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Mechanical properties of cancer cytoskeleton depend on actin filaments to microtubules content: Investigating different grades of colon cancer cell lines

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ABSTRACT

Biomechanical properties of cancer cells have been proposed as promising biomarkers to investigate cancer progression. Cytoskeletal reorganization alters these characteristics in different grades of cancer cells. In the present study based on the micropipette aspiration method, whole body evaluation for two different colon cancer cells was performed to determine viscoelastic parameters of the cells. A finite element model was developed for verification of experiments and predicting some behaviors of cells. Western blot analysis and fluorescence intensity for actin microfilaments and microtubules were performed to measure cell content of the proteins. It was illustrated that the proportion of microtubules and actin microfilaments is different in grade I and grade IV colon cancer cells in a manner that microtubules attain an effectual role in progressive reorganization of cytoskeleton in transition from nonaggressive to malignant phenotypes in cancer cells. Furthermore, it was concluded that larger instantaneous Young's modulus value for high grade cells is related to the existence of extensively build-up actin networks at the cell cortex. Based on the cell mechanics results, a simple parameter is suggested for sorting different grades of colon cancer cells.

1. Introduction

Cancer cells are classified into four distinct grades based on how severe they are and how quickly they can grow and spread. Grade I tumor cells are generally believed to be the least aggressive in behavior and the fourth ones tend to grow more quickly with the highest malignancy (Sun et al., 2006). Local invasion, intravasation, circulation, arrest and extravasation, proliferation and angiogenesis are the steps that are involved in cancer cell metastasis (Talmadge and Fidler, 2010). Metastatic cells need to pass through capillaries which are rigid with diameters smaller than tumor cells (Coughlin et al., 2013; Sato and Suzuki, 1976). Deformability play a critical role in the potency of tumor cells to form neoplastic foci (Ochalek et al., 1988). In general, cancer cells are more deformable than normal cells and indeed, it is believed that nontumorigenic cells are stiffer than malignant cancerous cells (Abdolahad et al., 2012; Rebelo et al., 2013).

As cancer progresses, cytoskeleton proteins transform leading to changes in mechanical properties of the cancer cells in contraction, stretchability, deformability and therefore in viscoelastic parameters (Kumar and Weaver, 2009). These cells structure regulations are strongly dependant on the redistribution and the activity of the three main filamentous biopolymers: microtubules, microfilaments, and intermediate filaments (Suresh, 2007).

Up to now, several techniques have been introduced to measure cellular mechanical properties quantitatively or qualitatively. They can be roughly divided into two major approaches. The first method probes only small parts of cells to provide a quantitative analysis and their results depend significantly upon the measurement location. Accordingly, the data obtained in the approaches generally show a large cell-to-cell variation. An Atomic Force Microscope (AFM) is the most applicable device in this category. In the second method, cells are investigated as a whole body. Micropipette aspiration (MA) is the most feasible and convenient ones in this category. Several experiments have been performed by

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implementing each of these two methods to evaluate mechanical features of cells (Evans and Yeung, 1989; Hochmuth, 2000; Suresh, 2007). Compared to AFM, the MA approach seems to be more reliable in representing the whole mechanical properties of a cell since it produces a deformation of entire suspended cell, and it eliminates undesirable effects of cell–matrix interaction and more importantly the stiffness of substrate (Lekka et al., 2012; Lim et al., 2006).

Assessing the cytoskeletal reorganization of cells during cancer progression could be a good indicator of cell state and provides valuable information for new developments in cancer diagnostics, prophylactics, therapeutics and drug efficacy assays (Bao and Suresh, 2003; Cross et al., 2008; Pokorný et al., 2011). Reducing complexity and costs towards clinical applications makes heavy demands to detect and isolate metastatic grades of cancerous cells (Hur et al., 2011). Single-cell stiffness has recently been recognized as a promising feature for assessment of the cell invasiveness and can provide new insights into the process of cancer metastasis (Guck et al., 2005). Several studies have been done to find out the amount of contribution for any of the skeletal biopolymers specially microtubules and microfilaments on mechanical properties of cancer cells (Guck et al., 2005; Ketene et al., 2012).

In the present study, differences in viscoelastic behavior of two distinct grades of colon cancer cells have been appraised by utilizing the MA technique. A computational model based on the finite element method (FEM) has been developed to validate actual experiments results and to predict some behaviors of cells. Western blotting and fluorescence intensity measurement for two major cytoskeletal components were performed and their relative alterations were correlated to mechanical properties of the cells. Furthermore, the effect of a microtubule-targeted drug (albendazole) on creep behavior of non-aggressive cells was studied. Finally, a simple parameter was suggested for recognition of different grades of colon cancer cells. To the best of our knowledge, no other study has ever been performed to evaluate alterations of colon cancer cells mechanical properties and their association to the ratio of cytoskeleton components.

2. Materials and methods

2.1. Cell culture

Two different grade tumor cell lines were obtained from the National Cell Bank of Iran, Pasteur Institute. HT29 and SW48 cell lines had been isolated from grade I and grade IV colon tumors respectively. Cells were maintained at 37 °C (5% CO_2, 95% air) in RPMI-1640 medium (Sigma) supplemented with 5% fetal bovine serum, and 1% penicillin/streptomycin (Gibco). The fresh medium was replaced every other day.

2.2. Micropipette aspiration experiment

MA is able to quantitatively measure mechanical properties of a cell with a good reproducibility. The following procedure was performed according to previous work on micropipette aspiration (Sato et al., 1996; Trickey et al., 2000). In brief, cells were detached from the substrate by 0.25% trypsin (Invitrogen) and suspended in the culture medium. This procedure took less than 4 min to minimize the effect of trypsinization (Badley et al., 1980) at room temperature around 20–22 °C. The step constant negative pressure imposed on each cell was maintained between 500 and 600 Pa in all experiments.

Exerting controlled suction pressure on the cell surface through the pipette causes the cell body to be aspirated into the pipette. Meanwhile the leading edge of cell surface was monitored by an inverted microscope (Nikon Eclipse) and captured with a digital camera (Nikon DXM1200). Internal diameters of micropipette were within the range of 4.5–5 μm. Before each experiment, micropipettes were coated with Sigmacote chemical agent (Sigma) to avoid adhesion of cells to inner wall of the micropipette (Tan et al., 2008). Each micropipette was under the control of a micromanipulator (Transfer Man Nk2, Eppendorf), to adjust its position. Analysis of images was performed by Axiovision LE Software (Zeiss).

2.3. Theoretical model of MA

A theoretical model for MA had been previously developed to extract the mechanical properties of cells. Indeed, it is a generalized Maxwell model which includes a spring (k_1) that provides the restoring force necessary to recover the initial shape after the release of the stress in parallel with series of a damper (μ) and a spring (k_2) as shown in Fig. 1B. These parameters are all assumed to be constant. In this model, the cell is supposed to be a homogeneous, half-space, incompressible and viscoelastic material subjected to a uniform axisymmetric aspiration pressure as depicted in Fig. 1A (Guilak et al., 2000; Sato et al., 1996; Theret et al., 1988). The relation between elastic and viscous parameters is described by Eq. (1) in which, boundary condition of no axial displacement of the cell at the micropipette end is assumed.

$$\frac{L(t)}{a}=\frac{2\Delta p}{k_1\pi}\left(1+\left(\frac{k_1}{k_1+k_2}-1\right)e^{-\frac{t}{\tau}}\right)h(t) \tag{1}$$

where ΔP is the applied pressure, $L(t)$ is the aspirated length, $h(t)$ is the unit step function, and parameter a is considered as the inner radius of the micropipette. The time constant (τ) of the viscoelastic model is calculated by the following equation:

$$\tau=\frac{\mu}{k_1}\left(1+\frac{k_1}{k_2}\right) \tag{2}$$

Viscoelastic parameters of cells are obtained by curve fitting of experimental data (L/a) with time using the least square method in Matlab according to the following equations:

$$y=Ae^{-Bx}+C \tag{3}$$

$$\frac{2\Delta p}{k_1\pi}=C,\quad \frac{2\Delta p}{k_1\pi}\left(\frac{k_1}{k_1+k_2}-1\right)=A,\quad \frac{1}{\tau}=B \tag{4}$$

These elastic constants k_1 and k_2 are related to standard elasticity coefficients by the following relationships where E_0 is the instantaneous Young modulus and E_∞ is the equilibrium Young modulus.

$$E_0=\frac{3}{2}(k_1+k_2) \tag{5}$$

$$E_\infty=\frac{3}{2}k_1 \tag{6}$$

2.4. Western blot analysis

To quantify relative content of actin and microtubule proteins, Western blot analysis was performed (Mahmood and Yang, 2012). Both cell lines were (70% confluent) growing in cell culture flasks. RIPA buffer with 1 × complete protease

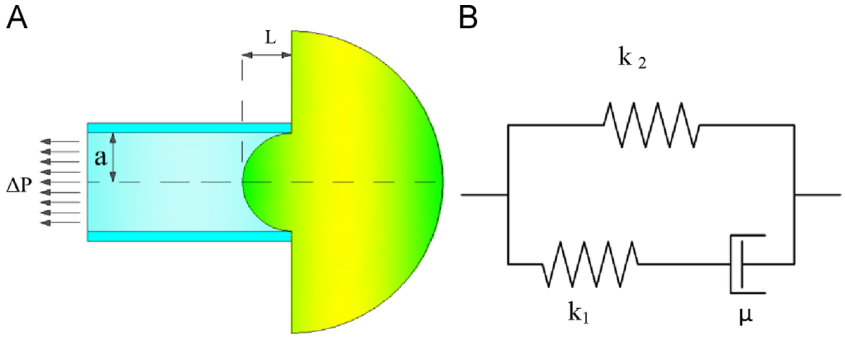

Fig. 1. (A) The theoretical representation of the micropipette aspiration experiments. The cell is modeled as an axisymmetric elastic half-space. A negative pressure, ΔP, is applied through the micropipette. (B) A three-parameter viscoelastic model (standard linear solid) was used to represent the material behavior of the cell.