1 The National Cancer Institute Investment in Biomechanics in Oncology Research

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Abstract

The qualitative description of tumors feeling stiffer than surrounding normal tissue has been long appreciated in the clinical setting. These empirical observations have been corroborated by the precise measurement and characterization of mechanical properties of cancerous tissues. Much of the advancement in our understanding of mechanics in oncology has been enabled by the development of innovative technologies designed to probe cells and tissues as well as integrative software analysis tools that facilitate biological interpretation and generation of testable hypotheses. While some mechanics in oncology research has been investigator-initiated and supported by the National Cancer Institute (NCI), several NCI programs described herein have helped to foster the growth of the burgeoning field. Programs highlighted in this chapter include Innovative Molecular Analysis Technologies (IMAT), Physical Sciences–Oncology Network (PS-ON), Tumor Microenvironment Network (TMEN), Integrative Cancer Biology Program (ICBP), and the Cancer Systems Biology Consortium (CSBC). This chapter showcases the scientific contributions of these programs to the field of biomechanics in oncology.

Keywords

National Cancer Institute · National Institutes of Health · Government programs · Funding · Physical Sciences-Oncology Network · Innovative Molecular Analysis Technologies Program · Mechanobiology

What is biomechanics in oncology? It is indeed a broad field, encompassing the study of how mechanical properties of cells and tissues are altered during cancer progression and the dynamic, multi-scale feedback loop where these changes synergize with other physical and chemical factors to impact cancer cells and the tumor microenvironment. Mechanics is an important contributing factor during all stages of tumor progression, including initiation, migration, metastasis, plasticity, treatment response, dormancy, and recurrence.

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The qualitative description of tumors feeling stiffer than surrounding normal tissue has been long appreciated in the clinical setting. These empirical observations have been corroborated by the precise measurement and characterization of mechanical properties of cancerous tissues. Much of the advancement in our understanding of mechanics in oncology has been enabled by the development of innovative technologies designed to probe cells and tissues as well as integrative software analysis tools that facilitate biological interpretation and generation of testable hypotheses. While some mechanics in oncology research has been investigator-initiated and supported by the National Cancer Institute (NCI), several NCI programs described herein have helped to foster the growth of the burgeoning field. Programs highlighted in this chapter include Innovative Molecular Analysis Technologies (IMAT), Physical Sciences-Oncology Network (PS-ON), Tumor Microenvironment Network (TMEN), Integrative Cancer Biology Program (ICBP), and the Cancer Systems Biology Consortium (CSBC). This chapter showcases the scientific contributions of these programs to the field of biomechanics in oncology.

1.1 Innovative Molecular Analysis Technologies Program

Scientific research is simultaneously enabled and limited by the tools available for exploring compelling questions. The potential for progress and the associated rate of discovery for any given field is often reliant on the development of new and better-suited technologies to pursue these questions. This is especially true for cancer research given the complexity of cancer biology and our ever-expanding appreciation for the broad diversity of cellular features and biological constituents that contribute to its development and progression. The NCI employs a variety of funding mechanisms for spurring development of new technologies, and the strategy for this broadly evolves with the ever-changing landscape of both science and technology. Since 1999, the NCI has maintained the Innovative Molecular Analysis Technologies (IMAT) program for supporting highly innovative technology concepts relevant to the full breadth of the cancer research spectrum.

The IMAT program is focused on supporting the development of highly innovative technologies that promise new capabilities for probing, targeting, or otherwise assessing molecular and cellular aspects of cancer biology. Tools for evaluating the mechanical properties that distinguish cancer cells from non-cancer cells and how the mechanical properties of those cells and of surrounding tissue affect tumor progression are all well within the scope of the program's interest. The breadth of the competitive landscape for IMAT awards and the program's longevity allows the program itself to serve as a useful window into how the NCI has considered contributing to advances in mechanobiology.

Applications specifically proposing to investigate mechanobiology features of cancer were received by the program as early as 2006, with the first award given in 2008 to develop a new optical technique to study the architecture of extracellular matrices [1, 2]. The development of the optics associated with this project led to the integration of quantitative fluorescence lifetime imaging microscopy (FLIM) and second harmonics generation (SHG) for label-free, noninvasive metabolite imaging of tumor-associated macrophages in the intact tumor microenvironment [3]. IMAT also supported the development of a high-throughput ballistic injection nanorheology platform to quantitatively measure intracellular mechanical properties by particle tracking methods [4].

Consistent with other fields of technology development and cancer research, a great deal of interest and growing excitement exists for more appropriately recapitulating and modeling the complexity of different tumor microenvironments (TME). Applications to develop imaging or other mechanical probing capabilities for rheological assessment of the TME, and more recently to leverage emerging materials and techniques to more accurately model the TME in vitro, have grown significantly in the last several

years. There is also growing interest in advancing our capabilities to detect and track cancer progression and response to treatment by evaluating cells collected from blood, also known as liquid biopsies. The ability to rheologically assess individual cells, often in addition to other techniques (e.g., size or cell surface marker labeling), has also seen substantial growth. The need and the enthusiasm by the cancer research community for such tools suggest further technology development may occur in this area.

The IMAT portfolio includes tools for direct interrogation of cell plasticity and deformability as well as the mechanics of cell migration through tissue. The biology of individual cells continues to hold many unknowns, and peripheral advancements in single-cell analysis (e.g., single-cell whole-genome and transcription analysis) suggests that more appropriate tools for integrating the rheological assessment to provide a more complete understanding of cell biology will continue to be needed. It is reasonable to anticipate that better tools will be needed to study cellular migration mechanisms for at least two reasons: first, as cancer research advances to offer a more accurate accounting of the TME, better tools will be needed to study invasive tumor cell migration in those environments; and second, exciting new capabilities for conscripting a patient's immune system to fight the disease will require a better appreciation of native and engineered immune cell migration into and through solid tumors and any treatment resistance mechanisms employed by cancer cells.

The IMAT program has supported ten distinct technologies through 2017 that offer new assessment capabilities for the field of cancer mechanobiology. The overall growth trend and enthusiasm for such applications within the IMAT program suggest that this will continue to serve as a useful window into tracking evolving interests and NCI priorities in this field.

1.2 Physical Sciences – Oncology Network Program

Recognizing the importance of the broad area of convergence in physical sciences in cancer research, in 2009 the NCI launched the Physical Sciences in Oncology Initiative to foster the integration of physical sciences perspectives and approaches in cancer research [5]. One area of emphasis the initiative supports is the study of physical laws and principles of cancer, notably how physical properties spanning length scales from subcellular to tissue level can be integrated with the molecular and genetic understanding of cancer to generate a more comprehensive view of the complex and dynamic multi-scale interactions of the tumor-host system. Techniques from the physical sciences are used to measure physical properties of single cells, discrete multicellular structures, and tissues. These measurements are being integrated with orthogonal data using high-dimensional analysis and computational modeling approaches. PS-ON research is being conducted via both multi-project Physical Sciences-Oncology Centers (PS-OCs) and single Physical Sciences-Oncology Projects (PS-OPs). An important element of the PS-OCs is the education and outreach component that focuses on training the next generation of transdisciplinary cancer researchers who bring physical sciences perspectives (including mechanobiology) into basic cancer biology and oncology. Moreover, the PS-ON awards have funds to support trans-network projects that may be used to advance novel, collaborative studies related to biomechanics in oncology.

Since 2009, the PS-ON program has supported research in this broad area of cancer mechanobiology to over 20 transdisciplinary research teams spanning more than ten US institutions. This section will describe the research advances in cancer mechanobiology that were made with support from the PS-ON program.

1.2.1 Cornell University

The Cornell University PS-OC examines the multi-scale biological and physical (structural, mechanical, and solute transport) mechanisms regulating tumor metabolism and function. They test the physical mechanisms by which the microenvironment regulates tumor metabolism and how obesity affects this interplay, investigate the role of altered metabolism and the physical microenvironment in modulating the biogenesis and function of microvesicles, and evaluate the integrated effects of physical and metabolic constraints on tumor cell migration and invasion.

Cornell University PS-OC researchers recently showed that cancer cells with high levels of chromosome instability can withstand migration through small, 1 μm constrictions due to more efficient repair of the nuclear membrane via activation of the STING pathway [6]. A mechanistic computational model was developed to predict the ability of cells to pass through small constrictions and thresholds for nuclear envelope rupture [7]. The model parameterizes actin contraction and cytosolic back pressure, and the nucleus is modeled as an elastic shell nuclear envelope with poroelastic material for the nucleoplasm and recapitulated nuclear envelope rupture found in experimental models of cancer cell migration [8]. If cancer cells are deficient in nuclear structural proteins lamins A and C, then they experience increased shear stress-induced apoptosis and are not as proficient at surviving the circulation during metastasis [9].

TGF-β-induced epithelial-to-mesenchymal transition of basal-like breast cancer cells resulted in more deformable nuclei that facilitate cell migration through constrictions and metastasis [10]. In this study, a computational motor-clutch model of cellular tractions suggests that this is due to larger numbers of both myosin II motors and integrin-mediated adhesion clutches. The shift to where the clutch strength matches that of the motors results in slower actin flow, enhanced cell spreading, and higher traction forces, which was experimentally observed in breast cancer cells with increased metastatic potential.

Cancer cells in fibrotic tumors characterized by collagenous stroma often have increased surface expression of α5β1 integrin, which is a fibronectin receptor [11]. Fibronectin being important for collagen cross-linking is an important signaling factor for downstream PI3K-dependent invasion. The nonlinear elasticity of the 3D fibrous extracellular matrix was shown to permit a positive feedback loop where cells pulling on collagen locally align and stiffen the matrix, and stiffer matrices promote greater cell force generation [12]. Also, cell force transmission distance increases with the degree of strain-induced fiber alignment and stiffening of the collagen matrices. Obesity was shown to play a role in increased fibrotic remodeling in breast cancer patient samples, and caloric restriction in obese mouse models resulted in decreased tissue fibrosis [13]. Early matrix stiffening is attributed in part to a stiffer fibronectin matrix and increased molecular unfolding of fibronectin that is secreted by pre-adipocytic stromal cells [14].

1.2.2 Johns Hopkins University

The Johns Hopkins University PS-OC develops an integrated approach for an in-depth understanding of the physical and chemical cues mediating local cancer cell invasion from the hypoxic primary tumor to distant organs, through single and collective invasion into the extracellular matrix (ECM) and confined migration along narrow tracks, which represent early steps in the metastatic cascade. They are testing the hypothesis that the physical microenvironment induces a signaling cascade of events that transforms collective to single-cell invasion, which may be facilitated by hypoxia-induced ECM remodeling. And they want to understand which forces are critical for the collective migration of tumor cells, whether the forces are passive (elastic and adhesive forces), frictional (resistance to cells sliding past one another and cells sliding across a substrate), active (protrusive and contractile forces), and traction forces upon the underlying or surrounding ECM.

Johns Hopkins University PS-OC team members showed that cancer-associated fibroblasts (CAFs) are mechanically active cells in the tumor microenvironment that regulate vascular growth. Using a 3D experimental model of vasculogenesis, it was shown that breast CAFs increased vascularization compared to normal breast fibroblasts by generating significantly larger deformations in the matrix [15]. By blocking several soluble factors, they demonstrated that the CAF-supported vessel growth is not completely attenuated, thereby demonstrating that the CAF-mediated mechanical activity is an important contributor as well.

Cell invasion and motility were modeled by a mechanochemical computational model specifically to study cell invasion from tumor clusters. The nonlinear mechanical properties of the ECM were shown to augment cell contractility, thereby providing the driving force for invasion [16]. Key findings of the model, which were corroborated experimentally in a 3D collagen melanoma model, were a biphasic relationship between the invasiveness and the matrix concentration. These data suggest that cancer cells have a context-dependent optimal stiffness for efficient migratory function in a context-dependent manner. Further, collective invasion was shown to be induced by anisotropic contractile stresses exerted on the ECM [17]. The fibrosarcoma cells in this study displayed highly aligned and elongated morphology at spheroid peripheries, which was shown to depend on β1 integrin-mediated cell adhesion and myosin II and ROCK-based cell contractility.

Aberrant nuclear morphology in cancer cells could be dictated by the pressure difference across the nuclear envelope, which is influenced by changes in cell volume and regulated by actin filaments and microtubules [18]. The osmotic pressure across the nuclear envelope is unequal due to its high concentration of genetic material and nuclear chromatin. A theoretical model demonstrates that when a cell is attached and spread on a substrate, the osmotic pressure inside the nucleus is larger than that of the cytoplasm, and the nucleus is inflated as opposed to becoming buckled and invaginating laterally. It was estimated that microtubules can apply a compressive force on the nucleus on the order of 10–100 Pa. A perinuclear actin cap that has been observed in polarized cells can exert tension on the apical surface of the nucleus [19].

Mechanical properties of cancer cells important for cell motility work in concert with their metabolic phenotype. Higher levels of glycolysis were shown to promote increased rates of cytoskeletal remodeling, greater traction forces, and faster cell migration [20]. These enhancements could be blocked by inhibiting glycolysis, but not by blocking mitochondrial ATP synthesis. The energy dependence of cancer cells on aerobic glycolysis rather than oxidative phosphorylation suggests that ATP localization with sites of active cytoskeletal remodeling is necessary for cell motility. Moreover, intratumoral hypoxia which promotes HIF production leads to cell and matrix contraction, focal adhesion formation, and breast cancer cell motility via phosphorylation of MLC, FAK, Rho, and ROCK [21].

1.2.3 Massachusetts Institute of Technology and The Methodist Hospital Research Institute

The PS-OCs at both the Methodist Hospital Research Institute and Massachusetts Institute of Technology use integrated analysis of patient and animal tumor models to understand physical factors in tumor architecture that influence heterogeneous drug distribution and the resulting biology. Mathematical models of abnormal interstitial fluid flow and the associated interstitial fluid pressure which mediates vascularized tumor growth demonstrate negative effects on the transport of therapeutic agents during chemotherapy [22]. Also, to better understand the emergence of drug resistance, a key factor under consideration is local drug concentrations within the tumor microenvironment, which has been shown to play a significant role in disease progression [23].

The development of high-throughput technologies to measure functional, phenotypic alterations in blood circulating tumor cells is

a promising area due to the paucity of predictive genetic biomarkers for many cancers. At the Massachusetts Institute of Technology PS-OC, they have developed a novel cantilever capable of measuring mass accumulation by shifts in resonance frequency that has been engineered and utilized to predict drug response [24]. Results indicated that cancer cells with reduced mass accumulation rates upon drug treatment predict drug sensitivity to targeted therapy. A modification to the cantilever whereby a 6-μm wide constriction is integrated into the 20-μm wide device allows for characterizing differences in deformability between tumor cells and blood cells, based on the duration of their passage through the constriction [25]. Cell types with metastatic potential are capable of transiting through the constriction at higher velocity, perhaps suggesting that the reduced friction associated with higher transit velocity may be a factor in cancer cell invasion through tight spaces [26].

1.2.4 University of Minnesota

The University of Minnesota PS-OC integrates modeling and experiments to investigate the molecular mechanics of cell migration and how the tumor microenvironment regulates disease progression as a function of the underlying cancer genomics. In a biophysical model for cell migration, it was shown that the survival of high-grade glioma patients is biphasically correlated with cell surface expression levels of CD44 [27]. CD44 is being explored as a potential molecular clutch that mediates cell migration, whereby cells with intermediate levels of CD44 exhibit the fastest migration rates and could be best suited for anti-CD44 therapy. It was also demonstrated both computationally and experimentally that many cell types are most migratory on an optimum stiffness, which is dictated by the number of active molecular motors (e.g., f-actin) and clutches (e.g., integrins) [28]. Further studies of forces exhibited during single-cell migration showed that force anisotropy is predominant in cancer cells that exhibit directional persistence when migrating along aligned matrix fibers [29]. The force anisotropy, which is the ratio of forces along the direction of cell alignment to the orthogonal direction, is associated with an increased number of larger and longer focal adhesions in the direction of matrix alignment.

1.2.5 Northwestern University

One focus area of the PS-OC at Northwestern University seeks to analyze the variation in chromatin structure—from the fiber level to chromosomes to the whole cell nucleus—using physical science-based tools such as spectroscopic imaging in combination with state-of-the-art cell biological approaches. The nucleus, often measured as the stiffest organelle in the cell, is also frequently abnormally shaped in cancer cells. *In vivo* the cell nucleus resists and responds to mechanical forces. When stretched, the nucleus exhibits buckling transitions, both in micromanipulation experiments where single nuclei are stretched with a micropipette and computational models that simulate the nucleus as a biopolymeric shell [30]. The model indicates that when extended beyond the initial linear elastic regime, the shell undergoes a hysteretic, temperature-dependent buckling transition. Furthermore, the nucleus appears to lack shape relaxation, implying that nuclear shape in spread cells does not store elastic energy and that dissipative rather than static cellular stresses deform the nucleus. It is suggested that nuclear shape changes occur at constant surface area and volume [31]. Finally, it has also been demonstrated that the rigidity of the cell nucleus is dictated by chromosome histone modification state, whereby increasing euchromatin or decreasing heterochromatin resulted in softer nuclei and nuclear blebbing [32].

1.2.6 University of Pennsylvania

The University of Pennsylvania PS-OC tests the hypothesis that intra-tumor heterogeneity can arise from physical properties of microenvironments and that mutations might

also be caused directly by physical properties of microenvironments to drive cancer. They are examining the physical biology of liver cancer cell membranes and how membrane biophysics affects cell signaling and how nuclear deformation impacts DNA stability in cancer cells. Based on current measurements for tissues, meta-analysis of genomics demonstrates that cancers originating in stiff tissues, such as the lung and skin, display 30-fold higher somatic mutation rates compared to cancers originating in soft tissues, such as the marrow and brain [33]. The nucleus when modeled as an elastic-fluid system, with chromatin as the elastic component and a fluid component that can be squeezed out when the nucleus is deformed, can predict that the fluid extraction is sufficient to account for the extent of DNA damage and genomic variation observed experimentally in controlled migration through constrictions [34, 35].

1.2.7 University of Maryland

A project at the University of Maryland, which also has partial support from the NCI IMAT program, has developed a microscopy technique, Brillouin spectroscopy, that interrogates mechanical properties of material via light scattering [36]. This technique based on flow cytometry methods is a label-free, non-contact, and noninvasive approach to characterize cell stiffness at a throughput of nearly 200 cells/h. Several regions can be measured within each cell as they flow through, including the nucleus. There is sufficient sensitivity of the imaging approach to detect changes in nuclear stiffness after treatment of cells with a histone deacetylase inhibitor which causes chromatin decondensation.

1.2.8 Georgia Institute of Technology

The Georgia Institute of Technology project uses mechanics-based methods for analyzing T-cell receptor-peptide-major histocompatibility complex interactions. They found melanomas to substantially alter the force-dependent T-cell receptor-peptide-major histocompatibility complex bond durability [37]. T cells can use mechanical forces to amplify antigen discrimination. T-cell receptors bind immobilized ligands and are subject to mechanical forces, unlike receptors for soluble agonists. Therefore, signaling by T-cell receptors can be modulated or triggered by force. The study of T-cell mechanoimmunology could shed new insight into cancer-immune stroma interactions.

1.2.9 Harvard School of Public Health

At the Harvard School of Public Health, a project is being pursued to derive data from a comprehensive suite of novel experimental probes—cellular motions, traction stresses, intercellular stresses, and cellular shapes—that are critically examined through the lens of a novel quantitative theory of cell jamming. The cell jamming theory suggests an opposing view from the conventional wisdom that adhesion molecules tether a cell to its immediate neighbors and thus impede cellular migration. In the mechanistic theory of cell-cell interaction, cell shape in an epithelial layer becomes less elongated and less variable as the layer becomes more jammed [38]. In a jammed state, a collection of cells is rigid like a solid, and in an unjammed state, the collective flows like a liquid. These theoretical frameworks are being tested in conjunction with our knowledge of the cell-cell adhesions to better understand cell migration in development, cancer, and other diseases such as asthma.

1.3 Other NCI-Supported Programs and Grants

In addition to IMAT and the PS-ON, other NCI-sponsored programs have supported the field of biomechanics in oncology. For example, the Tumor Microenvironment Network (TMEN) which was established by the NCI in 2006 to encourage fundamental research on the tumor microen-

vironment focused on the role of the human microenvironment to generate a comprehensive understanding of stromal composition in normal and cancer tissues and how the stroma affects tumor initiation, progression, and metastasis. Similarly, the NCI Integrative Cancer Biology Program (2004–2015) supported integrated experimental and mathematical modeling approaches to understanding cell migration and invasion, key cell properties underlying cancer metastasis.

During the duration of each program, a few of the supported groups incorporated mechanobiology into their studies. In a landmark paper supported by the TMEN program, it was found that in breast tumors, malignant cells actively modulate the mechanical properties of the ECM through secretion of enzymes such as lysyl oxidase [39], a protein that mediates collagen cross-linking. This study provided *in vivo* support to earlier work suggesting that collagen cross-linking and alignment increased local invasion and might contribute to metastatic spread [40]. Subsequently, research supported both by TMEN and by traditional NCI investigator-initiated research grants, demonstrated that the alignment of collagen fibers within and surrounding a breast tumor is a robust biomarker indicative of poor disease-specific and disease-free survival [41]. Recent work initiated within the PS-ON program using engineered tissues demonstrated that strain generated through cell-cell interaction appears to dictate the dynamics and extent of extracellular matrix alignment across a range of breast cancer models [42]. Studying mechanical behavior using engineered systems allows for careful investigation of the timescale of matrix reorganization, which at approximately 6 h appears to occur significantly faster than the time required to induce collective migration (~12 h), suggesting that alignment is a precursor of cell migration [42]. Alignment of extracellular matrix and the ensuing alteration of matrix stiffness can modulate the inside-out signaling of integrin engagement, with increased stiffness-associated stabilization of the vinculin-talin-actin structure leading to PI3K-mediated PI(3,4,5)P3 accumulation and Akt activation, thus promoting tumor cell survival and invasion [43].

TMEN investigators showed that in addition to promoting invasive behavior, very rigid microenvironments, such as the bone, can modulate gene expression in metastatic cancer cells promoting osteolysis and conditioning the metastatic niche for colonization and outgrowth [44, 45]. The mechanical properties of common sites of metastasis [46] have been linked to maintenance of dormancy [47] and drug resistance [48], suggesting that studies not accounting for the biophysical properties of the metastatic microenvironment may miss important predictors of disease progression.

Due to the multi-scale complexity of cancer mechanobiology, computational modeling approaches are needed to provide a better understanding about how mechanics affects molecules, cells, and tissues at differing biological scales. By the mid-1990s, predictive mathematical models of cell migration in two dimensions were well-developed and generally included terms accounting for generation of the cellular forces through integrin engagement required to propel cells forward on uniform surfaces or on those with gradients of ligand [49, 50]. Expanding these models to three dimensions, in work supported by the NCI Integrative Cancer Biology Program, required consideration of the multivariate nature of the microenvironment, including how the mechanics of the microenvironment are modulated by tumor cells [51]. A true understanding of how the mechanical microenvironment modulates cell migration and invasion requires a multi-scale modeling approach, the details of which have been extensively reviewed elsewhere by NCI-supported investigators [52]. In recent work completed by investigators within the NCI Cancer Systems Biology Consortium (CSBC), it was suggested that feedback mechanisms initiated through engagement of integrin receptors in response to dynamic and differential mechanical cues within the tumor microenvironment may underlie aspects of intratumoral heterogeneity and contribute to phenotypic plasticity [53].

1.4 Conclusion

Biomechanics in oncology is multi-scale from the level of single molecules and proteins to the cellular and tissue scales. The NCI demonstrates its interest in supporting the mechanobiology field in the context of cancer through continued support of the IMAT, PS-ON, and other targeted programs. Importantly, the NCI is supporting the field through investment in investigator-initiated projects as well. Support for the field across the NIH in general is also demonstrated through the incorporation of investigators with expertise in mechanobiology serving as grant reviewers on NIH study sections. Currently, there are a few study sections that have mechanics included in their keywords which describe the grants that they review. This is another step toward the general support of the field of mechanobiology.

The NCI recognizes the importance of clearly delineating the role of mechanics in the pathogenesis and progression of cancer. Further development of innovative technologies to probe, image, and precisely measure the mechanical properties of cells and tissues at different length scales will aid in the ability to expand the exploration of the mechanisms by which mechanics affects cancer processes. As the field of mechanobiology in cancer continues to grow, it will be important to integrate findings across multiple biological length scales using computational modeling approaches and novel experimental platforms.

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