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Konstantinos Konstantopoulos *Editors*

Biomechanics in Oncology

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Preface

A decade ago, the National Cancer Institute (NCI) launched a new program called the Physical Science-Oncology Network (PS-ON; https://physics.cancer.gov) in order to broadly support the integration of physical sciences perspectives and theories in cancer research using new and perhaps nontraditional approaches.

The overarching theme of the PS-ON program, which is to explore and uncover the physics and physical sciences principles underlying cancer-relevant perturbations, remains virtually unexplored and not understood. The physical principles and laws that define the behavior of matter are profoundly important in developing an understanding of the initiation and evolution of cancer at all length scales (i.e., submolecular, molecular, cellular, tissues, organisms, and populations). The goal is to unravel the complicated and multifaceted cancer disease process through the application of approaches from the physical sciences that are traditionally used to comprehend complex problems. There remained an opportunity to bring principles and approaches to bear from physics and engineering to cancer research. Embracing novel tool and technology development from the physical sciences into biology has therefore become a new challenge to many physical scientists and a new adaptation by many biologists.

Biomechanics represents an extremely important branch of the physical sciences. In the mid-1960s, Professor Y.C. Fung pioneered his vision for applications of traditional engineering mechanics and techniques to medicine, physiology, and biology, which was a beginning era of biomechanics. Over the past several decades, biomechanics has already grown into a mature discipline in engineering and physical sciences. Investigators in the field of biomechanics have recently had a vested interest in conducting transdisciplinary research in physical sciences-oncology. In 2012, the United States National Committee on Biomechanics (USNCB) sponsored its national Frontiers Symposium and, for the first time, focused on "Mechanics in Oncology," chaired by Cheng Dong from Penn State, Fan Yuan from Duke, and Lance Munn from MGH/Harvard (http://usncb.org/frontiers). This series of symposia and workshops of *Bioengineering in Oncology* has become a sustained event at the Biomedical Engineering Society (BMES) annual conferences.

This is certainly an exciting time to be studying *Biomechanics in Oncology*. To maintain a vision on the horizon of where the biomechanics in oncology field will need to go, we brought several leading scientists

to contribute to this book that is centered on discussing our emerging challenges and identifying context for the current state of biomechanics in oncology. Most importantly, this book highlights the aspects of biomechanics at different biological length scales, from inside and outside the cancer cell as well as in the circulation, all in the context of tumor initiation, progression and metastasis, and treatment. Many of the challenges in studying biomechanics in oncology have been tempered by the development of novel technologies for imaging and precisely measuring and quantifying cellular and extracellular mechanical forces. The book also discusses those technological approaches for studying biomechanics in oncology. In every aspect of biomechanics, it critically evaluates where we are and where we need to be to understand the significance and impact of mechanics in the context of cancer.

This book is most appropriate for anyone who wants to keep abreast of this new, converging field and the ever-changing applications since Professor Y.C. Fung started in the mid-1960s. We hope you enjoy this book highlighting the latest and greatest in biomechanics, and we look forward to your contributions to the future of *Biomechanics in Oncology*.

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1 The National Cancer Institute Investment in Biomechanics in Oncology Research

Anthony Dickherber, Shannon K. Hughes, and Nastaran Zahir

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.

References

1. Rueden CT et al (2009) Nonlinear optical microscopy and computational analysis of intrinsic signatures in breast cancer. Conf Proc IEEE Eng Med Biol Soc 2009:4077–4080
2. Provenzano PP, Eliceiri KW, Keely PJ (2009) Shining new light on 3D cell motility and the metastatic process. Trends Cell Biol 19(11):638–648
3. Szulczewski JM et al (2016) In vivo visualization of stromal macrophages via label-free FLIM-based metabolite imaging. Sci Rep 6:25086
4. Wu PH et al (2012) High-throughput ballistic injection nanorheology to measure cell mechanics. Nat Protoc 7(1):155–170
5. Zahir N (2018) The NCI physical sciences - oncology network. Trends Cancer 4(4):e1–e6
6. Bakhoum SF et al (2018) Chromosomal instability drives metastasis through a cytosolic DNA response. Nature 553(7689):467–472
7. Cao X et al (2016) A Chemomechanical model for nuclear morphology and stresses during cell transendothelial migration. Biophys J 111(7): 1541–1552
8. Denais CM et al (2016) Nuclear envelope rupture and repair during cancer cell migration. Science 352(6283):353–358
9. Mitchell MJ et al (2015) Lamin A/C deficiency reduces circulating tumor cell resistance to fluid shear stress. Am J Physiol Cell Physiol 309(11):C736–C746
10. Mekhdjian AH et al (2017) Integrin-mediated traction force enhances paxillin molecular associations and adhesion dynamics that increase the invasiveness of tumor cells into a three-dimensional extracellular matrix. Mol Biol Cell 28(11):1467–1488
11. Miroshnikova YA et al (2017) alpha5beta1-Integrin promotes tension-dependent mammary epithelial cell invasion by engaging the fibronectin synergy site. Mol Biol Cell 28(22):2958–2977
12. Hall MS et al (2016) Fibrous nonlinear elasticity enables positive mechanical feedback between cells and ECMs. Proc Natl Acad Sci U S A 113(49): 14043–14048
13. Seo BR et al (2015) Obesity-dependent changes in interstitial ECM mechanics promote breast tumorigenesis. Sci Transl Med 7(301):301ra130
14. Wang K et al (2015) Stiffening and unfolding of early deposited-fibronectin increase proangiogenic factor secretion by breast cancer-associated stromal cells. Biomaterials 54:63–71
15. Sewell-Loftin MK et al (2017) Cancer-associated fibroblasts support vascular growth through mechanical force. Sci Rep 7(1):12574
16. Ahmadzadeh H et al (2017) Modeling the two-way feedback between contractility and matrix realignment reveals a nonlinear mode of cancer cell invasion. Proc Natl Acad Sci U S A 114(9):E1617–e1626
17. Jimenez Valencia AM et al (2015) Collective cancer cell invasion induced by coordinated contractile stresses. Oncotarget 6(41):43438–43451
18. Kim DH et al (2015) Volume regulation and shape bifurcation in the cell nucleus. J Cell Sci 128(18): 3375–3385
19. Kim DH, Wirtz D (2015) Cytoskeletal tension induces the polarized architecture of the nucleus. Biomaterials 48:161–172
20. Shiraishi T et al (2015) Glycolysis is the primary bioenergetic pathway for cell motility and cytoskeletal remodeling in human prostate and breast cancer cells. Oncotarget 6(1):130–143
21. Gilkes DM et al (2014) Hypoxia-inducible factors mediate coordinated RhoA-ROCK1 expression and signaling in breast cancer cells. Proc Natl Acad Sci U S A 111(3):E384–E393
22. Wu M et al (2014) The effect of interstitial pressure on therapeutic agent transport: coupling with the

tumor blood and lymphatic vascular systems. J Theor Biol 355:194–207
23. Gampa G et al (2017) Drug delivery to melanoma brain metastases: can current challenges lead to new opportunities? Pharmacol Res 123:10–25
24. Stevens MM et al (2016) Drug sensitivity of single cancer cells is predicted by changes in mass accumulation rate. Nat Biotechnol 34(11):1161–1167
25. Shaw Bagnall J et al (2015) Deformability of tumor cells versus blood cells. Sci Rep 5:18542
26. Byun S et al (2013) Characterizing deformability and surface friction of cancer cells. Proc Natl Acad Sci U S A 110(19):7580–7585
27. Klank RL et al (2017) Biphasic dependence of glioma survival and cell migration on CD44 expression level. Cell Rep 18(1):23–31
28. Bangasser BL et al (2017) Shifting the optimal stiffness for cell migration. Nat Commun 8:15313
29. Ray A et al (2017) Anisotropic forces from spatially constrained focal adhesions mediate contact guidance directed cell migration. Nat Commun 8:14923
30. Banigan EJ, Stephens AD, Marko JF (2017) Mechanics and buckling of biopolymeric shells and cell nuclei. Biophys J 113(8):1654–1663
31. Tocco VJ et al (2018) The nucleus is irreversibly shaped by motion of cell boundaries in cancer and non-cancer cells. J Cell Physiol 233(2):1446–1454
32. Stephens AD et al (2018) Chromatin histone modifications and rigidity affect nuclear morphology independent of lamins. Mol Biol Cell 29(2):220–233
33. Pfeifer CR et al (2017) Genome variation across cancers scales with tissue stiffness - an invasion-mutation mechanism and implications for immune cell infiltration. Curr Opin Syst Biol 2:103–114
34. Bennett RR et al (2017) Elastic-fluid model for DNA damage and mutation from nuclear fluid segregation due to cell migration. Biophys J 112(11):2271–2279
35. Irianto J et al (2016) Nuclear constriction segregates mobile nuclear proteins away from chromatin. Mol Biol Cell 27(25):4011–4020
36. Zhang J et al (2017) Brillouin flow cytometry for label-free mechanical phenotyping of the nucleus. Lab Chip 17(4):663–670
37. Chen Y et al (2017) Receptor-mediated cell mechanosensing. Mol Biol Cell 28(23):3134–3155
38. Park JA et al (2016) Collective migration and cell jamming in asthma, cancer and development. J Cell Sci 129(18):3375–3383
39. Levental KR et al (2009) Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell 139(5):891–906
40. Provenzano PP et al (2006) Collagen reorganization at the tumor-stromal interface facilitates local invasion. BMC Med 4(1):38
41. Conklin MW et al (2011) Aligned collagen is a prognostic signature for survival in human breast carcinoma. Am J Pathol 178(3):1221–1232
42. Piotrowski-Daspit AS et al (2017) Dynamics of tissue-induced alignment of fibrous extracellular matrix. Biophys J 113(3):702–713
43. Rubashkin MG et al (2014) Force engages vinculin and promotes tumor progression by enhancing PI3K activation of phosphatidylinositol (3,4,5)-triphosphate. Cancer Res 74(17): 4597–4611
44. Ruppender NS et al (2010) Matrix rigidity induces osteolytic gene expression of metastatic breast cancer cells. PLoS One 5(11):e15451
45. Johnson RW et al (2014) Wnt signaling induces gene expression of factors associated with bone destruction in lung and breast cancer. Clin Exp Metastasis 31(8):945–959
46. Jansen LE et al (2015) Mechanics of intact bone marrow. J Mech Behav Biomed Mater 50:299–307
47. Ghajar CM et al (2013) The perivascular niche regulates breast tumour dormancy. Nat Cell Biol 15(7):807–817
48. Schwartz AD et al (2017) A biomaterial screening approach reveals microenvironmental mechanisms of drug resistance. Integr Biol (Camb) 9(12): 912–924
49. Tranquillo RT, Lauffenburger DA, Zigmond SH (1988) A stochastic model for leukocyte random motility and chemotaxis based on receptor binding fluctuations. J Cell Biol 106(2):303–309
50. Palecek SP et al (1997) Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. Nature 385(6616): 537–540
51. Zaman MH et al (2006) Migration of tumor cells in 3D matrices is governed by matrix stiffness along with cell-matrix adhesion and proteolysis. Proc Natl Acad Sci U S A 103(29):10889–10894
52. Mak M et al (2015) Multiscale mechanobiology: computational models for integrating molecules to multicellular systems. Integr Biol (Camb) 7(10):1093–1108
53. Park J et al (2017) Mechanochemical feedback underlies coexistence of qualitatively distinct cell polarity patterns within diverse cell populations. Proc Natl Acad Sci U S A 114(28):E5750–e5759

DNA Mechanics and Topology

2

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Abstract

We review the current understanding of the mechanics of DNA and DNA-protein complexes, from scales of base pairs up to whole chromosomes. Mechanics of the double helix as revealed by single-molecule experiments will be described, with an emphasis on the role of polymer statistical mechanics. We will then discuss how topological constraints—entanglement and supercoiling—impact physical and mechanical responses. Models for protein–DNA interactions, including effects on polymer properties of DNA of DNA-bending proteins will be described, relevant to behavior of protein–DNA complexes in vivo. We also discuss control of DNA entanglement topology by DNA-lengthwise-compaction machinery acting in concert with topoisomerases. Finally, the chapter will conclude with a discussion of relevance of several aspects of physical properties of DNA and chromatin to oncology.

Keywords

DNA mechanics · DNA–protein interactions · Supercoiling · Plectoneme · Braid · Worm-like chain · DNA topology · Linking number · Lengthwise compaction.

2.1 Overview of DNA Mechanics and Nuclear Function

Over the past several decades our understanding of the cell has become increasingly based on the concept of "molecular machines" that groups of enzymes associate together to accomplish specific tasks. In many cases, these enzyme machines perform "mechanical" functions, for example, transporters that actively push a specific "cargo" across a cell membrane. Many of the most impressive examples of active biomolecular machines are found in the cell nucleus, where very highly processive enzyme motors are involved in transcription, replication, and repair of double helix DNA molecules. Given that the DNAs in human cells are on the order of centimeters in length, the physical properties of DNA are essential to understanding how cell nuclear machinery operates. Proper regulation of DNA transcription, replication, and repair is essential to controlling cell behavior and development, and dysfunction of these processes is the root of many genetic diseases including many cancers.

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The mechanics of DNA and DNA–protein complexes (notably chromatin, i.e., strings of nucleosomes formed on DNA as occur in eukaryote chromosomes) affects many different aspects of nuclear function. For example, the flexibility of DNA and its modification by DNA-binding proteins affects how DNA bends and fluctuates, and therefore the probabilities and rates at which DNA sequences along the same molecule can "meet": this meeting of distant sequences occurs when distant sequences regulate genes. In some cases, it is known that gene activation repositions genes in the nucleus, another process which is affected on DNA mechanics. Homologous-sequence-based DNA repair depends on the transport together of sequence-matching DNA segments from different homologous chromosomes, a process which is still only partially understood, but which undoubtedly depends on DNA mechanics. Perhaps most impressive is the process by which chromosomal DNAs are replicated, and then the duplicated sister chromatids are physically and topologically separated from one another, culminating in mitosis and cell division, perhaps the most mechanically impressive feat carried out by eukaryote cells.

This chapter will focus on the mechanics of DNA and DNA–protein structures, focusing on the behavior of the double helix at scales from base pairs up to whole chromosomes. As might be expected, different force scales and descriptions are relevant at microscopic (few nanometer [nm]/single-molecular) and at mesoscopic (micron[μm]/chromosome-cell nucleus) scales. We will begin by focusing on the microscopic scales, discussing mechanics of the double helix as revealed by single-molecule biophysics experiments; we will then discuss how the topological properties of DNA impact its thermodynamics and mechanics. We will then discuss how proteins which bind to DNA can change its mechanical properties, which is the situation we find in vivo and in particular in chromosomes throughout the cell cycle. Finally we will conclude with a very brief summary of the chapter and a very brief discussion of relevance of DNA and chromatin mechanics to cancer.

Before launching into quantitative aspects of DNA mechanics, we begin with a few words about DNA chemical structure (Fig. 2.1) and basic physical properties. DNA molecules in cells are found in double helix form, consisting of two long polymer chains wrapped around one another, with complementary chemical structures (Fig. 2.1b). The double helix encodes genetic information through the sequence of chemical groups—the bases adenine, thymine, guanine, and cytosine (A, T, G, and C). Corresponding bases on the two chains in a double helix bind one another according to the complementary base-pairing rules A=T and G≡C. These rules follow from the chemical structures of the bases, which permit two hydrogen bonds to form between A and T (indicated by =), versus three that form between G and C (indicated by ≡). Each base pair has a chemical weight of about 600 Daltons (Da). The presence of the two complementary copies along the two polynucleotide chains in the double helix provides redundant storage of genetic information and also facilitates DNA replication, via the use of each chain as a template for assembly of a new complementary polynucleotide chain.

Inside the double helix, the two polynucleotide strands wrap around one another, forming a structure which has on average about 0.34 nm of helix length ("rise") per base pair, and with one helix repeat per 10.5 base pairs (a good scale to keep in mind is that there are approximately three base pairs per nm along the double helix axis). Now, double helix DNAs in vivo are long polymers: the chromosome of the bacteriophage (a virus that infects *E. coli* bacteria) is 48,502 base pairs (bp) or about 16 μm in length; the *E. coli* bacterial chromosome is 4.6×10^6 bp (4.6 Mb) or about 1.5 mm long; small *E. coli* "plasmid" DNA molecules used in genetic engineering are typically 2–10 kb (0.7–3 μm) in length; and the larger chromosomal DNAs in human cell nuclei are roughly 200 Mb or a few cm in length.

A key physical feature of DNA that should be kept in mind is that in physiological aqueous solution (e.g., under conditions similar to those found in the human cell nucleus: 150 mM of univalent cations, predominantly K^+; 1 mM of Mg^{2+}; pH 7.5) the phosphates along the backbones (see Fig. 2.1a; shown as the dark groups in Fig. 2.1b) are ionized, giving the double helix a

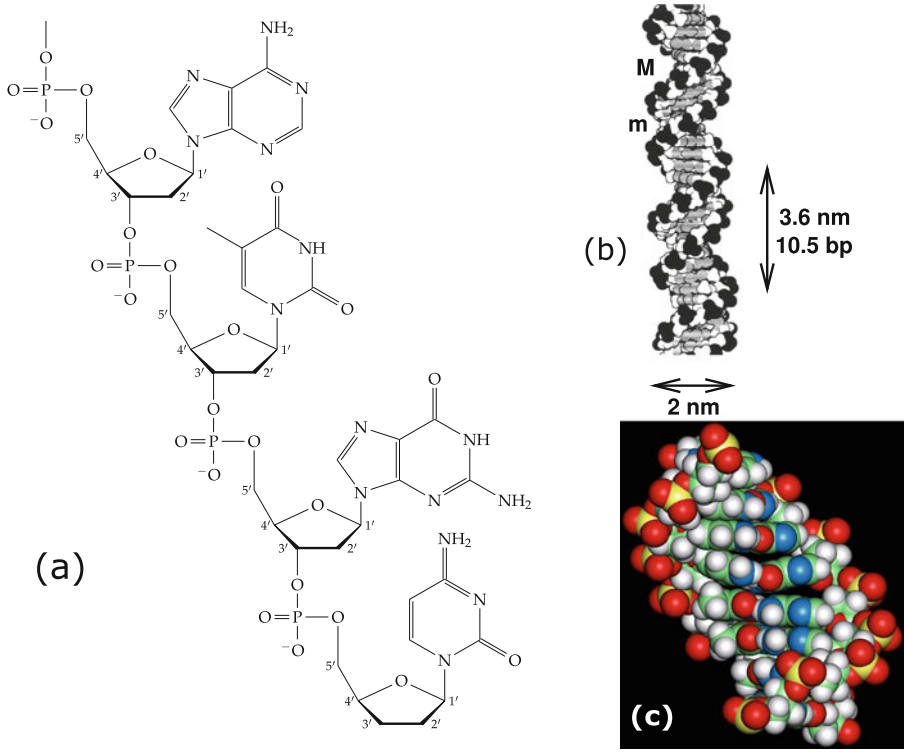

Fig. 2.1 DNA double helix structure. (**a**) Chemical structure of one DNA chain, showing the deoxyribose sugars (note numbered carbons) and charged phosphates along the backbone, and the attached bases (A, T, G, and C following the 5 to 3 direction from top to bottom). (**b**) Space-filling diagram of the double helix. Two complementary-sequence strands as in (**a**) noncovalently bind together via base-pairing and stacking interactions, and coil around one another to form a regular helix. The two strands can be seen to have directed chemical structures, and are oppositely directed. Note the different sizes of the major (M) and minor (m) grooves, and the negatively charged phosphates along the backbones (dark groups). The helix repeat is 3.6 nm, and the DNA cross-sectional diameter is 2 nm. Image reproduced from [1]. (**c**) Molecular-dynamics snapshot suggestive of a typical double helix DNA conformation for a short 10 bp molecule in solution at room temperature. Reproduced from [2]

linear charge density of about 2 e^- per base pair or about 6 e^- per nm. DNA under cellular conditions is therefore a strongly charged polyelectrolyte and has strong electrostatic interactions with other electrically charged biomolecules at short ranges. At ranges beyond the Debye length ($\lambda_D \approx 0.3\,\text{nm}/\sqrt{\text{M}}$, where M is the concentration of 1:1 salt in mol/litre = M), univalent ions in the cell screen electrostatic interactions, cutting it off beyond a distance of about 1 nm. Thus electrostatic repulsions between DNA molecules can be thought of as giving rise to an effective hard-core diameter of dsDNA of ≈3.5 nm under physiological salt conditions [3].

In the nm-scale world of the double helix (note that the "information granularity" of cells, the size of nucleotides, amino acids, nucleotides, and other elementary molecules is about 1 nm), thermal fluctuations excite individual mechanical degrees of freedom with energy $\approx k_\text{B}T \approx 4 \times 10^{-21}$ J (at room temperature, $T \approx 300$ K). This energy scale of thermal motion is well below that associated with covalent bonds ($\approx 1\,\text{eV} \approx 40\ k_\text{B}T$), which is good—thermal fluctuations by themselves can't easily break the covalently bonded DNA backbone! A second physical consequence of the thermal energy scale is that combined with the 1 nm length of molecular structure, one obtains a molecular-biological force scale of $1\ k_\text{B}T/\text{nm} = 4 \times 10^{-12}$ Newtons (4 piconewtons, or pN). This force scale is what must be used to hold a molecule in one place to nm