resistance, which is related to the extracellular pressure on the cell (unpublished data). Thus, it remains to be established whether the osmotic engine model operates in vivo where tissues and extracellular matrices are soft, porous, and permeable to water in all directions. In light of the plasticity of the different migration mechanisms, it is still unclear how the osmotic engine model of confined migration is related or convertible to other migration mechanisms discussed in previous sections or whether it represents an auxiliary mechanism. It is noteworthy that ROCK1, which phosphorylates myosin light chain, has been reported to be an upstream activator of NHE1 and could potentially serve as a functional switch between actomyosin-mediated migration and the osmotic engine model [76].

The osmotic engine model of migration may be relevant to cancerous cells which typically overexpress aquaporins, ion channels, and pumps [77–79] and can thus uptake and/or expel water more effectively than their normal counterparts. If cells cannot uptake water, then they need to push against a column of water during migration in stiff, confined microchannels. This so called barotaxis mechanism was demonstrated for differentiated HL60 neutrophil-like cells [80] as evidenced by the fact that the bulk velocity of the moving fluid anterior to the cell is identical to that of moving cells. When HL60 cells encounter an asymmetric bifurcation of different hydraulic resistances, cells tend to follow the path of lower resistance. The leading edge of HL60 cells protruding into the lower resistance channel extends at significantly faster rate than the other competing edge, eventually causing the losing edge to retract, thereby precipitating the final cell decision to the lower resistance channel. This directional bias becomes more evident as the hydraulic resistance difference increases to the point that almost no HL60 cells are able to enter a dead-end branch where it presents infinite hydraulic resistance. In marked contrast, about 20% of MDA-MB-231 breast cancer cells, which employ the osmotic engine model, enter the dead-end branch channel (unpublished data). Taken together, cells, and in particular cancerous cells, may both push and take up water concurrently when moving in stiff, confined channels, and thus the two mechanisms are not mutually exclusive. Cells may use hydraulic resistance to probe the path of least resistance in order to determine the most efficient path of migration, and directed flow of water from the osmotic engine model could serve as additional "fuel" to facilitate cell translocation.

8.7 Conclusion

Cell migration is a complex process which necessitates the interplay of various intrinsic and extrinsic factors. Confinement further contributes to the complexity of cell migration mechanisms by providing a physical cue that cells have to integrate and alter intracellular signaling to ensure optimized and efficient cell migration. Recent breakthroughs in bioengineering and microfabrication techniques have provided researchers with various useful tools to orthogonally control biochemical and physical inputs and recapitulate physiologically relevant microenvironments encountered in vivo in order to systematically investigate the effects of physical confinement on cell signaling and motility. These studies have provided us with invaluable insights on how confined cell migration occurs. Several intrinsic cellular factors, such as actomyosin contractility, integrin expression, MMP activity, actin, and microtubules, as well as extrinsic characteristics of surrounding matrix, such as adhesiveness, porosity, stiffness, elastic property, and osmolarity, contribute to this intricate network that controls the mechanism of confined migration. Cells choose their preferred mode of migration depending on the physicochemical properties of the local microenvironment and the cellular contractile state. Cells display high plasticity and are

capable of switching from one migration mode to another with ease. Understanding the mechanisms of confined cell migration thus offers promise for the development of novel therapeutic strategies that can target the different facets of cell motility, for diseases arising from dysregulated cell migration like cancer metastasis.

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