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Reprint of: Connections between single-cell biomechanics and human disease states: gastrointestinal cancer and malaria

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ABSTRACT

We investigate connections between single-cell mechanical properties and subcellular structural reorganization from biochemical factors in the context of two distinctly different human diseases: gastrointestinal tumor and malaria. Although the cell lineages and the biochemical links to pathogenesis are vastly different in these two cases, we compare and contrast chemomechanical pathways whereby intracellular structural rearrangements lead to global changes in mechanical deformability of the cell. This single-cell biomechanical response, in turn, seems to mediate cell mobility and thereby facilitates disease progression in situations where the elastic modulus increases or decreases due to membrane or cytoskeleton reorganization. We first present new experiments on elastic response and energy dissipation under repeated tensile loading of epithelial pancreatic cancer cells in force- or displacement-control. Energy dissipation from repeated stretching significantly increases and the cell's elastic modulus decreases after treatment of Panc-1 pancreatic cancer cells with sphingosylphosphorylcholine (SPC), a bioactive lipid that influences cancer metastasis. When the cell is treated instead with lysophosphatidic acid, which facilitates actin stress fiber formation, neither energy dissipation nor modulus is noticeably affected. Integrating recent studies with our new observations, we ascribe these trends to possible SPC-induced reorganization primarily of keratin network to perinuclear region of cell; the intermediate filament fraction of the cytoskeleton thus appears to dominate deformability of the epithelial cell. Possible consequences of these results to cell mobility and cancer metastasis are postulated. We then turn attention to progressive changes in mechanical properties of the human red blood cell (RBC) infected with the malaria parasite *Plasmodium falciparum*. We present, for the first time, continuous force–displacement curves obtained from in-vitro deformation of RBC with optical tweezers for different intracellular developmental stages of parasite. The shear modulus of RBC is found to increase up to 10-fold during parasite development, which is a noticeably greater effect than that from prior estimates. By integrating our new experimental results with published literature on deformability of *Plasmodium*-harbouring RBC, we examine the biochemical conditions mediating increases or decreases in modulus, and their implications for disease progression. Some general perspectives on connections among structure, single-cell mechanical properties and biological responses associated with pathogenic processes are also provided in the context of the two diseases considered in this work.

1. Introduction

Many cellular functions, such as cell division, motility, gene expression, signal transduction, wound healing and apoptosis (programmed cell death), critically depend on mediation and regulation of stress as well as elastic and viscoelastic properties of cell membrane and intracellular proteins and fluid (e.g., [1–3]). The

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changes in the elastic and viscoelastic properties of living cells are also linked strongly to the manner in which the cells respond to structural and molecular alterations induced by the onset and progression of diseases and to invasion by foreign organisms, such as parasites (e.g., [4–7]). Such changes in mechanical response are also known to play key roles in pathogenesis and pathophysiology [2,6,7].

The elastic modulus of a living cell can either increase or decrease depending on the biochemical origins of molecular reorganization occurring in conjunction with the developmental stages of pathogenic processes (e.g., [8]). Molecular architecture, transport properties and mechanical responses of the cytoskeleton, which is a dense intracellular biopolymeric network, are mediated and regulated by associated proteins. Strong chemomechanical coupling elicits reactions from the cytoskeleton [9]. For example, cellular signalling generated by surface recognition via integrins can cause a series of biochemical reactions which, in turn, lead to precise control and regulation of cytoskeleton structure, effective elastic response of the cell and its adhesion properties. In many cases, changes to cytoskeleton structure and mechanical properties are also accompanied by changes in cell shape and mobility [10]. Understanding the mechanisms associated with the connections among molecular reorganization in diseased cells and the resultant changes to the mechanical properties can, therefore, be critical to developing a complete knowledge of the developmental processes underlying disease progression.

Advances in experimental biophysics and bioengineering in the past two decades have enabled direct, real-time mechanical probing and manipulation of single cells and molecules. Such methods are now capable of imposing and sensing forces and displacements with resolutions as fine as a picoNewton and a nanometer, respectively, in a well-controlled manner. Available experimental techniques to probe single cells include micropipette aspiration, optical tweezers (also known as optical or laser traps), magnetic tweezers, atomic/molecular force probes, nanoindenters, microplate manipulators and optical stretchers. Descriptions of these techniques can be found in recent reviews [2,5,11–17].

In this paper, we explore connections among molecular structure of the cytoskeleton, cellular and subcellular elastic response, and human disease states. For this purpose, we consider experimental observations of single-cell mechanics associated with two very different human diseases: gastrointestinal tumor and malaria induced by the parasite *Plasmodium falciparum*. By integrating new experimental observations presented in this paper with our recent results [16,17], we identify critical elements of the structure–property–function connections underlying single-cell mechanical response in the context of these diseases. For the two disease states examined, we focus on the biochemical conditions for which the elastic modulus of the affected cell can either increase or decrease in the diseased state as compared to the healthy cell. We link, wherever possible, such changes in mechanical response with the underlying changes in molecular architecture as a consequence of disease development and to changes in cell shape and mobility. We further describe how such differences in alterations to mechanical properties regulate different biological and physiological responses during the developmental stages of the disease.

The paper is arranged in the following sequence. Section 2 deals with the single-cell mechanics of epithelial pancreatic cancer cells (Panc-1). We first present new experimental observations, obtained under physiological conditions (37 °C, 5% CO_2-containing air), of force-controlled and displacement-controlled tensile loading of single Panc-1 cell, using the microplate mechanical stretcher method [14–16]. These results illustrate how specific biochemical factors either increase or decrease the elastic stiffness. Results of evolution of hysteretic energy dissipation from force–displacement loops during repeated tensile cycling of the cell under force-control and displacement-control are also obtained so as to examine possible effects of biochemical factors. We then examine the underlying reorganization of molecular assembly in response to controlled biochemical modifications. Possible links among such structure and property changes and metastasis are postulated. Section 3 deals with another very different process that can lead to disease state involving human red blood cells (RBCs) parasitized by *Plasmodium* (*P.*) *falciparum* malaria parasites. Here the effects of infestation on the mechanical response of the single cell are investigated in vitro by recourse to direct large deformation tensile stretching with optical tweezers. Direct force–displacement curves are described for the first time for different developmental stages of the parasite inside the RBC and the results are compared with similar experiments performed on reference conditions involving healthy RBCs and uninfected RBCs exposed to the parasite. This information is then combined along with three-dimensional computational simulations of optical tweezers stretching to demonstrate how parasitization causes a marked increase in elastic stiffness.[1] Possible causes of this stiffening arising from transport of specific proteins from the parasite to the cell membrane or cytoskeleton are described. Micromechanical assays of RBCs parasitized by *P. falciparum* using the present optical tweezers studies are compared with those from other independent experimental methods [8,18], and the differing effects on mechanical response of RBCs from infestation by different parasites, viz., *P. falciparum* and *P. vivax*, are addressed. The paper concludes with some general perspectives on possible connections among mechanical properties, intracellular molecular reorganization and disease characteristics in the context of the differing situations involving gastrointestinal epithelial cancer and malaria.

2. Mechanics of human Panc-1 pancreatic cancer cells

Cytoskeletal components of human epithelial cells include filamentous biopolymers such as actin, microtubules and intermediate filaments. About 5% of the total protein structure of epithelial cells comprises keratins which are organized into biomolecular bundles, constituting chiefly the intermediate filaments of the cells and determining the mechanical characteristics of the cytoplasm. While human pancreatic cancer[2] cells express keratins 7, 8, 18 and 19, the subline of Panc-1 cells are known to express primarily K8 and K18.

2.1. Effects of SPC on the subcellular structure of Panc-1 cells

Sphingosylphosphorylcholine (SPC) is a bioactive lipid which promotes anti-apoptotic effects in human blood components such as blood plasma and high density lipoprotein (HDL) particles where it occurs naturally [19]. Elevated levels of SPC are also found in the brains of people afflicted with type A form of Niemann–Pick disease [20] (which is an inherited metabolic disorder caused by the accumulation of a fatty substance in the spleen, liver, lungs, bone marrow, or brain) and in blood and malignant ascites of ovarian cancer patients [21]. Because of its propensity to mediate cell proliferation [22] and cell migration [23], SPC is also considered to play a key role in cancer metastasis.

[1] Details of materials and experimental and computational methods used to obtain the results reported in both Sections 2 and 3 are summarized in the supplementary material accompanying this paper in the journal website.

[2] The human pancreas serves to accomplish two major functions: secretion of pancreatic juice into the duodenum along the pancreatic duct and regulation of blood glucose levels by secreting insulin and glucagons. Cancer of the pancreas is one of the leading causes of death due to cancer in the industrialized world, with an annual death toll exceeding 31,000 in the United States.