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Directed migration of mesenchymal cells: where signaling and the cytoskeleton meet

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Abstract

Cell migration directed by spatial cues, or taxis, is a primary mechanism for orchestrating concerted and collective cell movements during development, wound repair, and immune responses. Compared with the classic example of amoeboid chemotaxis, in which fast-moving cells such as neutrophils are directed by gradients of soluble factors, directed migration of slow-moving mesenchymal cells such as fibroblasts is poorly understood. Mesenchymal cells possess a distinctive organization of the actin cytoskeleton and associated adhesion complexes as its primary mechanical system, generating the asymmetric forces required for locomotion without strong polarization. The emerging hypothesis is that the molecular underpinnings of mesenchymal taxis involve distinct signaling pathways and diverse requirements for regulation.

Introduction

Chemotaxis, or cell migration directed by an external chemical gradient, is a primary means of intercellular communication. For example, two very different examples of chemotaxis are encountered during the inflammatory and proliferative phases of cutaneous wound healing [1]. During the inflammatory phase, neutrophils and macrophages are recruited from the circulation by gradients of soluble and immobilized chemokines, and once in the wound, these cells move chemotactically to ingest debris and bacteria. This is a rapid process, established within hours. By comparison, the proliferative phase spans days to weeks and is characterized by the proliferation and relatively slow chemotactic migration of fibroblasts, which are recruited from the collagen-rich dermis into the fibrinogen- and fibronectin-rich provisional matrix of the clotted wound. The primary chemotactic signal for the invading fibroblasts is platelet-derived growth factor (PDGF), released by platelets and macrophages [2]. The role of PDGF as a chemoattractant generally translates to other mesenchymal

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tissues (stroma), as seen in embryogenesis [3] and cardiovascular function [4,5]. PDGF signaling also plays a prominent role in tumorigenesis [6]. It is established that chemotactic signals influence cancer cell invasiveness, and thus metastasis, and growth factor signaling has been implicated in aggressiveness of mesenchymal tumors [7-10] and in reciprocal communication between carcinomas and nearby stromal cells [11,12]. In carcinomas, PDGF receptor signaling emerges in cancer stem cells following the epithelial-to-mesenchymal transition, a program associated with invasiveness [13•]. From these indications it is apparent that directed migration of mesenchymal cells is fundamentally important in both normal tissue homeostasis and in progression of disease.

Here, we examine evidence that characterizes mesenchymal chemotaxis, and other forms of directed migration exhibited by mesenchymal cells, as distinct from directed migration of leukocytes and other amoeboid cells. Whereas a common theme in cell locomotion is the generation of force applied in an asymmetric fashion, a mesenchymal cell exhibits unique architectures and dynamics of the actin cytoskeleton (and associated adhesion complexes) as its primary mechanical system. Accordingly, recent studies on mesenchymal cells suggest that signal transduction linking PDGF gradients and other spatial cues to local control of the actin cytoskeleton involves distinct molecular pathways and/or diverse requirements for regulation.

Mesenchymal versus amoeboid migration

Despite its pervasiveness in tissue development, homeostasis, and cancer, mesenchymal chemotaxis is poorly understood. Indeed, the bulk of the chemotaxis literature has focused on amoeboid cells such as neutrophils and the amoeba, *Dictyostelium discoideum* [14,15]. Amoeboid and mesenchymal motility modes lie at opposite extremes of cell migration phenotypes [16] and reflect the coordinated functions of the respective cell types (**Fig. 1**). The amoeboid migration phenotype is characterized by rapid locomotion (cell speed ~ 10 μm/min), a property attributed to the strong polarization that allows these cells to efficiently protrude via pseudopods and blebs and squeeze through pores in the connective tissue, largely unfettered by interactions with extracellular matrix (ECM) [17]. Amoeboid motility reflects the roles of neutrophils and lymphocytes as ‘professional migrators’ that must rapidly respond to crawl out of the circulation and then across great distances in secondary tissues to mediate innate and adaptive immunity, respectively [18]. In contrast, mesenchymal cells move slowly (cell speed < 1 μm/min) and are weakly polarized, typically exhibiting multiple, competing protrusions (lamellipodia and filopodia) [19]. Another characteristic feature that limits the efficiency of mesenchymal motility is strong, integrin-mediated adhesion to ECM. This ‘friction’ is tuned by the cells’ ability to degrade matrix, through expression of matrix metalloproteinases, and to disassemble otherwise stable focal adhesions [20]. This reflects the intimate relationship between mesenchymal cells and matrix in general, exemplified by the role of fibroblasts in secretion and mechanochemical remodeling of ECM during wound repair.

Chemotactic gradient sensing is generally mediated by chemoattractant receptors of different types in amoeboid and mesenchymal cells. In neutrophils and lymphocytes, gradients of chemoattractants (e.g., chemokines such as IL-8, LTB4, CXCL12, and

CXCL13, and N-formyl peptides shed by bacteria) are sensed by cognate receptors of the G protein-coupled receptor (GPCR) class. In fibroblasts, chemoattractants (certain growth factors, PDGF most notably but also fibroblast growth factors and epidermal growth factor (EGF)) are sensed by receptors of the receptor tyrosine kinase (RTK) class. Although it is true that the two classes of receptors largely access many of the same signaling pathways, i.e., those mediated by small GTPases and lipid second messengers, there are substantial differences in the dynamics of the two receptor types. A hallmark of GPCR regulation is desensitization, whereby agonist exposure results in rapid attenuation of the response. The theory of GPCR desensitization and adaptation as it relates to chemotaxis of amoeboid cells is well established. Adaptation is considered important for amoeboid cells' ability to sense the relative steepness of a chemoattractant gradient, allowing cells to respond over a broad range of absolute concentration [21,22]. Moreover, amoeboid cells are able to prioritize multiple chemoattractant cues and respond to them in sequence [23]. In a recent demonstration of this concept, primary neutrophils responding to gradients of IL-8 and LTB4 oriented in opposite directions oscillate/vacillate between the two, suggestive of switching between distinct sensing states [24•].

These receptor-level complexities do not have a known analog in RTK signaling. RTKs are subject to downregulation through the endosomal and ubiquitin-proteasome degradation pathways. As a consequence, in fibroblasts responding to a high dose of PDGF, tyrosine phosphorylation of its cognate receptors is transient on a time scale of ~ 1 hour [25]. Yet it is well known that prolonged exposure to growth factors is required to render cells competent for cell cycle progression, suggesting that receptor downregulation is simply a mass-action effect by which receptor expression is dampened when the growth factor concentration is far above physiological. Accordingly, it has been shown that multiple signal transduction pathways are prominently activated at sub-nanomolar concentrations of PDGF [25,26], which are far below receptor saturation and thus predicted to yield minimal receptor downregulation. Another established consequence of RTK endocytosis is growth factor (i.e., ligand) clearance. Mathematical modeling suggests that receptor-mediated clearance of PDGF might be important for maintaining a sharp gradient of the chemoattractant as fibroblasts collectively invade a wound [27]. Experimental evidence in a different chemotactic context, midbrain development in zebrafish [28•,29•], supports this concept.

Mesenchymal and amoeboid cells show certain similarities but also striking differences in their cytoskeletal organization that directly relate to their different modes of migration. Both cell types use the Arp2/3 complex to build dendritic arrays of actin filaments at their protruding edge(s). The Arp2/3 complex nucleates new filaments as branch points from existing ones and is a primary means of actin polymerization in lamellipodia [30,31]. Interspersed with the branched actin filaments are bundled, unbranched actin arrays that often protrude as finger-like filopodia. Alternate actin assembly pathways, such as those orchestrated by formins and Ena/VASP proteins, produce these structures [31,32]. These non-Arp2/3-based actin assembly pathways have been functionally linked to chemotaxis in neutrophils and tumor cell lines [33,34,35•]. Away from the leading edge, actin structures such as acto-myosin contractile arrays are quite different between mesenchymal and amoeboid cells. In amoeboid cells, Myosin II is primarily confined to the rear of the cells in a structure known as the uropod and is thought to provide a squeezing force that is both

functionally coupled to actin polymerization-based protrusion at the front of the cell [36] and critical for bleb-based protrusions [16]. In mesenchymal cells, Myosin II is associated with bundled actin stress fibers; whereas the Myosin IIB isoform is located mainly in retracting regions of the cell, Myosin IIA is found throughout the cell [37]. Contractile acto-myosin stress fibers are required for the strong substrate adhesion observed in mesenchymal cells and play a central role in regulation of membrane protrusion and overall cell migration [37-39]. Yet, the contribution of acto-myosin regulation to chemotaxis is poorly understood.

Role of the PI3K/Rac/WAVE/Arp2/3 circuit: directional sensing or efficient movement?

One mechanistic insight that initially seemed to unify the gradient sensing mechanisms of eukaryotic chemotaxis is receptor-mediated recruitment and activation of type I phosphoinositide 3-kinases (PI3Ks). These enzymes produce the lipid second messenger PIP_3 at the plasma membrane, and PIP_3 is readily dephosphorylated to form other phosphoinositides. Although GPCRs and RTKs mediate activation of differentially regulated isoforms of the PI3K enzyme, a common feature of the pathway in chemotaxing cells is formation of a spatially asymmetric pattern, with higher densities of 3' phosphoinositides biased in the direction of the chemoattractant gradient [22,40,41]. Another common signaling intermediate that generally exhibits bias in the direction of cell locomotion is the active, GTP-bound form of Rac [42,43]. PIP_3 and Rac-GTP each target multiple effectors and thus can promote cell migration in both subtle and direct ways; arguably the most direct is their synergistic recruitment and activation of the WAVE regulatory complex, which in turn activates the Arp2/3 complex [44]. Among the other targets of PIP_3 are certain guanine nucleotide exchange factors (GEFs) that mediate increases in Rac-GTP, and in turn Rac-GTP can promote PI3K signaling [43,45,46], in theory suggesting a complex signaling circuit with coherent feedforward and/or positive feedback loops that are thought to endow amplified sensitivity and robust polarization of signaling during chemotaxis [36,47,48,49•]. Based on these insights it has been assumed that activation of this signaling circuit leading to focal enhancement of Arp2/3-mediated F-actin polymerization is a common basis for gradient sensing, i.e., the chemotactic 'compass', perhaps with subtle variations across cell types and chemoattractant gradient conditions [40,50] (**Fig. 2A**).

In the context of mesenchymal chemotaxis in particular, various lines of evidence challenge this model. One is the use of direct observation chemotaxis chambers and cell tracking, which, together with advances in molecular interventions such as RNA interference, trump previous methods such as Boyden chamber assays. Recent studies indicate that neither PI3K [51] nor Rac [52] is absolutely required for PDGF chemotaxis. Modulation of these signaling intermediates affects cell morphology and migration speed, and even subtle changes in Rac signaling alters persistence of randomly migrating fibroblasts [53,54•]. But the ability to sense and respond chemotactically to a PDGF gradient is not grossly affected. Even more compelling is the observation that fibroblasts depleted of Arp2/3 complex, which completely lack dendritic F-actin and lamellipodia, show reduced cell speed but no change in fidelity of PDGF chemotaxis [55••]. Although another study implicated the Arp2/3 complex as essential for EGF chemotaxis [56•], the discrepancy with the abovementioned PDGF study can be attributed to differences in chemotactic chamber design and a non-

autonomous effect of factors secreted by Arp2/3-deficient cells [57•]. In weakly polarized mesenchymal cells, the signaling circuit leading to Arp2/3 activation is apparently required for efficient locomotion but not for gradient sensing (**Fig. 2B**).

Studies employing live-cell imaging of fluorescent protein biosensors support this alternative view. Localization of active Rac consistently accumulates after, not before, the onset of leading-edge protrusion [58,59]. Local Rac signaling is clearly sufficient to drive membrane protrusion and directed migration [46,60], so how are these observations reconciled? We recently showed that the role of PI3K signaling in fibroblasts is not to initiate protrusion but rather to stabilize nascent lamellipodia; the propagation of this process manifests as branching of lamellipodia and large-scale reorientation of migration directionality, which allows fibroblasts to efficiently align their locomotion towards a PDGF gradient [61•]. The implication is that PI3K and Rac signaling are important amplifiers that drive the engine of cell motility, but in mesenchymal cells they take their cue from a different process. As the search continues for signaling pathways that are required for mesenchymal chemotaxis, what is clear is that PI3K and Rac signaling are not simply redundant, i.e., replaceable ways to achieve gradient sensing by converging on the Arp2/3 complex. A wholly different means of asymmetric force generation must be at play. For example, PDGF receptor signaling might mobilize actin nucleators other than Arp2/3 complex or regulate myosin contractility (**Fig. 2B**). A clue supporting the latter possibility is the observation that PDGF stimulation of fibroblasts reduces RhoA activity at the cell front [62].

Directed migration towards a diverse set of spatial cues

While recent studies have seemingly yielded more questions than answers about the mechanisms of mesenchymal chemotaxis, even less is understood about other forms of directed migration that mesenchymal cells exhibit [63] (**Fig. 3A**). Given the aforementioned importance of ECM in their physiology, it is not surprising that mesenchymal cells engage in haptotaxis, or migration biased by a gradient of immobilized ligands. Adhesive ligands in ECM are recognized by various integrins that cluster to form nascent adhesion complexes under lamellipodia, some of which grow to form mature focal adhesions that are mechanically coupled to large, contractile actin stress fibers. In addition to these differences in mechanical linkages between the ECM and F-actin, nascent and mature adhesions have different signal transduction properties, with nascent adhesions mediating Rac signaling and thus Arp2/3-based lamellipodial protrusion [64-66]. Interestingly, whereas the Arp2/3 complex is dispensable for PDGF chemotaxis in fibroblasts, it is absolutely required for haptotaxis towards a variety of ECM cues [55••]. In haptotaxis, the cell must actively send out protrusions to encounter anchored ligands, while in chemotaxis, ligand molecules are encountered passively by diffusion. This difference might explain the requirement for proper protrusive structures and dynamics for successful haptotaxis. A hybrid form of chemotaxis and haptotaxis also exists, in which growth factors and chemokines that can function as soluble cues also bind to ECM and direct migration as immobilized ligands [67•,68••]. Whether this mode of directed migration is more similar to chemotaxis or haptotaxis in terms of molecular requirements remains to be determined.

Cell migration is also directed by mechanical and electrical cues. Certain cell types have the ability to sense and respond to a gradient of mechanical stiffness, a process known as durotaxis or mechanotaxis [69-71]. Mesenchymal cells are unique because the variable stiffness of their microenvironment is largely a property of the ECM (thus, durotaxis and haptotaxis are related), and because of the magnitude of the traction forces that they exert [72]. Recent work points toward the mechanical sensitivity of focal adhesions in durotactic responses [73••]. Cyclic fluctuations in force produced at focal adhesions allow cells to tug on flexible substrates and gauge relative stiffness. The regulation of several focal adhesion components, including FAK, vinculin, and paxillin, are critical for this durotactic sensing. A possible mechanotransduction mechanism is the activation of Rho, which is mediated by adhesions under tension, leading to activation of myosin contractility [74•]. Indeed, in mesenchymal stem cells migrating from soft to stiff matrix, there is a dramatic change in Myosin IIA/B organization [75•]. In future work, it will be exciting to see the process of durotaxis examined in more physiological settings and in other cell types, particularly those lacking classical focal adhesions. In addition, it will be interesting to study the interplay between haptotaxis and durotaxis when both ECM ligand density and mechanical compliance are being sensed. Cells also respond to electrical gradients (electrotaxis or galvanotaxis) in situations such as wound healing. Sensing this type of cue does not require ionic flux across the membrane, but it does seem to require electrophoretic displacement of membrane components and some intracellular signaling pathways such as PI3K [76,77•].

It is important to note that cells engaged in directed migration *in vivo* likely encounter multiple types of cues that they must simultaneously evaluate and prioritize to achieve an appropriate physiological response. For mesenchymal cells, two situations where directed migration plays a significant role are cutaneous wound healing and tumor cell invasion following epithelial-to-mesenchymal transition (**Fig. 3B,C**). During wound healing, dermal fibroblasts migrate into the clotted wound in order to reorganize and resynthesize the matrix. PDGF emanating from platelets and macrophages in the provisional matrix is a critical directional cue for these cells, but it seems likely that haptotactic, durotactic, and galvanotactic cues also play a role in fibroblast recruitment. How these cues might act in concert remains an open question, but some evidence indicates that direct crosstalk between integrin and PDGF signaling regulates mesenchymal stem cell migration [78]. Similarly, during tumor progression, a subpopulation of tumor cells adopt an invasive, mesenchymal phenotype and migrate away from the primary tumor [79]. These cells migrate towards blood and lymphatic vessels as part of the metastatic cascade of tumor dissemination. The directional migration cues in these situations are incompletely understood, but in the case of mammary adenocarcinoma these cells are responding to EGF cues released by tissue macrophages [80] as well as mechanical stiffness of the surrounding matrix [81,82]. Much remains to be learned about directed migration during pathophysiological situations such as metastatic cancer.

Unifying principles of directed migration

A guiding principle that unifies all forms of directed migration is asymmetric force generation aligned with the extracellular cue. In some cells, this is likely achieved by localized actin polymerization at the leading edge. However, other sources of asymmetric

force, such as differential adhesion, myosin motor activity, or osmotic pressure [83•] could serve this purpose. A second principle is the ability to spatially or/and temporally sense variations in the external environment, and to link that sensing via signal transduction to actuate a mechanical response. Hence, understanding directed cell migration will require greater focus on the interface between signaling and cytoskeletal networks.

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• Special interest

•• Outstanding interest

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thus provides a foundation for understanding the coupling between signaling and cytoskeletal dynamics.]

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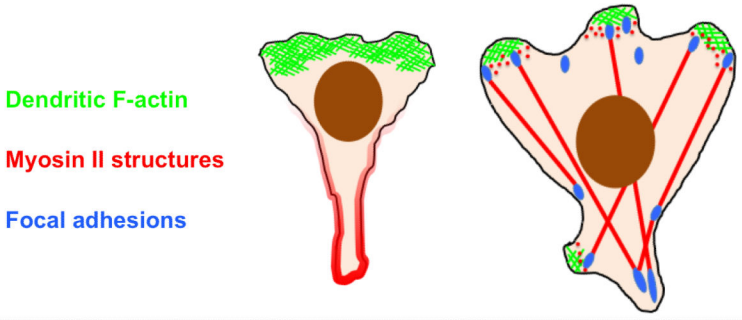

	Amoeboid	Mesenchymal
Migration speed	Fast, ~ 10 μm/min	Slow, < 1 μm/min
Polarity	Well-defined front and rear	Multiple, competing lamellipodia
Adhesion	Relatively weak, mostly intercellular	Strong, mostly ECM with well-defined adhesion complexes
Migration mechanics *in vivo*	Squeezing through pores in matrix/stroma	Traction via adhesion to ECM, matrix degradation as necessary
Organization of actin cytoskeleton	Thick dendritic actin network at the cell front; elsewhere, cortical actomyosin mediates contractility beneath the plasma membrane	Dendritic F-actin in lamellipodia; acto-myosin minifilaments mediate contractility behind the leading edge(s) and form thick stress fibers attached to focal adhesions
Chemoattractant receptors	GPCRs	RTKs

Figure 1. Mesenchymal vs. amoeboid motility and chemotaxis

The illustrations and table compare the structural and dynamic features of mesenchymal migration to those of amoeboid cells such as neutrophils and lymphocytes.

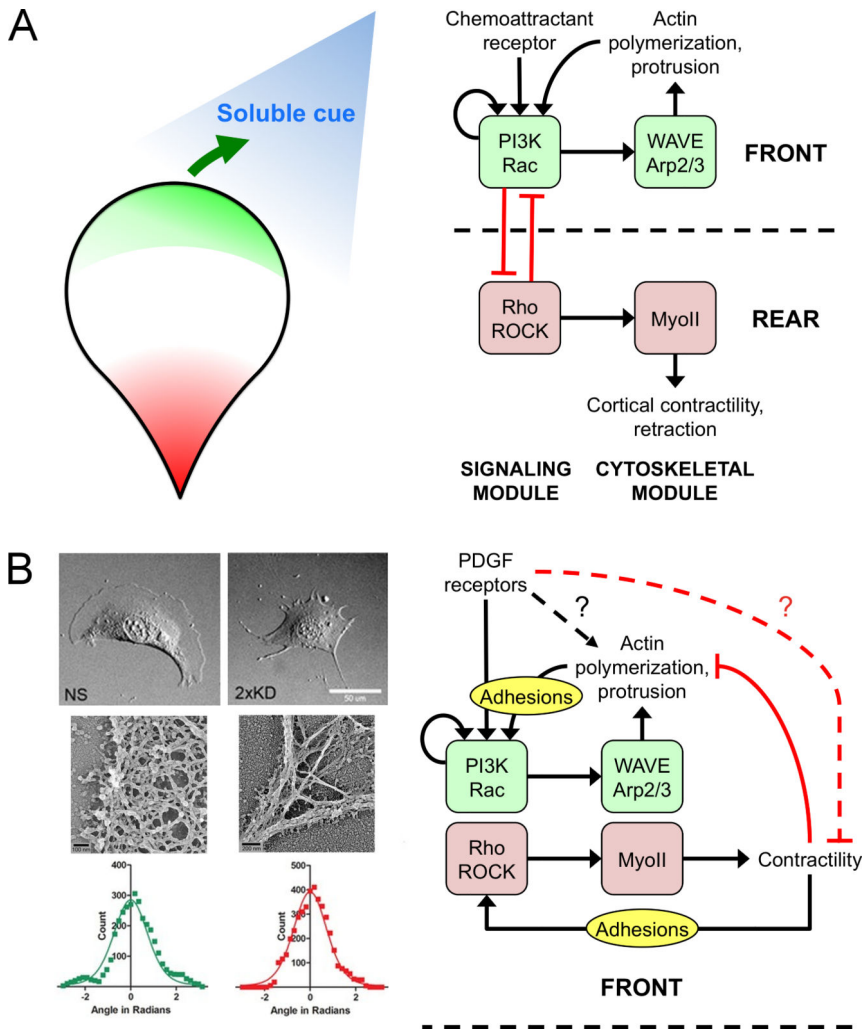

Figure 2. Clarifying the role of the PI3K/Rac/WAVE/Arp2/3 circuit in mesenchymal cells

(A) In the conventional model of gradient sensing in amoeboid cells, PI3K and Rac are engaged in a signaling module that controls Arp2/3-mediated actin polymerization at the front of the cell. Cells establish and maintain polarity through positive feedback in this circuit, combined with its functional incompatibility with Rho signaling and active Myosin II (MyoII) at the cell rear. An external cue simply introduces a bias of the Arp2/3 circuit towards the left or right of the migration axis. (B) The alternative model of mesenchymal chemotaxis is spurred by the observation that depletion of Arp2/3 complex in fibroblasts results in loss of dendritic F-actin arrays associated with lamellipodia but does not affect chemotactic fidelity. Panels at left adapted from Wu et al. [55••] (Copyright 2012 Elsevier Inc., used with permission). In this model, the Arp2/3 circuit follows the cue of an as yet uncharacterized gradient sensing mechanism and is important for agile cell movement during chemotaxis as well as random migration. This functional distinction is consistent with the relative lack of polarization in mesenchymal cells, in which Arp2/3 and myosin modules are neighbors, and the role of adhesion complexes in activating them to elicit protrusion and retraction of lamellipodia, respectively.

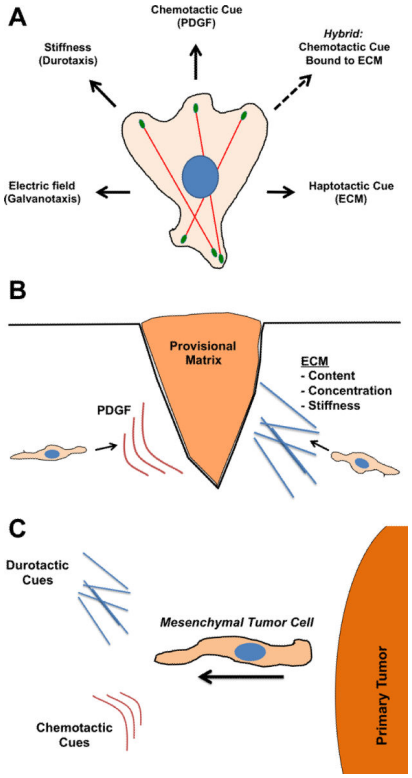

Figure 3. Directed migration cues for mesenchymal cells

(A) Diagram illustrating the diverse types of directional cues that mesenchymal cells respond to. Of note is the hybrid cue where chemotactic cues (e.g., growth factors) are bound to ECM scaffolds. (B) During cutaneous wound healing, fibroblasts (prototypical mesenchymal cells) respond to both PDGF (chemotaxis) and ECM cues (haptotaxis/durotaxis). (C) Likewise, mesenchymal tumor cells emerging from primary tumors sense multiple directional cues.