Acta Biomaterialia 55 (2017) 239–248

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Viscoelastic properties of normal and cancerous human breast cells are affected differently by contact to adjacent cells

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ARTICLE INFO

Article history:
Received 21 December 2016
Received in revised form 14 March 2017
Accepted 5 April 2017
Available online 7 April 2017

Keywords:
Atomic force microscopy
AFM
Power-law rheology
Cell mechanics
Cell-cell contact

ABSTRACT

Malignant transformation drastically alters the mechanical properties of the cell and its response to the surrounding cellular environment. We studied the influence of the physical contact between adjacent cells in an epithelial monolayer on the viscoelastic behavior of normal MCF10A, non-invasive cancerous MCF7, and invasive cancerous MDA-MB-231 human breast cells. Using an atomic force microscopy (AFM) imaging technique termed force clamp force mapping (FCFM) to record images of the viscoelastic material properties, we found that normal MCF10A cells are stiffer and have a lower fluidity at confluent than at sparse density. Contrarily, cancerous MCF7 and MDA-MB-231 cells do not stiffen and do not decrease their fluidity when progressing from sparse to confluent density. The behavior of normal MCF10A cells appears to be governed by the formation of stable cell-cell contacts, because their disruption with a calcium-chelator (EGTA) causes the stiffness and fluidity values to return to those at sparse density. In contrast, EGTA-treatment of MCF7 and MDA-MB-231 cells does not change their viscoelastic properties. Confocal fluorescence microscopy showed that the change of the viscoelastic behavior in MCF10A cells when going from sparse to confluent density is accompanied by a remodeling of the actin cytoskeleton into thick stress fiber bundles, while in MCF7 and MDA-MB-231 cells the actin cytoskeleton is only composed of thin and short fibers, regardless of cell density. While the observed behavior of normal MCF10A cells might be crucial for providing mechanical stability and thus in turn integrity of the epithelial monolayer, the dysregulation of this behavior in cancerous MCF7 and MDA-MB-231 cells is possibly a central aspect of cancer progression in the epithelium.

Statement of Significance

We measured the viscoelastic properties of normal and cancerous human breast epithelial cells in different states of confluency using atomic force microscopy. We found that confluent normal cells are stiffer and have lower fluidity than sparse normal cells, which appears to be governed by the formation of cell-cell contacts. Contrarily, confluent cancer cells do not stiffen and not have a decreased fluidity compared to sparse cancer cells and their viscoelastic properties are independent of cell-cell contact formation. While the observed behavior of normal cells appears to be crucial for providing the mechanical stability and therefore the integrity of the epithelial monolayer, the dysregulation of this behavior in cancer cells might be a central aspect of early stage cancer progression and metastasis in the epithelium.

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1. Introduction

At the cellular level, cancer is strongly associated with altered mechanical properties. Numerous studies have shown that cancer cells are mechanically softer than their benign or "normal" phenotype [1–6], while a few have reported the opposite behavior [7,8]. There is strong evidence that a dysregulation of the actin cytoskeleton, which primarily determines the complex mechanical behavior of living cells [9–11], accounts for the softening in cancer cells [12]. In recent years it was realized that cues of the cellular environment such as biochemical (e.g. different ECM proteins) and physical (e.g. stiffness or topography) cues strongly influence the actin cytoskeletal organization and thus, in turn, the mechanical state of the cell [13–17]. The majority of human cancers arise from the epithelial monolayer, a multicellular system that is formed by a continuous layer of cells [18]. In an epithelial monolayer the cell is embedded in a complex environment composed

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http://dx.doi.org/10.1016/j.actbio.2017.04.006
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of adjacent cells, which are tightly connected to each other via adherens junctions [19]. In normal epithelial cells the actin cytoskeleton of adjacent cells is mechanically coupled to the adherens junctions to provide mechanical stability by counterbalancing internal and external mechanical stress [20]. In cancerous epithelial cells stable cell-cell coupling is often disrupted by alterations of either the actin cytoskeleton [21,22] or the adherens junctions [23,24]. Loss of cell-cell coupling is a critical step during cancer progression and for the transition of cancer cells from an epithelial to an invasive mesenchymal phenotype (so-called epithelial-mesenchymal-transition, EMT) [25], a process proposed to cause cancer cells to proliferate continuously and to penetrate the physical barrier built up by their adjacent cells [26].

Recently, several studies have hinted that contact to adjacent cells alters the cell stiffness of normal and cancer cells differently: While normal kidney epithelial cells (MDCK) became stiffer [27], cancerous kidney epithelial (Vero) cells became softer [28] when progressing from sparse to confluent density. For lymph node metastatic cells (LNCaP), which only form intermittent cell-cell contacts, a slight increase of the cell stiffness was observed [29]. In these studies, atomic force microscopy (AFM) was used to perform single, quasi-static force-indentation curves, which were then analyzed by applying a Hertzian contact model, giving a single characteristic parameter for the cell stiffness: the Young's (or elastic) modulus. Although the Young's modulus is a suitable parameter for a quantitative comparison between different cell lines [30] including monitoring drug-induced changes [31,32], it assumes the cell as a purely elastic body. However, the actin cytoskeleton is not a static, but rather a dynamically remodeling structure crosslinked by a diversity of actin crosslinkers and motor proteins such as myosin [33,34]. Therefore, living cells have both elastic and viscous properties and hence exhibit the characteristics of a viscoelastic body [35–39].

Here, we applied force clamp force mapping (FCFM), a recently developed AFM imaging technique [36], to spatially resolve viscoelastic material properties of normal and cancerous cells at different cell culture densities to assess whether the cellular environment in an epithelial monolayer has an impact on the viscoelastic behavior of cells and whether this behavior is different for cells of different malignancy. As cell models we used the human breast epithelial cell lines MCF10A ("normal"), MCF7 ("non-invasive"), and MDA-MB-231 ("invasive"), common models for breast cancer [40], all three at sparse (no contact to adjacent cells) and confluent (full contact to adjacent cells) densities. Our data show that the viscoelastic properties of normal MCF10A and cancerous MCF7 or MDA-MB-231 cells are affected differently when comparing sparse to confluent cells: While confluent normal MCF10A cells were stiffer and had a larger fluidity than sparse MCF10A cells, non-invasive MCF7 and invasive MDA-MB-231 cancer cells did not stiffen and did not decrease their fluidity from sparse to confluent density. These findings provide new evidence for the importance of cellular mechanics and monolayer integrity for cancer progression and metastasis.

2. Material and methods

2.1. Cell culture

The non-tumorigenic human mammary epithelial cell line MCF10A was cultured in DMEM/Ham's F12 medium with stable glutamine (Biochrom, Berlin, Germany) supplemented with 5% horse-serum, 0.5 μg/ml hydrocortisone, 5 μg/mL insulin and 20 ng/mL epidermal growth factor (Sigma-Aldrich, St.Louis, MO). The mammary human breast cancer cell lines MCF7 and MDA-MB-231 were cultured in MEM (Eagle) medium with stable glutamine (Biochrom) and Leibovitz L-15 medium (Biochrom), respectively. Both mediums were supplemented with 10% fetal calf serum, 1% penicillin/streptomycin (Bichrom). MEM was additionally supplemented with 1% non-essential amino acid (Biochrom) and Leibovitz L-15 medium with 2 mM L-glutamine (Biochrom). The cell lines were maintained at 37 °C. MCF10A and MCF7 were incubated in a 5% CO_2, MDA-MB-231 in a 0% CO_2 humidified atmosphere. To compare sparse with confluent cells, cells were seeded on fibronectin-coated (5 μg/mL, Sigma-Aldrich) petri-dishes at low (0.5×10^4 cells/cm^2) or high (5×10^4 cells/cm^2) density one day before AFM measurements. Prior to the measurements, the cell culture medium was replaced with CO_2-independent Leibovitz L-15 medium, containing the same supplements as the respective culture medium for MCF10A, MCF7, and MDA-MB-231. For the EGTA experiments the cells were measured before and 1 h after incubation in L-15 medium supplemented with 2 mM of calcium-chelator EGTA (Sigma-Aldrich). Measurements were carried out at 37 °C.

2.2. Force Clamp Force Mapping (FCFM)

Force clamp force mapping (FCFM) on live cells was performed with a commercial AFM setup (MFP3D-Bio, Asylum Research, Santa Barbara, CA) mounted on an inverted microscope (Ti-S, Nikon, Tokio, Japan). Measurements were carried out using a pyramidal tip cantilever (MLCT-C, Bruker, Camarillo, CA) with a nominal spring constant of 0.02 N/m, calibrated by the thermal noise method [41]. The principle of FCFM is described in detail by Hecht

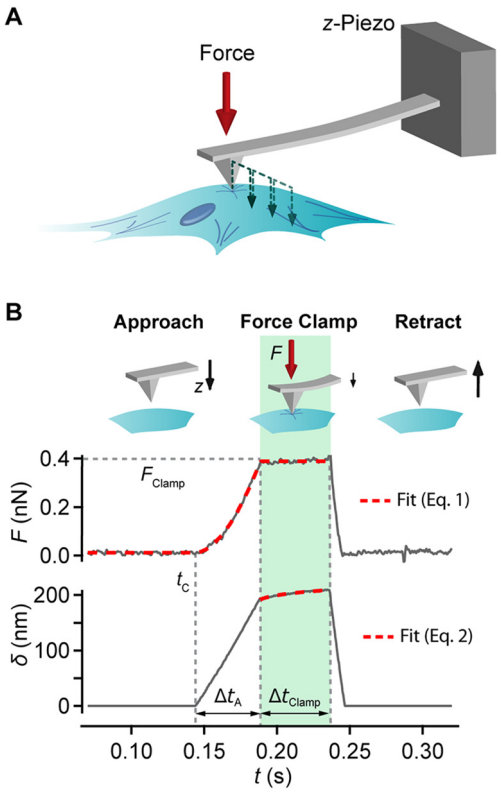

Fig. 1. Force clamp force mapping (FCFM). (A) Schematic of the FCFM experimental procedure of recording force-distance curves with an added force clamp period. (B) Time course of the force and the indentation during one force-distance curve recorded on a live cell. After the tip of the cantilever contacts the cell's surface, the force increases up to the predefined clamp-force F_{Clamp}. The force is then kept constant ("clamped") at F_{Clamp} for the dwell time Δt_{Clamp} before retracting the cantilever. The indentation δ creeped during the force clamp period owing to the viscoelastic behavior of the cell.

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