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The relative composition of actin isoforms regulates cell surface biophysical features and cellular behaviors

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ABSTRACT

Background: Cell surface mechanics is able to physically and biomechanically affect cell shape and motility, vesicle trafficking and actin dynamics. The biophysical properties of cell surface are strongly influenced by cytoskeletal elements. In mammals, tissue-specific expression of six actin isoforms is thought to confer differential biomechanical properties. However, the relative contribution of actin isoforms to cell surface properties is not well understood. Here, we sought to investigate whether and how the composition of endogenous actin isoforms directly affects the biomechanical features of cell surface and cellular behavior.

Methods: We used fibroblasts isolated from wild type (WT), heterozygous (HET) and from knockout (KO) mouse embryos where both β-actin alleles are not functional. We applied a combination of genome-wide analysis and biophysical methods such as RNA-seq and atomic force microscopy.

Results: We found that endogenous β-actin levels are essential in controlling cell surface stiffness and pull-off force, which was not compensated by the up-regulation of other actin isoforms. The variations of surface biophysical features and actin contents were associated with distinct cell behaviors in 2D and 3D WT, HET and KO cell cultures. Since β-actin in WT cells and smooth muscle α-actin up-regulated in KO cells showed different organization patterns, our data support the differential localization and organization as a mechanism to regulate the biophysical properties of cell surface by actin isoforms.

Conclusions: We propose that variations in actin isoforms composition impact on the biophysical features of cell surface and cause the changes in cell behavior.

1. Introduction

Actin cytoskeleton is the major filamentous network which functions to dynamically control cell polarity, membrane dynamics, cell movement and nuclear organization in eukaryotic cells [1,2]. In mammals, six actin isoforms are expressed in a tissue-specific manner, varying from the well-organized contractile apparatus consisting of skeletal muscle α-actin to the highly versatile actin cytoskeleton containing β-actin and γ-actin in non-muscle cells [3]. Functional studies of actin isoforms using knockout mice demonstrate distinct phenotypes, implying tissue-specific roles of each isoform in development [4]. Rescue experiments further reveal the functional convergence or divergence of specific isoforms. For example, transgenic expression of cardiac α-actin can fully rescue the lethality and muscle defects of skeletal α-actin knockout mice [5]. However, the expression of cytoplasmic γ-actin fails to rescue the lethality of skeletal α-actin knockout, even though the forced expression of γ-actin in wild-type can substitute 40% of skeletal α-actin in muscle thin filament [6]. Interestingly, in all knockout mouse models, there is a compensatory up-regulation of a subset of other actin isoforms [7], suggesting preferential functional interactions of certain isoforms.

Different tissues have different biomechanical properties corresponding to their physiological functions. There is evidence that actin cytoskeleton dynamics and alignment are linked to tissue-specific mechanical properties [8]. For example, myoblasts (C2C12 cells), which usually take an elongated shape, are more sensitive to growth geometry change than endothelial cells (HUVECs) or fibroblasts (NIH 3T3 cells) [9]. This is associated with specialized α-actin arrangement in elongated muscle cells. It is therefore likely that actin isoforms with specific subsets of actin-binding proteins contribute differentially to the

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mechanical properties of the cell [4], by regulating the biophysical properties of specialized organelles such as membranes. This in turn is likely to impact on cellular behavior.

Biophysical properties of cell surface can influence a variety of cellular processes and behaviors, because cell surface mechanics is related to the force or tension during membrane deformation in cell migration and tissue morphogenesis [10–12]. Cell surface features such as actin cortex mechanics control animal cell shape [13]. Additionally, mechanical stimuli from cell surface can impact on a variety of cellular processes inducing cell shape change, migration and differentiation [14]. Change in cell surface tension such as exposure to osmotic stress also induces actin cortex reorganization [15]. It is known that cell membrane tension and bending stiffness is strongly influenced by cytoskeletal elements [16–20]. However, the relative contribution of individual actin isoforms to cell surface mechanics has not been investigated.

In this study, we sought to investigate whether and how the composition of endogenous actin isoforms directly affects the biomechanical features of cell surface and cellular behaviors. For this, we used fibroblasts isolated from wild type (WT) mouse embryos (β-actin$^{+/+}$) as well as from heterozygous (HET) mouse embryos with only one functional β-actin allele (β-actin$^{+/-}$) and from knockout (KO) mouse embryos where both β-actin alleles are not functional (β-actin$^{-/-}$) [21]. Transcriptome analysis of the three cell types showed that WT, HET and KO cells expressing different amounts of β-actin mRNA exhibit varying levels of other actin isoforms such as smooth muscle α-actin and cytoplasmic γ-actin mRNAs. Endogenous β-actin in WT cells and the smooth muscle α-actin up-regulated in KO cells show different organization patterns. Interestingly, in HET and KO cells loss of β-actin leads to a decrease in cell surface stiffness and an increase of surface pull-off force in comparison to WT cells, indicating an essential role of β-actin in controlling cell surface biophysical properties. This is further supported by the observation that expression of exogenous β-actin in KO cells increases cell surface stiffness. The changes of biophysical properties of cell surface were associated with distinct cellular behaviors in 2D and 3D cultures among WT, HET and KO cells. Taken together, we show that variations in the composition of actin isoforms impact on cell surface mechanics, leading to changes of cellular behaviors.

2. Materials and methods

2.1. Antibodies and reagents

Anti-mouse IgG Alexa Fluor 647 (ab150115), Anti HA antibody (ab9110), Phalloidin-iFluor555 (ab176756), Y-27632 dihydrochloride (ab120129) were from Abcam. Antibody against β-actin (clone AC-74) was from Sigma-Aldrich. Antibodies of smooth muscle α-actin (α-SMA) (MA5-11547), rabbit IgG Dylight 550 (84541), mouse IgG Dylight 550 (84540), Hoechst 43222 (H1399), Wheat Germ Agglutinin (WGA) Alexa Fluor 647 conjugate (W32466) were purchased from Thermal Fisher Scientific. Cultrex 3D culture matrix rat collagen I (3447-020-01) is from R&D systems. Propidium iodide solution (J66584) was purchased from Alfa Aesar.

2.2. Cell culture

The β-actin$^{+/+}$ MEFs (WT), β-actin$^{+/-}$ MEFs (HET) and β-actin$^{-/-}$ MEFs (KO), and mouse endothelial cell line C166 (ATCC) were maintained and cultured with Dulbecco's modified Eagle medium (DMEM) with high glucose (Sigma), 10% fetal bovine serum (Sigma) and 100 units/mL penicillin and 100 μg/mL streptomycin (Sigma), in a humidified incubator with 5% CO_2 at 37 °C. For AFM measurement, fibroblast was seeded on glass slide and cultured in CO_2-independent L-15 Medium (Leibovitz, Sigma) supplemented with 1× GlutamMAX, 10% fetal bovine serum (Sigma) and 100 units/mL penicillin and 100 μg/mL streptomycin (Sigma).

2.3. Atomic force microscopy (AFM)-based force measurements

Fibroblasts were cultured on fibronectin-coated microscope glass slides for overnight in L-15 Leibovitz medium. All AFM measurements, lasting no > 3 h per experiment, were performed with 5500 AFM from Keysight Technologies using ~6 μm-diameter colloidal silicon spherical tips, which are attached to cantilevers with 0.08 N/m nominal spring constants. The tips were uncoated while the detector sides of the cantilevers were coated with ~70 nm gold (CP-PNPL-SiO-C-5 from NanoAndMore, Germany). Custom gold-coated silicon chips covered with hydrophobic Teflon membranes were used as “liquid cells”. The cantilever spring constants were determined from the power spectral density of the thermal noise fluctuations [22] before each experiment. Tip approach and retract velocity was set at ~5 μm/s. Prior to any measurements, cells of interest were carefully located using a video camera attached to the system (Fig. S2A, upper inset). Once cells have been located, force measurements were performed in the force-volume (FV) mode, in which the approach-retraction process (Fig. S2A) is repeated over cells, at a resolution of 8 × 8 pixels per FV image. For each set of experiment, the AFM probe was aligned at the center of a cell and subsequently 64 pairs of force versus displacement curves were acquired from a 10 × 10 μm^2 cell surface area in the FV mode. The force curves on all the pixels in the FV image were then analyzed with a home-made software [23] to obtain the distribution of the elasticity and maximum pull-off forces, as explained in detail below. Force profiles with unclear approach and/or retraction curves were excluded from the analysis. The number of investigated cells were 19, 17, 17, 20, and 18 cells for WT, KO, HET, KG, and KA, respectively. Every culture sample was measured using a new tip.

Quantitative information on cell elasticity was obtained by modeling the measured loading force versus surface indentation data using the classic Hertz model of contact mechanics [24]. According to the model, the loading force (F_L) applied by a non-deformable sphere indenter (the AFM tip) required to indent a distance (δ) into an infinitely deformable elastic half space (the cell surface) is given by:

$$F_L = \frac{4}{3}\frac{E}{(1-\nu^2)}R^{1/2}\delta^{3/2} \quad (1)$$

where E is the Young's modulus (i.e. elasticity modulus) of the cell, R is the radius of the spherical indenter, and ν is the Poisson ratio of the cell, which was set to 0.5 assuming cell's incompressibility. All force measurements were performed under a trigger force of about 1.5 nN, which reflected a cell surface indentation of approximately 2 μm. Fit range was chosen to be 400 nm (Fig. S2A, lower inset). The adhesion forces, comprising all interaction forces (specific and nonspecific) between the tip and cell surface, are defined as pull-off forces [25]. Quantitative analysis on maximum pull-off force was performed by first identifying local minima events on the retraction curves. In addition, force curves that possessed pull-off force values below 10 pN cut-off force were discarded from the analysis due to experimental noise during measurements. The data sets taken on WT cells in 64 × 64 pixel-resolution FV mode served as control of how the maximum pull-off forces are distributed over the cells interacting with AFM tip (Fig. S2C). Origin software (OriginLab, Northampton, MA) was used to evaluate the arithmetic mean and the standard deviations of the histograms by fitting the data to the LogNormal or Gauss probability density functions. This way, the average elasticity/maximum pull-off force based on the total number of indentation locations and adhesion/tether events investigated for cells of each condition were identified, which are expected to yield accurate estimate [26]. Unless otherwise specified, all the quantitative analysis related to elasticity and adhesiveness properties was conducted from the same set of force data collected on the cells.