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Cell shape dependent regulation of nuclear morphology

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ABSTRACT

Recent studies suggest that actin filaments are essential in how a cell controls its nuclear shape. However, little is known about the relative importance of membrane tension in determining nuclear morphology. In this study, we used adhesive micropatterned substrates to alter the cellular geometry (aspect ratio, size, and shape) that allowed direct membrane tension or without membrane lateral contact with the nucleus and investigate nuclear shape remodeling and orientation on a series of rectangular shapes. Here we showed that at low cell aspect ratios the orientation of the nucleus was regulated by actin filaments while cells with high aspect ratios can maintain nuclear shape and orientation even when actin polymerization was blocked. A model adenocarcinoma cell showed similar behavior in the regulation of nuclear shape in response to changes in cell shape but actin filaments were essential in maintaining cell shape. Our results highlight the two distinct mechanisms to regulate nuclear shape through cell shape control and the difference between fibroblasts and a model cancerous cell in cell adhesion and cell shape control.

1. Introduction

Normal physiological aging [1,2] and pathological situations [3,4] alter the size and shape of the nucleus. The geometry of the nucleus is known to have a significant influence on cell proliferation [5], gene expression [6], and protein synthesis [7] but the mechanisms through which nuclear morphology alters cell function remains unclear. The nucleus is enclosed by the nuclear envelope that isolates the chromosomes from the cytoplasm and typically has either an oval or round shape. The nuclear envelope is a double membrane with the outer membrane connected externally to the endoplasmic reticulum (ER), and to the inner nuclear membrane at each nuclear pore complex. Under the inner membrane lies a meshwork of nuclear lamina proteins composed of intermediate filament lamins that provide mechanical support and anchor the nuclear pore complexes [8,9]. The nuclear size is not directly related to the DNA content as the nuclei of cells in different tissue differ in size, but have identical DNA content. The nuclear size is influenced by transport between the nucleus and cytoplasm through nuclear pores [10], other cellular structures such as ER [11], lamin [12,13], nuclear envelop associated proteins [14], and lipid biosynthesis [15,16]. In different species, the nuclear size is found to be proportional to the overall size of the cell [17,18]. Previous studies of nuclear morphology have focused on the structural role of the nuclear lamina and nuclear envelope [2,19]. It is known for example, that alterations in lamina proteins in mutated cells [20,21] and cancer cells [22] are associated with abnormally shaped nuclei. However, the role of physical factors transduced through the cell membrane and cytoplasm in altering the shape of the nucleus have been the subject of only more recent investigations [6].

Recent studies have shown that perinuclear actin networks, consisting of actin filament bundles called transmembrane actin-associated nuclear lines [23] or actin cap [24], together with their regulatory proteins [25] exert significant control on nuclear shape. Molecules referred to as linkers of nucleus to cytoskeleton (LINC) anchor actin cap fibers directly to the nucleus [26–29]. Micromanipulation techniques have demonstrated further that compressive forces, driven by cytoskeletal tension in endothelial cells on elongated adhesive islands, alter the morphology of the nucleus [5] and offer insights to the spatial coordination between cell and nuclear shape. However, questions remain on the role of membrane tension. For example, in previous investigation confining cells to rectangular shapes, the membranes are locally physical compressing the nucleus as well as the global compressive forces driven by actomyosin tension, it is unknown the individual effect of each.

Changes in the nuclear size and shape have been associated with cancer and enlarged nuclei typically represent more metastatic [22,30–32]. However, it remains unknown if the coordination

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between cell and nuclear shape is different in cancer cells. In this study, we investigate nuclear shape remodeling on a series of rectangular shapes that allow direct membrane tension or without membrane lateral contact with the nucleus. The roles of cytoskeleton and tension in these patterned cells on nuclear shapes were investigated by various pharmacological agents while maintaining the cell shape independently by culturing cells on single adhesive islands of different rectangular shapes. Finally, the nuclear shape remodeling was investigated in a model adenocarcinoma cell to investigate the regulation of nuclear shape in response to changes in cell shape in cancer cells.

2. Materials and methods

2.1. Materials

Tissue culture dishes were purchased from Fisher Scientific (catalog no. 430166) and used as received. Polydimethylsiloxane (PDMS; Sylgard 184) was obtained from Dow Corning (Midland, MI) and used at 10:1 (w: w) base: curing agent. Cytochalasin D and nocodazole were obtained from Sigma Co. Y27632 was purchased from Calbiochem. Alexa 488 or 594-phalloidin, 4′, 6-diamidino-2-phenylindole (DAPI), and Alexa Fluor® 488 goat anti-mouse IgG1 (γ1) (secondary antibody) were purchased from Molecular Probes (Eugene, OR). Phosphate buffered saline (PBS), Iscove's modified Dulbecco's medium (IMDM) or Dulbecco's Modified Eagle Medium (DMEM), and Fetal bovine serum (FBS) were purchased from Invitrogen (Carlsbad, CA).

2.2. Preparation of poly (OEGMA-co-MA)

Random copolymers of Oligo (ethylene glycol) methacrylate (OEGMA) and methacrylic acid (MA) (Scientific Polymer Products, NY) were prepared by free radical polymerization of 10 wt % methanolic solutions of the two monomers (80:20 OEGMA to MA mass ratio) at 60 °C using an electric-heated thermostatic water bath. Polymerizations were initiated with 1 wt % (with respect to monomer) 2,2′-azobis (2-amidinopropane) dihydrochloride (Wako, VA) and allowed to react for 24 h.

2.3. Preparation of patterned tissue culture dishes

Micropatterns consisting of two different areas (900 and 2500 μm^2) of various geometric forms (square and rectangle) were fabricated on silicon wafers using standard photolithographic techniques. From this silicon master, complementary PDMS replicas were prepared following the soft lithography procedure developed by Whitesides and co-workers [33] and used as stamps in subsequent microcontact printing steps to form background of poly (OEGMA-co-MA) copolymer directly on cell culture dishes. Patterned dishes were sterilized under UV for at least 1 h before plating cells.

2.4. Cell culture

NIH 3T3 fibroblasts and A549 cells (Human lung carcinoma-derived alveolar epithelial cell line) were purchased from ATCC and cultured in IMDM and DMEM respectively, supplemented with 10% FBS, penicillin (100 U/ml), streptomycin (100 μg/ml) at 37 °C in a humidified atmosphere containing 5% CO_2. Sub-confluent monolayers were dissociated with 0.25% trypsin-EDTA solution, re-suspended in IMDM or DMEM with 10% FBS, and then plated on micropatterned culture dishes.

2.5. Treatment with pharmacological agents

Cells plated on the patterned culture dish were allowed to attach and spread to the adhesive island for 15–18 h prior to incubation with various pharmacological agents. Cells were incubated with 1.0 μM cytochalasin D for 35 min, 20 μM Y27632 for 30 min and 5.0 μM nocodazole for 1 h at 37 °C prior to being fixed and stained.

2.6. Immunofluorescence staining

Cells were plated at a density of approximately 8000-cells/cm^2 and allowed to reach confluence within the cell-adhesive patterns on the tissue culture dishes. After drug treatment for some cases, cells were fixed with 3.7% paraformaldehyde for 15 min, washed in phosphate buffered saline, permeabilized with 0.2% Triton X-100 for 5 min and then blocked with 3% BSA (bovine serum albumin) for 30 min. Samples were then rinsed with PBS and incubated with Alexa 594-phalloidin and DAPI to stain for F-actin and nuclei respectively. Phase-contrast and fluorescence micrographs were collected using a CCD camera (SPOT CAM, Diagnostic Instruments Inc.) mounted on a Nikon TE-2000 inverted microscope with 40 × objective lens. Confocal images were obtained from Zeiss LSM 710 NLO Confocal Microscope under 63 × or 40 × plan apochromat water objective lens. Images were processed with Metamorph software (Ver 6.0r4, Universal Imaging, Westchester, PA) or Zeiss Meta software version 3.5. Multiple Z-section images (0.2 μm thick sections) were analyzed and processed using a combination of Zeiss LSM Image Browser, Metamporph, and ImageJ (http://rsbweb.nih.gov/ij/). The nuclear height on the micropatterns for different aspect ratios were measured by staining the nuclei with DAPI and imaged confocally. Only a single cell confined within each pattern was considered for analysis.

2.7. Assessment of cell and nuclear morphology

Cells were fixed and stained with DAPI and phalloidin to show nucleus and F-actin. Cells with spreading area within ±15% of the adhesive island area were included in the quantification of cell and nuclear morphology. Projected cell and nuclei length and width was calculated by interactive tracing the maximum intensity projection image of a cell [F-actin or nuclei (DAPI)], thresholding, and applying the Integrated Morphometry Analysis (IMA) routine. The length was calculated by IMA as the span of the longest chord through the object; the width is the dimension perpendicular to the length. Quantification of cell nuclear elongation and determination of orientation were performed on an image analysis routine in Metamorph or by using NIH ImageJ software.

2.8. Statistical analysis

All the data are shown as the mean ± the standard deviation (SD). Pair-wise comparisons between means of two different groups were performed using a Student t-test. The differences between two groups of data are considered statistically significant or statistically highly significant if the Student t-test gives a significance level P (P value) smaller than 0.05 or 0.001, respectively. Multiple comparisons were performed using analysis of variance (ANOVA), followed by Tukey's post test to determine differences.

3. Results and discussion

3.1. Changes in cell morphology influence nuclear shape

We cultured cells on adhesive islands of equal areas (900 μm^2 and 2500 μm^2) with different aspect ratios to examine if and how