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Guiding cell migration by tugging

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The ability of cells to move directionally toward areas of stiffer extracellular matrix (ECM) via a process known as 'durotaxis' is thought to be critical for development and wound healing, but durotaxis can also drive cancer metastasis. Migration is driven by integrin-mediated focal adhesions (FAs), protein assemblies that couple contractile actomyosin bundles to the plasma membrane, transmit force generated by the cytoskeleton to the ECM, and convert the mechanical properties of the microenvironment into biochemical signals. To probe the stiffness of the ECM, motile fibroblasts modulate FA mechanics on the nanoscale and exert forces that are reminiscent of repeated tugging on the ECM. Within a single cell, all FAs tug autonomously and thus act as local rigidity sensors, allowing discernment of differences in the extracellular matrix rigidity at high spatial resolution. In this article, we review current advances that may shed light on the mechanism of traction force fluctuations within FAs. We also examine plausible downstream effectors of tugging forces which may regulate cytoskeletal and FA dynamics to guide cell migration in response to ECM stiffness gradients.

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Introduction

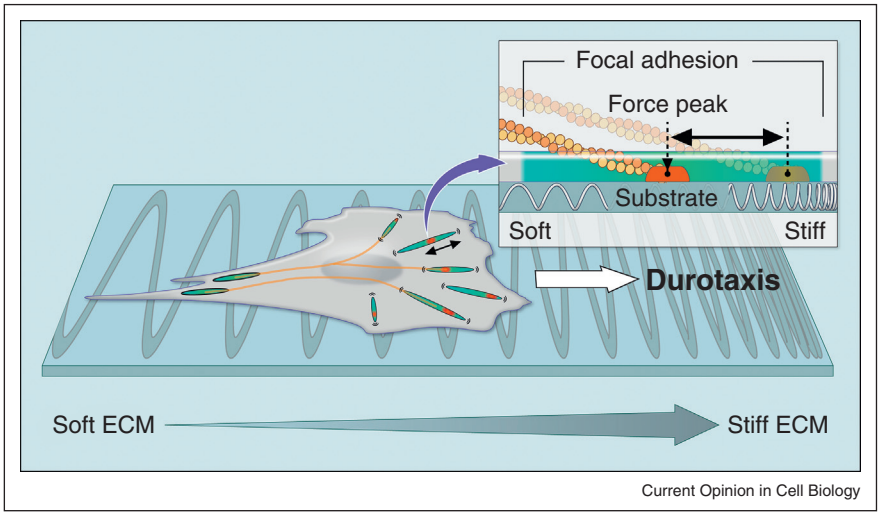
Directional cell movement is critical to embryonic development, immune system function, angiogenesis, and wound healing, as well as cancer metastasis. Cell migration is induced by a variety of signaling mechanisms that receive and process information from the cell's environment and provide specific control of cytoskeletal and adhesion machineries within the cell [1]. Historically, attention has been focused on understanding how diffusible or ECM-associated biochemical cues are transduced into activity of intracellular signaling networks that regulate cytoskeletal and adhesion dynamics. However, recent studies have highlighted the importance of physical cues such as ECM topology or rigidity in guiding cell migration. In particular, the propensity of cells to migrate toward areas of higher ECM rigidity via a process known as 'durotaxis' has garnered interest [2••]. Durotaxis is thought to contribute to physiological processes including stem cell differentiation [3,4], epithelial-to-mesenchymal transition [5,6], development of the nervous system [7,8], innate immunity [9], as well as promoting breast cancer or glioblastoma metastases [10,11].

The ability of cells to durotax in response to rigidity gradients requires mechanisms for constant surveillance of the variability in the stiffness landscape of the ECM in the cellular microenvironment. Several cellular structures have been proposed as force or rigidity sensors, including the plasma membrane [12], actin filaments [13••,14•], the cortical cytoskeleton [15,16], the nucleus [17], and cadherin-based adherens junctions [18]. However, there is extensive evidence that actomyosin-based contractility and integrin-based FAs are essential for ECM rigidity sensing [19,20]. Durotaxis is known to require myosin contractility [21], and the activity of FA proteins including FAK [22], paxillin, and vinculin [23], suggesting that integrin-based FAs serve as the rigidity sensors that specifically guide durotaxis. In this review, we focus on recent observations of the spatial and temporal dynamics of forces exerted by FAs during ECM rigidity sensing. We discuss possible molecular mechanisms that could mediate force dynamics in FAs and how force dynamics could be translated into polarized regulation of cytoskeletal and FA dynamics that drive directed cell migration.

Traction force fluctuations guide durotaxis

We recently used high-resolution traction force microscopy to characterize the nanoscale dynamics of cell-generated forces on the ECM [23]. Our studies revealed that mature FAs which appear static by other methods of microscopy may actually possess internal fluctuations in mechanics. FAs within a single cell were found to adopt one of two states: a stable state where traction was spatially and temporally static, and a dynamic state in which the pattern of traction fluctuations was reminiscent of repeated, centripetal tugging on the ECM. The choice between tugging and stable FA states could be predictably controlled by modulating ECM rigidity, myosin contractility, or a FAK/phosphopaxillin/vinculin pathway. Tugging traction in FAs was found to be dispensable for FA maturation, chemotaxis and haptotaxis, but critical for directed cell migration toward stiff ECM, i.e. durotaxis (Figure 1). Repeated FA tugging on the ECM suggests a means of regularly testing the local ECM rigidity landscape over time. ECM rigidity sensing by individual tugging FAs could allow tight control of directional

Figure 1



Nanoscale fluctuations of traction forces mediate ECM rigidity sensing and guide directed cell migration. Dynamics of traction forces within individual FAs are essential to direct cells toward stiff ECM. Zoomed insert depicts repetitive movement of force peak along individual FA (shown in green).

migration to guide cells along highly localized or dynamically changing ECM rigidity gradients during development or in tumors. FA-mediated sensing of local stiffness cues may also be utilized in addition to biochemical gradient sensing of diffusible and immobilized cues to fine-tune cell path-finding during development, morphogenesis, and pathological processes such as metastasis.

Mechanistic basis of force fluctuations

There are three basic components contributing to force on the ECM at an FA: (1) myosin II, which produces force on (2) actin filaments, which act as a conduit of the force to (3) FA proteins and integrins, which comprise the linkage between actin and the ECM through the plasma membrane. Dynamic changes in assembly/disassembly, activity, or protein–protein interactions within any of these three components could be responsible for mediating the fluctuations in force transmission seen in FAs (Figure 2).

Fluctuations in myosin contractility

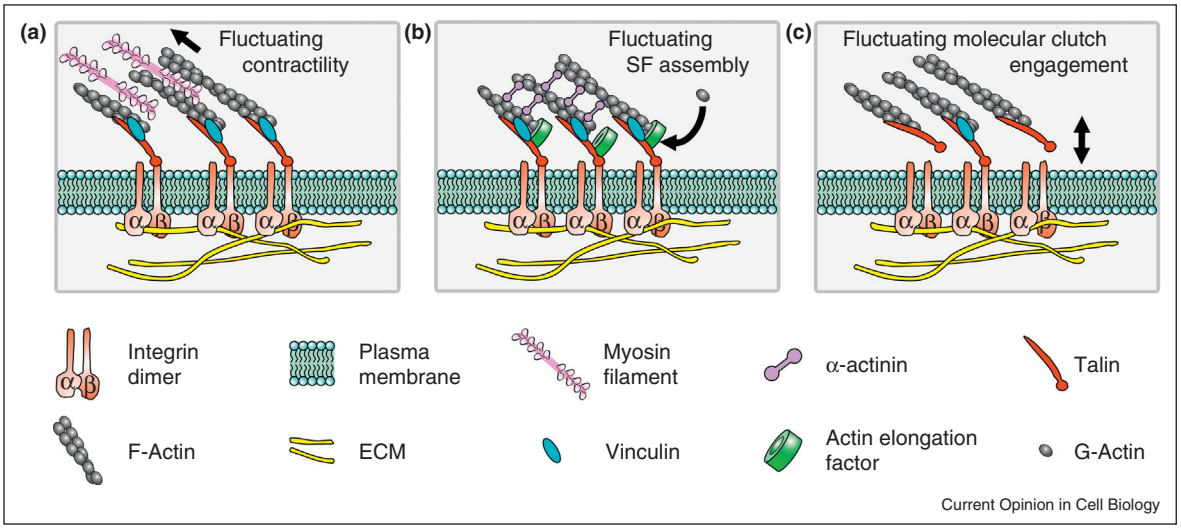
Temporal variations in myosin II contractility represent the most obvious mechanism for applying fluctuating pulling forces to the ECM. At the whole cell level, oscillating cell contractions driven by cyclical accumulation of myosin II on the actin network have recently been observed in non-excitable cells [24,25••,26]. However, global changes in actomyosin contractility are likely not responsible for fluctuations in traction force in FA, since force fluctuations in neighboring FAs, which are mechanically coupled to each other via the actin cytoskeleton [27], are not temporally correlated [23]. This suggests that dynamics of traction force are regulated locally, within single FA. Myosin II is closely associated with FAs [28] and interacts with FA components such as Rac guanine exchange factor β-pix [29]. Yet, myosin II is generally not considered a *bona fide* FA protein, because it tends to localize behind FAs within stress fibers and it is thought to transmit force from a distance through the stress fiber to the FA [30].

Mathematical modeling predicts that the activity of local contractile units could oscillate spontaneously due to the collective activity of motors acting on an elastic material [31]. Remarkably, such oscillations may be mechanosensitive, as for any given myosin activity the predicted oscillation frequency is determined by the elasticity of the FA and the ECM [32]. A direct demonstration that myosin accumulation at a FA affects the dynamics of its traction force is required to validate the notion that local fluctuations in contractility produce tugging forces at FA.

Fluctuations in actin mechanics

Since actin serves as the mechanical conduit between force generated by myosin and ECM-bound integrins, it is possible that cyclic variation in the mechanics of the actin cytoskeleton underlies traction fluctuations. Thus, cyclic variations in the assembly/disassembly of actin to modulate actin mechanics are a plausible mechanism for the origin of traction fluctuations at FA. There is mounting evidence that lamellipodial actin assembly occurs in cycles [33–35]. However, force fluctuations observed recently in fibroblasts occurred in mature FAs that are not associated with lamellipodial actin, but which are instead linked to actin stress fibers.

Figure 2



Possible molecular mechanisms mediating dynamics of traction forces within FAs. **(a)** Fluctuations of traction forces due to oscillations in local actomyosin contractility. The model shows minifilaments of non-muscle myosin II closely associated with FAs. Mathematical modeling predicts that activity of these local contractile units could oscillate spontaneously in a stiffness-dependent manner. **(b)** Fluctuations of traction forces due to cyclic variation in actin assembly at stress fibers. The model shows actin filaments within a stress fiber undergoing cycles of elongation mediated by a putative actin elongation factor, possibly Ena/VASP or formin family proteins. **(c)** Fluctuations of traction forces due to temporal variation in engagement between the actin cytoskeleton and the ECM. The model shows actin filaments being variably engaged to integrins via talin and vinculin.

Actin filaments within stress fibers terminate with their assembly-competent barbed ends linked to the FA [36]. These filaments could undergo cycles of assembly that would allow cyclic slack to develop in the stress fiber, and thus cyclic reduction in myosin-generated force transmitted to the ECM. Members of the Ena/VASP and formin families have been implicated as the main molecular players in the assembly of stress fibers at FAs [37–39] and could mediate cyclic assembly of F-actin at FAs. Ena/VASP proteins localize to FAs [40], and the related protein Mena binds directly to integrins [41]. Formins have been identified in FA proteomic screens [42–44], and inhibiting the activity of formin proteins disrupts FA traction and maturation [45••,46]. It was also recently demonstrated that VASP proteins are implicated in force-dependent stress fiber remodeling via interactions with zyxin [38,47••], and that formins allow accelerated barbed end elongation when under tension [48]. This suggests the intriguing possibility that fluctuations in contraction and actin assembly in stress fibers could cooperate via a positive feedback loop to mediate traction fluctuations at FAs [49•,50].

Fluctuations in the FA 'molecular clutch'

If contraction and actin polymerization are constant, tugging traction at FAs could also result from temporal variations in the strength or number of linkages between actin and the ECM via changes in protein–protein interactions within FAs. The putative chain of protein–protein interactions making up this linkage has been termed the 'molecular clutch' based on its role in regulating transmission of force from actomyosin to the ECM, analogous to the clutch of a car that regulates engagement or disengagement of forces generated by the engine to the mechanism that turns the wheels. There is extensive evidence for transient, regulatable interactions between actin and the ECM through FA proteins. Studies conducted by using FRAP (fluorescence recovery after photobleaching) imaging and fluorescent speckle microscopy have demonstrated that FA proteins bind and dissociate from FAs on a much faster time-scale than FA turnover and exhibit partial coupling to the actin cytoskeleton [51–53]. In particular, talin and vinculin are extensively implicated as load-bearing components mediating the regulatable, tunable link between actin and ECM-bound integrins [54–56]. Talin and vinculin interactions with actin, integrins, and other FA proteins are highly regulated through phosphorylation, lipid binding, and small GTPase signaling pathways [57,58]. Thus, it is possible that collective regulation of the integrin-binding or actin-binding affinity of talin and/or vinculin through oscillations in signal transduction could produce tugging force dynamics in FAs.

An alternative mechanism for generating tugging traction force from a simple 'motor — clutch' system that does not require positive or negative feedback or signaling pathways for regulation was recently predicted by a stochastic mathematical model [59••]. The model consisted of actin undergoing retrograde flow, 'clutch molecules' whose

engagement to actin and the ECM was dictated by association rate constants and whose disengagement was dictated by both rate constants and force (i.e. breaking strength), and ECM stiffness. The model predicted two distinct regimes of force transmission to the ECM within an individual FA: static and oscillating. The static force regime was promoted by stochastic association and disassociation of many clutches across a FA resulting in even, constant traction force centered within the FA. This state occurred when engaged clutch lifetime was short and ECM stiffness was high. The oscillating regime, which was promoted by long engagement lifetime and low ECM stiffness, was the result of cooperative engagement and simultaneous failure of local clusters of clutches within a FA, generating oscillations in traction magnitude and peak traction force position within the FA. Thus, the simple motor-clutch model generally accounts for stiffness-dependent changes in traction dynamics that have been observed in neurons and fibroblasts [23,59••]. However, order-of-magnitude differences between model predictions and experimental measurements in the rate of traction force increase call in to question whether this simple mechanism operates in vivo. Furthermore, this model does not account for the polymerization of actin that is known to occur at FAs.

Decoding traction dynamics by downstream effectors

To migrate directionally along gradients of ECM stiffness, cells require a mechanism to continuously measure variability in the stiffness landscape of the ECM and control cytoskeletal and adhesion dynamics. Cells sample stiffness by exerting actomyosin-generated pulling forces on the surrounding ECM through FAs [19,20]. Fluctuations of traction stress within FA may be a means by which cells repeatedly tug at the ECM to detect spatial and temporal changes in rigidity. But why is fluctuating FA traction required for durotaxis on a spatially and temporally *stable* ECM rigidity gradient but not for chemotaxis or haptotaxis [23]? Tension on FA proteins is thought to drive conformational changes including stretching or unfolding, which alter protein–protein interactions, induce recruitment of cytosolic proteins, and activate signaling pathways [60]. This suggests that fluctuating or oscillating signals may be specifically required for durotaxis. Although we still do not know which signaling pathways transduce dynamics of tugging forces into cellular behavior, only those which are: firstly, mechanically activated; secondly, originate from integrin FAs; and thirdly, exhibit fluctuating activity could be regulated by the dynamics of traction forces. Among known signaling pathways involved in the regulation of cell migration, integrins, their effectors and stretch-activated ion channels satisfy these criteria and will be discussed below.

Cyclic activation of integrins

One obvious candidate for an effector of tugging traction dynamics that may mediate directed cell migration is integrins themselves. Indeed, it is well established that integrin affinity for ECM is enhanced by tension [61–63]. However, a recent elegant study showed that while a single tug on an α5β1 integrin increased its affinity for ligand, the cyclic application of force reinforced the integrin–ligand bond, prolonging bond lifetime by two orders of magnitude to maintain single bonds for minutes [64••]. This suggests that repeated application of tension on integrins by FA tugging could be a mechanism to prolong adhesion lifetime. Thus, in a cell on an ECM stiffness gradient, tugging on the FAs coupled to a stiffer ECM region may overcome some threshold for cyclic mechanical reinforcement, while this threshold may not be reached in FAs coupled to the softer matrix area. This would result in preferential reinforcement of FAs in the direction of greater stiffness to promote directed migration.

Several signaling cascades downstream of integrin activation have been shown or predicted to exhibit oscillating activity and are known to regulate cytoskeletal and adhesion dynamics. Thus, in addition to reinforcing integrin adhesion, repeated tugging on integrins could induce cyclic activation of integrin signaling. FAK and Src tyrosine kinase activities are induced by integrin activation and force [65] and are known to play an important role in cell mechanosensing, migration, and invasion [22,66]. Although oscillations of Src/FAK activation have been predicted by mathematical modeling [67,68], they have not been observed experimentally. The Rho-family GTPases RhoA, Rac1, and Cdc42 have been implicated as master regulators of cell motility by controlling cytoskeleton and FA dynamics and are known to be activated by integrins and mechanical stimuli [69]. Live-cell imaging of biosensors has recently revealed coordinated cyclic activity of these three GTPases at the leading edge of migrating fibroblasts [70]. Although these oscillations could be induced by force fluctuations on integrins, the presence of both positive regulation by GEFs and negative regulation by GAPs and GDIs suggests that a robust biological oscillator in GTPase activity could arise in the absence of external triggers [71,72]. Indeed, RhoA activity oscillations that are critical to leading edge protrusion have been shown to be dependent on cycles of PKA-mediated RhoA phosphorylation, which induces a RhoA-RhoGDI interaction to inhibit Rho activity [73••]. Since PKA activation is integrin-dependent, this suggests that cyclic force on integrins could be the upstream activator of these signal oscillations. However, whether signal oscillations are required for motility and how they are decoded to produce directional migration in response to a rigidity gradient is not known.

Cyclic activation of stretch-activated Ca^{2+} channels

Regulation of calcium signaling by tugging forces is also a plausible mechanism for how traction dynamics direct cell migration toward stiff ECM. Ca^{2+} is a well-known

master regulator of cell migration, and front-to-rear gradients of intracellular Ca^{2+} underlie polarization and migration during chemotaxis [74,75]. Cytoplasmic Ca^{2+} controls actomyosin by activating myosin II [76], as well as the gelsolin/villin family of F-actin severing proteins [77]. Ca^{2+} also promotes FA turnover by activating calpain family proteases [78], which cleave several FA proteins including talin [79]. Thus, it is possible that a gradient of ECM stiffness could give rise to a gradient of local calcium influx, which in turn could promote local contraction as well as actin filament and FA turnover to cause cells to move toward stiffer ECM.

It is tempting to speculate that intracellular calcium gradients could be produced by stretch-activated calcium channels (SACs), whose activity is induced by repeated tugging on the ECM [80]. SACs are activated by mechanical stimulation, which triggers transient opening and ion flux [81]. Blocking SAC activity suppresses several mechanically induced cellular responses, including cell migration, FA maturation and traction force development [82,83], suggesting that SACs could both cause traction fluctuations as well as mediate their downstream effects. Importantly, sustained mechanical stimulus does not maintain SAC activity due to channel adaptation [81]; thus, maintenance of a localized Ca^{2+} gradient in cells via SACs would require repeated local mechanical stimulus. Recently, striking transient calcium 'flickers' mediated by the TRPM7 SAC were visualized at the front of migrating fibroblasts [84••]. Indeed, TRPM7 has been implicated in FA function, as it localizes to FAs and promotes calpain protease activity and FA turnover when overexpressed, while TRPM7 silencing increases FA strength [85]. Thus, fluctuations in traction force at FAs could promote repeated activation of SACs, possibly TRPM7, to maintain locally increased Ca^{2+} at the leading edge, thereby affecting FA and cytoskeletal dynamics to direct cell migration.

Open questions and future perspectives

Although transient changes in FA mechanics have a role in ECM-rigidity sensing and durotaxis, whether this mechanism contributes to other mechanosensitive processes, such as cell differentiation or epithelial-to-mesenchymal transition, remains to be investigated. Demonstrating that tugging forces regulate a specific cellular function, such as durotaxis, without affecting other mechanosensitive responses would be an important advance for future development of therapeutics aimed at modulating ECM-guided cell migration. Another open question is whether soluble factors can modulate migratory or invasive properties of cells by regulating the dynamics of traction forces at FAs. Indeed, although traction fluctuations are required for durotaxis, they strongly decrease the velocity of random cell migration [23]. In addition, growth factors may impact on cell motility through suppression of tugging traction, either by activating actomyosin contractility or by decreasing the strength of FAs [86,87]. In agreement with this hypothesis, decreased FA strength correlates with the onset of cancer cell motility, suggesting a functional link between cell invasiveness and the dynamics of traction forces at FAs [87]. It remains to be determined if traction fluctuations are required for metastasis, in which case they could become a therapeutic target.

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