Biochimica et Biophysica Acta xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Distinct impact of targeted actin cytoskeleton reorganization on mechanical properties of normal and malignant cells ☆

Yu.M. Efremov *, A.A. Dokrunova, A.V. Efremenko, M.P. Kirpichnikov, K.V. Shaitan, O.S. Sokolova

M.V. Lomonosov Moscow State University, Faculty of Biology, 119234, 1/73 Leninskie Gory, Moscow, Russia

ARTICLE INFO

Article history:
Received 23 January 2015
Received in revised form 23 April 2015
Accepted 5 May 2015
Available online xxxx

Keywords:
AFM
Force spectroscopy
Prostate cancer
Formin
Arp 2/3
Cortical actin

ABSTRACT

The actin cytoskeleton is substantially modified in cancer cells because of changes in actin-binding protein abundance and functional activity. As a consequence, cancer cells have distinctive motility and mechanical properties, which are important for many processes, including invasion and metastasis. Here, we studied the effects of actin cytoskeleton alterations induced by specific nucleation inhibitors (SMIFH2, CK-666), cytochalasin D, Y-27632 and detachment from the surface by trypsinization on the mechanical properties of normal Vero and prostate cancer cell line DU145. The Young's modulus of Vero cells was 1300 ± 900 Pa, while the prostate cancer cell line DU145 exhibited significantly lower Young's moduli (600 ± 400 Pa). The Young's moduli exhibited a log-normal distribution for both cell lines. Unlike normal cells, cancer cells demonstrated diverse viscoelastic behavior and different responses to actin cytoskeleton reorganization. They were more resistant to specific formin-dependent nucleation inhibition, and reinforced their cortical actin after detachment from the substrate. This article is part of a Special Issue entitled: Mechanobiology.

1. Introduction

One of the major characteristics of cancer cells is their ability to migrate to nearby tissues, blood or lymph vessels and to form secondary tumors at multiple distant sites. Tumor metastases are responsible for approximately 90% of all cancer-related deaths [1]. The mechanism by which malignant cells develop such ability involves changes in their mechanical properties and actin cytoskeleton dynamics. The ability to move is a basic property of living cells. The movement of cells and cell layers organizes and determines the formation of organs in embryogenesis, wound healing and the progress of inflammation. The actin cytoskeleton, together with other factors (nucleation promoting factors, microtubules, intermediate filaments, lipids), is responsible for these functions [2]. The dynamics of the cytoskeleton are regulated by actin-binding proteins (ABPs) promoting actin turnover, the key process in cellular motility [3]. The Arp2/3 complex is responsible for actin branching [4]. Cofilin and the Srv2 complex coordinate actin cable severing [5] and actin depolymerization [6,7]. Formin family proteins regulate the polarized growth of cells, stress fiber formation, and actin nucleation [8–10]. They also coordinate interactions between actin filaments and microtubules [11]. Cytoplasmic formins are autoinhibited [12] and activated upon interaction with Rho GTPases [13]. After activation, they processively nucleate long actin cables, thus generating leading edge protrusions [14]. Recently, a significant increase of formin expression in colorectal cancer [15–17] and leukemia [18] has been demonstrated. It has been hypothesized that formins may act as tumor suppressors [19], yet no such activity has been specifically identified.

Nucleation of the actin branch by the Arp2/3 complex occurs in combination with other proteins including the N-WASP/WAVE family. The activated complex binds to the side of the existing filament, aligns in a similar manner to an actin dimer and nucleates the formation of a new filament, which extends from the side of the existing filament at a 70° angle. Dense branched networks generated by Arp2/3 activity are essential for mesenchymal invasion and the formation of lamellipodia and invadopodia. Overexpression of WASP family proteins and the Arp2/3 complex has been associated with malignant phenotypes of several human cancers, including breast [20], colorectal [21], lung [22], and head and neck squamous cell carcinoma [23].

In cancer cells, the actin cytoskeleton is substantially modified, accompanied by changes in the expression and activity of ABPs [19,24,25]. As a result, cancer cells have distinctive mechanical properties from both normal and benign cells. With some exceptions [26–28], a low rigidity of cancer cells was reported in the majority of works [29–35]. It has been suggested that the elastic properties of cancer cells play a major role in the metastatic process, and the degree of deformability of cells has been proposed as a marker for metastatic potential [36,37]. Hypothetically, more aggressive cancer cells should

☆ This article is part of a Special Issue entitled: Mechanobiology.

* Corresponding author at: M.V. Lomonosov Moscow State University, Faculty of Biology, Department of Bioengineering, 119234, 1/73 Leninskie Gory, Moscow, Russia. Tel./fax: +7 4959395738.
E-mail address: yu.efremov@gmail.com (Y.M. Efremov).

http://dx.doi.org/10.1016/j.bbamcr.2015.05.008
0167-4889/

demonstrate enhanced mechanical deformability because these cells undergo substantial mechanical deformation during tissue invasion, intra- and extravasation. Yet results from recent studies show all possible types of correlation (direct, reversed, nonsignificant, and complicated) between the cell stiffness and the metastatic potential of cancer cells [30,34,35,38–40], meaning that the independent mechanical characterization is not sufficient to evaluate the metastatic potential of cells. Further investigations related to mechanical aspects, such as measurements in different conditions and after different treatments, are required to understand the connection of mechanical compliance with the metastatic potential.

Recently, several methods were suggested to characterize the mechanical properties of cells including different types of microrheology [41], optical tweezers [42], micropipette aspiration [43], magnetic tweezing cytometry [44], atomic force microscopy (AFM) [37,45,46] and others. Previously, we employed force spectroscopy, a commonly used AFM technique, to investigate the elastic properties of non-transformed epithelial Vero cells and showed that this cell line may be a good experimental model for studying the effects of different treatments on cell mechanical properties [47]. Here, we examined the mechanical properties (quasi-static and complex Young's moduli) of prostate cancer cells DU145, and compared them to normal Vero cells before and after different actin cytoskeleton reorganizations. Although the studied cell lines have different origins (both are epithelial, but Vero are from monkey healthy kidney and DU145 are from human prostate cancer), we showed here that they share some common properties with normal (Vero) and malignant (DU145) cells, which include cytoskeleton structure, migration abilities and mechanical parameters. The effects of inhibitors of the major actin nucleators, formin and Arp2/3, Cytochalasin D (CytD) and Y-27632 (ROCK inhibitor) treatments, as well as the detachment of cells from the culture substrate by trypsinization, were studied.

2. Materials and methods

2.1. Cell cultivation

Human prostate cancer epithelial cell line DU145 with medium invasiveness was chosen for the experiments [48]. A healthy African green monkey *Cercopitecus aethiops* kidney cell line (Vero) served as a control. Both cell lines were grown in DMEM (PanEco, Russia) with 10% fetal bovine serum (FBS) (HyClone, USA). Cells were grown at 37 °C in a humidified 5% CO_2 atmosphere in a CO_2-incubator (Sanyo, Japan) for 5–6 passages after being defrosted. For AFM imaging and force spectroscopy, cells were harvested from subculture, seeded on sterile glass coverslips (Menzel-Gläser, Germany) in sterile 35 mm Petri dishes and placed into the incubator at the same conditions as stated above for 1–2 days.

The cells were treated with the following compounds: SMIFH2 – an inhibitor of formin-mediated actin assembly [49]; CK-666 – an inhibitor of the Arp2/3 complex-dependent pathway of actin nucleation [50]; Cytochalasin D (all from Sigma-Aldrich, Germany) – an inhibitor of total actin polymerization; and Y-27632 (Abcam, UK) – inhibitor of the Rho kinase (ROCK) pathway of myosin light chain phosphorylation. Stock solutions were prepared in DMSO, and then reagents were diluted to final concentrations in a serum-free medium. Trypsinization was performed by the replacement of the cell culture medium with 0.25% Trypsin–EDTA in HBSS (PanEco, Russia).

2.2. Confocal microscopy

For confocal fluorescence imaging, cells were seeded on sterile 35 mm glass bottom Petri dishes (Greiner Bio-one, Germany) at a density of $3 * 10^5$ cells per dish and allowed to grow for the next 24 h. Then, the cells were fixed with 2% formaldehyde, permeabilized with 0.4% Triton-X100 in PBS, and stained. The filamentous actin (F-actin) was labeled with fluorescent phalloidin conjugate (with Alexa Fluor® 488 or Alexa Fluor® 546, Life Technologies, Europe). Cell nuclei were stained with DAPI (Life Technologies, Europe). The samples were mounted into the SlowFade® medium (Life Technologies, Europe). Fluorescence images were taken using Zeiss LSM510 Meta or Zeiss LSM710 Meta inverted confocal microscopes (Carl Zeiss, Germany) with a 63 × α (alpha)-Plan-Apochromat oil-immersion objective (NA = 1.46, Carl Zeiss AG).

For quantitative F-actin measurements after different treatments the staining procedure was slightly modified from the above. Control and treated cells were cultivated separately on the same glass slide (Menzel-Gläser, Germany) with attached Flexiperm silicone wells (Greiner Bio-one, Germany). After removing the silicone wells, the cells were fixed and stained in an identical way on the same slide, so the direct comparison of relative amounts of F-actin was possible. Fluorescence of Alexa Fluor® 546 phalloidin and DAPI were recorded using a Z-stack regime. The Image J software (National Institutes of Health, USA) was used for processing the fluorescent images. At least 40–50 cells were examined in each sample. The quantitative map of the Alexa Fluor® 546 phalloidin distribution was calculated for each measured cell and processed with the Image J software to estimate average cytoplasmic fluorescence intensity.

2.3. In vitro Matrigel invasion assay

Cell invasion experiments were performed using Bio-Coat cell migration chambers (BD Biosciences, USA) according to the manufacturer's protocol. Inserts were composed of 8 μm pore size filters coated with the Matrigel basement membrane matrix (BD Biosciences, USA). Briefly, detached cells ($2 * 10^5$ in 500 μl of serum-free DMEM/RPMI medium) were added to each insert (upper chamber), and the chemoattractant (5% FBS in DMEM/RPMI) was placed in each well of a 24-well companion plate (lower chamber). After 14-h incubation at 37 °C in a 5% CO_2-incubator, the noninvasive cells from the upper surface of the filter were thoroughly removed with a cotton swab. Invading cells on the lower surface of the membrane were fixed with methanol and stained with 1% Toluidine blue (Fluka) in 1% borax. For quantification, the membranes were mounted to glass slides, and the cells from 5 random microscopic fields (×200 magnification) were counted. Assays were performed in duplicate, and migration was expressed in terms of cells/field.

2.4. Young's modulus measurements.

AFM measurements were performed using a commercial Solver Bio atomic force microscope (NT-MDT, Russia), combined with an inverted optical microscope (Olympus, Japan) as described previously [47]. Cells, subjected to AFM measurements, were non-confluent (about 60%), and well attached to the Petri dish surface. Typically, 3–5 force curves were taken at a 2 μm/s rate from each of the studied cells near its center using tipless AFM probes CSG11 (NT-MDT, Russia) modified with a 9 μm diameter polystyrene bead. Hertz's contact model was applied for processing of the first 500 nm of indentations to calculate apparent Young's modulus (E). Experiments were conducted during a 30–40 min period, at 37 °C, after replacing the growth medium with the DMEM HAM/F12 medium with 15 mM HEPES (Sigma, United States). Three independent experiments were conducted for each cell line before and after treatment, with 10–25 cells analyzed in each experiment.

Viscoelastic behavior of cells was characterized by applying low-amplitude (10–15 nm) sinusoidal perturbations with a 0.3–230 Hz frequency (in a random order) to scanner Z-electrodes, when the cantilever was at a ≈500 nm indentation. An $E^*(f)$ was computed with correction for the tip-cell contact geometry (the first term of the Taylor expansion of the Hertz model was used) and for the hydrodynamic viscous drag [51]. At least 10 cells of each cell line were analyzed.

Please cite this article as: Y.M. Efremov, et al., Distinct impact of targeted actin cytoskeleton reorganization on mechanical properties of normal and malignant cells, Biochim. Biophys. Acta (2015), http://dx.doi.org/10.1016/j.bbamcr.2015.05.008