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Multiple Mechanisms of 3D Migration: The Origins of Plasticity

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Abstract

Cells migrate through 3D environments using a surprisingly wide variety of molecular mechanisms. These distinct modes of migration often rely on the same intracellular components, which are used in different ways to achieve cell motility. Recent work reveals that how a cell moves can be dictated by the relative amounts of cell-matrix adhesion and actomyosin contractility. A current concept is that the level of difficulty in squeezing the nucleus through a confining 3D environment determines the amounts of adhesion and contractility required for cell motility. Ultimately, determining how the nucleus controls the mode of cell migration will be essential for understanding both physiological and pathological processes dependent on cell migration in the body.

Introduction

How cells move has puzzled and bewitched multiple generations of life scientists [1–4]. Cell movement is an essential component of many physiological processes, such as the shaping of tissues and organs during development [5,6] and wound healing [7]. Tragically, cell movement drives the spread of tumor cells throughout the body [8].

Cell motility has been studied historically on two-dimensional (2D) tissue culture surfaces [9]. This model has yielded many fascinating molecular mechanisms that mediate and direct cell movement across 2D surfaces [10]. In particular, the small GTPase Rac1 has emerged as a central node in controlling cell polarity and directional migration [11, 12]. Localized activation of Rac1 at the plasma membrane directs the actin nucleator Arp2/3 to form the branched filamentous actin (F-actin) network which drives protrusion of the lamellipodium [13], a flat, fan-shaped structure often found at the leading edge of cells on 2D surfaces [14]. Integrin receptors then form small clusters termed nascent adhesions beneath the extending lamellipodium [15, 16]. The small GTPase RhoA helps to connect these nascent adhesions to myosin-containing actin (actomyosin) stress fibers by activating the formin family of actin

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nucleators, including mDia2 [17,18]. These force-generating machines respond to the rigidity of the 2D surface and provide the power to enlarge and strengthen the cell-matrix adhesions needed for moving the bulk of the cell body. The cell-matrix adhesions disassemble after the nucleus passes over them, and myosin II-mediated contractility squeezes the back of the cell forward [19,20].

The field of cell motility has focused increasingly on discovering how cells move in 3D extracellular matrix (ECM) environments, such as dermis and fibrillar collagen. Intriguingly, in addition to the well-described mode of lamellipodia-based motility, single cells can switch between several distinct 3D migration mechanisms, a phenomenon termed migratory plasticity (reviewed recently in [21,22]). Understanding how and why cells transition between multiple 3D migration mechanisms is emerging as one of the foremost challenges in understanding the control of physiological cell movement [23,24].

This review will describe the distinct migration mechanisms used by cells in 3D environments. We will highlight how Rac1-mediated lamellipodia formation, RhoA-mediated actomyosin contractility, and integrin-mediated adhesion dictate which mechanism a cell will use to move in 3D. Finally, we will suggest that the relative level of difficulty in moving the nucleus through a 3D matrix is the primary factor governing the choice of 3D migration mechanisms.

The plasticity of 3D cell movement

An early example of plasticity in the movement of cells was identified in developing Fundulus fish [25]. During gastrulation, Fundulus deep cells move in the space between two confining cell layers. Non-adherent deep cells possess large, stable blebs, which switch to flat lamellipodia or filopodia when the cells become more adhesive [26], similar to zebrafish progenitor cells [27]. More recently, studying changes in tumor cell morphology led to the discovery of the mesenchymal (elongated) and amoeboid (rounded) modes of 3D cell migration [28,29]. It is now clear that many cell types can use distinct mechanisms to move through diverse 3D environments [30]. These modes of 3D cell migration are most easily classified by their relative cell-matrix adhesion and actomyosin contractility (Figure 1).

Actomyosin contractility and pressure-driven protrusion

In covalently cross-linked matrices, such as dermis and fibroblast-derived matrix, adherent fibroblasts can use their nucleus like a piston to generate intracellular pressure to drive forward a blunt, cylindrical lobopodial protrusion (Figure 1a) [31,32]. These elongated cells are highly polarized and migrate directionally, despite their lack of lamellipodia and a non-polarized distribution of active Rac1 throughout the plasma membrane. In lobopodial fibroblasts, RhoA activity triggers actomyosin contractility to pull the nucleus forward via the nucleoskeleton-cytoskeleton linker protein nesprin 3. Due to the presence of a diffusion barrier around the nucleus, the forward motion of the nucleus pressurizes the cytoplasm to push the leading edge forward.

In contrast to fibroblasts, increasing RhoA activity in tumor cells moving in 2D and 3D results in a rounded cell shape [28,29] with actomyosin contractility localized to the back of

the cell (Figure 1c) [33]. Amoeboid tumor cells move using small, unstable blebs. These blebs may be generated by either weakened membrane-cortical cytoskeletal attachment, increased intracellular pressure, or both, and they help the cell to squeeze through the 3D matrix [4,34]. While some β1 integrin-mediated adhesion can be required for amoeboid tumor cell movement [29], the reduced focal adhesion size [31] and force generation [35] suggest that the adhesions in rounded, amoeboid cells are not nearly as strong as those found in more elongated cells, such as lobopodial fibroblasts.

Actin polymerization-driven membrane protrusion

In non-crosslinked 3D materials, such as type I collagen gels, adherent primary human fibroblasts and elongated (mesenchymal) tumor cells use low-pressure lamellipodial protrusions for migration (Figure 1b) [32]. Similar to lamellipodia on 2D surfaces, these protrusions use polarized Rac1 activity to direct Arp2/3-mediated actin-polymerization and push their plasma membrane through the matrix [31,36]. In contrast to lobopodial fibroblasts, intracellular pressure is lower and uniform throughout these cells. Some actomyosin contractility is still required for efficient 3D lamellipodia-based movment, however, most likely for aligning matrix fibers [37], enhancing protrusion stability [38], or moving the nucleus forward [39].

Inhibition of αvβ3 integrin-mediated cell-matrix adhesion reveals a second mechanism of actin-driven protrusion in breast cancer cells (Figure 1d). Normally, these cells utilize Arp2/3 to form lamellipodial protrusions in fibronectin-rich 3D collagen gels [36]. When Arp2/3 is inhibited, either directly or through increased α5β1 integrin recycling, these cells switch to using RhoA and the formin FHOD3 to help form distinctive spikes of F-actin at their leading edge. These actin spikes enable the aggressive invasive behavior of these elongated, metastatic cells [40].

Retrograde flow and 3D-dependent friction-driven movment

Interestingly, integrin-mediated adhesion, which is generally required for cells to move on 2D surfaces, is not always necessary for cells to migrate in 3D [27,41–43]. When non-adhesive cells are compressed between two surfaces, the rapid retrograde flow of actomyosin beneath the plasma membrane generates sufficient friction to propel the cell forward, despite the very low force produced (Figure 1e–g) [35]. Alternatively, the osmotic engine model of confined migration takes advantage of the semi-permeable nature of the plasma membrane to water, and it actively transports water from the front to the back of the cell to propel the cell forward (Figure 1h) [44]. Typically, cells that use these low-adhesion modes of friction-driven movement are unable to crawl along unconfined 2D surfaces, unlike amoeboid tumor cells [45]. Additionally, friction-driven, 3D cell movment generally does not require high actomyosin contractility, except to polarize the cells in some cases [43]. It remains unclear whether these forms of migration are efficient in fibrillar 3D matrices where contractility and adhesion are required [32,37,46].

Correlating the mode of migration with the 3D environment

The nature of the matrix material surrounding cells often determines how a cell will migrate in 3D [31,47–49]. Interestingly, the relative constraints placed on the machinery for moving the nucleus by a particular 3D environment (Box 1) appears to correlate with the level of adhesion and actomyosin contractility required for overall cell migration (reviewed recently in [50]).

When cells move through narrow, confined channels that lack fibrillar matrix, adhesion and contractility are generally not required for cell motility (Figure 1e–h) [27,43]. Instead, friction with the walls may be sufficient to generate movement since the channel area is significantly larger than the 3D matrix pore size that limits nuclear movement [49]. In non-crosslinked fibrillar 3D matrices such as collagen, however, adhesion and actomyosin contractility are required for cell movement, unless the nucleus is able to soften and change shape [51,52]. When the nucleus is rigid, such as in fibroblasts, adhesion and contractility are likely required to help the cell to apply traction force to align the matrix fibers and then to force the nucleus through the material [32,37,39,49]. Critically, low-pressure lamellipodial migration (Figure 1b) is not sufficient to move the nucleus through cross-linked, fibrillar matrix [31]. Instead, primary human fibroblasts, myofibroblasts, and de-differentiated chondrocytes use the power of myosin II to pull their nuclei through the cross-linked environment and generate the pressure necessary for lobopodial protrusion (Figure 1a) [32]. Thus, in this case, a cell can use a single mechanism to power both nuclear movement and protrusion of the leading edge.

Transitions between migration modes in a single cell

Interestingly, a single cell is capable of switching between different migration mechanisms in response to local changes to its soluble signaling and 3D matrix environments. The speed with which a cell can change to a new migration phenotype (≤ 15 minutes) [31] suggests these transitions do not require the expression of distinct sets of proteins, as needed at the onset of migration of heart precursor cells, for example [53]. Instead, cells likely transition between distinct migration modes by functionally rearranging the same core set of pro-migration proteins, such as integrins, actin, myosin, Rac1, and RhoA, in response to differences in local signaling and 3D matrix environments. Determining how a single cell can transition between two or more migration modes will help us to understand how these processes are related mechanistically. For example, treatment of primary human fibroblasts with platelet-derived growth factor (PDGF) is sufficient to trigger high-pressure lobopodial migration (Figure 1a) in a cross-linked 3D matrix [31]. Inhibiting actomyosin contractility switches these cells to the less efficient Rac1-mediated, lamellipodial migration mechanism (Figure 1b). If integrin-mediated adhesion is prevented, for example in confined channels, cells further revert to friction-driven amoeboid (A1) fibroblast migration (Figure 1f) [27]. These results suggest that even though PDGF receptor signaling is sufficient to trigger all three fibroblast modes of 3D migration, there is a hierarchy of migration mechanisms. Specifically, cells first employ high-pressure lobopodia, followed by low-pressure lamellipodia, and finally the amoeboid (A1) migration mode. It will be important to establish how this hierarchy of fibroblast migration mechanisms is related to the choice

between mesenchymal and amoeboid tumor cell 3D movement; this information could help establish what, if anything, is abnormal in the movement of single metastatic cells. We speculate that reducing cell-matrix adhesion will be sufficient to convert highly-contractile lobopodial fibroblasts (Figure 1a) to the amoeboid form of integrin-dependent tumor cell movement (Figure 1c), since cells become rounded after inhibiting integrin-based adhesion [54].

How do contractility and adhesion dictate the mode of 3D migration?

The mode of migration may be governed by the way in which cells produce and apply the force necessary to move through their environment [42,55]. When cells are confined and prevented from adhering, the molecular clutch [56] is likely not engaged. A disengaged molecular clutch allows polymerized actomyosin cortical fibers to flow rapidly from the leading edge towards the rear of the cell [57]. Because cell-matrix adhesion is low, cells using such weak friction-based migration exert very little force on their surroundings, consistent with the characteristic actomyosin-independence of this type of cell movement [27,43,44]. These cells can migrate using either flat lamellipodia or stable blebs. It remains to be determined whether intracellular pressure affects how these cells move, but the key feature may be the relative tension in the plasma membrane, either in response to intracellular pressure or cortical rigidity, which controls protrusion choice during low-adhesion 3D cell migration.

Once cell-matrix adhesion occurs, robust integrin engagement will trigger activation of the small GTPases Rac1 and RhoA [58,59], engage the molecular clutch, and transmit actomyosin-generated force from retrograde flow toward productive forward movment and matrix remodeling [60]. When the cell encounters cross-linked matrix, we speculate that an undiscovered mechanism detects the increased resistance to nuclear movement and activates the nuclear piston mechanism through increased RhoA signaling. This elevates actomyosin contractility in front of the nucleus, as discussed above, and generates the necessary force to support both traction and pressure increases. But why do these cells not continue to migrate using lamellipodia? One possibility is that elevated RhoA simply shuts off Rac1 signaling and lamellipodia formation through classical RhoA-Rac1 biochemical crosstalk [45,61]. Alternatively, membrane tension may be significantly elevated in high-pressure cells due to pushing of the cytoplasm against the plasma membrane. Since Rac1 activity is sensitive to membrane tension [62,63], this may help to prevent lamellipodia formation. It will be important to determine the relative contributions of physical mechanisms such as pressure and membrane tension versus intracellular signal transduction in determining whether cells form lamellipodia versus lobopodia.

Concluding Remarks

Despite the many examples of plasticity now reported for cells moving in 3D environments, several important questions remain to be addressed. It is still not clear how many different mechanisms can be used by cells to move. Automated, unbiased imaging approaches could more rapidly establish how many distinct mechanisms cells can use to migrate [30,64]. The function of each mode of migration is also unclear. Is each mode of migration simply the

most efficient way for a cell to move through a particular 3D environment, or does the particular mode help with other cellular functions, such as matrix remodeling? While investigating the movement of cells in 3D matrix has led to many unanticipated questions, it provides an important approach to answering the fascinating questions of how and why cells move in the body.

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Box 1

Nuclear movement as the rate limiting step of migration. The nucleus is the largest, stiffest component of a cell. When a cell is moving through a confined space, it is actually the resistance to migration imposed by the matrix on the bulky nucleus that can slow overall cell movement. A typical nucleus appears to be unable to travel through a pore less than 7 μm^2 in area [49]. As this size limit is approached, actomyosin contractility and cell-matrix adhesion become more and more essential, either acting at the rear of the cell to squeeze the nucleus through the pore [33] or pulling the nucleus from the front, as in lobopodial fibroblasts [32]. Alternatively, cells can change the structure of their nucleoskeleton to soften the nucleus and render it more deformable for easier passage through restricted spaces/pores within 3D matrices [51,52]. Additionally, up-regulation of extracellular proteases, such as MT1-MMP, may act to cleave the matrix fibers that impede nuclear movement, thus facilitating 3D cell migration [29,49].

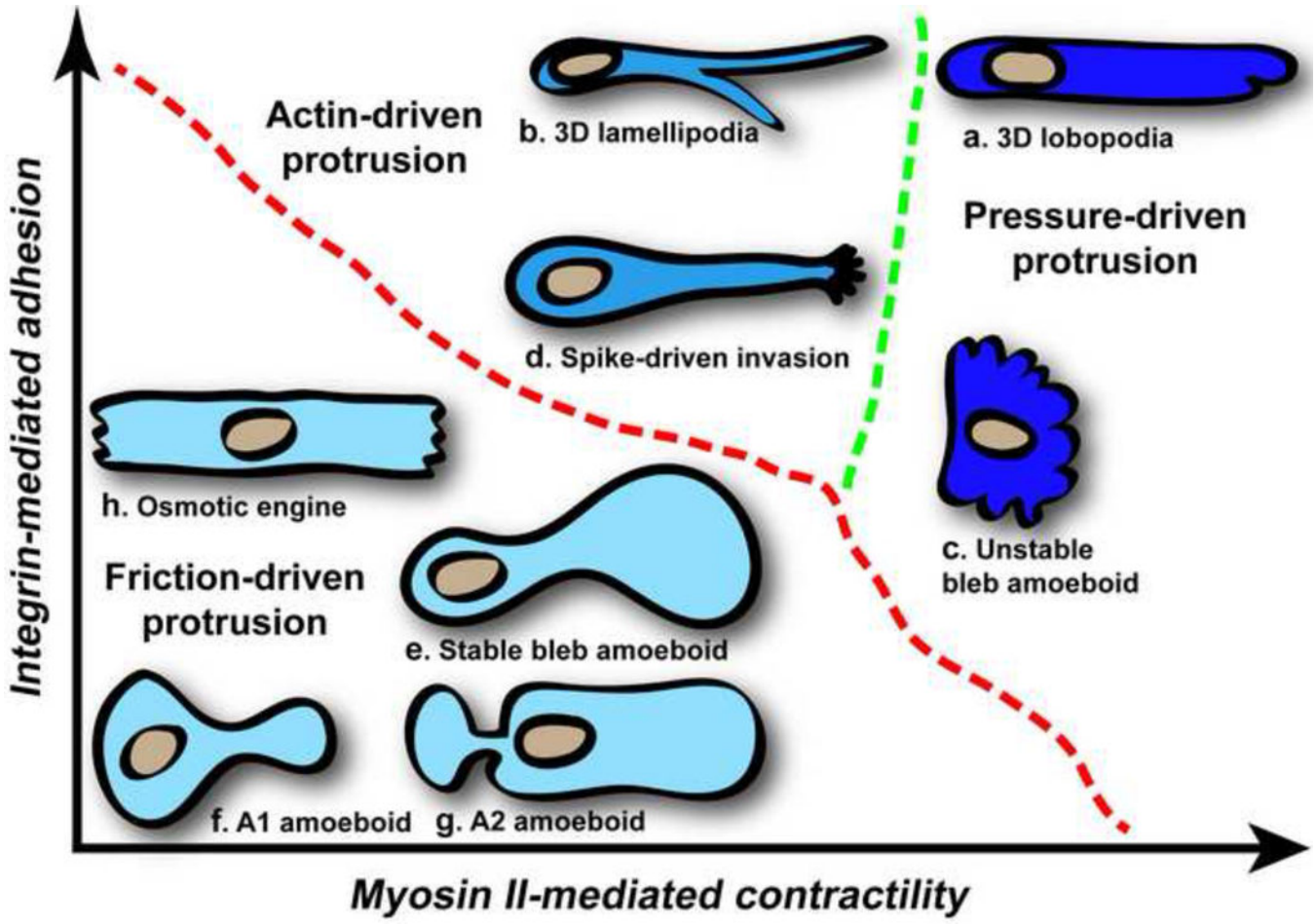

Figure 1.

Regulators of the plasticity of cell migration in 3D environments. The choice of each distinct mode of cell migration may require a combination of two variables, the strength of cell-matrix adhesion and the degree of actomyosin contractility. Primary human fibroblasts are currently the only cell type known to span the range of established cell migration phenotypes. **(a)** Lobopodial fibroblasts require cell-matrix adhesion and actomyosin contractility to move efficiently through cross-linked extracellular matrix. These cells use actomyosin contractility and robust integrin-mediated adhesion to pull the nucleus forward, like a piston, to pressurize the anterior cell and protrude the plasma membrane. **(b)** When contractility is reduced in fibroblasts, either by placing then in non-crosslinked matrix or inhibiting RhoA signaling, they switch to lamellipodia-based migration. When adhesion is prevented in confined 3D channels, fibroblasts shift to adhesion- and contractility-independent movement. In addition to fibroblasts, other cell types display similar patterns of motility. **(c)** Rounded, amoeboid tumor cells are highly contractile, but they rely less on cell-matrix adhesions compared to primary human fibroblasts. Amoeboid tumor cells migrate using small, unstable blebs, yet have enough cell-matrix adhesion to migrate across 2D surfaces. When RhoA is inhibited in amoeboid cells, they switch to a mesenchymal mode of motility, driven by lamellipodial protrusions. **(d)** When adhesion and adhesion trafficking are re-programed in certain breast cancer cells, they switch from Arp2/3 mediated

lamellipodia to formin-mediated, RhoA-dependent actin spikes. **(e–h)** Finally, many cells that are poorly adhesive and non-motile on 2D substrates can begin to move when confined between two surfaces; these cells are largely contractility-independent and adhesion-independent, migrate using large stable blebs, and exert very little force against the substrate. Future work should establish whether these modes of migration are universal or cell-type specific, as well as establishing if other mechanisms can generate distinct modes of 3D cell migration.