Mechanoregulation of Metastasis beyond the Matrix

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ABSTRACT

Epithelial transformation and carcinogenesis are characterized by profound alterations in cell mechanics that significantly affect multiple steps of the metastatic cascade. The ability of cancer cells to grow in the primary tumor, to locally invade through the confining extracellular matrix, to survive in circulation, and to extravasate into distant vital organs all depend on specific mechanical characteristics. Importantly, recent studies have shown that the mechanical properties of cancer cells also influence their interactions with immune and stromal cells. Here, we discuss the mechanical changes that cancer cells undergo during metastasis, how these changes affect immune and stromal responses, and the implications of these new insights for therapeutic intervention.

Introduction

Cellular transformation involves genetic and biochemical changes that rewire cellular metabolism, enhance cellular proliferation, and promote resistance to cell death (1). Proto-oncogenic events that lead to epithelial transformation also profoundly alter the mechanical properties of tumor cells (2, 3). These cell-intrinsic mechanical changes do not occur in a vacuum. Tumor growth is physically accommodated within the primary tissue of origin, creating various solid, liquid, and tensile stresses that impose physical forces on neighboring epithelial cells, stromal fibroblasts, vascular endothelial and smooth muscle cells, neurons, and immune cell populations such as macrophages and cytotoxic lymphocytes. Within this chaotic mechanical landscape, cancer cells locally invade into the neighboring tissues and intravasate into the lymphatic or blood circulation to leave their primary organ of origin. During hematogenous dissemination, cancer cells experience shear forces, which affect their interactions with platelets, neutrophils, and endothelial cells, ultimately affecting their chances of survival (4). At distant secondary organs, metastatic cells extravasate out of the blood vessels, but the vast majority of these disseminated cancer cells (DCC) are either killed by cytotoxic lymphocytes or enter a period of dormancy due to their inability to use niche resources (5, 6). The small minority of DCCs that evade immunity and adapt to the local microenvironment can eventually outgrow into lethal metastatic colonies (7, 8).

At every stage of this metastatic cascade, cancer cells must adapt to the biophysical properties of their microenvironment, navigating the complex web of forces imposed by the extracellular matrix (ECM), fluid flow, and passive or active interactions with other cells. In recent years, it has become clear that cell-intrinsic mechanical features such as cytoskeletal rigidity and membrane tension play central roles in this process. Here, we review our current understanding of how cellular mechanics shapes the behavior of metastatic cells and their interactions with immune and stromal populations within the tumor microenvironment.

Cell mechanics during primary tumor growth and local invasion

Biophysical properties of epithelial cells start changing at the initial precancerous stage. These changes have been linked to transcriptional programs characteristic of wound healing and tissue remodeling. During wound healing, epithelial cells at the wound edge show lower mechanical compliance (increased stiffness) in comparison with the rest of the epithelial cells that do not actively participate in edge closure dynamics (9). A similar stiffening phenotype is induced by TGFβ (10), a known driver of both fibrosis and malignancy. Oncogenes can also increase epithelial cell stiffness before full malignant transformation. HER2 and KrasG12D expression, for example, can rigidify epithelial cells cultured in biomimetic 3D microenvironments (2). Furthermore, polyomavirus-middle-T antigen (PyMT)–driven mammary tumorigenesis in genetically engineered mouse models (GEMM) is associated with a transient stiffening of the mammary epithelium (3). Collectively, these data indicate that mechanical change is an early event in epithelial transformation.

In situ carcinomas present with hypercellularity of the primary tumor that compresses the surrounding tissue, and this solid stress alters the physiology and fate of multiple cellular compartments within the tumor microenvironment (11). In a mouse model of colon tumorigenesis, for example, magnetically recreating solid stress through the use of ingested beads was found to trigger aberrant activation of the Wnt–β-catenin pathway in the colonic epithelium, even in the absence of a *de novo* tumor. This kind of mechanically induced hyperproliferation could potentially contribute to the progression and genetic heterogeneity of tumors. Solid stress and compression, along with tumor-derived cytokines, has also been implicated in the differentiation of fibroblasts into a chronically activated state known as the cancer-associated fibroblast (CAF). Once formed, CAFs generate excessive ECM and then drive pathologic ECM remodeling through cellular contractility and collagen fiber crosslinking (12). This stiffens the ECM and compounds solid stress. Concomitantly, CAFs deposit hyaluronan into the tumor microenvironment, generating repulsive electrostatic forces and retaining water, which further promotes swelling (13).

As tumor growth continues, the external forces created by solid stress, physical confinement, and swelling cause cellular jamming within the tumor mass (14). Tumor cells escape from these physical and topographical constraints and invade surrounding tissue by transitioning to a more deformable and viscous phenotype (14, 15). In MMTV-PyMT–driven GEMMs of breast cancer, these compliant cells form the invasion front of the tumor as the leader cells, which later

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Cancer Res 2022;82:3409–19

doi: 10.1158/0008-5472.CAN-22-0419

give rise to metastases in secondary organs (16). The enhanced deformability of these cells is induced by fluid transfer from the core of the tumor mass to cells at the leading edge of the invasion front via gap junctions (14). It is likely that elevated potassium ion levels in the tumor interstitial fluid of melanomas and glioblastomas (GBM) also promote mechanical compliance as extracellular potassium reduces stiffness of multiple cell types (17–19). DCCs collected from breast and lung of patients present with higher compliance than their normal counterparts, and many *in vitro* studies provide causal links between mechanical compliance and invasive outgrowth from the primary tumor (20). For example, stiffening breast cancer organoids in 3D culture by pharmacologically stabilizing actin filaments or stiffening pancreatic ductal adenocarcinoma and colon cancer cells in 2D culture by overloading actin–myosin contractility reduces invasion and migration, respectively, and these *in vitro* phenotypes translate to reduced metastatic burden in xenograft models (14, 21, 22). Increased cell stiffness presumably interferes with the cellular deformation necessary for navigation through confining ECM. Hence, pliability is directly linked to the capacity of cancer cells to escape the primary tumor microenvironment (21, 22).

The invasive behavior of leader cells is exacerbated by ECM deposition and remodeling by CAFs (23). Most prominently, in 3D spheroid models of early-stage breast cancer, tumor cells use physical force to align ECM fibrils, thereby enabling tumor derived factors to access and activate normal fibroblasts (24). Activated fibroblasts then apply contractile forces to remodel and stiffen the ECM, leading to feedback activation of the mechanosensitive yes-associated protein (YAP)–driven gene expression (25). YAP activity in fibroblasts re-enforces the pathological CAF phenotype characterized by excessive contractility, ECM deposition, and remodeling. This feedback between CAF mechanics and the ECM is one manifestation of mechanoreciprocity, a cyclical process whereby ECM remodeling by cells drives their own biophysical change. Adipocytes and myofibroblasts in breast cancer, hepatic stellate cells in liver cancer, and cancer cells themselves perpetuate this pathological biophysical landscape by contributing to ECM secretion (26–29). In invasive breast cancers, ECM remodeling through higher order filamentous bundling of collagen fibers by lysyl oxidase, along with an associated elevation in matrix stiffness, can initiate a variety of mechanotransduction pathways in cancer cells (30–32). Subsequent activation of focal adhesion kinase (FAK), Rho/Rac/Cdc42 GTPases, and extracellular signal regulated kinase (ERK) can ultimately lead to transcriptional activation of Myocardin-related transcription factor (MRTF), YAP, and TWIST1, all of which enforce migratory and invasive gene expression for subsequent dissemination (33–35). CAFs also use mechanical force for vascular remodeling, thereby opening the gates for hematogenous dissemination of cancer cells (**Fig. 1**; ref. 36).

Mechanics of entry into the vasculature

Cancer cell entry into the vasculature involves both the breaching of vessel walls and the pathological remodeling of vessel architecture. The latter is influenced by several physical factors within the tumor microenvironment. First, ECM stiffening, hyaluronan-driven water absorption, and hypercellularity lead to vascular compression in mouse and human tumors (36). In brain tumors, this compression can be deadly as it disrupts blood follow, creating regional hypoxia and ischemia. In extracranial tumors, however, compressive hypoxia is a strong promoter of distant metastases. Decreased tissue and thereby cellular oxygen prevents prolyl hydroxylation and subsequent degradation of HIFs (37). Once stabilized, HIF drives endothelial proliferation and vascular remodeling, during which, blood vessels transiently lose architectural integrity due to insufficient pericyte coverage (38, 39). This instability presents cancer cells with opportunities for intravasation into the blood circulation. Tumor hypoxia also activates HIF in cancer cells, inducing the expression of epithelial-to-mesenchymal transformation (EMT) genes that promote migration and metastasis (39). In mouse models of breast cancer, the EMT program is further strengthened by the canonical EMT transcription factor TWIST1, which is activated by ECM stiffening. Mechanistically, TWIST1 is sequestered in the cytoplasm by the anchoring protein G3BP2; ECM-driven mechanotransduction disrupts this interaction and allows TWIST1 to translocate to nucleus (34).

Vascular remodeling caused by ECM stiffening is also associated with macrophage and monocyte infiltration into breast tumors (31). Physiologically, macrophages resolve tissue damage, but in the context of tumorigenesis, they acquire phenotypes that promote tumor growth and invasiveness (40). For example, macrophages form nanotubes to contact neighboring mammary tumor cells that physically pull the cancer cells along ECM toward the endothelium (41). Concomitantly, these contacts induce RhoA GTPase signaling in cancer cells, enhance invasiveness through endothelial barriers *in vitro*, and promote dissemination in zebrafish models, suggesting that macrophage-mediated nanotubes enhance cancer cell intravasation (42). Indeed, intravital imaging of locally invading breast cancer cells shows that macrophages can create areas of transient vascular leakage and direct cancer cells into these zones (43).

Although intravital imaging has been instrumental for our understanding of physical interactions between migrating cancer cells and their stroma, *in vitro* modeling studies using Transwell assays and microfluidic channels with geometrically defined spatial constraints have illuminated the interplay between cancer cell mechanics and movement. Using these approaches, cancer cells have been shown to use cytoskeletal and membrane remodeling to modulate their cytoplasmic compliance, thereby enabling them to migrate through densely packed spaces (44). Unlike the cytoplasm, however, the nucleus is not easily deformable (45). Accordingly, cancer cell extrusion through tight apertures has been found to rupture the nuclear envelope, resulting in chromosomal breakage and DNA cleavage by cytoplasmic nucleases (44). Nuclear rupture likely represents a vulnerability for cancer cells that intravasate into circulation. The concomitant DNA damage, for example, could aggravate the impact of oxidative stress and contribute to cancer cell attrition during dissemination of melanoma and breast cancer cells in experimental models of metastasis in mice (46, 47). That being said, nuclear rupture in physically confined primary breast tumors in humans and mice has also been associated with enhanced local invasion by cancer cells, suggesting that mechanically induced DNA damage might promote metastasis either via damage-induced signaling pathways or by generating metastasis private oncogenic mutations (48). Both of these hypotheses remain untested, leaving the precise relationship between of nuclear rupture and metastatic evolution poorly defined (49).

Cell mechanics that promote cancer cell survival during transit through the vasculature

Once in the circulation, cancer cells lose adhesion to their ECM substrate and are deprived of the mechanical and biochemical inputs that normally maintain their survival (**Fig. 2**). In normal epithelium, loss of ECM contact leads to cell death by anoikis, a physiological process that maintains epithelial homeostasis. During epithelial renewal and regeneration, cell proliferation leads to overcrowding and mechanical compaction, which causes older epithelial cells to extrude and undergo apoptosis within the epithelial lumen. This

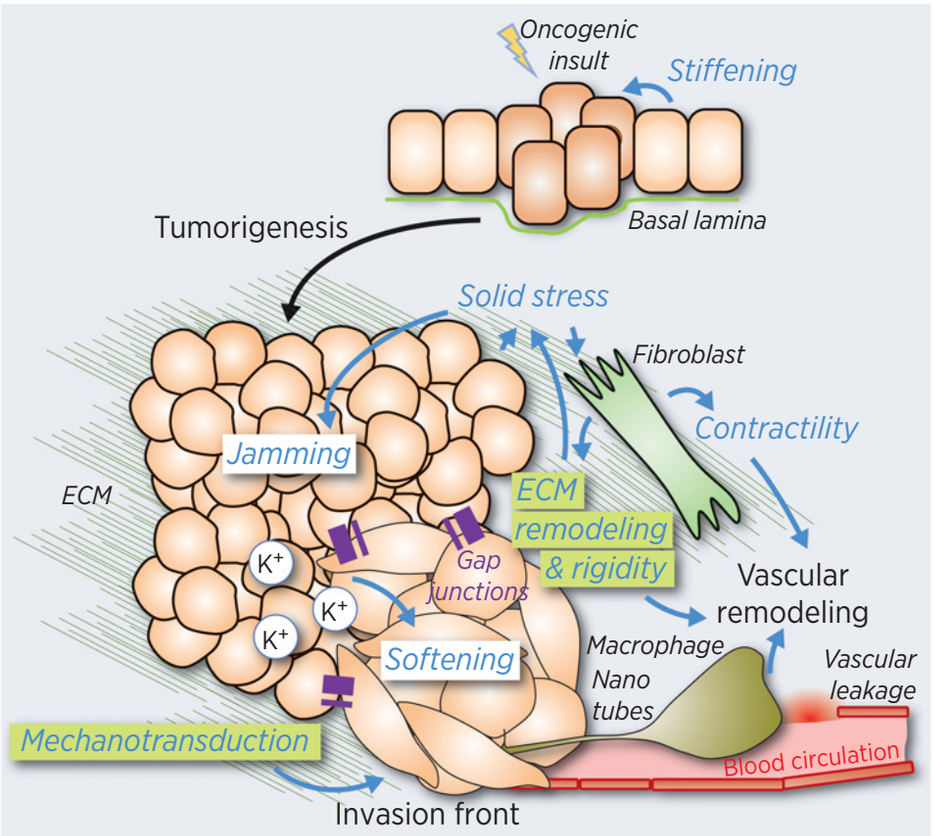

Figure 1.
Changes in mechanical and biophysical properties of epithelial cells (blue font) start at the precancerous stage and dynamically take place throughout from tumorigenesis to metastasis. Several components of the tumor microenvironment such as the fibroblasts, macrophages, and the ECM contribute to these changes.

progression is well-described for the colon epithelia (50). As cells extrude, cell surface integrins detach from the basal lamina, terminating survival signaling via PI3K, AKT, ERK, and YAP (51–53). Although loss of ECM contact causes anoikis and apoptosis in nontransformed cells, oncogenic activation of PI3K/AKT, ERK, and YAP enables cells to circumvent this process (51, 54). Interestingly, YAP can also be activated by calcium influx through mechanically gated Piezo channels (55). Mechanical compression within an epithelium is known to activate Piezo1/2 (56). It is tempting to speculate, therefore, that the mechanical compression experienced by cancer cells as they squeeze through a vascular endothelial layer might inhibit anoikis via the Piezo1/2-YAP axis.

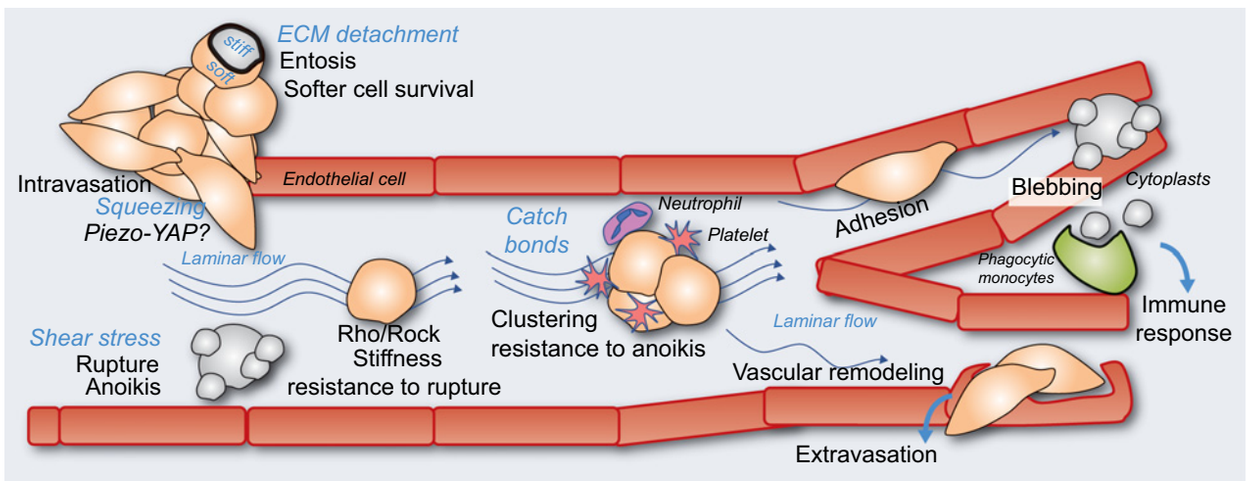

Figure 2.
Beyond the matrix, biophysical forces generated by laminar flow and loss of substrate attachment dictate the metastatic fate by affecting several processes such as resistance to anoikis, entosis, catch bond formation, clustering, extravasation, and the immune response.

Loss of ECM contact causes cancer cells to rely heavily on cancer cell–cancer cell interactions for survival (57). In some cases, this results in a cannibalistic process, called entosis, in which a "winner" cancer cell engulfs the adjacent "loser" cancer cell. Entosis allows cancer cells to resist detachment induced cell death and nutrient deprivation (58, 59). This process is driven by mechanical disparities; winner cells are softer than loser cells, and winner cells use actomyosin-driven contractility for engulfment (60). Entosis has been observed in primary and metastatic tumors and notably in pleural fluid in the lungs of patients with breast cancer and in urine samples of patients with bladder cancer (58, 61, 62). Thus, it seems possible that entosis might operate as a survival mechanism for winner cancer cells in fluid environments such as the circulation. Another consequence of enhanced cancer cell–cancer cell interaction is clustering. Breast cancer cells can invade into blood circulation collectively, even bringing CAFs and monocytes with them as they intravasate (63, 64). Although circulating cancer cell clusters are significantly rarer than isolated cancer cells in patients with breast cancer, their presence strongly correlates with distant metastases and poor survival (64). Consistent with these clinical observations, circulating cancer cell clusters drive more potent colonization of secondary organs in experimental models (64). In addition to promoting supportive cell–cell interactions, breast cancer cell clusters form nanolumens, which can concentrate growth factors and thereby promote juxtracrine survival signaling (65).

Although ECM detachment and intravasation share certain mechanical features, a major difference is that intravasating cells encounter powerful shear stress generated by laminar fluid flow in the blood circulation, which they must resist to avoid rupture. Many of the early steps in the metastatic cascade, such as tumor growth and local invasion, are facilitated by increased mechanical compliance, but cancer cells that enter into circulation need to have elevated cellular stiffness to avoid cell death by shear forces (66). This is accomplished via multiple mechanisms: For example, nuclear lamins promote nuclear stiffness, and their depletion sensitizes cancer cells to rupture by circulation mimicking shear stresses *in vitro* (67). Shear stress also triggers mechanotransduction in cancer cells, which culminates in Rho GTPase/Rho kinase (ROCK)–mediated F-actin polymerization and increased cortical stiffness. Inhibiting this pathway perturbs cancer cell survival in mouse models of experimental prostate cancer and breast cancer metastasis (66, 68–70).

Although laminar flow generates detrimental shear stress, it also provides tensile forces needed for strengthening intercellular "catch-bonds" between circulating cancer cells and platelets. Most molecular interactions behave as "slip-bonds," becoming weaker under tension. By contrast, catch bonds increase in affinity up to an optimal applied force (71, 72), typically in the 10–20 pN range. Adhesion molecules such as selectins and integrins have been shown to form catch-bonds with their cognate ligands (73–77), and it is generally thought that laminar flow in blood vessels provides the force required to trigger catch-bond engagement, thereby inducing clustering between cancer cells and platelets (78, 79). These heterotypic clusters are more resistant to fluid shear stress, anoikis, and NK cell–mediated cytotoxicity (80–82). Cancer cells also cluster with neutrophils through integrin mediated adhesions, and one would envision these catch-bonds would strengthen with laminar flow, as well (83). Finally, vascular endothelial cells also bind circulating tumor cells via integrins more strongly under laminar flow; this has been shown to facilitate cancer cell arrest in capillaries (4).

Cell mechanics during extravasation and metastatic seeding

Intravital imaging in mouse brain, lungs, and liver shows that cancer cells that survive circulation arrest in vascular forks or in areas of constriction in these secondary organs (84–87). Cancer cell arrest requires a permissive flow rate (88). After stable adhesions are formed between the circulating cancer cells and the luminal surface of the capillary endothelium, contiguous fluid flow activates mechanotransduction in the vascular endothelium, resulting in vascular remodeling. Flow induced vascular remodeling is critical for cancer cell extravasation as its pharmacological inhibition prevents transendothelial migration (89). In mouse models of melanoma lung metastasis, cancer cells that fail to extravasate are subject to continued exposure to fluid shear stress, which causes plasma membrane blebbing and the release of the blebs into the microenvironment (85, 90). Phagocytosis of these cancer cell derived blebs by tissue resident conventional dendritic cells and non-resident macrophages drives immune programming within the metastatic microenvironment, which influences the fate of the cancer cells that succeed in dissemination (85).

Mechanotransduction and the switch from metastatic dormancy to outgrowth

DCCs that complete extravasation can stay clinically undetectable for months and even decades and coordination of multiple metabolic, cell-cycle and immune surveillance mechanisms are involved in this state of metastatic dormancy (reviewed in refs. 91, 92). During metastatic dormancy, micrometastatic proliferation of DCCs is balanced by cancer cell death induced by the immune system (7, 93, 94). If immunity is compromised, unchecked DCC proliferation can lead to colonization and lethal outgrowth. The importance of immunosurveillance for metastasis suppression is perhaps most clearly seen in the context of organ transplantation, where immunosuppressive drugs intended to suppress tissue rejection can also promote metastatic tumor growth (95, 96). Some prominent examples are the emergence of melanoma in kidneys and of extracranial GBM metastases in kidneys, livers and lungs of transplant recipients. GBM cases are particularly interesting as brain tumors are traditionally thought of as non-metastatic. These observations highlight how cancer cells that are fully competent at performing all the earlier stages of the metastatic cascade, such as migration, invasion, and surviving in circulation, fail to form clinically meaningful metastatic colonies unless they can evade immune surveillance at secondary organs during the metastatic outgrowth phase. They also imply that the proliferative dormancy of DCCs might be coupled to immune evasion. Indeed, dormant lung, breast and pancreatic DCCs with slow cell-cycle kinetics downregulate activating ligands for T and NK cells, such as class I MHC I and MICA, thereby avoiding immune targeting by cytotoxic lymphocytes in mice (93, 94).

The emergence of DCCs from dormancy and their productive proliferation for colonization require the optimal use of the local resources. One of the more prominent microenvironments for metastatic outgrowth is the perivascular niche, which is located on the abluminal surface of blood vessels (84, 86, 87, 97). Breast, lung, melanoma, and colorectal cancer cells have been observed to spread and migrate on this surface in the bone marrow, lungs, and the liver in mouse models experimental colonization. Given that ECM rigidity promotes mechanotransduction and tumor progression, it is highly likely that DCCs seek out a mechanically optimal environment using durotaxis, a form of cellular migration that guides cells toward rigid substrates (98). Interestingly, even before cancer cells arrive at distant organs, factors secreted from the primary tumors can precondition secondary perivascular niches to form mechanochemically permissive

environments. For example, exosomes derived from orthotopic melanoma and breast tumors can induce phenotypic switching of distant pericytes and vascular smooth muscle cells in mice (99). Similarly, exosomes from pancreatic cancer cells have been found to drive resident Kupffer cells in the liver to secrete TGFβ (100). In both these cases, cancer-derived exosomes instruct stromal cells to secrete fibronectin, which is a precursor to tissue fibrosis, stiffening and hence a robust inducer of integrin-mediated mechanotransduction, all of which correlate with better metastatic seeding (12, 101). Disseminated breast cancer cells' mechanotransduction is also stimulated by the action of neutrophils, which secrete metalloprotease-containing extracellular DNA traps to cleave laminin and expose cryptic integrin-binding sites (102).

Interestingly, only DCCs with the appropriate machinery for mechanotransduction can form clinically meaningful metastatic colonies in secondary organs. For example, expression of TM4SF1, which is a membrane receptor that facilitates signaling downstream of the collagen receptor discoidin domain receptor 1, is sufficient to drive outgrowth of dormant breast cancer cells at secondary organs (103). DCCs also express urokinase-type plasminogen activator (uPA), a potentiator of integrin signaling. Integrin activation by uPA tilts the ERK/p38 growth/stress signaling balance toward the former to induce DCC proliferation (104). In models of experimental metastasis, mouse breast cancer cells use integrin-linked kinase (ILK) and beta-parvin for proper cytoskeletal metastatic outgrowth in the lungs (105, 106). Similarly, neuronal cell adhesion molecular L1 (L1CAM) expressed by DCCs binds to vascular basal lamina and amplifies integrin–ILK signaling to activate mechanosensitive transcription factors, including YAP and MRTFA/B, during breast and lung cancer metastasis to the brain and the lungs (107, 108). Conversely, astrocytes in the brain induce metastatic dormancy in DCCs by expressing lamin-211, which suppresses YAP-mediated metastatic outgrowth (109). These studies suggest that mechanotransduction is a driving force behind metastatic awakening from dormancy.

Fundamental mechanisms that regulate mechanosensitive transcription factors necessary for colonization

Metastases use specialized gene expression modules to occupy and expand within their new niches. Interestingly, several transcription factors that are critical for metastatic colonization, such as β-catenin, YAP, and MRTFA/B, are mechanosensitive (110, 111). Although the mechanical forces that drive β-catenin activation specifically during metastasis remain elusive, YAP and MRTF are known to be activated by cancer cell spreading on the abluminal surface of blood vessels in secondary organs (108). This process depends on integrin-based cell adhesion, cell polarization, and the elaboration of F-actin–based structures. YAP and MRTFA/B physically interact with each other to promote transcription of a partly overlapping set of genes for metastatic outgrowth (35, 112).

YAP and the related TAZ are well known as the targets of the developmental Hippo signaling cascade, which is primarily responsible for attenuating cell growth and proliferation during contact inhibition (113). During epithelial wound closure, for example, cell–cell contacts between confluent cells at the wound site activate Hippo kinases, which phosphorylate YAP/TAZ. These inhibitory phosphorylation events lead to cytoplasmic sequestration of YAP/TAZ and thereby inhibition of their transcriptional function, which they carry out in association with TEAD family of transcription factors. In addition to regulation by the Hippo pathway during development and disease, YAP/TAZ activation is also coupled to adhesion signaling and the cytoskeletal remodeling that flows from it. Proteins activated by integrin engagement, such as Rho/Rac family GTPases, FAK, p21-activated kinase (PAK), ILK, and Src are known to promote the nuclear localization of YAP/TAZ (114–116). F-Actin polymerization is a critical component of this process, as high levels of monomeric G-actin in the cytoplasm promote the association of YAP/TAZ with sequestering factors in the cytoplasm, such as ARID1a, a SWI/SNF chromatin remodeling complex member, and angiomotin family of proteins (117–119). Further highlighting the role of F-actin/G-actin ratios in the cell, YAP/TAZ transcriptional activity can be inhibited by actin capping and severing proteins (120). Finally, mechanotransduction from the cell surface to the nucleus via the linker of nucleoskeleton and cytoskeleton complex (LINC) significantly contributes to YAP/TAZ nuclear import by inducing the opening nuclear pore complexes (121). Once in the nucleus, YAP/TAZ upregulate expression of cyclins for cell-cycle progression, promote metabolic programming for cellular proliferation, and contribute to cytoskeletal and ECM remodeling through induction of a target gene set that partly overlaps with that of MRTFA/B (112, 122, 123)

MRTFA and B are two related transcription factors that are necessary for myoepithelial differentiation during lactation/involution cycles, cardiovascular development, and smooth muscle cell migration and investment (124, 125). As with YAP/TAZ, MRTFA/B nuclear import is regulated by the LINC complex (126, 127) and it is also highly responsive to the F-actin cytoskeleton. In this case, the relationship is quite straightforward: Monomeric G-actin binds directly to MRTF isoforms, sequestering them in the cytoplasm (127–129). The consumption of G-actin during cytoskeletal growth relieves this inhibitory interaction, enabling MRTFA/B to enter the nucleus. Thus, many of the structural and signaling proteins that deplete G-actin by incorporating it into F-actin, such as Rho/Rac GTPases, ROCK, and Formins, activate MRTFA/B. Upon entering the nucleus, MRTF isoforms associate with serum response factor (SRF), a ubiquitously expressed DNA-binding protein that is involved in both proliferative and morphologic responses to growth factor signaling. MRTF–SRF complexes specifically enhance the transcription of actin, actin-bundling proteins and other genes involved in cellular contractility. Thus, MRTF signaling feeds the very cytoskeletal response that drives it.

The close associations between activation of mechanosensitive transcription factors, such as YAP and MRTF, DCC proliferation, and cytoskeletal remodeling suggest that the biophysical state of DCCs changes during metastatic outgrowth (102, 108, 109). Malignant transformation is generally associated with an increase in mechanical compliance (see prior sections). This situation is reversed, however, in the perivascular niche, where the requirements of colonization and outgrowth demand cancer cell stiffening due to F-actin polymerization during adhesion to the abluminal surface of blood vessels. As described below, this microenvironmentally induced transition appears to be particularly important for the recognition of incipient metastases by the immune system (130–132).

Mechanoregulation of antitumor immunosurveillance

Structural plasticity and motility underlie multiple aspects of the immune cell lifestyle, including migration, intercellular communication, and phagocytosis. All of these processes are intensely physical, and in recent years it has become clear that they are subject to mechanical control (133). With respect to antitumor immunity, the mechanoregulation of cytotoxic lymphocytes, comprising cytotoxic T cells and natural killer (NK) cells, is particularly important. T cells and NK cells identify tumors cells by recognizing cell surface molecules indicative of stress and transformation. This induces the

formation of a highly dynamic cell–cell interaction, called an immune synapse (134, 135). The lymphocyte then secretes toxic granzyme proteases and the pore forming protein perforin into the interface, leading to tumor cell death. This killing response plays a central role in routine immunosurveillance and is a critical component of anti-tumor immunotherapy (136).

Immune synapses exert nanonewton scale forces, which regulate lymphocyte cytotoxicity in at least two ways. First, they enhance the pore forming activity of perforin by physically straining and distorting the target cell membrane (137). To achieve synergy between secretory and mechanical output in this way, lymphocytes use interfacial actin-based protrusions, which spatiotemporally coordinate the delivery of force and perforin within the synapse. The Wiskott–Aldrich Syndrome (WASP) protein, an actin nucleating factor, plays a particularly important role in this process (138). Second, synaptic forces also influence cytotoxicity by regulating mechanotransduction. Several critical lymphocyte immunoreceptors, including the T-cell antigen receptor and the $\alpha_L\beta_2$ integrin LFA1, form catch-bonds with their respective ligands that only achieve high affinity when placed under pulling force (73, 74, 76, 139, 140). Similarly, the signaling capacity of these receptors also appears to be dependent on mechanically gated conformational changes (141–143). Importantly, both of these mechanoregulatory processes are modulated by the physical properties of the target cell. The efficiency of perforin pore formation depends on the tension of the target membrane, which is itself supported by the underlying cortical cytoskeleton. Similarly, the mechanical requirements for immunoreceptor activation place physical demands on the opposing cell surface, which must be rigid enough to resist synaptic pulling forces. Indeed, multiple studies have shown that the rigidity of a target substrate or cell dictates the degree of lymphocyte activation through the synapse, with stiffer surfaces inducing stronger responses (144–146).

Hence, the biophysical status of cancer cells is emerging as a key regulator of their sensitivity to patrolling cytotoxic lymphocytes. In general, the enhanced pliability typically associated with malignancy would be expected to protect cancer cells from immune-mediated attack during metastatic dissemination. In certain specialized contexts, however, mechanoregulation could cut the opposite way by allowing lymphocytes to identify metastases by their specific biophysical traits. This idea was recently explored in the context of metastatic outgrowth in the perivascular niche. As described above, this process depends on actin polymerization, which enables cell spreading and migration along the abluminal surface of blood vessels. Although this architectural change is necessary for metastasis, it also increases cancer cell stiffness, leading to enhanced mechanotransduction and the activation of interacting CTLs and NK cells. Accordingly, MRTF overexpression in cancer cells was found to reduce their colonization of immunocompetent mice, while increasing colonization in mice lacking $CD8^+$ T cells or NK cells (132). Importantly, the immune sensitization induced by MRTF was reversed by selective depletion of F-actin in cancer cells, validating the biophysical basis of these phenotypes. These results have revealed a biophysical form of immunosurveillance, termed "mechanosurveillance" that enables cytotoxic lymphocytes to target metastatic outgrowth.

Could cancer cells exhibit biophysical immune vulnerabilities at other stages of the metastatic cascade? The increased osmotic swelling associated with local invasion from primary tumors could potentially make them more sensitive to cellular cytotoxicity by increasing membrane tension. Indeed, osmotic swelling *in vitro* has been found to enhance lysis by purified perforin (137). Similarly, ECM-dependent stiffening of the primary tumor, which is known to drive proliferative responses via YAP (117, 147), could also promote mechanosurveillance via the MRTF pathway. The effects of microenvironmental mechanics on cytotoxic lymphocyte function is a rich area for future research. Whether immune synapses exhibit the same biophysical capacities and requirements in circulation, in pleural or peritoneal fluids, or in highly compliant or very rigid tissues remains to be seen. The extent to which biophysical immune vulnerabilities affect anti-tumor immunotherapy is another interesting and largely unexplored topic. In this regard, it is interesting to note that high levels of MRTF signaling correlate with enhanced responsiveness to immune checkpoint blockade therapy in the context of melanoma (132). Future work in this vein should reveal additional prognostic markers and treatment opportunities.

Targeting and exploiting the mechanoregulation of metastasis

Drugs that target various signaling intermediates in the mechanotransduction cascade, such as integrins, FAK, and PAK, have been the subject of recent clinical trials (148–150). The molecular redundancy of mechanotransduction, however, poses a potential roadblock to this approach. As such, it is perhaps unsurprising that many clinical trials that use, for example, integrin-blocking therapeutics have thus far failed to show any progression free or overall survival (OS) benefit for patients with cancer (reviewed in ref. 151). As an alternative, one could imagine inhibiting upstream regulators of mechanotransduction in the tumor microenvironment. Fibroblasts, for example, express angiotensin receptors that activate Rho GTPase and thereby downstream adhesion signaling. This drives the accumulation of fibrotic tissue that strongly promotes mechanotransduction in the tumor microenvironment (152). Inhibiting this angiotensin pathway has been found to reduce ECM stiffness in the liver and in experimental liver metastases. Furthermore, retrospective analysis of patients with colorectal cancer shows that angiotensin inhibitors confer a survival benefit for patients with liver metastases. Beyond inhibiting proliferative mechanotransduction responses, reducing ECM stiffness could also improve drug perfusion and antitumor immune influx into the tumor microenvironment by releasing solid pressure on the vasculature (38). However, therapies that target the ECM and its mechanical properties can also dissolve the physical constraints on tumor growth and can permit cancer cells to dissemination from the primary tumor. For example, clinical trials using sonic hedgehog inhibition to reduce CAF-derived desmoplastic ECM showed that this approach results in poorer OS in patients with pancreatic ductal adenocarcinoma, and CAF and ECM-targeting correlates with increased metastasis in animal models (153–155). These studies highlight the need for designing specific ECM targeting or softening approaches for distinct pathological contexts.

Targeting mechanosensitive transcription factors such as YAP, MRTF, and TWIST1 is also a potential strategy for circumventing molecular redundancy at the level of intermediate signaling. This approach has its own complications, however. First, transcription factors are dynamically regulated, and those that mediate the initiation of metastases may not operate during the later stages of metastatic outgrowth. TWIST1, for example, drives EMT in response to mechanical stimuli during early metastatic dissemination, but it is shut down during colonization to enable metastatic outgrowth in secondary organs (34, 156, 157). By contrast, YAP is expressed and operational in established micrometastases (108). Even when an appropriate candidate transcription factor is present, however, developing a specific inhibitor can be stymied by the absence of obvious small-molecule binding sites (158). An alternative is to target regulatory processes for these transcription factors. For example, inhibiting

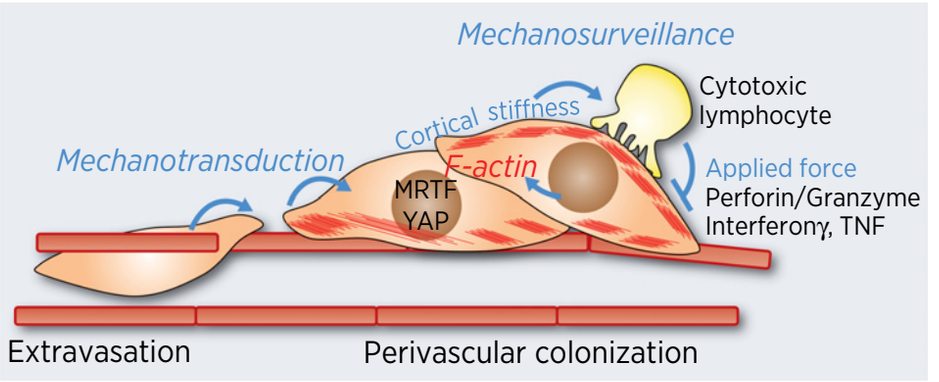

Figure 3.

Once in secondary organs, DCCs engage signaling and mechanotransduction to activate transcription factors such as YAP and MRTF for overt outgrowth. However, mechanotransduction, F-actin cytoskeleton remodeling, and the associated changes in cortical stiffness of DCCs expose them to mechanosurveillance by cytotoxic lymphocytes. Cytotoxic lymphocytes' recognition of cancer cells' cortical stiffness triggers force exertion and amplifies their own mechanotransduction for increased cytotoxicity through lytic granule and cytokine secretion. This paradox severely affects metastatic progression and it partly explains why the metastatic cascade is extremely inefficient, with less than 1% of the disseminated cancer cells successfully colonizing distant organs.

auto-palmitoylation of TEAD prevents its association with YAP/TAZ and inhibits cancer cell proliferation (159). It may also be more desirable to target the vulnerabilities associated with the transcription factor activation rather than the transcription factor itself. Indeed, MRTF activity in cancer cells increases stiffness and thereby facilitates mechanosurveillance, which can be further enhanced by immune checkpoint inhibition (132).

Targeting the mechanical properties of cancer cell cytoskeleton is also an intriguing option, and this has been attempted in some preclinical settings. For example, locking the acto-myosin contractility machinery in an activated state with the small-molecule 4-hydroxyacetophenone (4-HAP) impairs the invasiveness of colorectal cancer cells and reduces their ability to metastasize to the liver in mouse models (21, 22). That said, stiffening cancer cells may not always provide the same anticipated anti-invasion effect because various other treatments that reduce mechanical compliance of cancer cells can promote invasion *in vitro*. For example, β-adrenergic receptor stimulation of prostate cancer cells makes them more rigid and simultaneously more invasive (160, 161). Furthermore, ROCK-driven F-actin polymerization increases stiffness of solitary breast cancer cells as they invade collagen gels (162). As described above, the biophysical criteria for metastasis depend on the specific stage being considered. Hence, biophysical perturbations that inhibit one step (e.g., local invasion) may promote another (e.g., secondary seeding).

Although the impact of increasing cancer cell stiffness on invasion seems to be influenced by cell type and microenvironmental context, the impact on immune surveillance (i.e., mechanosurveillance) appears to be conserved across multiple models. Increasing the stiffness of breast cancer, melanoma, and hepatocellular carcinoma activates mechanosurveillance by NK cells, $CD8^+$ T cells and adoptively transferred T cells (130–132). In theory, increasing cancer cell stiffness could also promote their engulfment by macrophages (**Fig. 3**; refs. 163, 164). At this stage, however, attempts to expose metastatic cells to mechanosurveillance by increasing cancer cell stiffness face multiple challenges in clinical translation. First, the cell stiffening treatments that have so far been used in mice, such as myosin-activating 4-HAP, the actin cross-linking agent jasplakinolide, and the cholesterol depleting agent methyl-β-cyclodextrin, are too nonspecific to be used in humans (130, 131). Second, compounds that increase cancer cell stiffness could also potentially trigger mechanotransduction pathways that promote metastatic colonization. Thus, the ultimate translational challenge is to identify signaling intermediates and molecular processes that generate cortical cell stiffness, but which are uncoupled from prometastatic mechanotransduction.

Conclusions and Perspectives

It is now clear that biophysical forces regulate multiple stages of the metastatic cascade and that this regulation involves more than bidirectional interplay between the ECM and cancer cells. Nevertheless, our understanding of this topic is still rudimentary. The molecular and cellular complexity of tumors is recapitulated in their mechanobiology. Fibroblasts use contractility to remodel ECM, vascular cells respond to shear and solid stresses, cancer cells adapt to the biophysical properties of diverse niches, and immune cells leverage mechanical forces to destroy target cancer cells. Understanding how these various processes evolve and interact during tumor growth and evolution will require more sophisticated experimental systems that can effectively delineate different microenvironmental, cellular, and subcellular compartments while also providing ways to measuring and perturbing specific mechanical parameters in physiologically relevant contexts (165–167). Furthermore, the biophysical crosstalk between cancer cells and other prominent cells in the tumor microenvironment, such as pericytes and neurons, remains uncharacterized. A deeper understanding of these and other relationships should provide new avenues for therapeutic intervention that target mechanobiological vulnerabilities in cancer.

Another growing area of interest concerns the effects of general health on the biophysical landscape in cancer. Obesity, for example, is highly prevalent in countries that follow a western diet, and it profoundly affects the mechanical landscape of the tumor microenvironment. Obesity can lead to dramatic changes in ECM composition and thereby influence mechanotransduction in cancer cells and stromal cells (29, 168). Obesity is also associated with high cholesterol levels, which can affect immune cell, cancer cell, and vascular cell phenotypes through mechanoregulation of membrane fluidity and rigidity (131, 169, 170). During natural aging, the loss of arterial elasticity causes vascular stiffness, which affects the perivascular metastatic niche (171). Finally, physical exercise, which increases

hydrodynamic sheer stress in circulation, could regulate the survival of circulating tumor cells (172, 173). Studying these systemic level processes and their mechanobiology in the context of metastasis has the potential to guide clinical management of metastatic disease and offer new therapeutic strategies.

In conclusion, a basic understanding of the mechanobiology driving the growth and suppression of metastasis offers valuable insights into how the metastatic cascade is regulated and reveals novel nodes of therapeutic intervention. Merging these biophysical insights with data from multiomics, intravital imaging, and clinical studies will allow investigators to place mechanoregulatory pathways in their proper physiological context, laying the groundwork for translationally relevant progress in the field.

Authors' Disclosures

No disclosures were reported.

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Received February 4, 2022; revised June 24, 2022; accepted July 20, 2022; published first July 25, 2022.

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