Physical Biology in Cancer. 4. Physical cues guide tumor cell adhesion and migration

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Stroka KM, Konstantopoulos K. Physical Biology in Cancer. 4. Physical cues guide tumor cell adhesion and migration. Am J Physiol Cell Physiol 306: C98–C109, 2014. First published October 16, 2013; doi:10.1152/ajpcell.00289.2013.—As tumor cells metastasize from the primary tumor location to a distant secondary site, they encounter an array of biologically and physically heterogeneous microenvironments. While it is well established that biochemical signals guide all stages of the metastatic cascade, mounting evidence indicates that physical cues also direct tumor cell behavior, including adhesion and migration phenotypes. Physical cues acting on tumor cells in vivo include extracellular matrix mechanical properties, dimensionality, and topography, as well as interstitial flow, hydrodynamic shear stresses, and local forces due to neighboring cells. State-of-the-art technologies have recently enabled us and other researchers to engineer cell microenvironments that mimic specific physical properties of the cellular milieu. Through integration of these engineering strategies, along with physics, molecular biology, and imaging techniques, we have acquired new insights into tumor cell adhesion and migration mechanisms. In this review, we focus on the extravasation and invasion stages of the metastatic cascade. We first discuss the physical role of the endothelium during tumor cell extravasation and invasion and how contractility of endothelial and tumor cells contributes to the ability of tumor cells to exit the vasculature. Next, we examine how matrix dimensionality and stiffness coregulate tumor cell adhesion and migration beyond the vasculature. Finally, we summarize how tumor cells translate and respond to physical cues through mechanotransduction. Because of the critical role of tumor cell mechanotransduction at various stages of the metastatic cascade, targeting signaling pathways involved in tumor cell mechanosensing of physical stimuli may prove to be an effective therapeutic strategy for cancer patients.

tumor metastasis; extravasation; three-dimensional migration; matrix stiffness; cell adhesion

CELL ADHESION AND MIGRATION are ubiquitous events that underlie diverse physiological and pathological processes, including tissue morphogenesis, the immune response, and cancer metastasis. Recent experimental evidence has indicated that, in addition to chemical signals, physical cues from the cells’ microenvironment also influence cell adhesion and motility in a range of physiological contexts. Physical cues acting on cells in vivo include extracellular matrix (ECM) mechanical properties, dimensionality, and topography, as well as hydrodynamic shear stresses and local forces due to neighboring cells. The mechanisms of cancer metastasis, in particular, have been found to depend heavily on the physical cues from the many complex microenvironments experienced by tumor cells during their metastatic journey (147) (Fig. 1). This is largely due to the process of “mechanotransduction,” where cells translate mechanical forces into cellular responses through biochemical signaling pathways. Importantly, state-of-the-art technologies have recently allowed researchers to engineer microenvironments that mimic specific physical properties of the cellular microenvironment in the context of tumor cell metastasis (Fig. 2). While no in vitro assay will ever be fully capable of exactly replicating the full in vivo situation, the technologies discussed in this review have enabled the elucidation of how cells respond to various physical cues during metastasis. Hence, our understanding of cancer metastasis is moving forward due to the integration of physics, biology, and engineering strategies.

An early step in the metastatic cascade, after metastatic cells have dissociated from the primary tumor and invaded the local tissue, is intravasation into a blood vessel. During circulation in this vascular “highway” system, tumor cells physically and biologically interact with and adhere to other cell types, including the endothelium lining blood vessels, and they also experience significant hydrodynamic shear forces due to blood flow. Subsequently, the tumor cells extravasate across the endothelium to exit the circulatory system. From there, tumor cells navigate the complex, heterogeneous ECM of the neigh-

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boring tissue before finally localizing to the site where a secondary tumor will form. This review focuses on the range of mechanical and dimensional cues experienced by tumor cells specifically during the extravasation and migration periods of the metastatic cascade (Fig. 1) and how the tumor cells translate and respond to these signals through mechanotransduction.

Tumor Cell Extravasation

After initial interactions with the adhesion proteins on the surface of the vascular endothelium (74), tumor cells must cross the endothelial cell (EC) barrier during the next step of the metastatic cascade, i.e., extravasation. Given that the circulation is a harsh environment, subjecting tumor cells to a range of forces, including hemodynamic stress, collisions with other cell types and the vascular wall, and physical confinement in the smallest blood vessels, it is likely that extravasation from the blood vessel represents a key step during the metastatic cascade, after which tumor cells have access to ECM-rich tissues and other survival factors. However, some tumor cells may form a secondary tumor without ever leaving the vasculature, forming an “intravasculature metastasis” (1). Initially, it was believed that tumor cell extravasation could be mechanistically similar to leukocyte extravasation, which occurs during an immune response. Indeed, cancer cells have been reported to migrate through the body of ECs via the “transcellular” route (57, 73) and also between EC junctions via the “paracellular” route (145), two courses commonly used by leukocytes (2, 17, 18, 121, 130, 144, 148). During extravasation via the paracellular route, a leukocyte creates a small, localized gap in the vascular endothelial cadherin within the endothelium (121, 130). After the leukocyte has passed through the endothelium, the gap closes within several minutes, and additional leukocytes frequently pass through at the same location, where they form a secondary tumor. CEA, carcinoembryonic antigen; MUC16, mucin 16; PODXL, podocalyxin.

1 This review is part of a five-article theme series on Physical Biology in Cancer in this issue.
tumor cells cause significant disruption to the endothelium (10, 38, 57, 93, 159). As such, the mechanisms by which tumor cells cross the endothelium seem to be fundamentally different from immune cells, although much more research is necessary to fully understand this process.

Conventional methods for studying tumor cell extravasation have relied on 1) tail vein injection of tumor cells, followed by in vivo imaging and subsequent analysis (1, 140), 2) the Boyden assay (32, 56, 85), or 3) a three-dimensional (3D) collagen gel assay (43). While each of these methods offers its own advantages, none presents an optimal combination of visualization ability and biophysical parameter control, thus limiting the degree of spatial and temporal quantitative information that can be derived from such experiments. Recently, microfluidic technologies have been utilized to address the growing need for better models of tumor cell extravasation (24, 70, 124, 141, 157, 159). In addition, optical transparency of the zebrafish has aided in real-time observation of tumor cell extravasation in vivo. Using a combination of these methods, we are beginning to understand the mechanisms by which tumor cells cross the EC barrier and the mechanical role of the endothelium during this process.

**Physical role of the endothelium during tumor extravasation.** The endothelium is an important transducer of hemodynamic forces during blood vessel homeostasis and also in cardiovascular disease (29) and is far from a simple passive lining of the vasculature. Even before extravasation takes place, tumor cells physically interact with the vascular endothelium through a variety of proteins, including glycoproteins (48, 74). Similar to leukocytes, there is substantial evidence that these initial adhesive interactions are mediated by molecules expressed on the surface of tumor cells, which bind to E-, L-, or P-selectins on
the endothelium (Fig. 1). We have summarized the current mechanistic knowledge of these interactions in a previous review (74). Our laboratory has reported that sialofucosylated carinoembryonic antigen (136), podocalyxin (27, 135), and mucin 16 (22), which are expressed by numerous metastatic tumor cells, are functional E- and L-selectin ligands. Meanwhile, sialofucosylated CD44 variant isoforms represent the primary functional P-selectin ligand on the tumor cell surface (53, 101).

Not only does the endothelium facilitate initial adhesive events between the tumor cells and the vascular wall, but its mechanical state also regulates tumor cell extravasation. Endothelial cell-cell junction stability is associated with myosin light chain kinase (MLCK-) and Rho kinase-dependent EC contractility, which depends on subendothelial matrix stiffness (64, 78, 128, 131) (Fig. 1). Furthermore, the cytokine TNF-α, which is released by stromal cells during an immune response, and also oxidized LDL, which accumulates during cardiovascular disease, induce EC contractility and trigger actin polymerization and reorganization into stress fibers (12, 14, 122, 128, 131, 132). Consequently, increased EC contractility promotes leukocyte transmigration in the context of a normal immune response (128), cardiovascular disease (54, 131), and aging (64). Thus the integrity and mechanical properties of the endothelium represent key parameters in determining the efficiency of leukocyte extravasation. Mounting evidence suggests that this relationship also holds for tumor cell extravasation and that tumor cells are able to produce biological factors that optimally tune EC contractility to promote tumor cell extravasation.

One study evaluated the effectiveness of the endothelium as a vascular barrier against the invasion of 51 tumor cell lines, ranging from noninvasive to invasive, into a 3D collagen matrix (93). Only 9 tumor cell lines demonstrated decreased invasion in the presence of an EC monolayer; 17 cell lines became invasive or demonstrated significantly enhanced invasion. These results suggest that, for some tumor cells, the endothelium actually promotes invasion, rather than acting as a physical barrier and preventing tumor cell extravasation.

How then does the endothelium become hyperpermeable? Do tumor cells selectively adhere and extravasate in regions of inflamed vasculature? Alternatively, at any point in the vasculature, are tumor cells capable of producing biochemical factors that upregulate EC adhesion molecules and increase EC permeability, without a prerequisite for prior endothelium activation? While it is plausible that either scenario may occur, it is known that tumor cells secrete large amounts of VEGF (118), which increases vascular permeability, tumor cell-EC adhesion, and transendothelial migration of breast cancer cells through human brain microvascular ECs (83). Other vasoactive soluble factors secreted by metastatic tumor cells to increase vascular permeability include 12(S)-hydroxyeicosatetraenoic acid (59, 60), angiopoietin-2 (58, 62), chemokine (C-C motif) ligand 2 (150), O-glycosylated factors (100), fibrinogen (119), hepatocyte growth factor/scatter factor (90), 2,4,6,2-penta-chlorobiphenyl (36), and stromal cell-derived factor-1α (82; for review see Ref. 47). Thus the tumor cells are able to change the physical state of the endothelium to promote subsequent extravasation.

In addition to soluble factors secreted by tumor cells, EC mechanical properties also change as tumor cells begin to invade the endothelium. MLCK and downstream myosin II become locally activated when MDA-MB-231 breast cancer cells begin to invade calf pulmonary artery ECs, leading to increased EC contractility, according to a 3D Förster resonance energy transfer-based study (73). Coculture of ECs with MDA-MB-231 breast cancer cells decreases EC stiffness, as measured by magnetic tweezers, and also induces EC cytoskeletal remodeling (92). These mechanical changes lead to significant endothelial damage and monolayer disruption, which likely further facilitate extravasation of tumor cells.

**Tumor cell contractility in extravasation.** During transendothelial migration, neutrophils, monocytes, and T cells exert traction forces that are necessary for completion of the transmigration process (55, 130, 151). Inhibition of myosin II-, MLCK-, or RhoA-mediated contractility in these cells prevents full retraction of the leukocyte or lymphocyte cell body under the endothelium, resulting in incomplete transmigration and, thus, reduced extravasation efficiency. Meanwhile, very little is known about the role of tumor cell contractility during the extravasation step of the metastatic cascade. However, there exists some evidence that tumor cell contractility may be specifically important for extravasation. For example, inhibition of Rho-associated protein kinase (ROCK-) and MLCK-mediated traction forces in tumor cells decreases tumor cell invasion through human pulmonary microvascular ECs (92).

Furthermore, tumor cell expression of Gro-β and the IL-8 receptor (CXCR2) results in increased tumor cell force generation and cytoskeletal remodeling dynamics (93), thereby physically weakening the EC barrier to promote extravasation. This process is reminiscent of how ovarian cancer spheroids produce traction forces to physically clear away the mesothelium; this process requires αβ3-integrin, talin 1, and myosin II (67). Hence, tumor cell extravasation involves contractility and retraction in the endothelium and also in the tumor cells themselves.

**Migrating Beyond the Vasculature**

Upon exiting the blood vessel, tumor cells must navigate a complex tissue microenvironment while seeking a hospitable location to form a secondary metastatic tumor (Fig. 1). A significant amount of our understanding of tumor cell migration (and cell migration in general) has stemmed from studies utilizing two-dimensional (2D), homogenous, stiff, ECM-coated surfaces (44, 97, 112, 117). On a 2D substrate, cell migration is driven by actin-based protrusion at the cell’s leading edge, integrin-mediated adhesion to the substrate in distinct locations along the basal surface of the cell (i.e., focal adhesions), and myosin II-mediated contractile forces to promote de-adhesion at the trailing edge (81). However, the native tissue microenvironment in which tumor cells migrate in vivo is actually in 3D, or even one dimension (1D), as well as mechanically and biologically heterogeneous (44, 149). In the following sections, we review the various physical cues that direct cell migration and adhesion, with a primary focus on matrix dimensionality and mechanics. We also discuss several state-of-the-art cell migration assays used to evaluate the effects of various physical parameters within the cell’s microenvironment (see Ref. 111 for a detailed review of traditional and recent developments in cell migration assays).
Themes

Microenvironment dimensional cues in tumor cell adhesion and migration. Longitudinal tracks with bordering 2D interfaces (i.e., channels) exist between the connective tissue and the basement membrane of muscle, nerve, and epithelium (44). Furthermore, spaces between adjacent bundles of collagen fibers in fibrillar interstitial tissues also create 3D longitudinal channels (44). Similar channels may also be created by fibroblasts or leader cancer cells that degrade the matrix through proteolysis, creating a void tunnel in which follower cancer blasts or leader cancer cells that degrade the matrix through channels (44). Similar channels may also be created by fibroblasts or leader cancer cells that degrade the matrix through proteolysis, creating a void tunnel in which follower cancer blasts or leader cancer cells that degrade the matrix through channels (44). Parallel to the basement membrane of muscle, nerve, and epithelium, 2D and longitudinal tracks with bordering 2D interconnections likely exist in complex environments beyond that which simple 2D assays can replicate. Cell-derived and reconstituted ECM gels have been used for analysis of 3D adhesion and migration. For example, the architecture of collagen and Matrigel shares some distinct features of the native in vivo microenvironment, including the presence of pores and fibrillar structures. On 2D surfaces, cells adhere to the matrix via focal adhesions (50, 96), which promotes cell migration. However, when cells are partially embedded into a 3D matrix, focal adhesions become smaller and possess altered composition compared with those on 2D substrates (25, 26, 89). Strikingly, focal adhesions are no longer detected when cells are completely embedded into a 3D matrix (43, 45, 109), suggesting that cell adhesion to the surrounding matrix plays a reduced role during migration in 3D environments. Furthermore, in a study relating metastatic and migratory potential to adhesion strength on 2D substrates, the least adhesive (but most metastatic) cells exhibited the slowest migration speed; meanwhile, in 3D matrices, the lack of adhesion in highly metastatic cells allowed for the greatest migration speed (66). Thus, adhesion seems to affect cell migration in a dimension-sensitive manner.

In addition to altered adhesive phenotypes in 3D matrices, cells also exhibit different migration patterns as a result of matrix dimensional cues. For example, fibrosarcoma cells in 3D matrices undergo highly regular, periodic oscillatory migration patterns upon depletion of zyxin (or its binding partners α-actinin and p130Cas) (42), a protein found in focal adhesions, stress fibers, and the leading edge of many motile cells. Therefore, zyxin ensures that cells undergo random migration in 3D ECMs, although the exact mechanism is unknown. The oscillatory migration in zyxin-depleted cells is not observed on conventional 2D substrates, although it can be recapitulated on 1D micropatterned ECM stripes.

Other studies have similarly observed remarkable resemblances between migratory phenotypes in 1D and 3D, while 2D and 3D cases do not seem to be comparable. Microphotopatterning has been used to create complex ECM patterns on the surface of materials (33). This technique can be used to create 1D micropatterened lines that mimic the aligned fibrillar architecture in ECM matrices and that are relevant to mouse tumor (113) and in vivo metastasis models (125). In contrast to 2D, NIH 3T3 fibroblast migration along 1D lines and in 3D matrices is rapid, uniaxial, independent of ECM ligand density, and more dependent on microtubule polymerization (21, 33). Also, fibroblast adhesions along 1D lines are small, punctate, and polarized (21, 33) and are reminiscent of the diminished adhesive structures in tumor cells in 3D matrices (43) or in longitudinal channels (5). These experiments have been extended to include H-ras-transformed mouse fibroblasts (the PAP2 line, with NIH 3T3 fibroblasts as the parental cell line), which seem to be defective in dimension sensing. For example, H-ras-transformed mouse fibroblasts are more likely to exit 2D areas of matrix into 1D lines than are normal 3T3 cells; therefore, transformed cells do not accumulate on 2D regions over time, as the normal cells do (21). Thus, dimensional cues can significantly alter cell adhesion and migration phenotypes.

New microfluidic strategies to explore effects of physical confinement on tumor cells. A more controlled and systematic approach to studying tumor cell migration in such complex settings is by engineering the cellular microenvironment using sophisticated microfabrication techniques, such as microfluidics or microcontact printing. Microfluidic devices are particularly useful, since they allow multiple biological and physical cues (e.g., chemotactic gradient, biological soluble factors, surface ligand density, physical confinement, topography, microenvironment stiffness, and hydrodynamic forces) to be presented and controlled simultaneously within the same experiment. A microfluidic device with tapered channels has recently been used to show that human metastatic MDA-MB-231 breast cancer cells enter physically restrictive spaces at a greater frequency than nonmetastatic cells (88). We recently developed a chemotaxis-based microfluidic device containing channels of varying width (137) and used this device to study the mechanisms of tumor cell migration in varying degrees of physical confinement (5, 23). These devices are formed from polymethylsiloxane and coated with ECM protein (e.g., collagen or fibronectin). Tumor cell migration through wide channels (50 μm wide × 10 μm high) replicates the earmarks of typical 2D migration and depends heavily on actin polymerization, adhesion to the substrate, and contractility (5). Conversely, MDA-MB-231 metastatic breast cancer cell migration through narrow channels (3 μm wide × 10 μm high), where cells are completely confined, persists even in the absence of actin polymerization and myosin II-mediated contractility (5). When each of these processes is inhibited, cells are still capable of migrating up the chemotactrant gradient through the narrow confining channels (Fig. 3). These results align with in vivo data, where invasion of the leading fibroblasts or cancer cells requires not only proteolysis, but also Rho/ROCK activity, while migration of the following cancer cells through the precarved space is independent of Rho/ROCK activity (46). Similarly, a microfabricated collagen track assay has also demonstrated that MDA-MB-231 cell migration through void microtracks can occur independently of matrix metalloproteinase (MMP) production; these results suggest that pharmacological foci on MMPs may not inhibit all modes of tumor cell migration in vivo (77).

As in 3D matrices, cell migration in longitudinal channels is also less dependent on cell adhesion than is 2D migration (5, 63). As previously mentioned, tumor cells are still capable of migrating efficiently through narrow channels in the absence of ECM ligand (5, 63) or when treated with an anti-β1-integrin antibody (5). Furthermore, tumor cells completely confined in narrow channels do not form large focal adhesions at the interfaces with the four walls of the channel but, rather, form small nascent adhesions (5), reminiscent of observations in 3D matrices (43, 45, 109).
**Dimensional control of cell traction forces.** Because the localization of cell traction forces usually correlates with the presence of focal adhesions, we hypothesized that tumor cell traction forces become less important during migration in confined spaces. To address this idea, we developed a novel microfluidic device that incorporates deflectable ECM-coated microposts onto the bottom surface of the four-walled (wide or narrow) channels (114); cells migrating through the channels deflect the pillars as they exert traction on the bottom wall of the channel, thus providing a way to quantitatively measure cell contractility in confined spaces. Using this device, we found that, consistent with a decreased dependence on adhesion, tumor cell migration in narrow channels leads to decreased cell traction forces. On 2D surfaces, focal adhesion formation and traction force generation are integral steps in the cell migration process (98); however, human osteosarcoma cells migrating in narrow channels (3 μm wide × 10 μm high) compared with wide channels (50 μm wide × 10 μm high). In contrast, in confined spaces, myosin II-mediated contractility (blebbistatin) or actin polymerization (latrunculin-A (LA)) has a more pronounced effect on traction stress magnitude in 2D than 1D lines (21). Thus, tumor cell migration in confined spaces may occur by a divergent mechanism compared with migration on 2D surfaces, and tumor cells may be able to adapt their mode of migration in response to the physical properties of the microenvironment.

One such “plasticity” strategy could involve cross talk between Rac1 and myosin II. In normal fibroblast-like cells expressing α4β1-integrin, unconfined (2D) migration requires enhanced Rac1 activity at the leading edge of the cell, which is achieved by prevention of α4-integrin/paxillin binding (63).
α₁-integrin/paxillin binding. Consistent with this cross-talk mechanism, inhibition of Rac1 reduces migration in wide channels, while inhibition of myosin II (or Rho)-dependent contractility reduces migration in narrow channels. In general, expression of α₁β₁-integrin promotes cell migration in unconfined and confined spaces (63). Invasive A375 melanoma cells also express α₁β₁-integrin and demonstrate a cross-talk mechanism similar to that of normal α₁β₁-integrin-expressing fibroblast-like cells. Moreover, in A375 melanoma cells in which α₁β₁-integrin is blocked or in normal fibroblast-like cells devoid of α₁β₁-integrin (e.g., NIH-3T3 and Chinese hamster ovary parental cells), unconfined migration is rescued by inhibition of myosin II contractility, which enhances Rac1 activity; meanwhile, confined migration is rescued by inhibition of Rac1, which enhances myosin II activity (63). It remains to be determined if the cross talk between Rac1 and myosin II activity in tumor cells devoid of α₁β₁-integrin regulates unconfined and confined migration in a similar fashion.

Matrix mechanics direct tumor cell adhesion and migration. In addition to matrix dimensionality and microstructure, heterogeneities also exist in matrix mechanical properties in vivo (84). This is evident in the varying elastic moduli of different tissues, including breast (167 Pa) (105), brain (260–490 Pa) (94, 102), liver (640 Pa) (153), lung (5–6 kPa) (155), and skeletal muscle (100 kPa) (86). Furthermore, tissue stiffness often changes in pathological conditions. For example, while the healthy mammary gland is typically very soft, with an elastic modulus ~167 Pa, breast tumors are much stiffer, with an elastic modulus >4,000 Pa (105). Importantly, numerous cell types, including fibroblasts (49, 108, 127), ECs (13, 15, 16, 31, 64, 80, 115, 126, 128, 131, 152, 154), epithelial cells (30, 108), cardiomyocytes (8, 34, 68), dorsal root ganglia neurons (4, 146), neutrophils (103, 129), and stromal cells (35), are sensitive to matrix mechanical properties. Intriguingly, tumor cell migration and adhesion can also be driven by matrix mechanical properties on 2D substrates (75, 79, 139), which may induce metastasis (133), while some types of cancer cells may lose mechanosensitivity upon transition to a metastatic phenotype (134).

Matrix stiffness also influences migration in 3D matrices. For a given 3D Matrigel stiffness, the migration speed of DU-145 human prostate carcinoma cells is a balance between contractile and adhesive forces (156), which is similar to 2D substrates (110, 129). However, when integrins are inhibited on cells in 3D Matrigel matrices, maximal cell migration speed shifts to softer matrices (156); this is in striking contrast to current 2D models, where decreasing adhesiveness shifts maximal migration to a stiffer substrate. However, it seems that the role of matrix stiffness may also depend on the degree of transformation in the cells. MCF10A cells that co-overexpress the oncogenes ErbB2 and 14-3-3ζ (i.e., are fully transformed) are able to migrate more efficiently in stiffer 3D matrices than are partially transformed cells, which display significantly hindered migration in stiffer matrices (3).

Combinatorial effects of dimensionality and matrix stiffness. Mounting evidence indicates that tumor cell migration is guided by dimensional (i.e., physical confinement) and mechanical (i.e., matrix stiffness) cues, which we reviewed separately in the previous sections. In particular, it seems that the role of matrix stiffness in cell migration on 2D substrates is fundamentally different from its role on 3D substrates, suggesting that the dimensionality of a cell’s matrix dictates the way the cell perceives and responds to matrix stiffness. However, in 3D ECM matrices, perturbations to change matrix stiffness often concurrently change cellular confinement, making it difficult to reach firm conclusions on the combinatorial role of dimensionality and matrix stiffness in tumor cell migration. To address this issue, recent experiments have utilized a microfluidic approach to systematically and independently vary physical confinement and matrix stiffness (106, 107).

In these studies, three-walled (106) or four-walled (107) polyacrylamide-based devices were fabricated to contain channels of varying width (10, 20, or 40 μm) but with constant height (25 μm) and also varying wall stiffness (0.4, 10, or 120 kPa). For a given ECM stiffness, glioma cell migration speed increases as the cells undergo increasing physical confinement, possibly due to increased polarization of the actin cytoskeleton and traction forces (106). The ability of glioma cells to migrate through spatial restrictions has also been attributed to the volume-regulating ability of the sodium-potassium-chloride cotransporter isofrom 1 (52). However, it remains to be elucidated whether traction forces generated by glioma cells are relevant in channels of even smaller cross-sectional area, since we have observed a reduced role for traction forces during osteosarcoma cell migration in channels that are 10 μm wide × 4 μm high (114). Meanwhile, in wide channels (20 μm wide × 25 μm high or 40 μm wide × 25 μm high) or on 2D substrates, glioma cell migration speed is biphasic with matrix stiffness, with optimum migration at an ECM stiffness of ~10 kPa (106), a trend that has previously been reported for numerous cell types, including neutrophils (129), smooth muscle cells (110), and epithelial cells (30). However, in narrower channels (10 μm wide × 25 μm high), glioma cell migration speed is monotonic with matrix stiffness; that is, cells move with increasing speed as ECM stiffness increases (106). Inhibition of myosin II-mediated cell traction forces (via blebbistatin) in narrow channels abrogates this response and renders the relationship between migration speed and ECM stiffness relatively insensitive to matrix confinement. While these are interesting results, two major shortcomings are associated with this work: 1) cells are only physically confined on three sides, as the channels are actually “troughs,” rather than completely enclosed tubes; and 2) the smallest channel used in this study was 10 μm wide × 25 μm high, but future analyses should include even more physically restrictive channels (e.g., 3 μm wide × 10 μm high), as have been used in our polydimethylsiloxane-based devices (5, 63, 137), to reach a more comprehensive understanding of the connection between matrix stiffness and physical confinement. Recent improvements to this device have created a four-walled microfabricated polyacrylamide channel platform, thus enabling the discovery that MCF10A cell sensitivity to physical confinement is coregulated by transforming potential of the oncogenes ErbB2 and 14-3-3ζ and ECM stiffness (107). In other studies using 3D collagen gels vs. collagen-coated 2D substrates, cells overexpressing ErbB2 alone demonstrate the most significant decrease in cell migration speed in 3D matrices compared with 2D substrates (3). Meanwhile, cells overexpressing 14-3-3ζ alone exhibit the least sensitivity to matrix dimensionality. Thus, oncogenic lesions and ECM biophysical properties can synergistically interact to drive cell migration.

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**Tumor cell mechanics and metastatic potential.** On 2D substrates, human metastatic breast, prostate, and lung cancer cells exert significantly increased traction stresses compared with their nonmetastatic counterparts, and this correlation holds for a range of ECM densities and matrix stiffnesses (76). However, we previously discussed the reduced role of traction forces during migration of some tumor cell types through physically confining ECMS (114). Therefore, it is unknown whether the correlation between traction stresses and metastatic potential in breast, prostate, and lung cancer cells also applies to migration in physically confined spaces. It is also unclear whether enhanced contractility in these metastatic cells is more important for extravasation from the blood vessel or for migration through complex ECMS within tissues. However, it is noteworthy that another study found the reverse association, such that 2D traction forces were inversely correlated with metastatic potential in a series of murine breast cancer cell lines (66). Future work that measures traction forces in a 3D setting, either in an ECM containing fluorescent marker beads or in a channel with deflectable microposts, will be necessary to further elucidate the relationship between metastatic potential and traction force generation.

Until recently, it was believed that tumor cells are necessarily more deformable than the matched normal cells, in order to facilitate squeezing through confined spaces much smaller than the size of the cell body. Indeed, many studies have found a positive correlation between tumor cell deformability and metastatic potential (28, 51, 65, 104, 116, 143). However, recently, studies on prostate cancer cells, hepatocellular carcinoma cells, and human chondrosarcoma cells have shown an inverse, nonsignificant, or complicated correlation between mechanical compliance and metastatic potential (7, 28, 37, 158). However, it may be difficult to compare results using different experimental methods. For example, no cell-ECM adhesions exist when cell deformability is measured using optical tweezers; meanwhile, in atomic force microscopy experiments, cells are spread on and attached to a flat substrate. The presence of adhesions and altered cytoskeletal structure in this 2D setting may preclude measurement of a cell stiffness that is relevant to migratory ability. Thus, diagnostic tools that rely independently on cell compliance to predict metastatic propensity may not be the most effective; rather, diagnostic strategies based on migration ability seem to be more promising.

**Tumor cell mechanotransduction.** It is clear that cells respond to various physical stimuli, including matrix dimensionality and mechanical properties, and that mechanical signaling plays a critical role in disease (69). Mechanotransduction is the process by which cells convert these extracellular physical signals into intracellular biochemical activity. Integrins are transmembrane proteins that, when activated, link the cell’s ECM to the internal cytoskeleton, allowing mechanical signals from the outside to be propagated into the cell. This ECM-integrin-cytoskeleton complex acts as a molecular clutch in response to external mechanical forces (6, 20, 61). Focal adhesion kinase is recruited to the integrin complex, where it associates with other proteins, such as talin and paxillin, and is autophosphorylated at Y397 (138). Phosphorylation of focal adhesion kinase reveals a binding site for Src, leading to its activation. Indeed, Src has been implicated in cell mechanoresponses and force transmission (9, 39, 99, 123, 142), although Src’s mechanotransducing ability may be dependent on its coordination with its substrate, p130Cas (120), which enhances Src activity (11). Meanwhile, tyrosine phosphorylation of p130Cas regulates actin assembly/disassembly (72), which in turn allows the cell to regulate numerous behaviors, including cell shape, motility, and stiffness. (For a complete review of the role of Src and p130Cas in cancer cell mechanotransduction, see Ref. 91.) However, with growing evidence that integrin-based cell adhesion to the ECM plays a reduced role during tumor cell migration in confined microenvironments, the exact mechanisms by which mechanotransduction occurs may need to be redefined. In addition, further work is needed to understand how tumor cells transduce other physical stimuli such as matrix dimensionality.

**Summary and Future Perspectives**

Cell migration in narrow microchannels, which mimics migration between anatomic structures in vivo, involves distinct mechanisms compared with unconfined migration on 2D surfaces. As the cancer biology and cell migration fields move forward, it is necessary to explore alternative mechanisms of cell migration that diverge from the classical model of 2D migration. Various aquaporins and ion channels are upregulated in cancer (19, 71). Therefore, one such mechanism could rely on the activity of ion channels and aquaporins to direct water flux through the cell membrane, thus facilitating protrusion and retraction and driving migration. An integrated experimental and mathematical framework will likely be necessary to explore this water permeation-driven mechanism of cell migration in confined spaces.

It is becoming increasingly apparent that physical cues within the cell’s microenvironment directly impact tumor cell adhesion and migration during various points within the metastatic cascade. A range of physical forces act on tumor cells during circulation, extravasation through the endothelium, and migration through complex tissue architectures. Microfabrication techniques, including micropatterning and microfluidic technologies, have become valuable tools in mimicking distinct properties of the in vivo situation and have provided new perspectives on how tumor cells integrate multiple physical cues simultaneously and respond accordingly. In addition to acting as a multipurpose in vitro experimental technique for a range of applications, microfluidics may also become an important diagnostic and/or therapeutic analysis platform for metastatic cancer patients. Furthermore, because of the critical role of tumor cell mechanotransduction at various stages of the metastatic cascade, targeting signaling pathways involved in tumor cell mechanosensing of physical stimuli may prove to be an effective therapeutic strategy, although the effects on normal host cells will also need to be considered.

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K.M.S. and K.K. conceived the idea for the review; K.M.S. designed and drafted the manuscript; K.M.S. prepared the figures; K.M.S. and K.K. edited and revised the manuscript; K.M.S. and K.K. approved the final version of the manuscript.

REFERENCES


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