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Controversy and Consensus

Cancer Research

The Physics of Cancer

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Introductory Statement

While often described as a "disease of the genes," cancer is, in fact, a complex dynamic system in which evolving cells both affect and are affected by the physical properties of their environment. About 10 years ago, after a number of multidisciplinary workshops and meetings, the NCI leadership embarked on a bold program to systematically integrate physical sciences into cancer biology and treatment through formation of the Physical Sciences-Oncology Network (PS-ON). Here, we highlight key areas in which the two disciplines have been successfully integrated and lessons learned from the first decade of the PS-ON experiment.

Introduction

The societal and personal burden of cancer has stimulated decades of intense scientific effort that has resulted in many important insights and therapies. Yet, despite these advances, the improvement in mortality rates for patients with cancer still lags behind that of cardiovascular and cerebrovascular diseases. Research in cancer biology has been greatly accelerated by new experimental technologies and the revolution in genomics and bioinformatics. These new methodologies have generated overwhelming amounts of biomolecular data. Often lacking, however, are the conceptual frameworks necessary to organize these data in ways that guide more significant advances in understanding of the disease. Furthermore, the common focus on genes and gene products associated with cancers often neglects the physical context in which clinical cancer cells grow. Yet, all stages of cancer are impacted by the 3-dimensional (3D) microenvironment in which cancer cells reside and where they are subject to complex mechanical forces and spatiotemporally varying gradients of biomolecules and nonorganic components such as oxygen and acid. Cancer cells can also deploy "niche construction" strategies, including extracellular matrix (ECM) remodeling, angiogenesis, and extracellular acidification, to make their microenvironment permissive to tumorigenesis.

The Physical Sciences-Oncology Network (PS-ON; https://physics.cancer.gov) was established in 2009 by the NCI after a series of community-driven workshops to identify themes that would benefit from applying principles and technologies from the physical sciences to cancer research. Initially, four topical areas were pursued: Physical Laws and Principles of Cancer; Evolution and Evolutionary Theory in Cancer; Coding, Decoding, Transfer, and Translation of Information in Cancer; Complexity of Cancer. These areas were broadened later to (1) Physical Dynamics of Cancer and (2) Spatio-Temporal Organization and Information Transfer in Cancer. By using novel tools and physics and engineering approaches to investigate the role of physical forces and microenvironmental factors in cancer, the PS-ON complements the Cancer Systems Biology Consortium of the NCI, which addresses challenges of cancer complexity by combining experimental biology with *in silico* modeling, multidimensional data analysis, and systems engineering.

Now, about one decade into this multidisciplinary experiment, several "lessons learned" and knowledge gained have emerged. Although PS-ON investigators have pursued multiple avenues of investigation, two general areas have emerged. One broad topic applies physical sciences techniques to measure key biomechanical forces that affect cancers across multiple length scales ranging from molecules to cells to actual tumor tissue. These properties are important causes and consequences of malignant transformation and tumor growth and, therefore, critical to cancer biology and therapy. A second broad topic embraces the physics research paradigm, dating back to Newton and Galileo, in which mathematically based theoreticians work closely with experimentalists to define the first principles of a system. Below, we highlight key accomplishments and outstanding opportunities in these specific areas.

Physical Properties of Cancer Cells and Their Microenvironment

While cancer is typically considered a genetic condition, the microenvironment in which tumor cells are located is similarly important. Historically, research has focused on identifying biological signatures of tumor-promoting versus suppressive microenvironments, but aberrant physical environmental properties can equally drive the disease. In fact, the observation that tumors are stiffer than normal tissues enabled cancer diagnosis by palpation for centuries; and that tumors exhibit aberrant transport properties and, thus, metabolize nutrients differently than their normal counterparts has contributed to Otto Warburg's Nobel Prize in Physiology or Medicine in 1931. That varied tissue mechanics and transport properties can independently stimulate malignant transformation has only become clear relatively recently due, in part, to pioneering work by the PS-ON (1).

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Key components of the physical tumor microenvironment include varied 3D tissue and ECM architecture and mechanics, physical properties of single cells, gradients of soluble factors, interstitial pressure, and transport processes due to convection and diffusion. While some of these properties are a consequence of host response, others represent active niche construction by the cancer cells to produce an environment that enhances their own fitness often at the expense of host cells. Importantly, the changes are not static but vary dynamically due to the complex "eco-evolutionary dance" of time-dependent, mutually influencing changes in cancer cell states and host responses. In turn, these deeply linked dynamics influence the observed molecular signatures during cancer progression and heterogeneity. For example, research from the PS-ON revealed that the physical properties and forces of individual tumor cells correlate with their malignant potential. Network-wide comparative studies of more or less aggressive breast epithelial cell lines identified dramatic differences in individual cell mechanical deformability, cytoplasmic viscoelasticity, cellular traction forces, and response to pH and hypoxic stress (2). These behaviors are further modified when cells are subjected to varied physical properties of their microenvironment (3, 4), suggesting a complex network of interactions that ultimately influences key phenotypic changes such as invasion and metastasis.

Another insight emerging from research in the PS-ON is that tumor cell physical properties differ over time as cells assume new functions such as squeezing through ECM pores and intra- or extravasating through blood vessels. Traditional histology or cutting edge -omic methods have relied on the analysis of nonviable cells, preventing investigation of the dynamic nature of cell mechanics and their respective molecular adaptations. Instead, integrating advanced imaging techniques (e.g., *in vivo* microscopy) with cells expressing engineered force sensors allows researchers to observe dynamic changes of cellular tension or compression as cancers invade or defeat host response such as immune attack. In combination with microfabrication and materials science approaches, PS-ON investigators have identified that varied tumor cell migration modes depend on the ability of cells to adjust their nuclear mechanics to bypass physical constrictions (5). In turn, these intracellular forces can alter DNA thus, directly linking physical changes with the molecular properties of cancer cells that are often used in the clinic to guide therapy (6).

Despite the novel insights gained from PS-ON efforts, significant challenges remain. For instance, improved *in vitro* models will be needed to uncover and ultimately target the molecular mechanisms affected by physical forces in the tumor microenvironment. Harnessing tissue engineering and microfabrication technologies enables construction of physiologically relevant 3D tumor models that not only capture the intrinsic biological, but also physical properties of tumors. In particular, integrating microengineered culture models, advanced imaging, and precision medicine promises to advance understanding of the functional coupling between the physical microenvironment, cancer and stromal cell signaling, and future treatment strategies in a patient-specific manner. Nevertheless, it is not possible to comprehensively address the tremendous complexity of the *in vivo* cancer microenvironment, including various signaling pathways and their integration with convective-diffusion-reaction processes, via wet lab experiments. To address this complexity, multiscale computational models (continuum to cellular to molecular) allow for quantitative predictions of physical coupling among cells and microenvironmental conditions on the macroscopic scale. Coupling such models with experimental approaches in an iterative manner will be critical to further improve the utility of *in vitro* models while advancing insights about how a given property is distributed throughout a heterogeneous population, and then considering the functional consequences of this heterogeneity on tumor malignancy.

In addition to combining *in vitro* and *in silico* approaches to simulate tumor complexity, characterizing banked tissue using physical sciences–based approaches could be an alternative strategy. For example, nanoindentation-based mapping of tumor sections by PS-ON investigators has revealed significant spatial heterogeneity of tumor mechanics (7). While there has been considerable investment in generating publicly available molecular databases from clinical tumor specimens, no comparable databases exist linking clinical data with tumor physical properties, such as mechanics, nano/microstructure, or transport phenomena. Combining the physical science analysis technologies within the PS-ON with existing resources such as specimens from the NCI's National Clinical Trials Network, it should be possible to correlate clinical data and reported patient outcome with specific physical characteristics of the tumor specimens, rather than being limited to molecular characterization alone. Such studies represent a paradigm-shifting opportunity to gain novel and potentially transformative insights into the role of tumor mechanical properties in response to therapy.

Applying Technology from the Physical Sciences to Measure Cancers at Large and Small Dimensions

Tumors are dynamic entities whose physical and molecular properties naturally adapt to perturbation. At the genomic scale, adaptation typically occurs through selection of resistant mutations in a process that can take weeks to months, depending on the prevalence of the preexisting population of cells expressing these mutant isoforms. Development of second- and third-generation therapeutics targeting these resistant isoforms has extended progression-free survival for some cancers.

Despite advances in therapy, most common disseminated cancers remain almost invariably fatal because malignant cells have a remarkable capacity to evolve mechanisms to evade therapy. Hence, there is a clear need for more frequent monitoring to identify clinical metrics that can define intratumoral evolution during therapy to optimize application of this growing number of treatment options. This has led to extensive investigation of "liquid biopsies," in which circulating tumor cells (CTC) are extracted, counted, and sequenced from a sample of the patients' blood. Research within the PS-ON has facilitated the real-time analysis of CTC response to therapy by quantifying cell mass, proliferation rates, and molecular characterization through single-cell RNA sequencing of 100s to 1,000s of CTCs from given patient tumors (8).

While these new technologies are providing insight into CTC response to therapy, solid tumors, including their metastatic progeny and disseminated cancer cells, are regulated by the physical and molecular parameters dictated by their microenvironments, which are likely poorly recapitulated by CTCs. In this context, adaptation to therapy at the transcriptional and

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posttranscriptional level can occur on the seconds-to-minutes timescale. Importantly, these rapid adaptive responses are both prevalent and functionally relevant, as they encode multiple resistance mechanisms that account for a large fraction of recurrent tumors, including altered cell state (e.g., epithelial-to-mesenchymal transition), increased expression of drug efflux pumps, or activation of bypass signaling pathways. To gain insight into therapeutic response of solid tumors *in vivo*, research within the PS-ON has combined spatially resolved tissue and molecular imaging techniques (e.g., MRI), stimulated Raman scattering (SRS), matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI)) with large-scale molecular characterization technologies (e.g., functional proteomics, transcriptomics, metabolomics) in solid tumors and surrounding tissues to define the interaction between therapeutic distribution, efficacy, and tumor cell adaptive response (9)

The multistage analysis employed within the PS-ON can provide unprecedented resolution and depth of information on solid tumor response to therapy, yet the intra- and intertumoral heterogeneity observed in these and other studies highlight our extremely limited ability to predict which adaptive response will be employed by a given tumor cell *in vivo*. This cell decision process is likely influenced by the physical parameters affecting the cell, drug exposure (dose and duration), as well as transcript, protein, and metabolite expression states. Although we have made advances in data acquisition, computational models to integrate these complex datasets are still needed. Ideally, multiscale models representing dynamic response to therapy at the molecular, cellular, and tissue scales while also integrating physical and chemical interactions from the microenvironment will enable the extrapolation of acquired data pretreatment to identify future adaptive response and optimal therapeutic options.

As we move forward with these efforts, one of the further challenges facing the field will be the development of technologies enabling nondestructive monitoring of tumor cell adaptive responses *in situ*, with rapid (second-to-minute time scale) dynamics. These data would inform the physician of the immediate/early changes in solid tumors or their disseminated progeny following treatment with a given chemotherapy and allow for rapid adjustment of the treatment to tackle adaptive response, thus eliminating tumor defense mechanisms prior to establishing full-scale resistance.

The Physics Research Paradigm—Bringing Theoreticians to Cancer

For centuries, physical scientists have routinely used the tools of mathematics to quantify the physical world—justifying Galileo: "The book of Nature is written in the language of Mathematics." In contrast, the biological sciences, faced with the remarkable diversity in the natural world, have developed a research paradigm and scientific tradition focused on observation, description, and classification. The formation of the PS-ON represents a systematic attempt to bridge this critical gap through the integrated multidisciplinary consortia.

Clearly, mathematical models without experimental data are of little practical value but, by the same token, the mere accumulation of data in the absence of models can be equally limited. Thus, the PS-ON combines biomolecular research, with hypothesis-driven, biologically informed mathematical models to provide theoretical frameworks to organize and understand data, and to guide new experiments.

The potential role of mathematics to clarify the complex dynamics of cancer is evident in the concept of evolution in cancer. First proposed by Nowell and extended by such pioneers as Knudson, Vogelstein, and Weinberg, the concept of "somatic evolution" was generally viewed as a genetic process. Application of evolution-based mathematical models has further extended the evolution model by integrating the key role of host environmental selection forces. The complexity of these interactions is extended full circle as cancer cells often use niche construction strategies in which their gene expression both affects and is affected by local extracellular conditions. Importantly, the application of therapy represents additional selection forces that elicit tumor cell death as well as complex molecular dynamics to deploy adaptive strategies and evolutionary interaction among the resistant subpopulations. Within the PSON, these evolutionary dynamics have been investigated and manipulated to improve responses to currently available treatment, resulting in a new generation of cancer treatment protocols explicitly guided by evolutionary principles framed in mathematical models (10).

Conclusion

Human cancers represent complicated heterogeneous systems governed by both biological and physical forces that are challenging to characterize. Furthermore, predicting the results from an iatrogenic perturbation of a cancer system remains a daunting task. An important first step is defining the initial conditions of the cancer system prior to treatment. Current concepts of "precision medicine" characterize pretreatment tumors exclusively through molecular data. However, PS-ON investigators have demonstrated that clinically important initial conditions also include spatial and temporal variations in environmental conditions, including mechanical properties. Importantly, these molecular, cellular, and tissue scale mechanical forces are coupled through eco-evolutionary principles with the genotypic and phenotypic properties of cancer cells.

The next step must include predictive models that can reliably anticipate the results of a specific therapy on a tumor given its initial conditions. These models require detailed understanding of the immediate effect of the treatment, as well as the adaptive strategies that are evolutionarily available to the tumor. Ultimately, as the number of treatment strategies continues to increase, cancer therapy must become more flexible and strategic as specific drugs, doses, and time of administration are constantly changed to anticipate and exploit or prevent the cancer cells' eco-evolutionary arc in response to the selection forces imposed by each treatment.

In summary, the PS-ON, by fostering sustained, systematic collaborations of cancer biologists and oncologists with physicists, mathematicians, and engineers, has brought new insights into complex physical–biological interactions that are both the causes and consequences of temporal and spatial variations in the molecular properties of cancer cells. Ongoing studies seek to integrate these data through eco-evolutionary first principles, to gain a better understanding of basic cancer biology, which can ultimately lead to optimization of therapeutic strategies.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: F.M. White, R.A. Gatenby, C. Fischbach
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Fischbach
Writing, review, and/or revision of the manuscript: F.M. White, R.A. Gatenby, C. Fischbach

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References

1. Madsen LD, Weaver L, Jacobsen SN. Influence of material properties on TEM specimen preparation of thin films. Microsc Res Tech 1997;36: 354–61.
2. Physical Sciences - Oncology Centers Network, Agus DB, Alexander JF, Arap W, Ashili S, Aslan JE, et al. A physical sciences network characterization of non-tumorigenic and metastatic cells. Sci Rep 2013;3: 1449.
3. DelNero P, Lane M, Verbridge SS, Kwee B, Kermani P, Hempstead B, et al. 3D culture broadly regulates tumor cell hypoxia response and angiogenesis via pro-inflammatory pathways. Biomaterials 2015;55:110–8.
4. Wu PH, Aroush DR, Asnacios A, Chen WC, Dokukin ME, Doss BL, et al. A comparison of methods to assess cell mechanical properties. Nat Methods 2018;15:491–8.
5. Cao X, Moeendarbary E, Isermann P, Davidson PM, Wang X, Chen MB, et al. A chemomechanical model for nuclear morphology and stresses during cell transendothelial migration. Biophys J 2016;111:1541–52.
6. Irianto J, Xia Y, Pfeifer CR, Athirasala A, Ji J, Alvey C, et al. DNA damage follows repair factor depletion and portends genome variation in cancer cells after pore migration. Curr Biol 2017;27:210–23.
7. Acerbi I, Cassereau L, Dean I, Shi Q, Au A, Park C, et al. Human breast cancer invasion and aggression correlates with ECM stiffening and immune cell infiltration. Integr Biol 2015;7:1120–34.
8. Stevens MM, Maire CL, Chou N, Murakami MA, Knoff DS, Kikuchi Y, et al. Drug sensitivity of single cancer cells is predicted by changes in mass accumulation rate. Nat Biotechnol 2016;34:1161–7.
9. Randall EC, Emdal KB, Laramy JK, Kim M, Roos A, Calligaris D, et al. Integrated mapping of pharmacokinetics and pharmacodynamics in a patient-derived xenograft model of glioblastoma. Nat Commun 2018;9: 4904.
10. Zhang J, Cunningham JJ, Brown JS, Gatenby RA. Integrating evolutionary dynamics into treatment of metastatic castrate-resistant prostate cancer. Nat Commun 2017;8:1816.

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