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Cell elasticity with altered cytoskeletal architectures across multiple cell types

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ABSTRACT

The cytoskeleton is primarily responsible for providing structural support, localization and transport of organelles, and intracellular trafficking. The structural support is supplied by actin filaments, microtubules, and intermediate filaments, which contribute to overall cell elasticity to varying degrees. We evaluate cell elasticity in five different cell types with drug-induced cytoskeletal derangements to probe how actin filaments and microtubules contribute to cell elasticity and whether it is conserved across cell type. Specifically, we measure elastic stiffness in primary chondrocytes, fibroblasts, endothelial cells (HUVEC), hepatocellular carcinoma cells (HUH-7), and fibrosarcoma cells (HT 1080) subjected to two cytoskeletal destabilizers: cytochalasin D and nocodazole, which disrupt actin and microtubule polymerization, respectively. Elastic stiffness is measured by atomic force microscopy (AFM) and the disruption of the cytoskeleton is confirmed using fluorescence microscopy. The two cancer cell lines showed significantly reduced elastic moduli values (~0.5 kPa) when compared to the three healthy cell lines (~2 kPa). Non-cancer cells whose actin filaments were disrupted using cytochalasin D showed a decrease of 60–80% in moduli values compared to untreated cells of the same origin, whereas the nocodazole-treated cells showed no change in elasticity. Overall, we demonstrate actin filaments contribute more to elastic stiffness than microtubules but this result is cell type dependent. Cancer cells behaved differently, exhibiting increased stiffness as well as stiffness variability when subjected to nocodazole. We show that disruption of microtubule dynamics affects cancer cell elasticity, suggesting therapeutic drugs targeting microtubules be monitored for significant elastic changes.

1. Introduction

The interdependence of cell elasticity and cytoskeletal components is a critical step toward understanding the mechanics of living tissue. Cellular responses and their microarchitecture react and adapt to their environment and disease state (Discher et al., 2005; Ingber et al., 1995; Bao and Suresh, 2003). Changes in cell elasticity have been implicated in the pathogenesis of many human diseases (Lee and Lim, 2007) including vascular disorders, (Qiu et al., 2010) malaria, (Glenister et al., 2002; Suresh et al., 2005) sickle cell anemia, (Nash et al., 1984) arthritis, (Trickey et al., 2000) asthma, (An

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et al., 2006) and cancer (Suresh et al., 2005; Suresh, 2007; Rebelo et al., 2013; Cross et al., 2007; Remmerbach et al., 2009; Wirtz et al., 2011; Yallapu et al., 2014). Therefore, there is a practical need to measure cell mechanics quantitatively to understand how diseased cells differ from healthy ones. In particular, investigating the mechanical properties of cancer cells may help to better understand the physical mechanisms responsible for cancer metastasis.

Common techniques to measure the mechanics of cells, include the use of magnetic beads (Ingber et al., 1995; Fabry et al., 2001), optical tweezers (Svoboda and Block, 1994; Dao et al., 2003), micropipette aspiration (Trickey et al., 2000; Thoumine et al., 1999; Reynolds et al., 2014; Jones et al., 1999) and atomic force microscopy (AFM) (Rebelo et al., 2013; Radmacher et al., 1996; Ketene et al., 2012a, 2012b; Darling et al., 2008). Since the application of AFM to living cells, it has readily been adapted to characterize cell topography (Henderson et al., 1992; Rotsch and Radmacher, 2000) as well as mechanical properties (Rebelo et al., 2013; Ketene et al., 2012a; Darling et al., 2006; Lekka et al., 1999; Li et al., 2008) with nanoscale precision. The AFM has been further adapted for microrheology (Rother et al., 2014; Nalam et al., 2015), frequency modulation (Raman et al., 2011; Caporizzo et al., 2015), and creep (Ketene et al., 2012b; Corbin et al., Bashir; Moreno-Flores et al., 2010) experiments to study the viscoelastic properties of various cell lines. Of interest to this work, is the evaluation of elasticity of endothelial cells (Ohashi et al., 2002; Mathur et al., 2000; Sato et al., 2004; Braet et al., 1998), fibroblasts (Rotsch and Radmacher, 2000; Park et al., 2005; Bushell et al., 1999; Wu et al., 1998), chondrocytes (Jones et al., 1999; Freeman et al., 1994; Koay et al., 2003), fibrosarcomas (Zaman et al., 2006; Moeendarbary et al., 2013; Efremov et al., 2014) and hepatocellular carcinoma cells (Wu et al., 2000). Kuznetsova et al. (2007) includes a table of expected moduli values for various cell lines. Based on elastic modulus measurements, many groups show that cancerous cells are softer than their non-transformed counterparts (Rebelo et al., 2013; Park et al., 2005). This stands to reason since increased deformability of cancer cells allows them to metastasize and infiltrate tissues (Ribeiro et al., 2014). Such behavior is attributed to modification in the cytoskeletal organization of the cells (Buda and Pignatelli, 2004; Lindberg et al., 2008).

The influence of cytoskeletal drugs on cell elasticity has allowed the decoupling of cytoskeletal components and their contribution to overall cell elasticity. Theoretically, actin filaments in the cell periphery are the most rigid of the main components of the cytoskeleton but due to their tubular structure, microtubules have a larger bending stiffness (Suresh, 2007). The resulting contribution of each cytoskeletal component toward cell elasticity does not depend solely on the bending stiffness of individual components, but will factor based on organization and concentration, which will be dependent on cell type. For example, Jackson et al. (2008) indicates that changes in cytoskeletal cross-linking proteins in response to mechanical loading would be sufficient to increase whole cell stiffness. AFM studies that investigate the influence of cytoskeletal drugs on cell elasticity have shown the elastic modulus to be highly affected by actin filament disruption, (Ketene et al., 2012a; Rotsch and Radmacher, 2000; Moreno-Flores et al., 2010; Cai et al., 2010; Takai et al., 2005; Titushkin and Cho, 2007) but there are groups that find cell elasticity is relatively unaffected by microtubule-targeted drugs (Rotsch and Radmacher, 2000; Takai et al., 2005; Titushkin and Cho, 2007) and some that find microtubules contribute greatly (Ingber et al., 1995; Wu et al., 1998, 2000').

While many AFM studies examine drug responses of one cell type, or one healthy and one cancerous cell type, our goal is to present a comprehensive AFM study across multiple cell lines, healthy and cancerous, that examines the effect of cytoskeletal destabilizers on cell elasticity using the exact same method. We find destabilizing the microtubule network produces no effect in healthy cell lines, while producing a significant increase in elastic moduli in the fibrosarcoma line. We aim to elucidate the functional relationship between cellular mechanics and cytoskeletal stability and whether that relationship is conserved across different cell types.

2. Experimental methods

The methods here are designed to probe the mechanical behavior across all cell lines providing a comprehensive head to head comparison across 15 conditions: control, actin destabilized and microtubule destabilized for each of 5 cell types. The same plating conditions (Section 2.1), cytoskeletal visualization technique (Section 2.2) and experimental test protocol (Sections 2.3–2.5) allow for direct comparison. Furthermore, the control of the AFM parameters, indentation depth and approach velocity, maintain the same probing frequency for each condition, which is critical for comparing the mechanical properties of materials that exhibit a frequency dependence.

2.1. *Cell culture*

Five cell lines were cultivated for this study: human dermal fibroblasts, bovine chondrocytes, HUVECs, HT 1080 (fibrosarcoma), and HUH-7 (hepatocellular carcinoma). Fibroblasts and HUVECs were cultured in Fibrolife (Lifeline Cell Technologies) and Vasculife (Lifeline Cell Technologies), respectively. HT 1080 fibrosarcoma cells, HUH-7 hepatocarcinoma cells, and chondrocytes were cultured in DMEM L-glutamine supplemented with 10% fetal bovine serum and 1% penicillin streptomycin. All cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2. Cells were grown to confluency and harvested by trypsinization. Coverslips were coated for 30–40 min with 5 μg/mL fibronectin (BD Biosciences) dissolved in PBS prior to plating. Cells were initially plated at 50–75 k density on glass coverslips (22 × 40 mm) for all studies, and were incubated for 48 h prior to experiments. Three conditions were studied: control (recording buffer only), 2.5 μM Cytochalasin D (Sigma Aldrich) in recording buffer for 30 min, and 10 μM Nocodazole (Sigma Aldrich) in recording buffer for 30 min. Recording buffer solution was made as follows: 10% 10 × HBSS (Gibco), 1% Heparin, 1% Glutamax, and 1% FBS in DI water. Solution was then pH-balanced to 7.4 using NaOH.