Biochemical and Biophysical Research Communications 415 (2011) 591–596

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Effects of micropatterned curvature on the motility and mechanical properties of airway smooth muscle cells

Jimin Xu [a], Cheng Chen [b], Xuemei Jiang [a], Rong Xu [a], Dhananjay Tambe [c], Xiaojuan Zhang [a], Lina Liu [d], Bo Lan [a], Kaiyong Cai [a], Linhong Deng [a,*]

[a] Key Laboratory of Biorheological Science and Technology, Ministry of Education, and Bioengineering College, Chongqing University, 174 Shapingzhengjie Street, Chongqing 400044, China
[b] Center for Joint Surgery, Southwest Hospital, The Third Military Medical University, Chongqing 400038, China
[c] Program in Molecular and Integrative Physiological Science, Department of Environmental Health, Harvard School of Public Health, Boston, MA 02115, United States
[d] Department of Mechanical Engineering, Chongqing Chemical Industry Vocational College, Chongqing 400020, China

ARTICLE INFO

Article history:
Received 30 September 2011
Available online 2 November 2011

Keywords:
Micropatterned curvature
Migration
Stiffness
Cytoskeleton
Airway smooth muscle cell

ABSTRACT

Geometric features such as size and shape of the microenvironment are known to alter cell behaviors such as growth, differentiation, apoptosis, and migration. Little is known, however, about the effect of curvature on cell behaviors despite that many cells reside in curved space of tubular organs such as the bronchial airways. To address this question, we fabricated micropatterned strips that mimic airway walls with varying curvature. Then, we cultured airway smooth muscle cells (ASMCs) on these strips and investigated the cells' motility and mechanical properties using time-lapse imaging microscopy and optical magnetic twisting cytometry (OMTC). We found that both motility and mechanical properties of the ASMCs were influenced by the curvature. In particular, when the curvature increased from 0 to 1/150 μm^{-1}, the velocity of cell migration first decreased (0–1/750 μm^{-1}), and then increased (1/750–1/150 μm^{-1}). In contrast, the cell stiffness increased and then decreased. Thus, at the intermediate curvature (1/750 μm^{-1}) the ASMCs were the least motile, but most stiff. The contractility instead decreased consistently as the curvature increased. The level of F-actin, and vinculin expression within the ASMCs appeared to correlate with the contractility and motility, respectively, in relation to the curvature. These results may provide valuable insights to understanding the heterogeneity of airway constrictions in asthma as well as the developing and functioning of other tubular organs and tissue engineering.

1. Introduction

Cells respond to physical cues of the microenvironment such as extracellular matrix (ECM) stiffness and geometry by altering a wide range of behaviors such as growth, differentiation, apoptosis and migration. Microfabrication technology has made it possible to study cells cultured on well defined micropatterns with controlled geometric features. This leads to extensive reports in recent years that demonstrate the shape and size of cell-adherent micropatterns can be determinants of cell migration, cell polarity, cell fate and even differentiation of mesenchymal stem cells [1–3].

One less-studied geometric feature, however, is the curvature of the microenvironment, which is not trivial because many types of cells reside in curved space of tubular organs, such as the blood vessels and bronchial airways. Take the latter as an example, along the bronchial airway tree, the caliber of the airway changes from one generation to the next, ranging from 3480 to 200 μm [4], corresponding to the curvature changing from 1/1740 to 1/100 μm^{-1}. Such great variation of the airway wall curvature apparently raises the question on whether it influences the behaviors of those cells living and acting within the airway wall, such as the airway smooth muscle cells (ASMCs).

In addition, ASMCs are thought to be the end effectors of asthma-associated excessive airway narrowing [5,6]. And the excessive narrowing may be attributed to either increased amount of ASMCs due to motility-regulate ASMCs migration, or enhanced contractility that is closely associated with the mechanical properties such as stiffness of the ASMCs [7–9]. On the other hand, the narrowing along the bronchial tree in asthma appears to be highly heterogeneous [10,11], which raises the possibility that the size/curvature of the airways may influence the motility and mechanical properties of the ASMCs.

To address these questions, micropatterned cell-adherent substrate strips were fabricated to mimic sections of airways with varying radii, or equivalently, curvatures. And ASMCs were cultured on these strips and then evaluated in terms of motility and mechanical properties [12,13]. The results showed that both

* Corresponding author. Fax: +86 2365112877.
E-mail address: denglh@cqu.edu.cn (L. Deng).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.10.111

motility and mechanical properties of the ASMCs were indeed influenced by the curvature, suggesting different physical behaviors for the ASMCs in different sized airways. This finding may not only be useful to better understand the role of airway size in determining the heterogeneity of airway constriction dynamics, and ultimately help in therapy to target the right airway segments, but also may benefit studies in other fields such as vascular diseases and tissue engineering.

2. Materials and methods

2.1. Materials

Soft printing stamp was made of poly-dimethylsiloxane (PDMS, Sylgard 184, Dow Corning, Midland, Michigan). Tissue culture reagents and collagen Type I were purchased from Sigma–Aldrich (St. Louis, MO). The synthetic arginine-glycine-aspartic acid (RGD) containing peptide was purchased from American Peptide Company (Sunnyvale, CA). The ferrimagnetic beads (dia. 4.5-μm) were kindly provided by Dr. J.J. Fredberg of Harvard School of Public Health (Cambridge, MA). Unless otherwise noted, all other reagents were obtained from Fisher Scientific (Newark, DE).

2.2. Fabrication of micropatterned strips with varying curvatures

In order to mimic bronchial airways from generation 1 to generation 28 [4], an array of substrate strips was fabricated with equal width of 20 μm but varying curvatures from 0 (being straight) to 1/50 μm^{-1} (being semicircular) using well established soft contact printing method [14]. A square ($1000 \times 1000\ \mu m^2$), and a circular (rad. 500 μm) pattern were also fabricated as controls adjacent to the array of strips.

The micropattern was coated with type I collagen (10 μg/mL, Sigma–Aldrich) on the inside bottom of a 35 mm Petri dish for cell culture (Greiner, German), and the rest of the Petrie dish area was incubated with 0.1% F127 Pluronic (Sigma–Aldrich) solution overnight in water at 4 °C so that the cells were prevented from adhering outside the micropattern.

2.3. Cell culture on the micropattern

ASMCs were freshly dissociated and harvested from trachea muscles of Sprague Dawley (SD) rats [15]. The grown cells were verified as ASMCs by staining with antibodies against α-smooth muscle actin and calponin [16]. The cells were seeded into the Petri dish and allowed to adhere to the micropatterned strips for 2 h, and those non-adherent cells were then washed away. Subsequently, the cells were kept on the micropattern for several days in the same medium but with only 2.2% fetal bovine serum so that the cells were adapted to the curved microenvironment [17]. Before experiment, the cells were serum deprived but supplemented with 5.7 μg/mL insulin and 5 μg/mL transferrin for 24 h in order to maintain the ASMCs in contractile phenotype.

2.4. Microscopy of ASMCs cultured on the micropattern

ASMCs cultured on the micropattern were observed for cell morphology and growth by phase contrast optical microscopy (Leica DMI6000, Germany). The immunofluorescence-stained cells grown on the micropattern were examined under the laser scanning confocal microscope (Leica TCS SP5 II, Germany). The mean optical density of a single cell's F-actin or vinculin fluorescence labeling was measured using Image-Pro plus software. To standardize the fluorescence intensity measurements among experiments, the time of image capturing, image intensity gain, image enhancement, and image black level in both channels were optimally adjusted at the outset and kept constant for all experiments [18].

2.5. Evaluation of cell motility

From each image of a time-lapse imaging sequence, the contour and the geometric center (in X, Y coordinates) of each cell on the micropattern was extracted quantitatively using Image J software (National Institutes of Health, Bethesda, MD, USA). And the velocity of the cell migration, V, was calculated as follows:

$$V = \frac{\sum \sqrt{(X_i - X_{i-1})^2 + (Y_i - Y_{i-1})^2}}{T} \tag{1}$$

where X_i, Y_i are the coordinates of the cell center at the i th time point, and T is the total time of observation.

The mean square displacement (MSD), $\langle d^2 \rangle$, was determined by the following equation:

$$\langle d^2(t) \rangle = \mathrm{MSD}(n\Delta t) = \frac{1}{N-n} \sum_{i=1}^{N-n} \left[(X_{i+n} - X_i)^2 + (Y_{i+n} - Y_i)^2 \right] \tag{2}$$

where Δt corresponds to the time interval between two frames, n and N denote the number of time steps and the frames, respectively.

The increase of the MSD can be characterized by the logarithmic derivative:

$$\beta(t) = \frac{d \ln \langle d^2(t) \rangle}{d \ln(t)} \tag{3}$$

leading to a time-dependent increase $\langle d^2(t) \rangle = \mathrm{MSD}(t) \approx t^{\beta(t)}$ [19].

2.6. Measuring cell mechanical properties

Mechanical properties of the ASMCs were assessed by optical magnetic twisting cytometry (OMTC), of which the details have been well described in previous publications [12,20]. Only cells located on the middle part of the micropatterned strips were selected in which case the bead twisting direction and the principal direction of the cell were parallel, and thus minimizing the error of the measurement [8].

3. Results

3.1. Effect of the curvature on cell shape and cytoskeleton organization

The images of the ASMCs were taken every 5 min up to 300 min after they were deposited onto the micropattern as shown in Fig. 1A. Compared to those grown on the square/circular micropattern, the ASMCs grown on the micropatterned strips generally oriented well along the curved geometry and seemed to be fatter and less stretched on the strips with greater curvature, while being most stretched on the strips with intermediate curvature. The F-actin filaments of the ASMCs also appeared to be bundled and oriented along the strips and the expression of vinculin exhibited a dependence on the curvature of the strips, indicating remodeling of the cytoskeleton and the focal adhesions as the curvature changed (Fig. 1B).

To evaluate the effect of curvature on the remodeling of the cytoskeleton and focal adhesions, the fluorescence intensity of F-actin and vinculin were analyzed of the ASMCs grown on three strips with curvatures of 0, 1/750, 1/150 μm^{-1}, representing low, intermediate, and high curvatures, respectively. The results showed that the F-actin expression decreased as the curvature increased, and in contrast, the vinculin expression was the greatest