Mechanosensing tensile solid stresses

Lance L. Munn[a,1] and Hadi T. Nia[b]

Experiments and modeling over the past decade have concluded that cells use sophisticated molecular "clutches" at focal adhesions to determine the stiffness of their supporting substrate (1). This mechanism has been shown to modulate a variety of cell functions, including differentiation and migration. In PNAS, Panzetta et al. (2) propose a modification to the existing models, adding mechanical solid stress as an important signaling property.

Ever since the discovery that stem cells differentiate into different lineages based on the material properties of their substrate (e.g., neurogenic markers are induced on soft substrates but osteogenic markers are activated on stiff substrates) (3), there have been intense efforts to understand the mechanisms by which cells probe their mechanical environment and respond appropriately. The physical microenvironment guides tissue organization during development but is also important in adult tissue, where cells need to constantly probe the mechanical environment and respond to physical insults or trauma. A familiar example is the response of fibroblasts and epithelium in the vicinity of a wound; these cells sense changes in the tissue mechanics and integrate this information with biochemical signals to migrate and produce tension, closing the wound and reestablishing mechanical homeostasis.

Such mechanical sensing and adjustments of cell phenotype contribute to tumor progression, where there are chronic changes in forces and mechanical structure that cause normal cells in and around the growing mass to react to the changing mechanical environment. In most solid tumors, they produce fibrosis (stiffness) and additional forces (solid stresses) that can fuel protumor processes. This is highlighted by recent studies showing that abnormalities in the physical tumor microenvironment contribute to cancer progression and hinder treatment response (4, 5). A better understanding of the genesis of these physical abnormalities has uncovered novel therapeutic targets and enabled new strategies (6, 7).

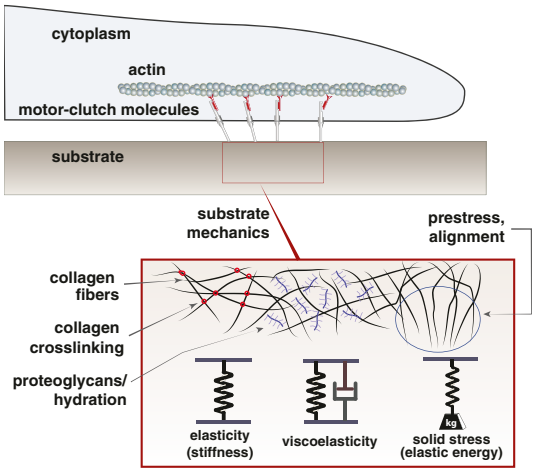

Fig. 1. The motor-clutch mechanism and substrate mechanics. The force-sensitive assembly and disassembly of focal adhesion elements is thought to allow the cell to respond appropriately to different matrices. Although originally formulated based on considerations of substrate stiffness, which is largely affected by collagen cross-linking, other properties or mechanical states may also affect the clutch mechanism. For example, incorporation of hydrated proteoglycans can confer viscous properties to the matrix, and mechanical deformation can create elastic energy (solid stress), which is generally anisotropic.

From the perspective of solid mechanics, tissues can have multiple properties or states that cells might sense and use as cues. The most well studied of these is elasticity or stiffness. Quantitatively, stiffness is determined by how much force is required to deform the substance by a given amount. Tissues such as brain and adipose are more compliant, while bone, muscle, and cartilage are more stiff. Most solid tumors are also stiff (8, 9), and this has long been used in diagnosis, as they can be distinguished

[a]Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114; and [b]Department of Biomedical Engineering, Boston University, Boston, MA 02215

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[1]To whom correspondence may be addressed: munn@steele.mgh.harvard.edu.

from normal tissue by palpation or elastography techniques. Because tissue mechanics play a central role in tumor progression, researchers are focusing on understanding the molecular mechanisms by which cells interact with the environment, and many of the key molecular sensors of stiffness have been identified (10). In 2008, Chan and Odde (1) formulated the "motor-clutch model" to explain how the cell's cytoskeletal machinery automatically changes focal adhesion forces on substrates with different elasticity. The model shows that cytoskeletal linking molecules with constant on rates but force-dependent off rates have the right properties to organize the focal adhesions appropriately in response to local substrate stiffness.

Another material mechanical property that may be relevant for cell mechanosignaling is viscoelasticity (11). While the elasticity in most mechanobiology studies is the equilibrium Young's modulus, which describes the resistance of the tissue to an applied force when the force is applied extremely slowly (quasi-statically), viscoelasticity describes the rate dependence of resistance of the tissue to an applied force. For example, stress relaxation, which describes how quickly the tissue relaxes after removing an external load, can be explained via viscoelasticity parameters of the tissue. Accordingly, others have modified the Chan–Odde model to include these viscous or viscoelastic properties of the substrate (12, 13) (Fig. 1).

In addition to elasticity or viscoelasticity, tissues can contain solid stresses (14), defined as mechanical forces transmitted and generated through the elastic and solid constituents of tissues. Common in tumors, solid stresses have received significant attention recently because they are mechanically distinct from stiffness or viscoelasticity, and likely affect biology through different mechanisms (15, 16). While stiffness is a material property dictated by the composition and structure of the matrix, solid stress depends only on strain energy stored in the tissue, and these forces can be compressive or tensile (16, 17). The solid stresses can be produced by uncontrolled growth in a confined space, by recruitment or production of excess cells or matrix, by osmotic forces, or by the contraction of matrix by myofibroblasts. Note that solid stresses are forces applied or transmitted through the elastic (solid) phase of the tissue, and hence can give rise to elastic (strain) energy; this is distinct from fluid pressure, which cannot store energy in the length and time scales relevant to tissue mechanics. Although the causes of tissue stiffening and how cells react to changes in stiffness are well understood, there is relatively little known about the origins and consequences of solid stresses.

Despite early evidence that solid stress collapses blood and lymphatic vessels (4), increases invasiveness of cancer cells (18), activates tumorigenic pathways (19), drives cell cycle entry (20, 21), and causes neurological dysfunction (22), the emerging subfield of cancer mechanobiology associated with solid stress is still developing, and there are many open questions about the nature of the solid stresses and their effects on biology. For example, it is not clear whether cells sense force (stress) or displacement (strain), or whether the known mechanosensitive structures such as integrin adhesions, cell–cell junctions, glycocalyx components (23), ion channels, and the cell nucleus are involved. The dynamics of the responses also warrant examination: Do the cells detect and respond to long-term solid stresses (e.g., in the tumor microenvironment) or only transient forces?

In PNAS, Panzetta et al. (2) address some of these key questions (2). By seeding cells on elastic substrates and monitoring the mobility of intracellular particles, they are able to determine how stresses in the substrate affect cytoskeletal microrheology. The authors compare cytoskeletal mechanics of cells grown on prestressed and unstretched substrates and find that they react differently to the stressed substrates, as indicated by their stiffer cytoskeletons.

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The results were similar whether they stretched the cells and substrate together or stretched the substrate first, before seeding the cells. The authors conclude that, because stiffness is not affected by prestretch, the cells must be sensitive to the intrinsic strain energy (solid stress) contained in the stretched substrate. Panzetta et al. also investigate the time scale at which solid stress is sensed and find changes in the cytoskeleton within 2 h of stressing the preseeded cells. Similar to stiffness sensing, Panzetta et al. hypothesize that the strain energy in stretched substrates is sensed through the dynamics of myosin motors pulling actin filaments. To provide further mechanistic insight into their observations, they suggest a modification to the mechanochemical clutch model (1), including an additional force term that is not changed by substrate strain. This is meant to represent the intrinsic solid stress, represented by a second spring in the perpendicular direction.

This study supplies more fuel to the discussion of the molecular clutch model but also raises a number of additional questions. For example, because tissues can contain both tensile and compressive stresses, it would be interesting to know whether these are sensed by the same cellular machinery, and whether they have different consequences. Most notably, tumors are generally in compression near the center but in tension at the periphery (16), and this should result in anisotropic stresses and asymmetric matrix organization that may provide additional signals for cells during dissemination. It is also interesting to speculate about the implications of solid stresses in the context of matrix metalloprotease activity. When migrating cells degrade collagen fibers, local stresses in the matrix are released, causing physical deformation of the matrix and stochastic changes in matrix stiffness. Similar nonlinear dynamics occur when cell-induced tension causes unfolding of matrix-associated protein domains. As we gather more information about these processes, they can eventually be incorporated into future models of mechanosensing.

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