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Genome variation across cancers scales with tissue stiffness – An invasion-mutation mechanism and implications for immune cell infiltration

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Abstract

Many different types of soft and solid tumors have now been sequenced, and meta-analyses suggest that genomic variation across tumors scales with the stiffness of the tumors' tissues of origin. Multiple 'mechanogenomics' mechanisms might explain this scaling of mutation rate with tissue stiffness. Since stiff solid tissues have higher density of fibrous collagen matrix, which should decrease tissue porosity, cancer cell proliferation could be affected and so could invasion into stiff tissues as the nucleus is squeezed sufficiently to enhance DNA damage. Although careful analyses continue to be required for rigorous conclusions about such DNA damage, diversification of a cancer genome after constricted migration is now clear *in vitro*. Understanding genome changes that give rise to neo-antigens is important to selection (immuno-editing) as well as to the development of immunotherapies, and engineered monocytes/macrophages seem particularly relevant to understanding infiltration into solid tumors.

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Introduction

Tumors are often palpably stiffer than nearby normal tissue [1], with stiffness of breast and liver, among other organs, correlating with cancer risk [2,3]. Tissue stiffness likely contributes in normal cells to motility [4] and differentiation [5], and in cancer cells to invasion [6] and various epigenetic mechanisms [7], including stiffness-dependent nuclear localization of oncogenic factors (e.g. YAP) [8]. It is unclear, however, if a physical attribute of the microenvironment such as stiffness could contribute—in a 'mechanogenomics' type of process—to any of the many genetic changes that typically occur in cancer.

Meta-analyses of recently published cancer mutation data begin to suggest that—beyond some initial driver mutation(s)—the large genomic variation across diverse cancers scales with stiffness of the normal tissue of origin. Stiffness-dependent cell biological mechanisms for genome variation are needed to establish any causality, and some molecular mechanisms are now emerging. We focus on one possible mechanism based on the fact that stiffer tissues, including tumors, are enriched in collagen [9], and many studies of collagen gels show that denser collagen has smaller matrix pores (eg. Ref. [10]). Thus, as cancer cells proliferate and/or invasively migrate into stiff, small-pore surroundings, the DNA can be damaged, which might ultimately contribute to genomic diversity.

Invasion and proliferation are defining tasks of any malignant cell; the equal but opposite challenge of an immune cell—therapeutic or otherwise—is to confront stiffness barriers and infiltrate a wound or disease site in order to attack 'non-self'. In the cancer context, genome variation can produce novel protein sequences that might be perceived by the immune system as 'neo-antigens'. Such sequences are by definition absent from normal cells, and so can be used to identify and eliminate (immune-edit) cancerous cells if the neo-antigen signals are sufficiently potent, accessible, and foreign to overwhelm 'self' recognition [11]. A moonshot-scale effort now seeks to employ neo-antigens in various immunotherapy approaches. Some therapies use engineered T-cells to target neo-antigens on the cancer cell membrane [12], while other therapies exploit the major histocompatibility complex (MHC)—class I and class II—to target nuclear and cytoplasmic neo-antigens [13–15]. Monocytes and macrophages are the focus here and are particularly interesting for targeting to neo-antigens because these phagocytic cells exhibit a robust ability to infiltrate solid tissues, including tumors. The microenvironment-dependent plasticity of such cells, which is now being mapped by modern systems biology

methods, could also be triggered, in part, by the stiffness or solidity of the tissue.

Genomic variation scales with tissue stiffness

Advances in genome sequencing have enabled cataloging of the genomic variations that occur in cancers of many different types [11,16,17], and although oxidation artifacts can complicate such methods [18], somatic mutation rates are being collected in databases such as The Cancer Genome Atlas (TCGA) run by the National Cancer Institute (NCI). For the healthy tissues of origin of 36 types of cancer, tissue microelasticity data were culled from numerous recent papers [11,19–38] that used a variety of physical methods, including atomic force microscopy (AFM), micro-indentation probes, micropipette aspiration, and imaging-based elastography (Table 1). Whereas AFM pushes on cells and tissues at the ~100-nm to multi-micron length scales in order to provide a measure of a microenvironment's stiffness, the larger length scale imaging-based elastography methods that perturb and monitor by magnetic resonance imaging, for example, typically probe on a millimeter length scale. The latter encompasses many cells and the matrix between them; in principle, all of these types of measurements should be made on fresh tissue, since the former add up to the latter. However, measurements on cultured cells are likely to have little relevance to the tumor, because culture conditions such as gel stiffness influence cell mechanics [5]. Importantly, based on current tissue measurements, meta-analyses of genomics indicate that cancers arising in tissues that are normally stiff to withstand mechanical expansion and distension, such as lung and skin, exhibit 30-fold higher somatic mutation rates (as median per sequenced megabase) than cancers arising in soft tissues, such as marrow and brain (Figure 1A). Importantly, the stiffness of a typical brain tumor or marrow tumor never increases to that of a typical bone tumor microenvironment even though tumors often stiffen—or, less frequently, soften—in tumorigenesis [1]. Accounting for tissue-dependent replication rates in the normal tissues (again) will no doubt be important as discussed below, but the normal hierarchy of tissue stiffness seems crucial, with brain being softer than liver, which is softer then bone, etc.—regardless of cancer or not.

Childhood muscle and bone cancers have only slightly elevated somatic mutation rates as compared to childhood marrow and brain cancers, but they have >10-fold more chromosome copy number changes and structural variants [23] (Figure 1B). This disparity suggests that large-scale, chromosome-level amplifications and deletions—more so than somatic mutations—are signatures of some mutational processes that associate with tissue stiffness. In adult melanoma, fibrotic skin tends to be stiffer and exhibit more chromosome copy number changes than softer, less fibrotic skin [24,39] (Figure 1C-*i*). Moreover, these copy number changes increase even faster with stiffness than do somatic mutation rates, and all mutations are most abundant in invasive melanoma [24] (Figure 1C-*ii*). The relationship between chromosome-level mutations and stiffness thus holds even within a given tissue type, suggesting a correlation between mutations and stiffness that cannot be entirely explained away by exposure to carcinogens.

Table 1

Cancer types and the microelasticities of the healthy tissues in which they arise.

Cancer type		Normal tissue stiffness (kPa)
Pilocytic astrocytoma		0.4 [9]
Acute myeloid leukemia (AML)		0.3 [25]
Acute lymphoblastic leukemia (ALL)		0.3 [25]
Chronic lymphocytic leukemia (CLL)		0.3 [25]
Medulloblastoma (MB)		0.4 [9]
Carcinoid		0.4 [9]
Neuroblastoma		0.4 [9]
Thyroid		2.2 [25]
Glioma low grade		0.4 [9]
Glioblastoma		0.4 [9]
Breast		0.4–1.1 [27]
Lymphoma B cell		0.3 [25]
Multiple myeloma		0.3 [25]
Kidney chromophobe		2.6 [9]
Prostate		3.0–3.8 [28,29]
Ovary		2.5 [30]
Kidney papillary cell		2.6 [9]
Kidney clear cell		2.6 [9]
Pancreas		2.7 [31]
Liver		1.3 [9]
Endometrium		1.3 [28]
Head and neck		
Uterus		1.3 [28]
Cervix		1.6 [32]
Colorectum		0.9 [33]
Esophagus		4.7 [34]
Lung small cell		5.9 [9]
Stomach		1.3 [35]
Bladder		3.2 [36]
Lung adenocarcinoma		5.9 [9]
Lung squamous		5.9 [9]
Melanoma		3.8–6.4 [9,37]
Squamous cell carcinoma		3.8–6.4 [9,37]
Basal cell carcinoma		3.8–6.4 [9,37]
Childhood cancers	ALL	0.3 [25]
	MB	0.4 [9]
	Rhabdomyosarcoma	11.9–25.7 [9,38]
	Osteosarcoma	34.3 [9]

Mechanical causes of mutation underlie genomic variation with tissue stiffness

Scaling of genomic variation with tissue stiffness could result from at least three possible mechanical sources of mutations. First, stiff matrix enhances cell proliferation,