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Fibroblasts lead the way: a unified view of three-dimensional cell motility

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Abstract

Primary human fibroblasts are remarkably adaptable, able to migrate in differing types of physiological 3D tissue and on rigid 2D tissue culture surfaces. The crawling behavior of these and other vertebrate cells has been studied intensively, which has helped generate the concept of the cell motility cycle as a comprehensive model of 2D cell migration. However, this model fails to explain how cells force their large nuclei through the confines of a 3D matrix environment and why primary fibroblasts can use more than one mechanism to move in 3D. Recent work shows that the intracellular localization of myosin II activity is governed by cell-matrix interactions to both force the nucleus through the extracellular matrix and dictate the type of protrusions used to migrate in 3D.

Keywords

Cell motility; actomyosin contractility; adhesion; extracellular matrix

Moving from 2D to 3D environments

The ability of cells to navigate diverse 3D environments is essential for many aspects of multicellular life. For example, immune cells patrol structurally diverse tissues to detect and fight infections, while fibroblasts move through the dermis to sites of tissue damage where they remake the matrix and help to restore the barrier function of the skin. Conversely, the inappropriate 3D migration of metastatic cancer cells can be lethal. Discovering the molecular mechanisms driving 3D fibroblast migration could improve our understanding of normal wound healing, as well as fibroblast-mediated pathologies, such as tissue fibrosis or tumor progression and metastasis. Additionally, by learning how normal, primary human cells move in 3D, we could establish if the motility mechanisms used by single invading cancer cells are abnormal. Such cancer-specific mechanisms of 3D movement might then be

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targeted therapeutically to reduce metastasis, while leaving the movement of untransformed cells, such as fibroblasts, relatively unaffected.

Ideally, cell movement should be studied in a physiologically relevant 3D tissue. The discovery that primary fibroblasts can crawl out of tissue explants and onto rigid 2D tissue culture surfaces enabled the pioneers of the field of cell behavior to infer underlying molecular mechanisms [1, 2]. The imaging of dynamic cell movements, along with biochemistry and genetics helped to establish the mechanistic basis of primary fibroblast motility as a conceptual cycle of four steps, known as the cell motility cycle [3, 4].

Together the steps of the 2D cell motility cycle generate directional movement (Figure 1). First, polarized signaling by phosphatidylinositol (3,4,5)-trisphosphate (PIP3) [5] and the small GTPases Rac1 [6] and Cdc42 [7] direct actin nucleating proteins, such as Arp 2/3 [8], to help polymerize branched actin filaments (F-actin) against the plasma membrane to initiate protrusion of a lamellipodium [9]. Second, integrin receptors in the protruding plasma membrane bind matrix ligands on the 2D surface and cluster to form small nascent adhesions [10]. Third, RhoA and the formin family of actin nucleators, such as mDia1 and 2, help assemble actin stress fibers to connect the nascent adhesions at the front of the cell to mature adhesions underneath the cell body [11, 12]. Actomyosin contractility then pulls on the nascent adhesions to enlarge and strengthen them, thereby increasing contractile tension between the front and rear of the cell. Finally, myosin II activity at the rear of the cell signals adhesions to disassemble, pulls the weakened adhesions off the 2D surface, and retracts the trailing edge [13, 14]. Once the back of the cell is detached, the cell body can move forward. Concurrently, polarized microtubules direct the transport of lipids and proteins to the leading edge along with polarity signals that localize Rac1 and Cdc42 activity to the front of the cell [15, 16]. Additionally, retrograde cortical flow of F-actin sweeps plasma membrane lipids and proteins rearward and helps position the nucleus at the back of the cell [17].

Advances in imaging technology and the use of in vitro 3D extracellular matrix (ECM) models have helped establish that the 2D cell motility cycle is not the only mechanism by which primary fibroblasts and cancer cells move (reviewed in [18]). Cells can use different protrusive mechanisms, such as lamellipodia [19], lobopodia [20], or membrane blebs [21], in response to the differing physical structure of the 3D matrix. This migratory plasticity has revealed novel cellular mechanisms that are essential for 3D motility, yet not required by cells crawling across a rigid 2D tissue culture surface.

This review discusses how the 3D ECM can impose unique structural advantages and constraints on moving cells. We then present recently discovered adhesion, signaling, and cytoskeletal mechanisms that occur only in 3D environments. We explain how these mechanisms may determine the mode of primary fibroblast 3D migration. Finally, we discuss how delineating the complexity of migration mechanisms in normal cells will permit direct comparisons with metastatic cancer cell migration and invasion.

The physical challenges and advantages of moving in a 3D matrix

Unlike a 2D matrix, a 3D matrix can facilitate the movement of non-adhesive cells [22]. For example, while stationary non-adherent cancer cells form membrane blebs on a 2D surface, they become motile when compressed between two surfaces [23]. This phenomenon can be recapitulated by compressing water droplets containing microtubules, kinesin, and ATP [24], which allows the friction between the flowing microtubules and the confining surfaces to propel the droplet forward. The compression-dependent migration of cells in a low-adhesion environment has been reported for multiple cell types, including primary human fibroblasts [25, 26]. The fibrillar structure of the 3D matrix can also act as a physical barrier to cell movement. Properties such as the distance between matrix fibers, their rigidity, and their cross-linking with each other can impose constraints on cell motility not present in liquid media (Box 1; for other reviews see [27, 28]).

Box 1

The physical structure of the matrix can act as a barrier to 3D cell movement

There are at least three distinct ways in which fibrous extracellular matrices can impede cell movement. Pore size (panel A) is indicated by the shortest distance between matrix fibers delineating a continuous space in the matrix [31]. Pore size in collagen gels can be manipulated by varying the polymerization temperature, but this can also change the relative stiffness of the material. The relative stiffness (panel B) of extracellular matrix fibers can be measured by atomic force microscopy (AFM) and is given by the Young's modulus in units of Pascals [68]. The rigidity of collagen gels can be increased by increasing fiber density, but doing so also decreases the apparent pore size of the material. Besides stiffness, the elastic behavior of a material can also be measured by AFM [20]. Elastic behavior (panel C) is affected, in part, by the extent of interconnection or crosslinking of individual matrix fibers [69]. In simple collagen gels, the individual fibers can move independently of one another, contributing to non-linear elastic behavior. Highly cross-linked material, like skin dermis and fibroblast-derived matrix, are linearly elastic matrices [20]. It is likely that in each case, the relative ability of a cell to remodel its matrix environment will help dictate its physical response. For example, fibroblasts could use traction force to pull apart unattached collagen fibers, or secrete proteases to sever collagen fibers in order to enlarge pores and move forward through the matrix. We speculate these mechanisms may not be sufficient in materials where the fibers are covalently bound together, necessitating the use of the nuclear piston mechanism to achieve efficient 3D cell movement.

Whether the 3D matrix impedes or facilitates cell movement depends upon the molecular mechanisms operating within a particular cell. Specifically, the degree of cell-matrix adhesion and the deformability of the nucleus are two cell-intrinsic factors that correlate with rapid 3D cell movement. Immune cells, such as dendritic cells, have soft-deformable nuclei and interact with the matrix weakly, allowing them to move rapidly ($\sim 240 \mu\text{m}/\text{hour}$) between matrix fibers [22]. In contrast, slower fibroblasts ($\sim 40 \mu\text{m}/\text{hour}$) have stiffer nuclei and interact strongly with the surrounding matrix as they move. Despite these cell type-specific differences, recent work indicates that the steric problem of moving the bulky nucleus through the fibrillar 3D matrix is a universal constraint on 3D migration [22, 29–31]. Whether cells move fast or slow, they rely on the power of non-muscle myosin II to overcome this fundamental barrier to their movement through tissues.

The myosin II-dependence of 3D cell migration

Imaging cell motility in 3D matrices shows that mechanisms essential for 3D cell movement are not always consistent with 2D migration models. Actomyosin contractility, triggered by the small GTPase RhoA, is required for 3D movement, but not necessarily for 2D motility [32, 33]. Though actomyosin contractility does play a role in other aspects of 2D cell biology such as producing fibrillar fibronectin [34], promoting retraction of the leading edge [35], and enlarging cell-matrix adhesions [11]. Myosin II has been proposed to apply

traction force to the 3D matrix to orient fibers and limit cellular protrusions to facilitate directional cell motility [36, 37]. Another important function of actomyosin contractility, and reportedly the myosin IIB isoform in particular, is to force the nucleus through narrow pores in 3D matrix [38]. The nucleus is the largest single organelle, and its ability to transit through the tight spaces in dense matrix can be the rate-limiting step during 3D cell migration of a diverse array of cell types [22, 29–31].

At least two distinct mechanisms are used by cells to apply the forces produced by myosin II to the nucleus. In strongly adherent fibroblasts, extensive mechanical connections exist between the lamin-based nucleoskeleton and the cytoskeleton, which facilitate force transmission between the nucleus and the extracellular matrix [39]. These mechanical connections and actomyosin contractility can function to pull the nucleus through narrow pores in the extracellular matrix [29, 31, 40]. In less adherent immune cells and certain cancer cells, myosin II-mediated contractility is localized behind the nucleus to squeeze the nucleus forward [22, 41, 42]. Interestingly, the choice between these two strategies for moving the bulky nucleus through dense 3D matrix may depend, in part, on the relative softness and deformability of the nuclei. In short-lived immune cells where myosin II activity is found behind the nucleus, the softer nuclei can pass more easily through narrow pores. This strategy may not be appropriate for longer-lived primary fibroblasts, however, because cells with softer nuclei are susceptible to increased DNA damage and cell death [30, 43].

Mechanical control of 3D migration by the small GTPase RhoA

Classically, RhoA and actomyosin contractility govern the size of cell-matrix adhesions and traction forces on 2D surfaces to control cell velocity [44]. In 3D, RhoA also responds to the elastic behavior of the surrounding matrix to control intracellular pressure and the mode of fibroblast migration [20, 40]. This pathway is distinct from the role of RhoA in controlling adhesion size in that it absolutely requires nesprin 3, while control of adhesion size is at least partially dependent on mDia1. These results imply that there may be two independent mechanical mechanisms downstream of RhoA, one of which only becomes apparent in 3D matrix.

Recent work presents new mechanisms for how the extracellular matrix can control RhoA activity that may not be evident on 2D surfaces. Increased contractility, such as that which occurs in fibroblasts migrating through stiff 3D matrix environments, decreases acetylated microtubules and thereby microtubule stability [45]. This functional relationship is required to maintain cell migration, in part by, increasing surface expression of integrins and promoting contractility-dependent extracellular matrix remodeling. This reciprocal relationship between microtubule modification and force generation may also function through release of the RhoA guanine exchange factor, GEF-H1, into the cytoplasm where it can bind and activate RhoA to facilitate cell migration in confined environments [46].

While the stability of cell-matrix adhesions contributes to efficient 3D migration [47], integrin signaling also regulates RhoA activity to maintain rapid cell movement. In soft collagen matrices, $\alpha 2 \beta 1$ integrin signaling suppresses actomyosin contractility by activating the guanine exchange factor β Pix and Cdc42 at the leading edge [48]. Active Cdc42 keeps

RhoA inactive via the RhoA inactivating protein srGAP1. Conversely, $\alpha 4 \beta 1$ integrin-mediated signaling decreases Rac1 activity to increase RhoA-dependent actomyosin contractility and cell migration in confined microchannels [49]. Finally, recycling of $\alpha 5 \beta 1$ between the surface and endosomes is restricted to a region in front of the nucleus near the leading edge in certain cancer cells [50]. This local recycling is required to increase RhoA activity and form elongated protrusions necessary to invade into the 3D matrix. It will be interesting to determine if these RhoA regulatory pathways also have a role in lobopodial fibroblasts or how they may be functioning in non-adherent amoeboid fibroblasts.

The plasticity of 3D fibroblast migration

Nineteenth century cell biologists recognized that single cell protists move using a variety of structurally distinct protrusions [51]. These distinct protrusions were first characterized based on morphology. The giant amoeba, *Amoeba proteus*, uses actomyosin contractility to drive cytoplasmic streaming to increase intracellular pressure and form blunt, cylindrical protrusions termed “lobopodia” [52]. In contrast, low-pressure protists, like *Vanella miroides*, crawling on 2D surfaces use actin polymerization to form broad, flat fan-shaped protrusions termed “lamellipodia” [53]. Migrating primary human fibroblasts can switch between low-pressure lamellipodia and high-pressure lobopodia, which requires myosin-II, in response to the structure of the 3D matrix [20, 40]. Moreover, cells can use a third type of migration mechanism classified as amoeboid fibroblast (A1) motility. The major similarities and differences between the lobopodial, lamellipodial, and amoeboid modes of fibroblast motility are summarized in Table 1. While the mechanism of pressure generation is not the same in protists and human cells, it is remarkable that the relationship between pressure and the type of protrusion formed is conserved.

Lobopodia-based migration

Fibroblasts migrating in linearly elastic 3D matrix tend to use blunt cylindrical lobopodia (Figure 1A), rather than the classical lamellipodia found on cells migrating on 2D surfaces. Lobopodia were first distinguished from lamellipodia by their lack of lamellipodial markers like cortactin; the signaling pathways (Rac1, Cdc42, and PIP3) that are polarized at the leading edge of lamellipodial cells are non-polarized in lobopodial cells [20]. Additionally, lobopodial protrusions use intracellular pressure to extend their leading edge in place of the actin polymerization and Brownian ratchet mechanisms classically associated with lamellipodia [40]. In cross-linked, linearly elastic matrix, high cell-matrix adhesion localizes myosin II in front of the nucleus to pull it forward like a piston to pressurize lobopodial protrusions [40]. To accomplish this, myosin II relies on the linker protein nesprin 3, which helps to connect vimentin intermediate filaments to the lamin-based nucleoskeleton. This nuclear pulling mechanism is supported by other evidence indicating strong association of active myosin II with vimentin [54] and vimentin filaments connecting the nucleus to the extracellular matrix [55, 56]. Additionally, a mechanism using vimentin filaments to pull the bulky, rigid nucleus through the confines of 3D matrix would take advantage of vimentin's resistance to breakage by tensile forces, a property not provided by either F-actin or microtubules [57], although actomyosin bundles are stronger than single F-actin filaments [58]. Vimentin and actomyosin contractility are also required to position the nucleus towards

the rear of the cell prior to leading-edge protrusion at the onset of 2D motility [17, 59]. Because the motion of the nucleus can be associated with actomyosin contractility and vimentin in both 2D and 3D contexts, we speculate that positioning the nucleus rearward on 2D and pulling the nucleus forward in 3D are manifestations of the same molecular mechanisms that are specifically required in fibroblasts moving through a 3D extracellular matrix. Testing this hypothesis and understanding why myosin II can move the nucleus rearward on 2D initially, but pulls the nucleus forward steadily in 3D will help reconcile 2D and 3D migration mechanisms.

3D Lamellipodia-based migration

In strain-stiffening, non-linearly elastic 3D material or when actomyosin contractility is reduced, migrating cells switch to low pressure lamellipodia (Figure 1B). 3D lamellipodia-based migration resembles the classical form of migration driven by the 2D cell motility cycle. In these cells, lamellipodial markers and filamentous actin are enriched at the leading edge, along with Rac1, Cdc42 and PIP3 signaling [20]. These cells are characterized by relatively low intracellular pressure, which is uniform throughout the cell. This uniform pressure distribution indicates that the nuclear piston mechanism is not active in lamellipodial cells, even though they continue to require cell-matrix adhesion to migrate efficiently. We speculate that less force is required to move the nucleus forward when cells are moving through non-linearly elastic 3D matrix compared to cross-linked, linearly elastic materials, such as dermis and fibroblast-derived matrix.

Amoeboid fibroblast migration

When primary fibroblasts are compressed between two surfaces chemically treated to prevent integrin-dependent adhesion, they revert to a third type of 3D migration recently defined as “fibroblast amoeboid” or A1 motility (Figure 1C) [25]. Indeed, cytoplasmic pressure in combination with weakening of the connections between the plasma membrane and underlying cortex can give rise to dynamic membrane blebs, which protrude the plasma membrane independently of lamellipodia formation [60]. Amoeboid metazoan cell migration is a property of single cells, such as neutrophils and dendritic cells, that move and change shape rapidly compared to adherent fibroblasts [61]. Amoeboid cancer cells are defined as round cells which form membrane blebs at their leading edge [62, 63]. Two adhesion-independent amoeboid forms of motility have been further characterized based on the degree of retrograde actomyosin flow. Amoeboid fibroblasts (A1) have a leading edge protrusion with a pattern of actomyosin flow that differs from cells using the A2 mode of amoeboid migration. A2 cells, such as blebbing Walker 256 carcinosarcoma cells and leukocytes, have a large stable bleb at their leading edge and require myosin II activity to sustain bleb-based migration when confined between two low-adhesion surfaces [23, 25, 64]. In contrast to lobopodia-based migration, during adhesion-independent amoeboid fibroblast migration, myosin II flows rapidly rearward along leading-edge protrusions and presumably accumulates uniformly over the cell cortex. Importantly, this myosin II is completely dispensable for the rapid movement of these cells [25]. Indeed, the integrin-independent movement of A1 amoeboid fibroblasts is likely driven by retrograde flow of the cortical cytoskeleton. Since the velocity of primary fibroblasts moving through fibrillar 3D ECMs is dramatically reduced upon integrin inhibition [20], it may be that this amoeboid

fibroblast myosin II-independent mechanism is not sufficient to migrate efficiently in more structurally complex fibrillar 3D ECMs, such as collagen and fibroblast-derived matrix. While this low-adhesion mode of 3D fibroblast migration is likely to be associated with very low traction forces [64], the associated intracellular pressure and protrusion identity remains to be determined. Moreover, it is not clear if pressure or reduced strength of cortical attachment is the critical parameter in formation of the various types of blebs characterized during amoeboid cancer cell migration. Additionally, it remains to be determined how the classical round amoeboid cancer cell mode of migration is mechanistically related to the recently described adhesion-independent A1 and A2 amoeboid forms of motility.

Concluding remarks

The diversity in the mechanisms used by primary fibroblasts to migrate in physiological 3D environments shows there is still much to discover in terms of the molecular mechanisms of cell movement. Because cells in 3D can use more than one mechanism to migrate, the precise number of these alternative modes of migration and their mechanistic interrelationships needs characterization [65]. Establishing the rules that dictate how cells migrate could narrow down the number of possible mechanisms, and it may pinpoint essential components required to sustain cell movement in different 3D matrix environments.

Blocking the inappropriate motility of metastatic cancer cells without affecting normal cells is a therapeutic goal. Establishing how primary, untransformed cells move in 3D will allow precise mechanistic comparisons to be made with cancer cells, and may lead to new drug targets to reduce cancer cell invasion and metastasis. For example, the mechanical response of cells to their environment may be defective in some cancer cells [66]. Restoring this response may reduce the inappropriate movement of the cancer cells [67]. Studying the molecular mechanisms of cell movement in 3D extracellular matrix, rather than on 2D tissue culture plastic, is likely to provide the most efficient way to address these and other important questions (see Outstanding Questions Box) in the future.

Outstanding Questions

- Why do primary fibroblasts use more than one mechanism to migrate? It may be that lobopodial, lamellipodial, and amoeboid fibroblast migration can each be particularly efficient in a given different 3D material. An alternative hypothesis is that the mode of migration is required for migration-independent fibroblast functions, such as matrix production and remodeling.
- How does the cell sense the elastic behavior of its 3D environment to activate the nuclear piston mechanism and trigger high-pressure lobopodial motility?
- How does the degree of cell-matrix adhesion alter the localization of myosin II activity within the cell?
- What are the similarities and differences between the modes of fibroblast and cancer 3D migration?

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Trends

- Primary human fibroblasts can transition between three distinct mechanisms of migration in three-dimensional extracellular matrix.
- The degree of actomyosin contractility and cell-matrix adhesion helps to dictate the mode of cell migration.
- The modes of migration are distinguished, in part, by the intracellular localization of myosin II and the type of protrusion used by the cell to migrate.
- The mechanism by which a cell moves its bulky nucleus through the fibrillar matrix also governs intracellular pressure and the type of protrusion used to migrate.

A. Lobopodial

B. Lamellipodial

C. Amoeboid (A1)

Figure 1.

(A) High-pressure 3D migration. In lobopodial cells, polarized myosin II acts through vimentin filaments and the nucleoskeleton-cytoskeleton linker protein nesprin 3 to pull the nucleus forward and raise intracellular pressure. This high pressure causes the membrane to protrude and allows new cell-matrix adhesions to form. We speculate that connections are then made to link the new adhesions to older adhesions in the cortex, and to the lamin-based nucleoskeleton. Myosin II-independent forces bring the cell rear forward as cell-matrix adhesions disassemble. In lobopodial fibroblasts, the nucleus can act as a piston, physically separating the cell into two compartments and raising the pressure in front to produce

lamellipodia-independent protrusion. While Rac1, Cdc42, and PIP3 signaling are non-polarized in these polarized cells, microtubules might provide polarity in response to matrix topography. (B) 2D and 3D lamellipodia-based movement. Membrane protrusion by actin polymerization allows the formation of new membrane-matrix contacts. Actomyosin contractility throughout the cell strengthens these adhesions and increases cellular tension without increasing intracellular pressure. Adhesions disassemble at the back of the cell, and actomyosin contractility retracts the trailing edge to help the cell body glide forward. Polarized signaling by Rac1, Cdc42, and PIP3 coordinates protrusion and adhesion formation. Microtubules deliver additional polarity cues to the front of the cell, along with membrane and lipid components. (C) Adhesion-independent “amoeboid” fibroblast 3D motility. When cells are not strongly adherent to the substrate, myosin II-driven retrograde flow of actomyosin occurs only in the very leading protrusions and likely leads to a relative uniform distribution of myosin II in the cell cortex. These cells are unable to generate productive forward movement on a 2D surface. However, when these cells are compressed between two surfaces, the resulting friction is sufficient to translate force, presumably from the retrograde flow of the cortical cytoskeleton, into forward movement of the cell. See Box 1 for a discussion of how the physical structure of the matrix can help determine 3D migration mechanisms.

Table 1

Key differences between the three modes of fibroblast migration

	Lobopodia	Lamellipodia	Amoeboid (A1)
Integrin-dependent	Yes	Yes	No
Myosin II-dependent	Yes	No	No
Cellular distribution of Myosin II	Forward of the nucleus	Throughout the cell	Rapid retrograde flow in leading protrusions
Intracellular pressure	High (~2000 Pa)	Low (~500 Pa)	?
Polarized signaling	No	Yes	?
Compartmentalization of the cytoplasm	Yes	No	?