At the leading edge of three-dimensional cell migration

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Summary

Cells migrating on flat two-dimensional (2D) surfaces use actin polymerization to extend the leading edge of the plasma membrane during lamellipodia-based migration. This mode of migration is not universal; it represents only one of several mechanisms of cell motility in three-dimensional (3D) environments. The distinct modes of 3D migration are strongly dependent on the physical properties of the extracellular matrix, and they can be distinguished by the structure of the leading edge and the degree of matrix adhesion. How are these distinct modes of cell motility in 3D environments related to each other and regulated? Recent studies show that the same type of cell migrating in 3D extracellular matrix can switch between different leading edge structures. This mode-switching behavior, or plasticity, by a single cell suggests that the apparent diversity of motility mechanisms is integrated by a common intracellular signaling pathway that governs the mode of cell migration. In this Commentary, we propose that the mode of 3D cell migration is governed by a signaling axis involving cell–matrix adhesions, RhoA signaling and actomyosin contractility, and that this might represent a universal mechanism that controls 3D cell migration.

Key words: Cell migration, Extracellular matrix, Intracellular signaling, Mechanotransduction, Pseudopodia

Introduction

Autonomous cell motility is found throughout the tree of life. For example, unicellular protozoa hunt their prey through mud and leaf litter along the pond bottom, whereas highly specialized immune cells seek and destroy microbial invaders within a complex three-dimensional (3D) extracellular matrix. The diversity in cell types and environments requiring 3D cell motility is associated with the wide diversity of molecular mechanisms used for migration (Allen and Allen, 1978; Friedl and Wolf, 2010; Haston et al., 1982).

In vitro studies of metazoan cell migration have established that cells use actin polymerization coupled with cell–matrix adhesion to generate thin and wide lamellipodial protrusions to crawl across rigid 2D tissue culture surfaces (Abercrombie et al., 1970a; Abercrombie et al., 1970b; Dipasquale, 1975). However, imaging of cells moving in 3D models of extracellular matrix, as well as *in vivo*, reveal that lamellipodia-based motility is only one of multiple migration strategies (Friedl and Wolf, 2010; Lämmermann and Sixt, 2009; Ridley, 2011). These distinct migration strategies, or modes, are distinguished by differences in cell morphology, the extent of adhesion to the surrounding extracellular matrix and the mechanics of leading edge protrusion.

Significantly, studies of both cancer cell lines and normal fibroblasts show that cells of the same type can use different modes of migration depending on the physical properties of the extracellular matrix, the degree of extracellular proteolysis and on soluble signaling factors (Petrie et al., 2012; Sanz-Moreno et al., 2011; Wolf et al., 2003). These demonstrations of mode switching, or plasticity (Friedl and Wolf, 2010), within a single cell type suggest that the apparent diversity of migration strategies might in fact be based on a common intracellular pathway that governs the switch between modes of cell motility. Although this type of regulation of cell morphology or migration mode had been suggested previously, the molecular mechanisms linking soluble signaling factors and matrix structure to the mode of 3D cell migration remained unclear (Albrecht-Buehler, 1980; Bovee, 1964; Friedl, 2004; Harris, 1994).

Cells use cell–matrix adhesions in combination with contractility of the actin cytoskeleton to sense and respond to changes in rigidity of the extracellular matrix in 2D and 3D as reviewed previously (Peyton et al., 2007; Roca-Cusachs et al., 2012). The small GTPase RhoA, Rho-associated kinases (ROCK1 and ROCK2, hereafter referred to as ROCK), and the actin-binding motor protein myosin II are important components of this process of mechanotransduction. Significantly, this RhoA–ROCK–myosin-II signaling axis also has a key role in dictating the mode of 3D cell migration (Petrie et al., 2012; Sanz-Moreno and Marshall, 2010). This Commentary will first briefly review mechanotransduction and the cell motility cycle, before describing the distinct modes of 3D migration on the basis of the type of protrusion that forms the leading edge. We will then present evidence supporting the concept that the mode of 3D cell migration depends on the response of the ubiquitous RhoA–ROCK–myosin-II signaling axis to adhesion-mediated mechanical signals during cell migration.

Regulation of actomyosin contractility by cell–matrix adhesion

Intracellular signal transduction, or mechanotransduction, in response to elevated matrix stiffness can increase actomyosin contractility to initiate adhesion maturation, modulate gene transcription or trigger directional cell migration (Choi et al., 2008; Dupont et al., 2011; Lo et al., 2000; for recent in-depth reviews of the mechanisms of integrin-mediated mechanotransduction, see Moore et al., 2010; Roca-Cusachs et al., 2012; Schwarz and Gardel, 2012). Cells are able to sense and respond to changes in matrix rigidity by several mechanisms (Fig. 1). β1 integrins are the primary plasma membrane

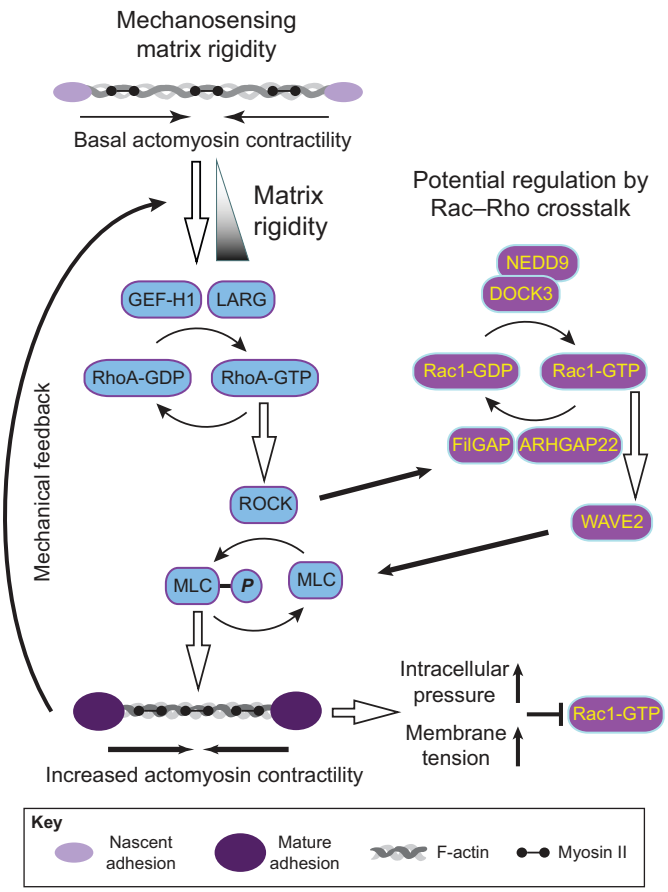

Fig. 1. Mechanosensing of matrix rigidity by RhoA, ROCK and myosin II and its potential regulation by Rho–Rac crosstalk. The RhoA–ROCK–myosin-II signaling axis is capable of sensing changes in the structure of the extracellular matrix and responding to it by increasing actomyosin contractility. The actin-binding motor protein myosin II maintains a low level of tension on actin fibers that are coupled to the extracellular matrix through cell–matrix adhesions. This basal tension enables myosin II to respond to changes in matrix rigidity or elastic behavior by increasing the tension on cell–matrix adhesions to activate the GEFs GEF-H1 and LARG. These GEFs activate RhoA, which in turn activates ROCK to increase the phosphorylation of myosin light chain (MLC), thereby further increasing myosin II activity and actomyosin contractility. This mechanical feedback loop can increase integrin clustering and adhesion maturation, and might increase intracellular pressure and plasma membrane tension to prevent lamellipodia formation and promote lobopodia- and bleb-based motility. Crosstalk between Rac1 and RhoA signaling potentially regulates the mechanosensing of matrix rigidity and the mode of 3D cell migration. During mesenchymal (lamellipodial) melanoma cell migration, NEDD9 forms a complex with the Rac1 GEF DOCK3 to activate Rac1 and suppress MLC phosphorylation through the Rac effector WAVE2, thereby suppressing amoeboid migration. Conversely, during amoeboid migration RhoA-dependent ROCK signaling can activate the Rac1 GAPs ARHGAP22 and FilGAP to inactivate Rac1 and suppresses mesenchymal migration in melanoma and carcinoma cells, respectively.

receptors transmitting tensional forces from the actin cytoskeleton to the extracellular matrix (Danen et al., 2002; Guilluy et al., 2011b). Myosin-II-mediated contractility is required for cells to actively sense changes in the rigidity of the extracellular matrix (Engler et al., 2006; Pelham and Wang, 1997). The action of myosin II along actin stress fibers maintains the basal tension on the cell-matrix adhesions. This basal tension enables mechanosensitive focal adhesion proteins to sense the increase in resistance, which results when the basal actomyosin tension pulls on a more rigid extracellular matrix. The increased tension at focal adhesions can cause calcium influx through stretch-activated calcium channels, trigger the integrin-dependent activation of focal adhesion kinase (FAK) and of Src, and change the conformation of certain mechanosensing proteins, such as p130Cas (also known as BCAR1), talin and vinculin, to initiate intracellular signaling and mechanotransduction (Moore et al., 2010).

Rho signaling also plays key roles in mechanotransduction. Activating signals or diminished microtubule stability recruit the Rho guanine-nucleotide-exchange factors (GEFs) GEF-H1 (also known as ARHGEF2) and leukemia-associated Rho GEF (LARG, also known as ARHGEF12) to β1-integin-containing focal adhesions (Guilluy et al., 2011b; Heck et al., 2012). These GEFs bind and activate RhoA by catalyzing the exchange of GDP for GTP. GTP-bound RhoA can activate many downstream targets, such as ROCK and the actin-nucleating family of mammalian homologues of Diaphanous (mDia) proteins. mDia increases actin stress fiber formation, and ROCK regulates phosphorylation of the regulatory myosin light chain to further increase actomyosin contractility (Fig. 1) (Nakano et al., 1999; Totsukawa et al., 2000). Thus, RhoA, ROCK and myosin II activity act in a mechanical feedback loop to respond to changes in extracellular matrix rigidity in 2D and 3D. In addition, an important feature of this mechanical signaling network is the contribution of extrinsic soluble factors, such as growth factors or cytokines, which can modulate RhoA activity to increase or decrease actomyosin contractility independently of matrix rigidity (Ridley and Hall, 1992).

The cell motility cycle

Cell migration depends on a series of discrete cellular mechanisms that function together during the cell motility cycle (Lauffenburger and Horwitz, 1996), a process that is best understood for metazoan cells adhering to and crawling over 2D surfaces (Abercrombie et al., 1970a; Ridley et al., 2003). Although the cell motility cycle and mechanotransduction share many components (e.g. regulation by Rho family GTPases, myosin II activity and cytoskeleton remodeling), these processes can be considered independently when the cell is moving across a structurally uniform surface.

The motility cycle begins when a stationary cell receives a motogenic signal, such as the growth factors or cytokines in serum, and becomes motile, forming distinct leading and trailing edges. Internal polarization of both microtubules and the secretory apparatus restrict lateral protrusions and facilitates the delivery of vesicular cargo to the leading edge (Bergmann et al., 1983; Gundersen and Bulinski, 1988; Kupfer et al., 1982; Vasiliev et al., 1970). The Rho family GTPases Rac1 and Cdc42 activate the actin nucleator Arp2/3 at the leading edge to polymerize actin and form thin, wide lamellipodial protrusions (Nalbant et al., 2004; Wu et al., 2012; Wu et al., 2009). The lipid second messenger phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P_3] is also enriched in lamellipodia during persistent migration in a given direction (Welf et al., 2012). Lamellipodia undergo cycles of protrusion and retraction, but they can be stabilized by the formation of nascent adhesions beneath the leading edge (Giannone et al., 2004). Arp2/3-dependent actin polymerization at the leading edge, in combination with myosin II activity, results in actomyosin contraction and the retrograde flow of filamentous actin (F-actin) towards the cell body (Ponti et al., 2004).

Actomyosin contraction leads to the enlargement and strengthening of nascent adhesions, which, through the process of adhesion maturation, then develop into focal adhesions (Choi et al., 2008; Vicente-Manzanares et al., 2007). Intracellular proteins in these maturing adhesions can act as components of a molecular clutch, linking the retrograde flow of F-actin to the extracellular matrix (Brown et al., 2006; Hu et al., 2007; Mitchison and Kirschner, 1988). Engagement of the molecular clutch slows the retrograde flow of F-actin and enables actin polymerization at the leading edge to push the plasma membrane forward. As the cell body and nucleus move over the mature focal adhesions, adhesion disassembly is initiated and actomyosin contracts the rear of the cell to form the trailing edge.

Although the basic components of the cell motility cycle (protrusion, attachment, contraction and detachment) are likely to be conserved among the different modes of 3D cell migration, the underlying molecular mechanisms can vary (for reviews, see Friedl and Wolf, 2009; Friedl and Wolf, 2010). These distinct mechanisms manifest as differences in overall cell morphology, the type and strength of cell–matrix adhesions, the speed of actin retrograde flow, or the directional persistence and velocity of migration (Danen et al., 2005; Doyle et al., 2012; Doyle et al., 2009; Lämmermann and Sixt, 2009; Renkawitz and Sixt, 2010). It is the structure of the leading edge, however, that appears to best define the mode of cell migration.

Protrusions define the mode of migration

The mode of cell migration has historically been classified on the basis of the morphology of the leading edge (Abercrombie et al., 1970b; Bovee, 1964; Trinkaus, 1973), and molecular studies have established that the different types of leading edge protrusions result from distinct combinations of signaling and actin regulatory proteins (recently reviewed in Charras and Paluch, 2008; Chhabra and Higgs, 2007; Ridley, 2011). Therefore, for the purposes of this Commentary, we classify the mode of 3D cell migration primarily by the type of structure that forms the leading edge (Fig. 2; Table 1).

The current nomenclature of cellular protrusions associated with cell motility is rooted in the morphological description of unicellular protozoa (Calkins, 1933). The term pseudopodium encompasses any dynamic structure extending from the cell body that is capable of extension and retraction. These pseudopodia, or more simply protrusions, occur as several functionally distinct types. Rhizopodia are anastomosing protrusions with retrograde transport towards the cell body, and they resemble retraction

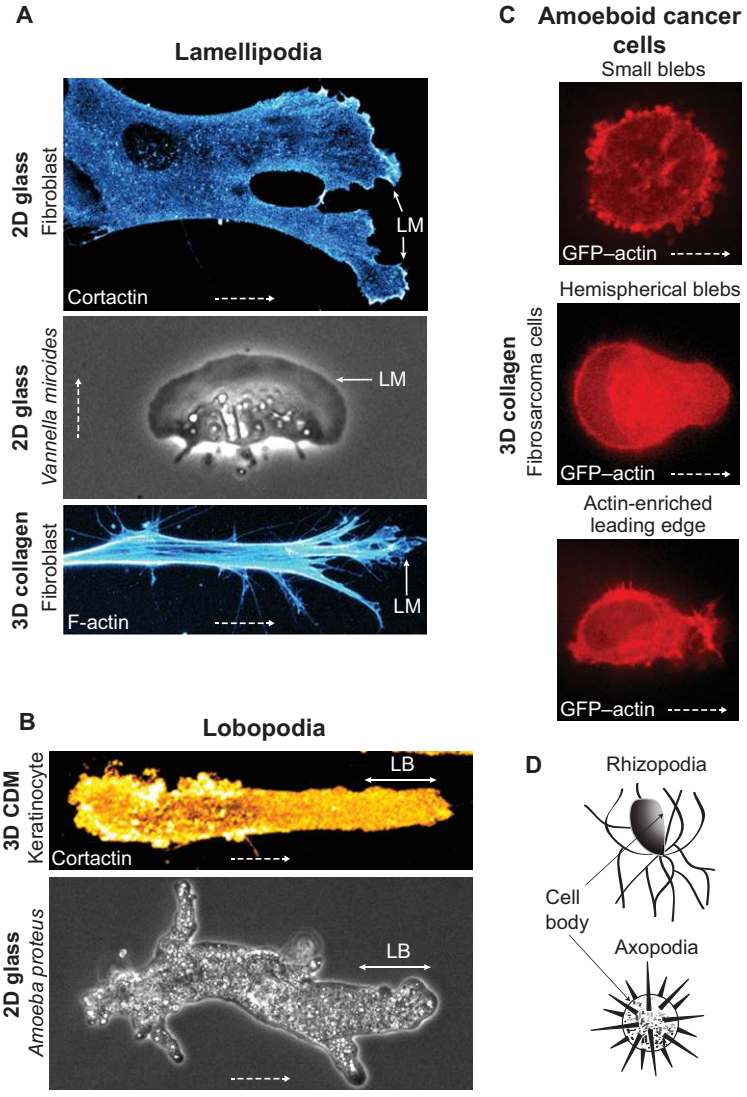

Fig. 2. Pseudopodium identity can define the mode of 2D and 3D cell migration. There is a wide diversity in the types of pseudopodia, or protrusions, that are used to extend the leading edge during cell migration on 2D surfaces and in 3D extracellular matrix. The type of pseudopodium can be used to define a specific mode of cell motility. (**A**) Lamellipodia-based migration is used by cells that migrate on 2D glass (upper and middle panels) and in a 3D collagen matrix (lower panel). Lamellipodia are thin fan-shaped protrusions enriched in F-actin and actin-binding proteins such as cortactin. (**B**) Lobopodia are blunt cylindrical protrusions that might be driven by intracellular pressure rather than actin polymerization. They are classically associated with giant amoeba (lower panel), but are also formed by metazoan cells migrating in linear elastic 3D material, such as cell-derived matrix (CDM) (upper panel). (**C**) Round amoeboid migration of cancer cells in 3D collagen comprises at least three distinct modes of migration that are characterized by multiple small blebs (upper panel), large hemispherical blebs (middle panel), or an actin-enriched leading edge (lower panel). (**D**) Rhizopodia and axopodia are pseudopodia used by certain protozoa to migrate and feed. LM, lamellipodium; LB, lobopodium. Broken white arrows indicate the direction of migration.

Table 1. The degree of adhesion and RhoA signaling can uniquely identify the mode of 3D migration

Mode of 3D migration	Cell shape	Mode switching	Adhesion	RhoA signaling	Polarized signaling	Rho and Rac cross-talk	References
Lobopodial	Elongated	Switch to lamellipodia	High	Required	No	Unknown	(Petrie et al., 2012)
Lamellipodial	Elongated	Switch to lobopodia (normal cells) and amoeboid (cancer cells)	High	Not required	Yes	In mesenchymal cancer cells Rac1 activity suppresses RhoA and amoeboid migration. Rac1 activity does not prevent normal fibroblasts from switching to lobopodial motility.	(Petrie et al., 2012; Sahai and Marshall, 2003; Sanz-Moreno et al., 2008)
Amoeboid (cancer cells)[a]	Round	Switch to lamellipodial migration	Low	Required	PtdIns(3,4,5)P_3 is not polarized	Activating Rac1 or inactivating RhoA will switch cells to lamellipodial movement.	(Lorentzen et al., 2011; Sahai and Marshall, 2003; Wolf et al., 2003)
Filopodial	Round or elongated	Can be found with lamellipodia, lobopodia, and blebs	High or Low	Not required	No	No	(Nalbant et al., 2004; Svitkina et al., 2003; Tomasek et al., 1982; Trinkaus, 1973)

[a]This term can refer to least three distinct modes of migration: small-bleb-based migration, hemispherical-bleb-based migration and migration with an actin-enriched leading edge. How these parameters compare amongst these different types of cancer cell migration is unclear.

fibers found on dividing or migrating tissue culture cells (Koonce and Schliwa, 1986; Mitchison, 1992). Axopodia are rigid radial projections that are enriched in microtubules (Tilney, 1968). Both rhizopodia and axopodia are used for movement and feeding by particular classes of protozoa (Lee et al., 1985). Lobopodia are blunt cylindrical protrusions driven by intracellular pressure, used by lobose amoeba (Kudo, 1977), and have been recently discovered in fibroblasts migrating in particular 3D environments (Petrie et al., 2012). Increased intracellular pressure, acting alone or in combination with local weakness in the cell cortex, can trigger the rapid expansion of spherical membrane blebs (Charras et al., 2005). These small cytoplasmic extensions are rapidly retracted by actomyosin contraction and can be seen on moving cells (Charras and Paluch, 2008). Lamellipodia are thin veil-like extensions of the cytoplasm that are driven by actin polymerization and are used by many tissue culture cells migrating in 2D and 3D environments (Abercrombie et al., 1970b; Petrie et al., 2012) and certain small amoeba (Lee and Jacobson, 1997). Finally, filopodia are actin-rich finger-like protrusions at the leading edge that act to sense the local microenvironment (Chhabra and Higgs, 2007). Filopodia can act alone or in combination with blebs, lobopodia or lamellipodia in 2D and 3D environments (Svitkina et al., 2003; Tomasek et al., 1982; Trinkaus, 1973). Although not directly associated with the leading edge, other types of pseudopodia are indirectly involved in cell motility. Invadopodia and podosomes are actin-rich structures, but they use integrin-dependent adhesion and proteolysis to degrade extracellular matrix proteins and penetrate 3D environments in normal and pathological settings (Chen, 1989; Linder et al., 2011).

The existence of so many functionally distinct leading edge structures might suggest that there are numerous independent mechanisms that are capable of sustaining cell movement. Such complexity would complicate our understanding of normal cell migration and the molecular defects leading to cancer cell invasion and metastasis. By contrast, individual metazoan cells have been observed to switch rapidly between different modes of 3D cell migration. This mode-switching behavior is often dictated by the level of activity of RhoA, ROCK and myosin II, suggesting that these ubiquitous components help to govern pseudopodium identity and the mode of 3D cell migration.

Mode switching during 3D cell migration

It is well known that cytoskeleton and actomyosin contractility can dramatically change cell morphology in response to extracellular cues. For example, the small amoeba *Vannella miroides* extends clear conical pseudopodia from a central cell body when it is floating in liquid, but forms flattened lamellipodia when migrating over 2D surfaces (Bovee, 1964; Lee and Jacobson, 1997). This adaptability is also well documented for ‘deep’ cells that migrate in the 2D space between the epithelium and the underlying internal yolk layer during embryonic development of the teleost *Fundulus* (Trinkaus, 1973; Trinkaus and Erickson, 1983); these rapidly migrating, weakly adherent cells use large hemispherical blebs for migration, similar to primordial germ cells (Goudarzi et al., 2012; Lämmermann and Sixt, 2009). The blebs bulge outwards in the direction of migration and fill with cytoplasm without the characteristic retraction phase that is associated with smaller blebs (Charras et al., 2005). During later developmental stages,

this distinct form of motility switches to a mode that uses a mixture of lamellipodia and filopodia. More recent studies have extended these observations of mode switching to normal and cancer cells moving through 3D extracellular matrix, and show how cells can integrate cues from the environment to trigger such mechanistically distinct forms of motility, as discussed below.

Mode switching by non-cancerous fibroblasts

Primary dermal fibroblasts can switch between lamellipodia- and lobopodia-based 3D migration (Petrie et al., 2012). Lobopodia are formed when fibroblasts in linear-elastic 3D materials (Box 1), such as dermal explants or cell-derived matrix, move in response to serum or platelet-derived growth factor (PDGF) and glucose. Fibroblast lobopodia are blunt cylindrical protrusions characterized by robust cell–matrix adhesions, non-polarized Rac1, Cdc42 and PtdIns(3,4,5)P_3 signaling, and, compared with those found in lamellipodia, low cortical levels of F-actin and actin-binding proteins. These cells also lack the extensive branched pseudopodia characteristic of fibroblasts in non-linear elastic materials such as a 3D collagen matrix (Bard and Hay, 1975). This adhesion-dependent mode of migration requires a high degree of actomyosin contractility because inhibiting RhoA, ROCK or myosin II prevents fibroblasts from responding to the linear elasticity of the 3D matrix and they then switch to a lamellipodia-based type of migration (Petrie et al., 2012). Soluble signaling factors can also regulate RhoA activity and the mode of 3D fibroblast migration. Treatment of primary fibroblasts with PDGF in the absence of glucose decreases RhoA signaling and causes cells to switch to lamellipodia-based migration in linear elastic 3D extracellular matrix.

In contrast to blunt cylindrical lobopodia, 3D lamellipodia are small fan-shaped structures at the tips of prominent pseudopodia (Grinnell et al., 2003; Petrie et al., 2012), although the length of the pseudopodia can vary depending on the motogen used to stimulate migration and the mechanical properties of the 3D collagen matrix (Kim et al., 2012). On 2D surfaces, however, lamellipodia tend to be wide flattened protrusions (Abercrombie et al., 1970b). Primary fibroblasts and many cancer cell lines use lamellipodial migration in non-linear elastic 3D environments, such as collagen gels. In cancer cells, this form of 3D cell migration is also known as the elongated or mesenchymal mode. Similar to the larger 2D lamellipodia, 3D lamellipodia are enriched in F-actin, the actin-binding proteins cortactin and the Wiskott–Aldrich syndrome protein family member WAVE, as well as active Rac1, Cdc42 and PtdIns(3,4,5)P_3 (Bass et al., 2007; Grinnell et al., 2003; Petrie et al., 2012; Sahai and Marshall, 2003; Wolf et al., 2003; Yamazaki et al., 2009). Although cells moving in 3D environments do not require high levels of RhoA, ROCK or myosin II activity to form lamellipodia, myosin II and ROCK can be required for rapid 3D cell migration. Myosin II inhibition significantly reduces the velocity of 3D cell migration, whereas ROCK inhibition only slows cells that move in pliable non-linear elastic 3D extracellular matrix (Doyle et al., 2009; Petrie et al., 2012; Provenzano et al., 2008). Similar to lobopodia, 3D lamellipodia are associated with the formation of robust adhesions to the surrounding matrix, with β1 integrin function required for rapid migration (Carragher et al., 2006; Deakin and Turner, 2011; Petrie et al., 2012; Wolf et al., 2003).

Box 1. Rigidity versus elastic behavior of 3D extracellular matrix

The rigidity or stiffness of a material can be represented by its Young's modulus (E), also termed the elastic modulus. In contrast, the elastic behavior of an extracellular matrix can be characterized as either non-linear or linear. Non-linear elastic materials undergo strain stiffening and become more rigid in response to increased force, whereas linear elastic materials do not (Storm et al., 2005). The rigidity and the elastic behavior of the extracellular matrix can modulate the velocity and the mode of 3D migration, respectively (Petrie et al., 2012; Zaman et al., 2006). Despite these differences, RhoA, ROCK and myosin II activity are required for both the response to 3D matrix rigidity and to elastic behavior. Although it is currently unclear whether the mechanisms governing these two unique forms of mechanotransduction are related, preliminary evidence shows the rigidity of an extracellular matrix, such as type I collagen, is responsive to changes in matrix density and cross-linking, whereas its elastic behavior might only be responsive to the degree of cross-linking (Petrie et al., 2012). We speculate that the actomyosin-contractility-dependent mechanosensing apparatus will be capable of discriminating between changes in matrix density and the degree of matrix cross-linking.

Mode switching by tumor cells

As with primary fibroblasts, many cancer cell lines are able to change their mode of migration, switching from the lamellipodia-based mesenchymal mode to the amoeboid or rounded form after extracellular proteolysis is inhibited (Wolf et al., 2003) or if the balance between RhoA and Rac1 signaling is shifted towards RhoA through Rho–Rac crosstalk (Fig. 1) (Guilluy et al., 2011a; Sanz-Moreno et al., 2008; Yamazaki et al., 2009). Although it seems reasonable to conclude that the motility of mesenchymal cancer cells corresponds to 3D lamellipodia-mediated migration, because there is a similar enrichment of F-actin at the tips of pseudopodia in these processes, it is less clear whether all cancer cells that are classified as 'amoeboid' due to their round morphology rely on a single type of protrusion to form the leading edge. The term amoeboid might in fact encompass at least three distinct modes of motility in rounded cancer cells (Fig. 2C) (Lorentzen et al., 2011). The adenocarcinoma cell line MTLn3 is morphologically round in 3D collagen, yet it has a leading edge that is enriched in F-actin (Wyckoff et al., 2006), similar to border cells migrating in *Drosophila melanogaster* egg chambers (Wang et al., 2010). Walker carcinosarcoma cells are round and protrude large hemispherical blebs in the direction of cell movement, which fill with cytoplasm without being retracted (Keller and Bebie, 1996), as described for *Fundulus* deep cells. Finally, several cancer cell lines move as round cells through 3D Matrigel and collagen by rapidly protruding and retracting multiple small blebs. These blebs can occur anywhere on the plasma membrane except at the rear of the cell, where ezrin links the plasma membrane to the actin cytoskeleton to inhibit blebbing by mediating the formation of a rigid uropod, thereby facilitating directional migration (Lorentzen et al., 2011; Poincloux et al., 2011). It will be important to distinguish between these different sub-types of amoeboid cancer cell migration in the future.

Despite these different leading edge structures, the rounded modes of cancer cell motility appear to share several characteristics. These cells generally require integrin-mediated adhesion to migrate and can deform the surrounding matrix as they move (Lorentzen et al., 2011; Wyckoff et al., 2006). However, their diffuse pattern of integrin localization in the plasma membrane might reflect weaker cell–matrix interactions

than those in normal cells undergoing 3D lamellipodial or lobopodial migration (Deakin and Turner, 2011; Petrie et al., 2012; Poincloux et al., 2011; Roca-Cusachs et al., 2009; Wolf et al., 2003). Amoeboid cancer cell migration is also strongly dependent on RhoA and ROCK signaling, along with actomyosin contractility (Sahai and Marshall, 2003; Sanz-Moreno et al., 2011). Reducing RhoA, ROCK or myosin II signaling by direct inhibition (Sahai and Marshall, 2003; Wilkinson et al., 2005) or indirectly through Rho–Rac crosstalk increasing Rac1 activity (Sanz-Moreno et al., 2008; Yamazaki et al., 2009) leads to a switch whereby round amoeboid cancer cells migrate using the elongated lamellipodial mode of 3D migration.

3D lobopodial, lamellipodial and amoeboid migration can be uniquely identified on the basis of a combination of only two characteristics: the degree of cell–matrix adhesion and the requirement for RhoA, ROCK or myosin II activity (Lämmermann and Sixt, 2009) (Table 1). Interestingly, the balance between cell–matrix adhesion and actomyosin contractility also governs the transition between lamellipodia- and bleb-based migration of Walker carcinosarcoma cells on 2D surfaces (Bergert et al., 2012). Given that these properties are also integral to the cellular response to matrix rigidity, it is possible to propose a general model in which mechanotransduction through the cell–matrix-adhesion–RhoA–ROCK–myosin-II axis dictates the mode of 3D cell migration (Fig. 3). It is possible that these distinct forms of migration are not mutually exclusive, but can be found in the same cell under some conditions.

Regulation of the mode of 3D migration by RhoA, actomyosin contractility and Rho–Rac crosstalk

Treating fibroblasts in 3D collagen with motogens triggers the cell motility cycle and lamellipodia-based migration. During migration in non-linear elastic 3D collagen matrix, RhoA activity and actomyosin contractility are relatively low, allowing Rac1-dependent signaling pathways to sustain lamellipodia-based migration (Petrie et al., 2012; Sanz-Moreno et al., 2008). This basal level of RhoA activity is likely to be sufficient for a response to changes in matrix structure and for regulating Rac1 activity to facilitate lamellipodia protrusions through the matrix (Guilluy et al., 2011b; Machacek et al., 2009). Reducing Rac1 signaling in normal (i.e. non-cancerous) fibroblasts that undergo 3D lamellipodial migration does not induce a switch to lobopodia-based motility (Petrie et al., 2012). This finding indicates that lamellipodial migration in normal fibroblasts migrating in 3D collagen is not maintained by Rac1 crosstalk-mediated inhibition of RhoA. We speculate that when fibroblasts sense linear elastic 3D material, such as cell-derived matrix or covalently cross-linked collagen, they respond by activating RhoA and increasing actomyosin contractility (Box 1). Although increased actomyosin contractility in fibroblasts on 2D surfaces can lead to cell rounding (Chartier et al., 1991), more effective cell–matrix adhesion in 3D environments could translate the increased actomyosin contractility into lobopodia-based migration by at least two mechanisms. First, increased RhoA activity could antagonize Rac1-dependent signal transduction and lamellipodia formation through biochemical crosstalk (for a review, see Guilluy et al., 2011a). A second possibility is that increased actomyosin contractility could physically alter the plasma membrane to dampen Rac1 signaling and lamellipodia formation. For example, localized Rac1 signaling is inhibited by increased tension in the plasma membrane (Houk et al., 2012; Katsumi et al., 2002). Therefore, elevated actomyosin contractility in a cell that moves through the 3D extracellular matrix could increase intracellular pressure and plasma membrane tension (Raucher and Sheetz, 1999) to prevent lamellipodia formation and lead to the cell switching to a

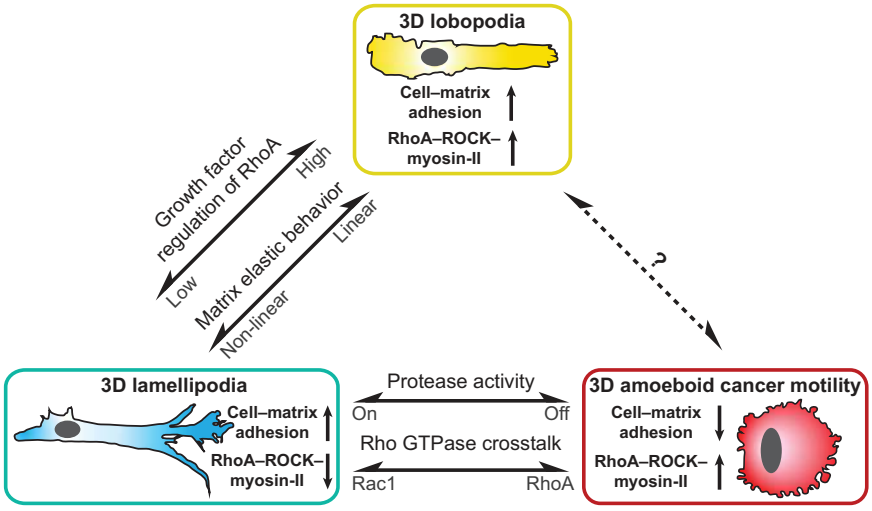

Fig. 3. Mechanical control of the mode of 3D cell migration. Three modes of 3D metazoan cell motility can be uniquely identified based on only two characteristics: their degree of cell–matrix adhesion and their requirement for RhoA, ROCK and myosin II activity. 3D lobopodia-based motility is associated with robust cell–matrix adhesion and requires RhoA signaling. Reducing RhoA activity, through soluble signaling factors or changes in the elastic behavior of the 3D matrix, causes adherent cells to undergo a transition to 3D lamellipodia-based motility. Cancer cells switch to the rounded low adhesion, high contractility mode of amoeboid cancer cell motility upon inhibition of protease activity or upon modulation of Rho GTPase crosstalk. Cells that use lamellipodia might have low membrane tension; therefore increased actomyosin contractility could elevate membrane tension and prevent lamellipodia formation during both lobopodial and round amoeboid cancer cell migration. It remains to be determined how cells capable of lobopodia-based migration transition to round bleb-based motility (indicated by the question mark).

lobopodia-based migration (Fig. 1). Rho protein crosstalk and plasma membrane tension could also cooperate during 3D cell migration to coordinate RhoA and Rac1 signaling in response to changes in actomyosin contractility (de Kreuk and Hordijk, 2012).

The transition from lamellipodia- to bleb-based cancer cell migration in a 3D collagen matrix also requires changes in Rac1, RhoA, ROCK and myosin II signaling (reviewed by Sanz-Moreno and Marshall, 2010). The balance between Rho and Rac signaling, as mediated by crosstalk, governs the shift between lamellipodial and round bleb-based migration of certain cancer cells (Fig. 1) (Sanz-Moreno et al., 2008; Yamazaki et al., 2009). In melanoma cells, the Crk-associated substrate (Cas) family member NEDD9 forms a complex with the GEF DOCK3 to activate Rac1 and promote mesenchymal cancer cell migration (Ahn et al., 2012; Sanz-Moreno et al., 2008). Active Rac1 suppresses amoeboid cancer migration through its effector WAVE2 (also known as WASF2), which acts through an unknown mechanism to reduce myosin light chain phosphorylation and actomyosin contractility (Sanz-Moreno et al., 2008). Similarly, active RhoA and ROCK can activate the GTPase-activating proteins (GAPs) and Rac1 antagonists ARHGAP22 (Sanz-Moreno et al., 2008) and FilGAP (also known as ARHGAP24) (Saito et al., 2012), possibly by increasing plasma membrane tension (Sanz-Moreno and Marshall, 2009) to decrease Rac1 signaling and lamellipodia formation. Because protease inhibition can also switch cancer cells to bleb-based motility (Wolf et al., 2003), it will be important to investigate whether it also increases the activity of RhoA, ROCK and myosin II.

The central role of RhoA signaling in promoting both lobopodial and amoeboid cancer cell migration might indicate that these two modes of 3D cell migration are closely related. It will be important to determine whether transformed cells utilize 3D lobopodia-based migration and conversely whether cells can directly switch between lobopodial and round-cell amoeboid 3D migration.

The relationship between the 3D motility of normal and cancer cells

Identifying how 3D cell migration becomes abnormally regulated in cancer cells is crucial for understanding cancer invasion and metastasis. The switch between lamellipodial and amoeboid 3D migration of cancer cells is likely to be related to a similar mechanism in normal untransformed cells (Sanz-Moreno and Marshall, 2010). However, the degree of similarity is difficult to establish without detailed comparisons between cancer cells and their normal counterparts. For example, primary fibroblasts and cancer cells share the ability to switch between different modes of 3D migration, yet N-Ras-transformed HT1080 fibrosarcoma cells form lamellipodia in linear elastic cell-derived matrix, in contrast to normal fibroblasts, which form lobopodia under these conditions (Petrie et al., 2012). We speculate these varying responses could be explained by the fact that HT1080 cells have an increased protease activity and reduced mechanotransduction compared with that in primary fibroblasts (Brenner et al., 2000; Jones and DeClerck, 1980). The increased proteolysis associated with HT1080 cells could locally reduce the rigidity and change the elastic behavior of the surrounding 3D matrix (Kirmse et al., 2011; Kirmse et al., 2012; Petrie et al., 2012). Such a matrix modification might diminish mechanotransduction and prevent HT1080 cells from properly responding to changes in matrix structure, thereby preventing adhesion-dependent lobopodia-based 3D migration. This hypothesis could be tested by experimentally manipulating the level of protease activity that is associated with normal and transformed cells and determining whether this resulted in a change in their mode of 3D migration.

Although the rounded hemispherical bleb-based mode of 3D migration is used by normal cells in some developmental settings, it remains to be established how these rounded bleb-based modes of cell migration are regulated in matched pairs of normal and cancer cells. Motility of rounded cancer cells is associated with an increased actomyosin contraction that is coupled with decreased integrin clustering and adhesion (Deakin and Turner, 2011; Lorentzen et al., 2011; Poincloux et al., 2011). Reduced adhesion and lower levels of integrin expression have also been associated with oncogenic transformation (Plantefaber and Hynes, 1989; Pylayeva-Gupta et al., 2011), and defective fibroblast adhesion has been correlated with a rounded cell morphology (Pouysségur and Pastan, 1976). We speculate that the diffuse localization of integrins in cell–matrix adhesions of migrating amoeboid cancer cells could be relevant to the characteristic behavior of their protrusions: cell extensions might be unable to resist the pulling force associated with increased actomyosin contractility, resulting in pseudopodia retraction and cell rounding (Maddox and Burridge, 2003). These differences might explain why increased RhoA, ROCK and myosin II signaling leads to motility by round cancer cells. whereas in primary fibroblasts with normal integrin expression and function it leads to elongated lobopodia-based migration.

Matrix adhesion and cell velocity

It is important to note that cell–matrix interactions and actomyosin contractility can regulate the speed of 3D cell migration without switching of the mode. Although leukocytes and fibroblasts can both use 3D lamellipodia-based migration, leukocytes use integrin-independent adhesion (Schmidt and Friedl, 2010) without actomyosin contraction to interact weakly with the surrounding matrix, whereas fibroblasts use integrins and actomyosin contraction to interact strongly with the matrix and produce high traction forces (Renkawitz and Sixt, 2010). During fibroblast migration in fibrillar environments, myosin-II-mediated contraction stabilizes integrin-based adhesions under the lamella, the region adjacent to the lamellipodium, to engage a molecular clutch and slow the retrograde flow of actin more efficiently than in fibroblasts migrating on a 2D substrate (Doyle et al., 2012). In combination, these factors enhance lamellipodial extension, stimulate forward progression of the lamella and facilitate rapid cell migration. In contrast, 3D lamellipodia-based leukocyte migration is associated with low actomyosin contractility, allowing for rapid integrin-independent low-traction movement (Lämmermann et al., 2008; Renkawitz and Sixt, 2010). Leukocytes can locally modulate the rate of actin polymerization at the leading edge to compensate for subcellular changes in matrix adhesion and increased traction forces, to maintain their rapid 3D migration (Renkawitz et al., 2009).

What is the function of mode switching?

Because mode switching occurs during 3D migration of both normal and cancer cells, it is likely to facilitate 3D motility directly or to participate in other aspects of cell function that are coupled with cell movement. For example, each mode of 3D migration might be particularly efficient in a given extracellular

matrix. This efficiency could manifest itself as either faster motility or increased directional persistence in response to an external cue. Although the functional relationship of the 3D migration to directionality is currently unclear, particular modes of 3D migration can be associated with more rapid migration, such as fibroblast migration through linear elastic 3D matrix in the absence of glucose (Petrie et al., 2012) and the migration of certain cancer cells through collagen (Pinner and Sahai, 2008; Sanz-Moreno et al., 2008; Wilkinson et al., 2005). However, there are examples in which switching to a different mode of migration is not associated with an accelerated migration. Protease inhibitors switch HT1080 cells from a lamellipodia-based migration to a round bleb-based amoeboid motility in loose 3D collagen without changing their velocity (Wolf et al., 2003). Similarly, inhibiting RhoA or ROCK activity during primary fibroblast migration in cell-derived matrix causes the cells to switch from lobopodia- to lamellipodia-based migration without reducing their speed (Petrie et al., 2012). If a specific mode of 3D cell migration is not always required for efficient migration, what other purposes might mode switching serve? There are at least two additional possibilities. First, the mode of 3D migration could have a role in other aspects of cell function that are linked with motility; in the case of normal fibroblasts it could facilitate the production and remodeling of extracellular matrix (Pattabiraman and Rao, 2010; Zhong et al., 1998), whereas the lamellipodia-based 3D migration of HT1080 cells might promote matrix degradation (Friedl and Wolf, 2009). Alternatively, rounded bleb-based and lobopodial migration could act to maintain leading edge protrusion at times when elevated intracellular pressure and membrane tension prevent the formation of lamellipodia.

Conclusions and perspectives

In this Commentary, we have reviewed accumulating evidence for the concept that the response of cells to the elastic behavior of the extracellular matrix and rigidity sensing by integrin-based adhesions, combined with the coordinated activity of RhoA, ROCK and myosin II, form the basis of a ubiquitous regulatory pathway governing the mode of 3D cell migration (Fig. 3). This model predicts that growth factor and cytokine regulation of RhoA signaling will be as important as the structure of the 3D extracellular matrix in determining the mode of cell migration. Therefore, triggering cell migration with a defined motogen, instead of a complex mixture of soluble factors as are found in serum, might be helpful in further exploring how cell motility depends on the dual influences exerted by the structure of the extracellular matrix and intracellular signal transduction (Grinnell and Petroll, 2010).

Precise regulation of the RhoA–ROCK–myosin-II axis and intracellular contractility mediated by the structure of the extracellular matrix and extrinsic soluble factors has an important role in many cellular processes. Elucidating how this universal mechanical signaling network governs the mode of 3D cell migration should help to reveal how it contributes to normal and dysregulated cell and tissue functions (Ingber, 2003; Rape et al., 2011).

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