BioArchitecture, 6:53–59, 2016
ISSN: 1949-09921949-100X online
DOI: 10.1080/19490992.2016.1171428

COMMENTARY

Post-polymerization crosstalk between the actin cytoskeleton and microtubule network

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ABSTRACT. Cellular cytoskeletal systems play many pivotal roles in living organisms by controlling cell shape, division, and migration, which ultimately govern morphology, physiology, and functions of animals. Although the cytoskeletal systems are distinct and play different roles, there is growing evidence that these diverse cytoskeletal systems coordinate their functions with each other. This coordination between cytoskeletal systems, often termed cytoskeletal crosstalk, has been identified when the dynamic state of one individual system affects the other system. In this review, we briefly describe some well-established examples of crosstalk between cytoskeletal systems and then introduce a newly discovered form of crosstalk between the actin cytoskeleton and microtubule network that does not appear to directly alter polymerization or depolymerization of either system. The biological impact and possible significance of this post-polymerization crosstalk between actin and microtubules will be discussed in detail.

KEYWORDS. branching morphogenesis, cellular contractility, cytoskeletal crosstalk, fibronectin, HDAC6, integrin, microtubule acetylation, MLC, salivary gland

INTRODUCTION

The three major cytoskeletal systems found in all animal cells—actin cytoskeleton, microtubule network, and intermediate filaments—appear to coordinate their functions in mediating numerous cellular processes.[1,2] In the case of the actin cytoskeleton and microtubules, their

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Received March 16, 2016; Accepted March 19, 2016.
Color versions of one or more of the figures in this article can be found online at www.tandfonline.com/kbia.
Commentary to: Joo EE, Yamada KM. MYPT1 regulates contractility and microtubule acetylation to modulate integrin adhesions and matrix assembly. Nat Commun 2014; 5:3510; PMID:24667306; http://dx.doi.org/10.1038/ncomms4510
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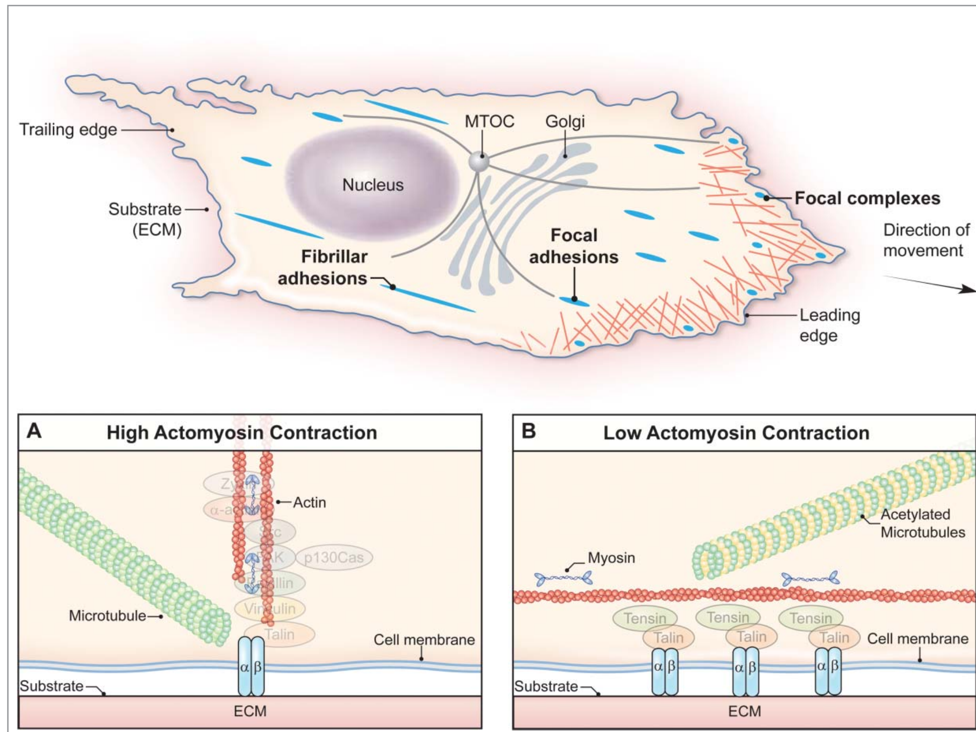
polymerization and depolymerization are coordinated. This coordination or crosstalk is achieved through a number of mechanisms. For example, many signaling components, such as Rac1 and Rho GTPases, their regulators including GEFs and GAPs, or their downstream effectors, associate with both the actin cytoskeleton and microtubules in a competitive manner, such that associating with one cytoskeletal system affects its function on the other system.[3-9] These signaling proteins alter the polymerization/depolymerization of either the actin cytoskeleton or the microtubule network, and their association controls the dynamics of these systems. Another example of crosstalk that controls the dynamics of these 2 cytoskeletons is the regulation of the molecular scaffolding platform that controls the polymerization/depolymerization of the actin cytoskeleton and the microtubule network.[10,11] However, in our recent paper, we described a novel form of crosstalk between the actin cytoskeleton and microtubule network at the post-polymerization stage of either system: this crosstalk does not depend on microtubule dynamics as determined by direct measurement of microtubule assembly and disassembly.[12] In this post-polymerization mode of crosstalk between actin cytoskeleton and microtubule network, we found that actomyosin contractility is inversely regulated by the acetylation of microtubules through a competitive interaction of myosin phosphatase with regulators of actomyosin contraction or microtubule acetylation. We found that this crosstalk governs both cell migration rate and embryonic branching morphogenesis.[12] In the following sections, we describe in greater detail the mechanisms by which this crosstalk influences or regulates 2 examples of major biological processes.

ACTOMYOSIN-MICROTUBULE CROSSTALK DURING CELL MIGRATION

Cell migration is a complex and dynamic process essential for the development and well-being of any complex organism. Broadly speaking, migration of an adherent cell can be viewed as a cycle of extension, attachment, and detachment of the cell relative to its surroundings, such as other neighboring cells or adjacent substrates, in order to create the required friction and motive force to move from one place to another.[13-16] A key determinant of efficient cell migration is the precise, well-coordinated attachment and detachment of the cell from its surroundings.[17-19] Typically, attachment of an adherent mammalian cell involves adhesion molecules, such as integrins, that interconnect the outside of the cell to its internal cellular machinery[20] (Fig. 1). Once these adhesions are formed, they undergo a maturation process by associating with different proteins, which results in alterations in attachment strength.[21-25] For detachment, cells forcefully pull on the adhesions or mechanically break down and/or internalize the adhesion molecules.[10,17,26-28] Interestingly, cytoskeletal systems appear to be involved in both of these processes: maturation of integrin-mediated adhesions is closely correlated with actomyosin contractility, and detachment/dissolution of adhesions is linked to the activity of the microtubule plus ends and/or hyperactivation or loss of actomyosin contractility.

Complex biochemical, biophysical, and genomic studies have established that a vast number of changes can occur to facilitate or regulate cell motility. These changes range from precise regulation of small signaling proteins to macromolecular rearrangements of the cytoskeletal systems.[1,29] Thus, it is not surprising that the well-coordinated polymerization and depolymerization of the actin cytoskeleton and microtubule network are necessary for any cell to migrate properly. However, it was a bit surprising to find that these 2 cytoskeletal systems are subjected to further regulation after polymerization. We found that a phosphatase composed of 3 subunits termed myosin phosphatase interacted competitively with myosin light chain (MLC) and a deacetylator of microtubules (histone deacetylase 6, HDAC6).[12] This interaction lead to dephosphorylation of the bound protein, i.e., either MLC or HDAC6, which then resulted in an alteration of the

FIGURE 1. Crosstalk between actomyosin contractility and microtubule acetylation. Actomyosin and microtubules regulate the rate of cell migration by controlling the assembly and disassembly of integrin-mediated adhesions. Typically, integrin-mediated adhesions are categorized as focal complexes, focal adhesions, or fibrillar adhesions. When cells are under high actomyosin contractility, there appears to be a low level of microtubule acetylation (panel A). However, when cells are under low actomyosin contractility, there appears to be an increase in the acetylation of microtubules and maturation of integrin-mediated adhesions to the fibrillar form (panel B).



contractile activity of the myosin bound to actin filaments or the amount of acetylated microtubules in cells. Interestingly, this inverse regulation of actomyosin contractility and microtubule acetylation was important for efficient attachment and detachment of fibroblasts to their surroundings as indicated by the maturation of adhesions, internalization of integrin receptors, and fibrillogenesis of fibronectin, an integrin ligand (Fig. 1). Thus, this additional regulation of actomyosin contractility and microtubule acetylation coordinated the attachment and detachment of cells from their surroundings by controlling integrin receptor density and activation/adhesion maturation to affect ligand interactions and matrix assembly.

ACTOMYOSIN-MICROTUBULE CROSSTALK DURING BRANCHING MORPHOGENESIS

As in fibroblasts, actomyosin-microtubule crosstalk was observed in salivary gland explants. Salivary glands have provided a powerful model system for elucidating branching morphogenesis. Salivary gland explants contain—at a minimum—mesenchyme, epithelium, and parasympathetic ganglion (PSG); these explants can be cultured on a filter in vitro for several days while retaining physiological functions, tissue morphology, and branching morphogenesis.[30] When the balance of actomyosin contractility and microtubule acetylation was disturbed using an experimental approach

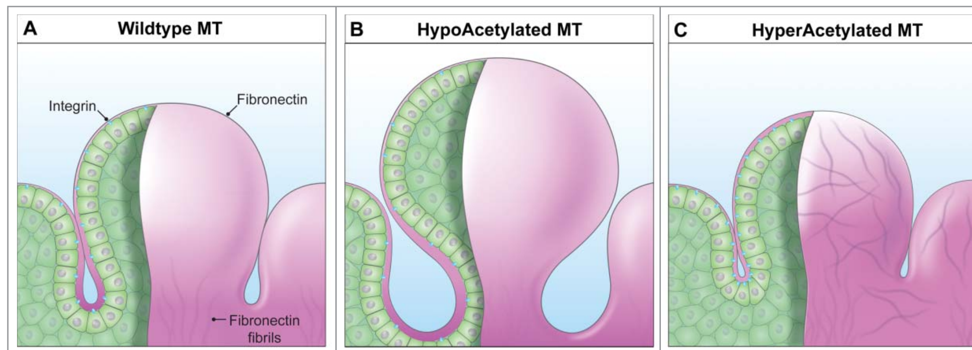
analogous to that applied to fibroblasts, some of the phenotypes of the latter were recapitulated in the salivary gland explants.[12] These alterations included increased density of fibronectin and its receptor $\alpha5\beta1$ integrin when the whole explants were infected with a virus that mimicked hyperacetylation of microtubules. Interestingly, the increase in fibronectin and its receptor integrin was mostly observed in the mesenchyme, particularly at the junction between mesenchyme and epithelium.

These experiments could not, however, clearly differentiate whether the virus infected primarily the mesenchyme or also the outermost epithelial cells, nor whether it was only the mesenchyme and not the epithelium that had responded in a manner similar to the fibroblasts. Nonetheless, the increase in fibronectin and integrins in the mesenchyme was accompanied by a decrease in epithelial morphogenesis. These findings raise 2 questions for the future: (1) does hyperacetylation of microtubules in the mesenchyme also decrease the 3-dimensional migration of mesenchymal cells in analogy to the effects on fibroblasts in cell culture? and (2) do changes in the mesenchyme by themselves affect epithelial branching morphogenesis of the salivary glands? To decipher the first question, the migration rates of mesenchymal cells can be examined by combining advanced live microscopy with advanced mouse genetics for tissue-specific manipulations. However, one caveat in interpreting such findings would be that cell migration in vivo can be influenced by multiple factors, including signaling from other cells and the heterogeneity of the environment, both of which can lead to competing signals and effects distinct from findings from studies of single cell migration in cell culture settings. In deciphering the mechanism of the decrease in epithelial branching morphogenesis upon disruption of the balance between acetylation and contractility, there are a number of published reports suggesting that cross-communication between the mesenchyme, epithelium, and parasympathetic ganglion can be important for controlling epithelial progenitor cells, which are key players in the epithelial branching morphogenesis of developing salivary glands (Fig. 2). Thus, further studies will be needed to dissect how altered acetylation of microtubules in the mesenchyme affects epithelial progenitor cells and its impact on epithelial branching morphogenesis.

POTENTIAL IMPLICATIONS OF THE CROSSTALK

The balance between actomyosin contractility and microtubule acetylation is important

FIGURE 2. Actomyosin contractility and microtubule acetylation govern the levels of fibronectin and $\alpha5\beta1$ integrins in developing salivary glands. Salivary glands explants infected with lentivirus expressing an acetyl-mimetic mutant of tubulin (HyperAcetylated MT; panel C) appear to have more fibronectin and $\alpha5\beta1$ integrins compared to glands expressing the acetyl-null mutant of tubulin (HypoAcetylated MT; panel B). Although more characterizations are needed, fibronectin polymerizes into filaments and is depicted in fibrous form at the basement membrane for clarity.



for both efficient migration of fibroblasts and epithelial morphogenesis of salivary glands. However, it is not yet certain whether this balancing occurs naturally in vivo during normal development and tissue remodeling, or during disease pathogenesis. It may be quite challenging to detect these types of post-translational modifications and altered biological functions using current technologies for acquiring and analyzing large-data sets in vivo. Nevertheless, some extrapolation from current data appears to support the validity of this post-polymerization crosstalk between the actin cytoskeleton and microtubule network. First, there appears to be an up-regulation of HDAC6 during salivary gland development that correlates with the initial stages of epithelial morphogenesis and rapid cell movement (http://sgmap.nidcr.nih.gov/sgmap/sgexp.html). This may imply that inverse regulation between actomyosin contractility and the microtubule network is necessary for rapid movement and/or early stages of epithelial morphogenesis. Second, in aging tissues, microtubule acetylation is increased (unpublished data). In fibroblasts or salivary glands, re-establishing actomyosin contractility or microtubule acetylation rescues migration rates or branching morphogenesis, respectively. It would be interesting to examine the long-term effects of an elevated level of either actomyosin contractility or microtubule acetylation, or both. Third, both FN and integrins are increased in the mesenchyme of the infected salivary gland, which suggests that the cellular niche is altered. Since the theory of reciprocity states that an abnormal cellular niche can cause abnormal cellular growth,[31,32] altered actomyosin contractility and microtubule acetylation may serve as a biomarker for certain disease states. These possibilities suggest that more experimentation should be performed to uncover the potential in vivo applications of this crosstalk between actomyosin and microtubule acetylation.

CONCLUSIONS

It is well-documented that actin and tubulin coordinate their polymerization and depolymerization into actin filaments and microtubules to regulate many fundamental biological processes. In our recent study, coordination between actin and tubulin was observed even after their polymerization, which can govern the efficiency of fibroblast cell migration and potentially to alter cellular niches. Interestingly, many current cancer therapies target microtubule polymerization to curtail cell division. Yet, resistance to these treatments often develops. Considering all of these observations, we cautiously suggest that even a small change in such cytoskeletal systems and their balance may have profound effects. It may, therefore, be useful to investigate the post-polymerization regulation of microtubules in order to develop more targeted control of microtubule function for inhibiting cancer cell growth.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

FUNDING

This work was supported by the Intramural Research Program of the NIH, NIDCR.

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