



Estimation of the mechanical connection between apical stress fibers and the nucleus in vascular smooth muscle cells cultured on a substrate



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ABSTRACT

Actin stress fibers (SFs) generate intercellular tension and play important roles in cellular mechanotransduction processes and the regulation of various cellular functions. We recently found, in vascular smooth muscle cells (SMCs) cultured on a substrate, that the apical SFs running across the top surface of the nucleus have a mechanical connection with the cell nucleus and that their internal tension is transmitted directly to the nucleus. However, the effects of the connecting conditions and binding forces between SFs and the nucleus on force transmission processes are unclear at this stage. Here, we estimated the mechanical connection between apical SFs and the nucleus in SMCs, taking into account differences in the contractility of individual SFs, using experimental and numerical approaches. First, we classified apical SFs in SMCs according to their morphological characteristics: one subset appeared pressed onto the apical surface of the nucleus (pressed SFs), and the other appeared to be smoothly attached to the nuclear surface (attached SFs). We then dissected these SFs by laser irradiation to release the pretension, observed the dynamic behavior of the dissected SFs and the nucleus, and estimated the pretension of the SFs and the connection strength between the SFs and the nucleus by using a simple viscoelastic model. We found that pressed SFs generated greater contractile force and were more firmly connected to the nuclear surface than were attached SFs. We also observed line-like concentration of the nuclear membrane protein nesprin 1 and perinuclear DNA that was significantly located along the pressed SFs. These results indicate that the internal tension of pressed SFs is transmitted to the nucleus more efficiently than that of attached SFs, and that pressed SFs have significant roles in the regulation of the nuclear morphology and rearrangement of intranuclear DNA.

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1. Introduction

Actin stress fibers (SFs) are contractile bundles of the F-actin cytoskeleton that are held together by α -actinin and contain non-muscle myosin and tropomyosin (Pellegrin and Mellor, 2007). Cells change their shape and function by assembling these SFs and exerting the contractile forces of SFs on extracellular matrices. This intracellular force transmission is critical for various biological events, including cell migration (Renkawitz and Sixt, 2010), proliferation (Chen et al., 1997), and differentiation (Chen et al., 2007). It has recently been suggested that the nucleus is connected to the F-actin cytoskeleton by a protein complex consisting of Sad1p, UNC-84 (SUN)/Klarsicht, ANC-1, Syne homology (KASH) domain

proteins, referred to as the linker of nucleus and cytoskeleton (LINC) complex (Crisp et al., 2006). This connection has been reported to play important roles in nuclear positioning during cell migration (Luxton et al., 2010; Lombardi et al., 2011) and in the mechanosensing of adherent cells (Kim et al., 2012), which may be deeply involved in intracellular force transmission from the F-actin cytoskeleton to the nucleus. From these viewpoints, we recently investigated the mechanical interaction between SFs and the nucleus in vascular smooth muscle cells (SMCs) cultured on substrates by using a laser-based nano-dissection technique (Nagayama et al., 2011, 2013): we dissected apical SFs running across the top surface of the nucleus at a point slightly outside the nucleus by laser irradiation to release fiber pretension. The fibers shortened following the dissection, and the nuclei significantly moved in the direction of shortening of the dissected fibers as if they were pulled by the contractile force of the SFs. These results indicated that apical SFs over the nucleus are connected to the nuclear surface. However, apical SFs exhibit significant

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morphological variation: some appear pressed onto the apical surface of the nucleus, and others appear smoothly attached to nuclear surface; the shortening of these fibers and the accompanying nuclear movements were also quite varied (Nagayama et al., 2013). The molecular components of SFs and their contractility vary with their intracellular location (Tanner et al., 2010; Kim et al., 2012); such contractile variation may be present even among apical SFs within the same cell, and may affect contact conditions between SFs and the nucleus. Furthermore, the extent of the

binding forces that may influence force transmission efficiency between SFs and the nucleus is also unclear at this stage.

In order to clarify these issues, we observed the dynamic shortening of apical SFs running across the top surface of the nucleus, and the movement of the nucleus, following laser dissection of SFs in SMCs on a substrate. Furthermore, we investigated differences in mechanical connections between SFs and the nucleus taking into account differences in SF contractility. We quantitatively estimated the pretension of SFs and the mechanical

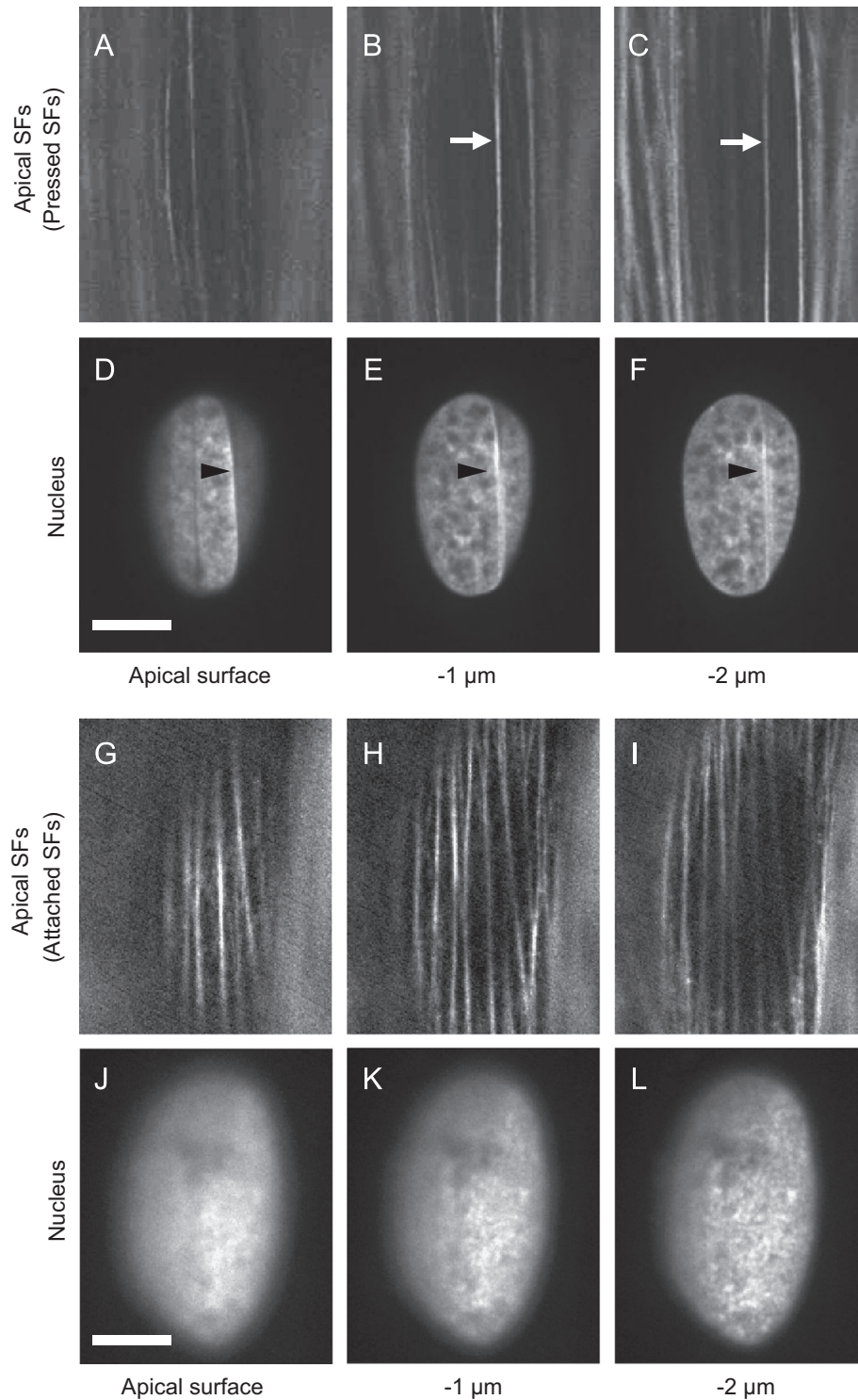


Fig. 1. Typical examples of fluorescence images of apical SFs and the nucleus in living SMCs. To visualize the cell nucleus, nuclear DNA was stained with Hoechst 33342 (Molecular Probes). The cells were classified into two types: one had line-like concentrations of intranuclear DNA along the apical SFs (pressed SFs: (D)–(F), black arrowheads), and the other did not have such concentrations of intranuclear DNA (attached SFs: (J)–(K)). Bar = 10 μm.

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