

REVIEW

Neural regulation of cancer: from mechanobiology to inflammation

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Despite recent progress in cancer research, the exact nature of malignant transformation and its progression is still not fully understood. Particularly metastasis, which accounts for most cancer death, is a very complex process, and new treatment strategies require a more comprehensive understanding of underlying regulatory mechanisms. Recently, the sympathetic nervous system (SNS) has been implicated in cancer progression and beta-blockers have been identified as a novel strategy to limit metastasis. This review discusses evidence that SNS signaling regulates metastasis by modulating the physical characteristics of tumor cells, tumor-associated immune cells and the extracellular matrix (ECM). Altered mechanotype is an emerging hallmark of cancer cells that is linked to invasive phenotype and treatment resistance. Mechanotype also influences crosstalk between tumor cells and their environment, and may thus have a critical role in cancer progression. First, we discuss how neural signaling regulates metastasis and how SNS signaling regulates both biochemical and mechanical properties of tumor cells, immune cells and the ECM. We then review our current knowledge of the mechanobiology of cancer with a focus on metastasis. Next, we discuss links between SNS activity and tumor-associated inflammation, the mechanical properties of immune cells, and how the physical properties of the ECM regulate cancer and metastasis. Finally, we discuss the potential for clinical translation of our knowledge of cancer mechanobiology to improve diagnosis and treatment.

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More than four decades ago in 1971, U.S. President Richard Nixon signed the National Cancer Act, resolving to find cures to combat this devastating disease. Thanks to improved funding for cancer research and tremendous research efforts, we now have a much deeper knowledge of cancer etiology, pathogenesis, treatment and prevention. Indeed, an increasing body of research enables us to better understand the hallmark features of cancer and to devise therapeutics that target those characteristics of the disease.¹ As a result, the number of cancer survivors in the U.S. has increased from 3 million in 1971 to 14.5 million in 2014.² Despite this significant progress, we are still far from curing most forms of cancer. This is in part because we still do not have a fully integrated knowledge of cancer. Although our understanding of how individual characteristics, such as angiogenesis and inflammation, contribute to cancer progression has improved, additional factors that affect cancer progression have emerged, such as the physical properties of tumor cells and their microenvironment (Figure 1). In addition, it is becoming apparent that cancer outcomes are influenced by factors on multiple levels that range from subcellular (genetics and gene transcription) to psychosocial (behavior, diet, lifestyle factors and environmental exposure). In this review, we

explore the influence of chronic stress as a physiological factor that influences cancer progression. We consider the impact of stress signaling through the sympathetic nervous system (SNS) on tumor cells and tumor-associated inflammation, and consider the possibility that stress regulates the physical properties of cells to influence metastasis and cancer progression.

THE SNS AND CANCER

Metastasis is a complex, multistep process in which tumor cells spread through the body via a process of detachment, intravasation, transit through systemic circulation, extravasation and colonization (Figure 2).³ Throughout these steps, the tumor microenvironment can impact tumor cell dissemination.⁴ Studies of physiological regulators of metastasis identify the SNS as a component of the tumor microenvironment that regulates multiple steps in metastasis.^{5,6}

The SNS mediates a stress response by releasing neurotransmitters, the catecholamines norepinephrine and epinephrine. These neurotransmitters are structurally similar and exert their effects by binding to adrenoceptors. Epinephrine is mainly secreted from the adrenal medulla, whereas norepinephrine is secreted from both the adrenal medulla and sympathetic nerve terminals.⁷ SNS nerve fibers can

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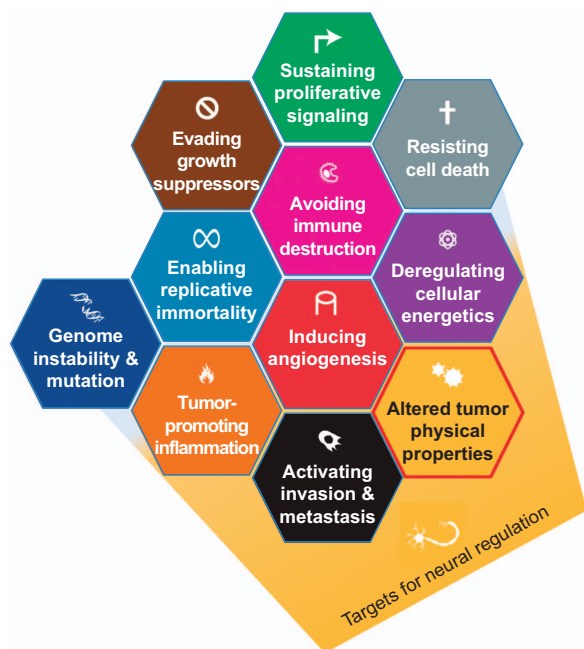


Figure 1 Hallmarks of cancer. The original six hallmarks of cancer: (1) sustaining proliferative signaling, (2) evading growth suppressors, (3) activating invasion and metastasis, (4) enabling replicative immortality, (5) inducing angiogenesis and (6) resisting cell death, were first proposed in 2000 by Douglas Hanahan and Robert Weinberg.⁹⁹ In 2011, four additional next-generation cancer hallmarks were added: (7) avoiding immune destruction, (8) tumor-promoting inflammation, (9) genome instability and mutation and (10) deregulating cellular energetics.¹ As we better understand cancer, additional characteristics are emerging. Here we focus on the physical properties of the tumor and microenvironment as an emerging new hallmark.^{3,100} We show how misregulation by neural signaling can impact these cancer hallmarks to promote disease progression. Permission to reuse symbols for The Hallmarks of Cancer was granted by Elsevier.

directly innervate tumor tissue and metastatic targets. When chronic stress activates the SNS, norepinephrine is released, which can bind directly to β -adrenoceptors on cancer cells.⁸ Various other types of cells are present in the tumor microenvironment, including immune cells, fibroblasts and endothelial cells.⁹ Each of those cell types also express β -adrenoceptors, allowing them to also respond to neurotransmitters.¹⁰ In addition to being released locally from nerve fibers in the proximity of cancer cells, epinephrine may be released systemically from the adrenal gland during times of stress¹¹ and delivered through the vascular system to the tumor microenvironment.¹²

Accumulating evidence from preclinical studies demonstrates the important role of SNS signaling in regulating multiple hallmarks of cancer. The SNS may impact tumor progression by modulating systemic release of growth factors and mobilization of inflammatory cells. SNS nerve fibers in the liver and white adipose tissue release norepinephrine to trigger secretion of glucose, adipokines and pro-inflammatory cytokines into the bloodstream.^{13,14} These factors may provide a growth stimulus to cancer cells and induce tumor-associated inflammation. In addition, SNS-mediated release of norepinephrine in the lymph nodes, spleen and bone marrow regulates differentiation and egress of immune cells. For example, SNS activation in the bone marrow stimulates myeloid lineage immune cells such as monocytes and neutrophils,^{15,16} which can migrate into the tumor and promote metastasis.⁶ Activation of β -adrenoceptors promotes tumor cell dissemination by increasing the density of intratumoral blood and

lymph vessels.^{17,18} Inflammation can also promote SNS-dependent angiogenesis.⁶ SNS signaling also directly affects cancer cell behavior by increasing invadopodia formation, which facilitates invasion and escape from the tumor.¹⁹

MECHANOBIOLOGY AND CANCER

Although much is understood about how neural signaling regulates many aspects of tumor biology, less is known about how the SNS regulates the physical and mechanical properties of tumor cells and other cell types in the tumor microenvironment. The mechanical properties of cells determine how they sense and respond to external mechanical cues and play crucial roles in myriad biological functions.²⁰ Mechanical phenotype or ‘mechanotype’ is the inherent property of a cell that determines its capacity to deform: stiffer cells deform less in response to mechanical load compared to softer cells. Cells have both elastic and viscous characteristics, which are largely determined by three main classes of cytoskeletal proteins: filamentous actin (F-actin), intermediate filaments and microtubules. The contribution of these cytoskeletal components to the cell mechanotype depends on their expression levels, structure and organization, which can be modulated by filament crosslinkers (fascin, fimbrin, α -actinin, spectrin, filamin and dystrophin), motor proteins (myosins), as well as other regulatory proteins (Rho GTPases, profilin and cofilin). The nucleus is also emerging as a major factor in regulating cell deformability.²¹

The physical properties of cells and extracellular components in the tumor microenvironment may have functional consequences for cancer progression. As summarized in Figure 2, the mechanical properties of cancer cells and their surrounding environment could be implicated in many steps of metastasis.³ However, there is still much to learn about which factors regulate the mechanotypes of cancer cells and cells in the surrounding environment including tumor-associated immune cells. Until now, only a short list defines critical genes and signaling pathways that regulate cell mechanotype.^{22–24} Even less is known about extrinsic factors, such as physiological influences and environmental cues, which can also trigger changes in the mechanical properties of cells.^{25,26}

Expanding our knowledge of the physical factors that impact cancer progression will help us to achieve a more integrated understanding of this disease, ultimately allowing us to develop novel, more efficient therapeutic drugs and preventative strategies. Here we explore the hypothesis that the SNS—a physiological factor present in the tumor microenvironment—may influence cell mechanotype to impact tumor cell behavior and cancer progression.

We will review how SNS signaling through β -adrenoceptors regulates cancer cell structure and how cell mechanotype is linked to a malignant phenotype. Physical changes that occur during cancer progression in immune cells and the ECM will also be discussed. Finally, we will describe how an understanding of neural regulation of cancer mechanobiology provides new opportunities for therapeutic intervention, and new strategies for cancer diagnosis and prognosis.

NEURAL SIGNALING REGULATES TUMOR CELL STRUCTURE AND METASTASIS

A growing number of preclinical studies support the notion that SNS signaling regulates metastasis. Metastasis is responsible for 90% of deaths from solid cancers;²⁷ once cancer metastasizes, there are limited treatment options. Studies using mouse models of cancer demonstrate that β -adrenergic signaling accelerates the progression of multiple types of cancer.^{5,6,17,28–31} For example, the experience of chronic stress

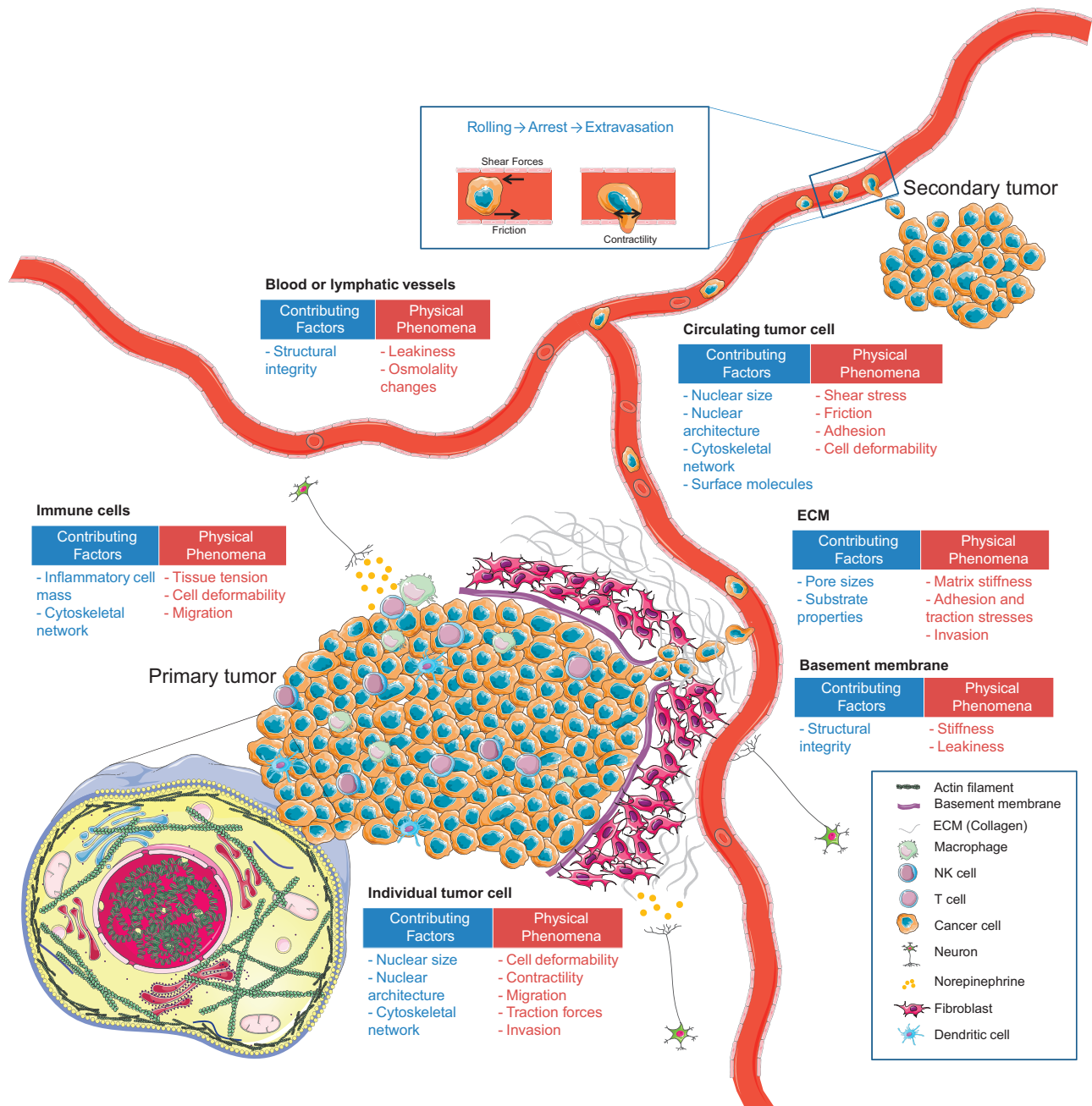


Figure 2 Mechanical properties of cancer cells and the tumor microenvironment. The mechanical properties of cancer cells and the tumor microenvironment may be implicated in various steps of tumor metastasis. There is an interplay between tumor cells in the primary tumor and their environment, as tumor cells communicate with both the extracellular matrix (ECM) and immune cells. Metastatic tumor cells become dissociated from primary tumor and move into blood vessels by passing through the basement membrane and ECM structures. Tumor cells in circulation are subject to various types of physical stresses such as shear forces and friction. Contributing factors (blue) that can increase or decrease metastatic or invasive potential of tumor cells. Physical phenomena (red) can be observed or measured during metastasis. The placement of sympathetic nerve fibers indicates cellular interactions that are regulated by neural signaling. Images were adopted from Servier Medical Art by Servier (<http://www.servier.com/Powerpoint-image-bank>) and modified by the authors under the following terms: CREATIVE COMMONS Attribution 3.0 Unported (CC BY 3.0).

or pharmacological activation of SNS pathways with β -adrenergic agonists, both increase tumor growth and metastatic dissemination. In many studies, metastasis is the key step in cancer progression, which is regulated by β -adrenergic signaling.^{5,6,17,28} Importantly, mechanistic studies also demonstrate that inhibiting β -adrenergic signaling with β -blockers prevents the adverse effect of SNS signaling on cancer progression.^{5,6,17,28,29} In accordance with those preclinical data, epidemiological studies support the idea that pharmacological

inhibition of β -adrenergic signaling improves cancer outcomes. Retrospective analyses of several cohorts show that cancer patients who are incidentally exposed to β -blockers for treatment of hypertension are likely to have reduced metastasis and improved survival (for summary of these studies, see ref. 32). Although these clinical findings are compelling, the results of these retrospective studies are limited to association and cannot demonstrate causality or define mechanism. Prospective randomized clinical studies will be essential to investigate

causal relationships between stress signaling and cancer outcomes in patients.

At the cellular level, SNS signaling may regulate cancer progression by inducing physical changes in cancer cells. For example, preclinical studies show that SNS signaling increases invadopodia formation.¹⁹ These structural changes induce protrusions from the plasma membrane that enable tumor cells to localize cell-derived proteases and increase their capacity to invade.¹⁹ The formation of invadopodia is accompanied by changes in focal adhesions that reduce cell migration on two-dimensional surfaces. β -adrenergic regulation of focal adhesion kinase—a key protein in the formation of focal adhesions and invadopodia—also protects tumor cells from anoikis, which increases their survival in circulation and promotes metastasis.³³

Although the effects of the SNS on cancer cell mechanotype remain to be investigated, activation of β -adrenoceptor signaling is known to regulate the mechanical properties of other cell types, including blood and muscle cells. Treating red blood cells and airway smooth muscle cells with the β -adrenoceptor agonist, isoproterenol, makes these cells more deformable.^{34,35} In contrast, treating primary cardiomyocytes with the same drug makes these cells stiffer.³⁶ These differences may reflect signaling from different β -adrenoceptor subtypes and/or the variations in readout from different measurement techniques that depend on time and length scales (Box 1). Taken together, these studies suggest that activation of β -adrenergic signaling plays a role in regulating the deformability of various cell types. Future studies to investigate the effects of β -adrenoceptor activation on the mechanical properties of cancer cells could provide insight into the mechanism of neural signaling in cancer progression.

CELL MECHANOTYPE IN MALIGNANT TRANSFORMATION

Although the effect of SNS activation on tumor cell deformability is unknown, cell mechanotype appears to relate to invasive status.^{37–39} Recent experiments show that the invasive potential of cells correlates with their deformability: softer or more deformable cells are more invasive.^{38,39} The increased invasive potential of softer cells may be explained by the idea that more deformable cells can move more easily through tight gaps, which could assist their escape from a primary tumor and invasion into surrounding ECM. There is also growing evidence from *in vitro* experiments that malignant cancer cells are softer or more deformable than benign cells. This is shown through experiments on human cell lines derived from various tissues using

Box 1 Measuring mechanotype

The mechanical properties of biological materials can be characterized using various techniques. Common methods to measure the mechanical properties of cells and protein networks are summarized in Figure 3 (for details, see review³). Many of these methods measure the displacement or change in shape of a cell or protein network in response to well-defined physical stresses. Atomic force microscopy is used to measure the viscoelastic properties of a single cell or protein network with displacements down to 1 nm. Other techniques, such as magnetic twisting cytometry and micropipette aspiration, can probe mechanical properties over length scales of 1–10 μm . The deformability of whole cells can also be measured by forcing cells to pass through smaller pores than cell size. For example, the parallel microfiltration method, which applies air pressure to a cell suspension to pass cells through a porous membrane, can measure the relative deformability of different cell samples.³⁷ Microfluidic deformability cytometry measures whole-cell deformability by applying stretching extensional flow to single cells.⁴² Active forces generated by the cell can be probed by traction force microscopy, where displacements of the substrate that result from contractile forces of the cell are measured.

different mechanotyping methods, such as atomic force microscopy,⁴⁰ optical stretching,⁴¹ deformability cytometry⁴² and parallel microfiltration³⁷ (Box 1, Figure 3). Cell deformability is associated with the aggressiveness of tumor cells. For example, overexpression of key transcription factors (Snail, Slug and Zeb1) that are involved in epithelial-to-mesenchymal transition makes ovarian cancer cells softer.³⁷ Human mammary carcinoma (MCF-7) cells are more deformable than their non-malignant mammary epithelial counterparts (MCF-10), and metastatic MCF-7 (modMCF-7) cells are even more deformable than the less invasive MCF-7 cells.³⁸ Human lung adenocarcinoma cells with greater metastatic potential are also more deformable than their less metastatic counterparts.³⁹ Human bladder epithelial cancer cells are more deformable than normal cells.⁴³ Similarly, transformed fibroblasts are significantly more deformable than normal untransformed fibroblasts.^{38,44} Taken together, malignant cells across various types of cancers are more deformable than normal cells. Moreover, more invasive tumor cells are softer or more compliant than less invasive tumor cells. However, it is still unclear whether increased cell deformability drives invasive potential and metastasis, or whether it is simply a byproduct of selective pressures that are applied during metastatic progression. Nevertheless, cell mechanotype provides information about the invasive capacity of cancer cells and is thus emerging as a complementary biomarker for malignancy; such information may be potentially important for cancer treatment and outcomes.

Deciphering the molecular origins of the altered mechanotype of malignant cells is a complex challenge. Mechanotype is an emergent property that can change as a result of multiple proteins, signaling pathways and other factors. Changes in the structure and organization of cytoskeletal networks are well known to alter cell mechanotype or deformability; such structural changes are also linked to malignant phenotype. For example, actin and microtubule content is increased in high grade colon cancer and ovarian cancer cells compared to low grade cancer cells.^{45,46} Differences in cytoskeletal architecture also underlie variations in the deformability of melanoma cells; these structural alterations are associated with *in vivo* metastatic potential in mouse models.⁴⁷ However, although changes in cytoskeletal architecture are often observed, they do not always correlate with the softer mechanotype of cancer cells: whereas mesenchymal-type ovarian cancer cells are consistently softer than their epithelial-type counterparts, there is no consistent pattern of actin distribution or microtubule organization that can explain the softer mechanotype.³⁷ Cell mechanotype can thus provide unique information as a biomarker to classify the malignant status of a cell.

Cancer cell deformability also changes after treatment with chemotherapeutic agents. Considering that many chemotherapy drugs are designed to target cytoskeleton components and arrest cell division, it is not surprising that anti-tumor drugs can alter cancer cell mechanotype. For instance, taxanes and vinca alkaloids disrupt the structure and dynamic of microtubules, and result in inhibition of mitosis. The common chemotherapy compound, paclitaxel or Taxol, is widely used in ovarian, breast and non-small cell lung cancers. By binding to microtubules, this drug inhibits cell division, and also makes cells stiffer or less deformable at concentrations of 0.1 to 1 000 nM.³⁷ Whereas other studies show that endometrial and cervical cancer cell lines treated with a much higher concentration (50 μM) of Taxol become softer, or more deformable.⁴⁸ These results suggest that alteration in mechanotype with Taxol treatment is concentration dependent. Moreover, different cell types (SKOV3 and OVCA433 vs Ishikawa and HeLa) can exhibit different responses to the same drug. Different measurement techniques (parallel microfiltration vs atomic

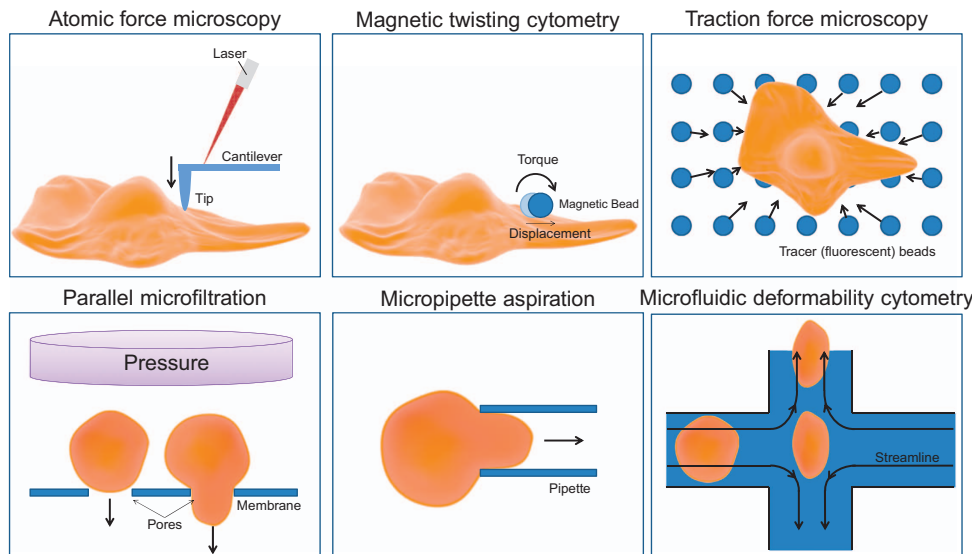


Figure 3 Methodology for measuring the mechanical properties of cells. Different methods are used for measuring mechanical properties of cells (See Box 1). Images are adapted from Servier Medical Art by Servier (<http://www.servier.com/Powerpoint-image-bank>) and modified by the authors under the following terms: CREATIVE COMMONS Attribution 3.0 Unported (CC BY 3.0).

force microscopy) can also yield varying results depending on the length scale of deformation (Box 1). Nevertheless, anti-tumor reagents that target cytoskeletal components consistently alter the stiffness of cancer cells. Interestingly, chemotherapeutic reagents that do not target subcellular structures can also change the deformability of cancer cells. For example, the DNA intercalating agent daunorubicin (an anthracycline) and the corticosteroid dexamethasone both stiffen acute lymphoblastic leukemia and acute myeloid leukemia cells.⁴⁹ Treatment of metastatic tumor cells from patient effusions and A549 human lung adenocarcinoma cells with green tea extract, which exhibits anti-cancer properties, causes these cells to be stiffer.⁵⁰

Although the molecular mechanisms of these clear differences in the cancer cell mechanotype with transformation and with chemotherapy exposure are still not completely understood, the physical property of cells is emerging as a useful label-free biomarker. Currently, cancer diagnosis and prognosis rely on various methods including screening strategies, such as positron emission tomography, magnetic resonance imaging and mammogram, immunohistochemical staining for various biomarkers and analysis of genome sequences. These traditional approaches may be complemented by analysis of tumor mechanotype. For example, magnetic resonance elastography probes the elastic properties of breast tissues *in vivo*.⁵¹ For analysis of excised tumor cells, rapid, simple and scalable methods such as parallel microfiltration or microfluidic deformability cytometry could be used for diagnosis, prognosis and detecting effects of drug treatments in cancer patients.^{37,42} High throughput analysis of cell mechanotype distinguishes circulating cancerous cells from normal cells in patients, and demonstrates how this approach can enhance the accuracy of pathological diagnosis, especially in borderline cases that are challenging to assess by visual inspection alone.⁵²

SNS REGULATES TUMOR-ASSOCIATED INFLAMMATION: THE POTENTIAL ROLE OF MECHANOTYPE

The tumor microenvironment is highly complex and dynamic. During tumor development, the local microenvironment evolves with crosstalk among various residing cells including tumor, endothelial, immune cells and fibroblasts. For example, tumor cells secrete

chemotactic factors that recruit immune cells into the tumor microenvironment.⁵³ The fate of carcinoma cells that leave the primary tumor is determined by the balance between tumor-promoting and tumor-suppressing immune cells, which regulate whether tumor cells will be eliminated by the immune system or escape immune surveillance. In the tumor microenvironment, heterogeneous populations of immune cells may be present that include both tumor-promoting immune cells (including M2 macrophages, myeloid derived suppressor cells and regulatory T cells) and tumor-suppressive immune cells (including natural killer cells and CD8⁺ T cells).⁵⁴ However, accumulating evidence shows that tumor-promoting immune cells are selectively recruited to the established tumor parenchyma and promote tumor progression.⁵⁴ Macrophages play a role in tumor progression as they are critical in inflammation, modulating tumor microenvironment and promoting metastasis.⁵⁵ In a highly pathogenic feedback loop, tumor cells respond to growth factors produced by immune cells—particularly M2-skewed macrophages—that drive tumor cell proliferation.⁵⁶ These interactions between tumor cells and subverted macrophages also drive invasion of tumor cells, leading to increased metastasis.⁵⁷ Hence, identifying factors that modulate the infiltration of immune cells into the tumor microenvironment is essential to eradicating cancer.

Recruitment of macrophages to tumors is regulated by SNS signaling, which accelerates metastasis.⁶ Myeloid cells in bone marrow express β -adrenergic receptors allowing them to recognize SNS-derived signals in the form of stress neurotransmitters.⁵⁸ β -adrenergic signaling thus induces recruitment of macrophages into the tumor parenchyma.⁶ Those tumor-associated macrophages facilitate pro-metastatic events, such as increased angiogenesis, remodeling of ECM, generation of pro-inflammatory environment, recruitment of immune and cancer cells, and inhibition of anti-tumor immune responses.^{6,17,59} Sensory neurons also play a well documented role in inflammation (see review⁶⁰ for details), however, this review is focused on the role of SNS in tumor-associated inflammation.

Macrophages are not the only type of immune cell that are regulated by SNS signaling and contribute to cancer progression. Other tumor-associated immune cells, such as natural killer cells and

T cells express β -adrenoceptors, and their function is sensitive to SNS signaling.^{61,62} Studies in rat models of cancer show that neural signaling suppresses natural killer cell activity and compromises host resistance to natural killer-sensitive tumors.³¹ β -adrenergic activation by endogenous neurotransmitters directly inhibits the generation of anti-tumor cytotoxic T cells,^{63,64} and blocks the recruitment of protective T cells into squamous cell carcinoma.⁶⁵ This may be due, in part, to the limiting effects of β -adrenergic signaling on the egress of T lymphocytes from lymphoid organs.⁶⁵ In addition, immune cells themselves synthesize and secrete catecholamines to communicate with other immune cells.⁶⁶ Taken together, these data suggest that β -adrenergic activation either by SNS signaling or immune cells plays a critical role in directing the immune response in ways that favor escape from immune surveillance and promote tumor progression.

The changes in immune cell migration and recruitment to tumors that are activated by neural signaling are associated with cytoskeletal changes in immune cells. For example, factors that regulate immune cell chemotaxis to promote migration also induce structural changes in immune cells. The formation of filopodia-like projections allows immune cells to migrate in response to chemotactic molecular gradients and interact with pathogens.^{67,68} Such changes in migration behavior are also associated with altered cytoskeletal structure,^{67,69–71} which can also impact the mechanical properties of neutrophils, monocytes, dendritic cells and lymphocytes. For example, micropipette aspiration experiments show that cytoskeletal actin regulates macrophage deformability: macrophages treated with cytochalasin B are softer compared to vehicle-treated cells.⁷² In contrast, treatment with colchicine, a microtubule-disrupting agent, has no observable effect on cell deformability.⁷² These observations suggest that actin is a predominant regulator of macrophage deformability. Disrupting the connectivity between integrin and actin results in induction of a transcriptional program characteristic of migratory dendritic cells,⁷⁰ suggesting that changes in gene expression underlie changes in immune cell migration. Given that the physical properties of immune cells are important for their function,⁷³ a more complete understanding of the impact of neural signaling on immune cell mechanotype will be important to understand the impact of this physiological regulator on cancer progression.

PHYSICAL PROPERTIES OF THE ECM IN CANCER PROGRESSION

The ECM is essential to every organ; this three-dimensional, non-cellular structure is generated during early developmental stages and plays a critical role in normal tissue development and function.⁷⁴ In mammals, the ECM is composed of about 300 proteins including collagen, proteoglycans and glycoproteins, and provides physical support for tissue integrity and elasticity. ECM composition and structure are constantly remodeled by neighboring cells. For example, the ECM is composed of both soluble and insoluble factors that are secreted by stromal cells. To maintain homeostasis, tight regulation of ECM degradation and generation is required.⁷⁵ However, an increasing number of studies suggest that dysregulated ECM remodeling, such as aberrant deposition and loss of ECM components, contributes to cancer progression.⁵⁵

ECM stiffness plays a pivotal role in cancer progression.^{75,76} In fact, palpation has been used for centuries to detect tumors, which are generally stiffer than normal tissue. The rigidity of tumors originates from the stiffer ECM due to desmoplasia.⁷⁷ Changes in the rigidity of ECM can activate integrins and growth factor receptors, which then further alter matrix stiffness.⁴⁰ This positive feedback loop can impact malignant transformation similar to oncogenic signaling pathways.⁷⁷

For example, interstitial fibrosis promotes breast tumorigenesis by altering mammary ECM mechanics,⁷⁸ and ECM stiffening is also implicated in human breast cancer invasion and aggression.⁵⁵ The increased density of tumor ECM may also be a physical barrier to effective treatment by chemotherapy.⁷⁹

The stiffness of surrounding ECM also provides mechanical cues to cancer cells. For example, cells alter their morphology and intracellular tension in response to the stiffness of their substrate. When normal mammary epithelial cells are cultivated in soft substrate, they form acinar structures, whereas these cells grow in a monolayer when they are cultured on a stiff surface.⁸⁰ The mechanical properties of the cell nucleus, another major determinant of cell mechanical properties, are also directly influenced by matrix stiffness.⁸¹

Changes in the physical properties of the ECM may also influence the behavior of immune cells. Aberrant ECM stiffness and changes to ECM components in cancers may restrict or enhance immune cell migration.⁷⁵ Lysyl oxidase secreted by hypoxic breast tumor cells mediates collagen crosslinking; and accumulated collagen deposition is found in cancers that have increased ECM stiffness.⁷⁵ Increased density of ECM facilitates infiltration of tumor-promoting immune cells (CD11b⁺ myeloid cells) as they adhere to cross-linked collagen.⁸² The density and orientation of the ECM fibers can also regulate lymphocyte trafficking. T cells can migrate faster in ECM with loose fibronectin and collagen, whereas they migrate more slowly in dense ECM.⁸³ Integrins that mediate cell–cell and cell–ECM adhesion are also expressed in immune cells and influence cell migration and retention. For example, CD8-T cells express integrin that bind to collagen and aid their retention in the tumor microenvironment.⁸⁴ Furthermore, proteases secreted by cancer cells result in proteolysis and subsequent release of ECM fragments, which are known as matrikines; these regulate immune and pro-inflammatory cell behavior including immune cell migration. Such fragments of ECM and collagen that are generated by matrix metalloproteinases attract neutrophils to the sites of inflammation and promote dendritic cell egress.⁸⁵ ECM molecules and their derivatives may also affect the function of immune cells to regulate tumor inflammation. Versican, a large ECM proteoglycan, is upregulated in many cancers and activates macrophages to produce interleukin-6 and tumor necrosis factor, thereby establishing a pro-inflammatory microenvironment.⁸⁶ Taken together, these observations illustrate that ECM physical properties can regulate immune cell migration and direct immune responses, and thereby also affect cancer progression by determining how effectively the immune cells can infiltrate into the tumor.

Although cancer progression can be modulated by both the physical properties of the ECM and the SNS, it is still unclear how signaling from SNS may affect the ECM.⁸⁷ But as SNS regulates growth factors and cytokines that can affect ECM remodeling, stress-responsive neural signaling may impact the physical properties of the ECM to affect cancer progression. For example, transforming growth factor- β (TGF β) is implicated in the production and crosslinking of collagen in rat muscle cells.⁸⁸ The expression of TGF β is regulated by norepinephrine, where it is implicated in interstitial fibrosis associated with increased expression of ECM components such as fibronectin and TGF β in rat hearts.⁸⁹ These observations suggest that β -adrenergic signaling may regulate the density of components of the ECM.

CLINICAL IMPACT AND POTENTIAL TARGETS FOR THERAPEUTIC BENEFIT

Our current body of knowledge on neural regulation of cancer and mechanobiology provides several potential therapeutic interventions. Links between neural signaling and cell mechanotype suggest that

modulating SNS signaling could be used to change the physical properties of tumor cells and tumor-associated immune cells to slow cancer progression and improve survival. This may be particularly useful in cancer subtypes with limited treatment options, such as triple negative breast cancer, in which β -blocker use is linked to improved outcome.^{90,91} The role of cell mechanotype and cell–ECM interactions in modulating cancer progression suggests that monitoring the mechanotype of tumor cells or tumor-associated immune cells could complement other readouts of treatment efficacy. Until now, evidence that β -blockers improve survival in cancer patients comes from retrospective cohort datasets. Moreover, *in vitro* studies show that inhibition of β -adrenergic signaling reduces the invasive potential of cancer cells. It will be important to evaluate the effect of β -blockers on cancer cell mechanotype and the physical properties of cancer cells in patients, as well as on other cells including immune cells. Understanding how β -adrenoceptor subtypes modulate the physical properties of cells in the tumor microenvironment will also provide deeper insight into cancer progression. For example, our recent studies show a critical role for β_2 -adrenoceptor signaling in SNS regulation of cell structure and invasion;^{19,92} The β_3 -adrenoceptor has also been implicated in cancer progression.^{30,93}

Pharmacological interventions that modulate cell or ECM mechanical properties could also be used to slow cancer progression. For example, ECM-modulating enzyme inhibitors (for example, inhibitors of matrix metalloproteases) effectively slow cancer progression in preclinical models of cancer, although their efficacy has been disappointing in the cancer clinic.⁹⁴ However, recent Phase 2 clinical trials have evaluated the effects of simtuzumab, an antibody that inhibits lysyl oxidase-like 2, in colorectal adenocarcinoma and pancreatic adenocarcinoma (NCT01479465 and NCT01472198). The TGF β -inhibitory antibody is also being evaluated in multiple cancers including breast, lung and glioma; TGF β is critical for regulating the expression of proteins that comprise the ECM (NCT01401062, NCT02581787 and NCT01472731).

New technologies that probe cell mechanotype may be used to identify patients who may benefit from interventions that target the physical properties of cancer cells. For example, deformability cytometry can define whether cells from pleural effusions are malignant or benign.⁹⁵ The observation that malignant cells are more deformable than normal cells^{38,52} suggests that mechanical biomarkers may enhance diagnosis, either in a label-free manner or in combination with current immunohistological methods of diagnosis.

In addition, the mechanical properties of cells can be used as an indicator of drug response. As discussed earlier, many cancer drugs target the cytoskeleton and alter the viscoelastic properties of cells.^{37,48,96} Moreover, cell mechanotype can identify cells that are resistant to common chemotherapy drugs. Cisplatin-resistant ovarian cancer cells are less deformable due to modification of their actin cytoskeleton.⁹⁷ The altered mechanotype of chemoresistant cells can be utilized in large-scale drug screening using cell deformability as a readout; mechanotype measurements are advantageous for high-throughput screening as an inexpensive, label-free approach. Indeed, we have identified compounds that reduce cell deformability from a small molecule mechanotype screen; these compounds also inhibit invasion and anchorage independent growth of tumor cells (unpublished data). Such a screening method may be adapted for use with clinical cancer samples to define the drug sensitivity of cancer cells from patients. This may help to guide choice of therapeutic strategies, which could address the challenge of chemoresistance in cancer treatment.

Cell mechanotype may also be used as a prognostic tool to identify patients with advanced disease. As metastasis accounts for most cancer deaths, secondary tumors are an important prognostic factor in cancer patients. As metastasizing cancer cells travel via the circulatory system, detection of circulating tumor cells (CTCs) in vasculature, is one way to predict the degree of metastasis. CTCs can be isolated based on their biophysical properties, as CTCs have greater nuclear to cytoplasmic ratio and are a larger size than normal cells.⁹⁸ Cell mechanotype may thus be an effective prognostic biomarker for CTCs.⁹⁸ In a similar way, detection of CTCs may be applied to determine the presence of residual cancer cells or relapse of tumor after treatment.

Significant advances in our understanding of cancer biology are emerging with expanded knowledge of cancer immunology and the development of techniques to evaluate the physical properties of tumor cells and their microenvironment. However, a deeper understanding of how physiological factors such as SNS signaling modulate mechanical properties of tumor cells, immune cells and the ECM will provide novel targets for new therapies that may help to treat metastasis and improve cancer survival. Cancer is a complex disease. Achieving a more integrated understanding of the relationships between physiological regulators and the physical properties of tumor cells and their environment will enable a more holistic perspective on cancer, and ultimately more effective treatments.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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