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The regulation of dynamic mechanical coupling between actin cytoskeleton and nucleus by matrix geometry

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ABSTRACT

Cells sense their physical microenvironment and transduce these signals through actin-nuclear links to regulate nuclear functions including gene expression. However, the spatio-temporal coupling between perinuclear actin and nucleus and their functional importance are still unclear. Using micropatterned substrates to control cell geometry, we show that perinuclear actin organization at the apical plane remodels from mesh-like structure to stress fibers. The formation of these apical stress fibers (ASFs) correlated with significant reduction in nuclear height and was found to exert an active compressive load on the nucleus via direct contact with mature focal adhesion sites. Interestingly, the dynamic nature of ASFs was found to transduce forces to chromatin assembly. In addition, geometric perturbations or using pharmacological drugs to inhibit actomyosin contractility of ASFs resulted in nuclear instability. Taken together, our work provides direct evidence of physical links between the nucleus and focal adhesion sites via ASFs, which modulate nuclear homeostatic balance and internal chromatin structure. We suggest that such direct links may underlie nuclear mechanotransduction to regulate genomic programs.

1. Introduction

Cells can transmit extracellular physical cues such as force, geometry and stiffness from substrate to the nucleus to regulate gene expression [1–7]. Such transduction involves both biochemical as well as physical signaling through the intricate cytoskeletal framework [8,9]. Studies have revealed that this intricate cytoskeleton surrounds the nucleus and maintains it in a prestressed state [10–12]. The prestressed nuclear morphology is tightly controlled by cellular geometry impinging on gene expression [13–15]. Previous experiments have demonstrated that the nucleus responds to force exerted at the cell membrane suggesting possible physical links between the cytoskeleton and the nucleus [16]. In support of such physical links, ablation of LINC proteins using RNAi altered nuclear morphology and function [17,18]. The maintenance of nuclear morphology and positioning through physical coupling to the cytoskeleton has also been found to be important in differentiation [19,20], aging [4,21], mechanical homeostasis [22] and is correlated with diseases [20,23,24].

An aligned actin filament structure has been observed at the apical plane of MCF-10A cells, which is altered in cancer cells (MCF-7) [25]. These apical actin filaments have been shown to form a “perinuclear actin cap” shaping the cell nucleus in mouse embryonic fibroblasts [26,27]. Importantly, similar dorsal actin filaments were also found to be part of transmembrane actin-associated nuclear (TAN) lines and drive nuclear movement [28]. Defects in anchorage of TAN lines to the nucleus, due to lamin A variants, have been shown to affect nuclear movement [24]. Though several studies suggest the coupling between actin cytoskeleton and the cell nucleus, the spatio-temporal organization of these links, its role in nuclear homeostatic balance and transduction of active stress to modulate internal chromatin structure has not been demonstrated.

In this study, we hypothesize that perinuclear actin may exert a direct compressive load on the nucleus and as a result indenting as well as stabilizing nuclear morphology. In addition, these dynamic indents could transmit force to chromatin assembly. To test this hypothesis, high resolution imaging was carried out on cells adhered to micropatterned substrates of various geometries. Multicolor confocal sections of labeled actin and nucleus were

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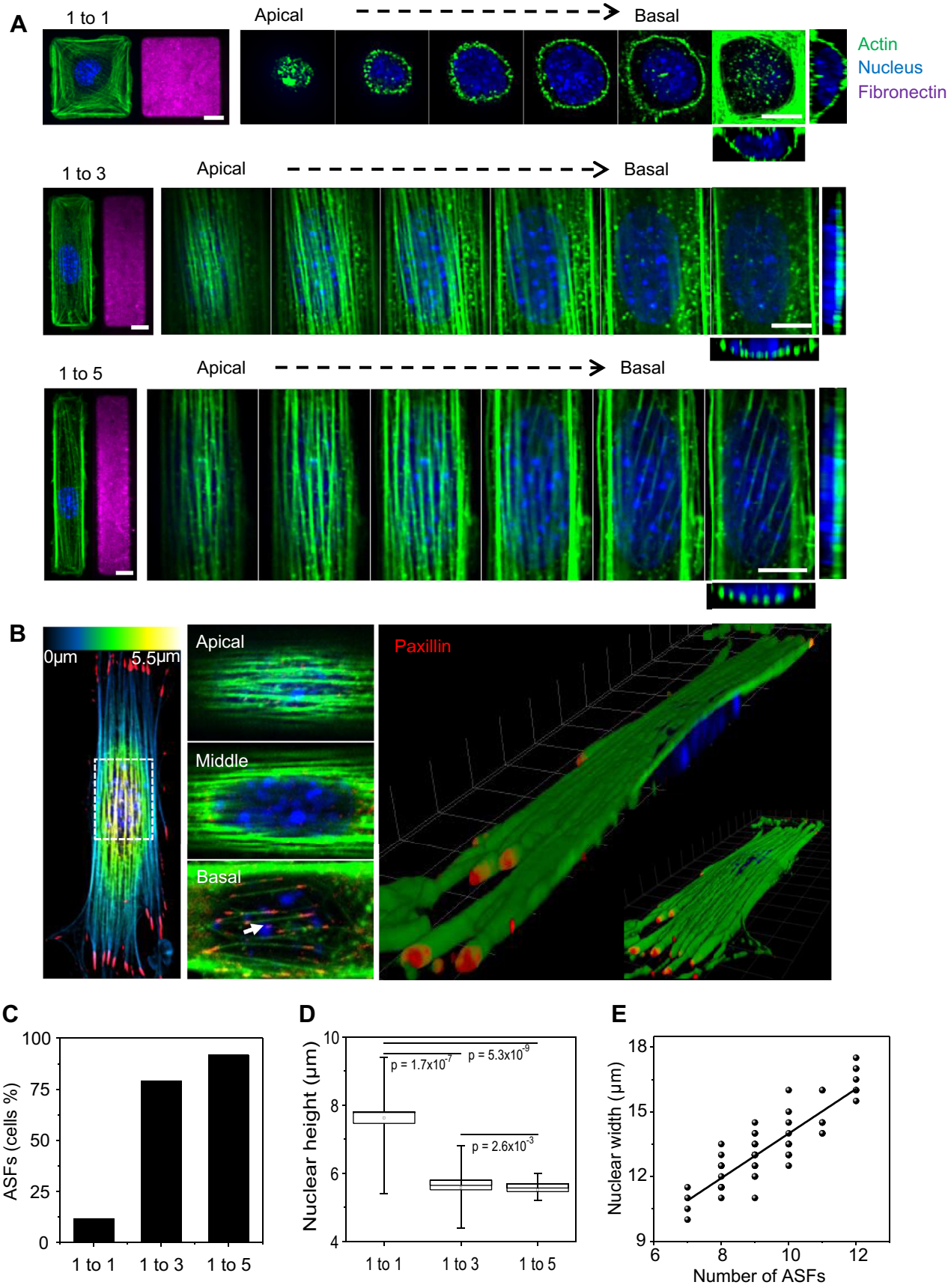

Fig. 1. Cell geometry regulates perinuclear actin organization and nuclear morphology. (A) Representative image of single cells grown on rectangular patterns of different aspect ratio – 1:1, 1:3 and 1:5 (area = ~1600 μm^2, coated with fibronectin, shown in purple color) and actin structure at different planes from the apical to basal plane along with its