ARTICLE ADDENDUM

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A novel role of actomyosin bundles in ERK signaling

Hiroaki Hirata[1,†,*] and Masahiro Sokabe[1,2,*]

[1]Mechanobiology Institute; National University of Singapore; Singapore; [2]Mechanobiology Laboratory; Nagoya University Graduate School of Medicine; Nagoya, Japan; [†]Current affiliation: R-Pharm Japan; Nagoya, Japan

Intracellular and extracellular mechanical environments have a significant impact on survival and proliferation of cells. While the extracellular signal-regulated kinase (ERK) subfamily of MAP kinases plays critical roles in regulations of these cellular behaviors, activation of ERK is affected by mechanical conditions of cells. We have recently found that ERK is activated on contractile actomyosin bundles. ERK activation on actomyosin bundles depends on tension in the bundles, which is generated by either myosin II activity of external forces. In this Addendum, we discuss a novel, potential role of actomyosin bundles in ERK signaling and mechanical regulation of cell survival and proliferation.

Keywords: actin cytoskeleton, cell survival, contractility, mechanotransduction, proliferation, signal transduction, stress fiber, tension

*Correspondence to: Hiroaki Hirata; Email: hhirata@med.nagoya-u.ac.jp; Masahiro Sokabe; Email: msokabe@med.nagoya-u.ac.jp

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A growing body of research shows that cell survival and proliferation are regulated not only by soluble chemical factors such as growth factors and cytokines but also by intracellular and extracellular mechanical environments. Adherent types of cells including fibroblasts, endothelial and epithelial cells adhere to extracellular matrix (ECM) substrates through integrin-mediated adhesion complexes. When ECM substrates are compliant, cell cycle progression and cell proliferation are inhibited, and the apoptosis rate is increased.[1-3] At the same time, cells on softer substrates generate smaller actomyosin-based contractile force, resulting in development of less mechanical tension in the actin cytoskeleton.[4] Therefore, potential involvement of cytoskeletal tension in the regulation of cell survival and proliferation has been discussed.[5] Consistent with this hypothesis, when the tension is reduced by disrupting the actin cytoskeleton or by inhibiting the RhoA-Rho kinase-myosin II cascade, cell cycle progression is hampered.[6,7]

ERK is a crucial regulator of cell survival and proliferation, and its activation (phosphorylation in the activation loop) is closely related to the level of cytoskeletal tension. Actomyosin activity[8] and stiff ECM substrates[9] are required for ERK activation. Mechanical stretching of cells upregulates ERK activity, which depends on the intact actin cytoskeleton.[10] Furthermore, ERK association with the actin cytoskeleton and activation of actin-associated ERK have been reported.[11] Finally, we have recently found that ERK is activated on actomyosin bundles in a tension-dependent manner.[12] ERK localizes to the actin cytoskeleton independently of myosin II activity. However, the actin-associated ERK is phosphorylated exclusively on actomyosin bundles called stress fibers, but not at lamellipodial or cortical F-actin accumulations, in a myosin II-dependent manner. Mechanical stretching of myosin II-inhibited cells restores ERK phosphorylation on stress fibers, strongly suggesting a crucial role of tension in ERK activation. Importantly, when quantified myosin II- or stretch-mediated tensile force in stress fibers, ERK phosphorylation was found to increase with tensile force on the fibers. This positive correlation between ERK phosphorylation and tensile force is observed in each stress fiber, indicating ERK phosphorylation is locally regulated on individual stress fibers. Thus, individual stress fibers are likely to work as a tension sensor and a platform for ERK activation. The myosin II-dependent ERK phosphorylation occurs not only on conventional stress fibers but also on actomyosin bundles connecting E-cadherin clusters in a keratinocyte

monolayer, suggesting a general role of actomyosin bundles in tension-dependent ERK activation.

ERK translocates to the nucleus upon phosphorylation and activates various transcription factors.[13] Nuclear localization of ERK is dependent on myosin II activity.[14,15] Furthermore, RSK, a major downstream effector of ERK, is phosphorylated in a myosin II-dependent manner, and mechanical stretching of myosin II-inhibited cells upregulates RSK phosphorylation.[12] However, disruption of stress fibers abolishes stretch-induced phosphorylation of RSK.[12] These results suggest that tension-dependent ERK activation on actomyosin bundles is involved in activating downstream signal cascades.

Sustained, basal ERK activity is necessary for survival of cells.[16] ERK phosphorylation on actomyosin bundles can be observed under the normal, static cell culture condition in the presence of serum.[12] Therefore, endogenous tension in actomyosin bundles under the static condition would contribute to cell survival through maintaining basal ERK activity. Consistent with this idea, disruption of the actin cyoskeleton, myosin II inhibition or soft ECM substrates, all of which decrease mechanical tension in actomyosin bundles and diminish ERK activity, induces apoptotic cell death.[2,17] Even in the context of multicellular systems such as epithelial cell monolayers, tension-dependent ERK activation is likely to contribute to cell survival. For example, keratinocytes die due to apoptosis within 24 h after inhibition of cell adhesion to ECM (the phenomenon called "anoikis").[18,19] By contrast, keritinocytes in a tensed cell sheet suspended over the ECM-devoid region, where cells are held together by actomyosin bundles interconnected through E-cadherin-mediated cell-cell junctions, are viable for > 40 h and even proliferate.[20] ERK activation on tensed actomyosin bundles in the suspended cell sheet may avert the cells from apoptosis.

Actomyosin bundles are major contractile force generators in cells. The bundles are linked to integrin-mediated (focal adhesion) or cadherin-mediated (adherens junction) cell adhesion complexes, and exert forces to these complexes. The transmitted forces drive various mechanotransduction events at the adhesion complexes.[21-23] Therefore, actomyosin bundles have been recognized as a critical mediator of mechanotransduction at focal adhesions and adherens junctions.[24,25] By contrast, above considerations reveal a novel role of actomyosin bundles; actomyosin bundles per se act as a mechanotransduction platform to activate ERK signaling (**Fig. 1**).

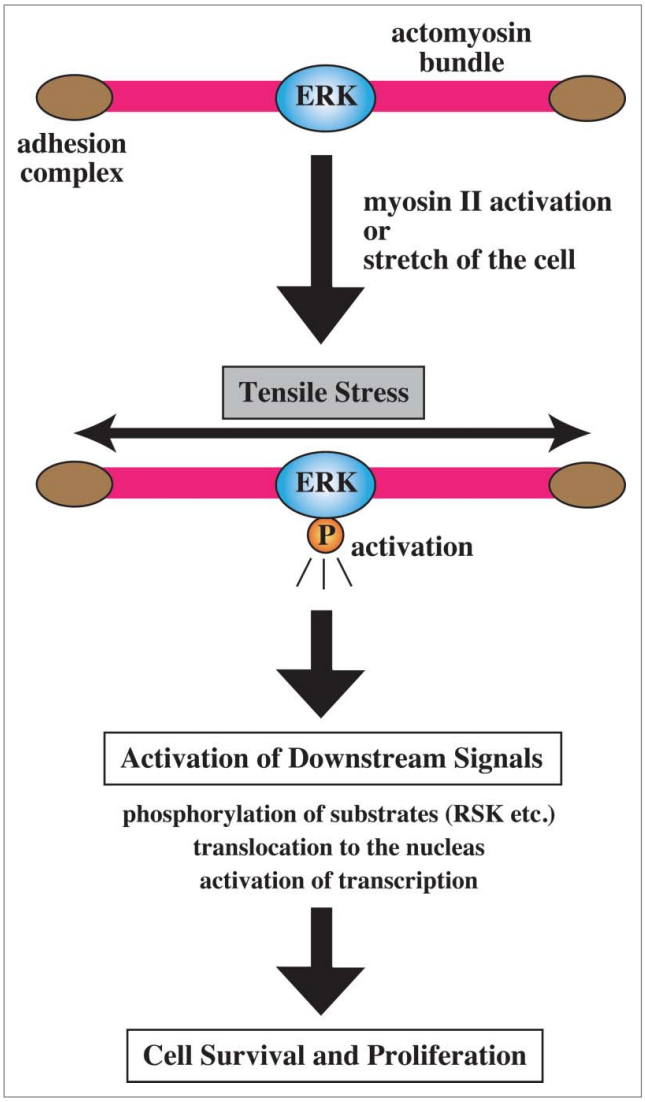

Figure 1. Hypothetical role of actomyosin bundles in tension-dependent ERK signaling. ERK localizes actomyosin bundles in a myosin II-independent manner. Once myosin II is activated, or cells are mechanically stretched, mechanical tension in actomyosin bundles is developed between adhesion complexes (focal adhesions or adherens junctions), which induces activation (phosphorylation) of ERK on the bundles. Through activating downstream signaling, tension-dependent ERK activation would ensure cell survival and proliferation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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