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Traction Forces and Rigidity Sensing of Adherent Cells

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Abstract—**This article is a concise review of our efforts in understanding the biological functions of traction forces, particularly in relation to the detection of rigidity of adhesive materials by fibroblasts.**

I. Introduction

Traction forces are mechanical forces exerted by cells to the adhesive surfaces, readily detectable as deformation of elastic polymer substrates or bending of microscopic elastomer pillars [1]. These forces are strongly dependent on type II myosin [2], consistent with the idea that they come from contractile interactions between actin and myosin II filaments.

For adherent cells that form focal adhesions, traction forces are most likely generated by the associated actin cytoskeleton. However, all focal adhesions are not equally involved in generating traction forces. Active force generation appeares to concentrate at the frontal region, where nascent focal adhesions form during cell migration [3]. The stress (force per unit area) is also the strongest at nascent focal adhesions in the frontal region [4]. Forces at mature focal adhesions appear to be passive in nature, to counter-balance forces at the front and/or to maintain the tension as the cell becomes stationary [4]. In stationary cells, focal adhesions appear to bear a relatively uniform stress such that the net force becomes proportional to the area of the focal adhesion [5].

As cell migration inevitably depends on mechanical interactions between cells and extracellular materials, initial investigations of traction forces were focused on their role in propelling cell migration. However it soon became clear that strong traction forces are generated only by relatively stationary cells that are tightly anchored to the substrate, while some highly motile cells such as amoebae show much weaker traction forces [6]. In terms of cell migration, traction forces are generated primarily for the purpose of overcoming strong adhesions. Their role may therefore be viewed as secondary and indirect. Supporting this notion, inhibition of myosin II and traction forces has at most a weak effect on the speed of cell migration [7].

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II. Diverse Potential Functions of Traction Forces

From the observations above, one may suspect that traction forces are involved in functions other than cell migration. A strong clue comes from the severe disruption of cell shape upon the inhibition of traction forces. While control adherent cells typically show a polygonal, convex shape, cells with compromised myosin II functions become elongated, with striking curvatures along the processes [7, 8]. In addition, while control cells have a limited capability to follow large curvatures on patterned substrates, those treated with myosin II inhibitors are much more adaptive [9]. A plausible explanation is that traction forces provide inward forces equivalent to surface tension for liquid drops, which serve to maintain the integrity of the cell body. This notion is supported by the frequent fragmentation of cells that lose myosin IIB [10].

An equally significant role of traction forces is to organize the extracellular matrix. Collagen gels become organized into a semi-aligned network that may extend for millimeters upon the exertion of forces [11], while the formation of fibronectin fibrils also requires contractile forces [12]. These fiber structures may then serve as guiding tracks for cell migration. As adherent cells respond to mechanical forces transmitted through adhesive structures [13], traction forces may function as regulatory signals that transmit through the extracellular matrix. The speed of such signals may be orders of magnitude higher than that for the diffusion of chemical signals, while the specificity may be controlled by the ability of targeted cells to bind differentially to different matrix materials.

III. Cellular Responses to Substrate Rigidity

Adherent cells proved to be highly sensitive to substrate rigidity, which affects cell growth, apoptosis, migration, and cytoskeletal structures. As rigidity requires an active mechanical mechanism for detection, traction forces exerted at focal adhesions likely play a key role. Cells may readout the rigidity based on either the resulting deformation or the resistive counter force. Experimental observations of the deformation of substrates of different rigidity appeared to favor the latter idea [14].

Additional understanding of rigidity sensing came from the recent application of photosensitive substrates that soften upon the exposure to UV [15]. Localized softening of the frontal region, where active traction forces are generated, cause retraction of the cell, reversal of cell polarity, or cell immobilization. In contrast, softening the rear region shows

no detectable effect. These results are consistent with a rigidity sensing mechanism driven by traction forces in the frontal region.

The response to substrate rigidity likely involves force-induced biophysical and biochemical events on the cytoplasmic side of focal adhesions. The components that respond directly to forces remain to be identified. Implicated mechanisms include stretched activated ion channels and force-induced changes in protein conformation or protein-protein topographic relationship [16-18], which may in turn modulate enzymatic activities such as tyrosine phosphorylation and small GTPases.

Imaging with GFP-tagged actin and the focal adhesion protein zyxin indicates that stiff substrates stimulate active assembly of actin filaments at focal adhesions, which generate a flux that carries other focal adhesions into the cytoplasm and lead to the formation of stress fibers [16]. This activity is much weaker on soft substrates, which may account for the small, dynamic adhesion structures and the lack of large actin bundles. Interestingly, responses similar to that on stiff materials were also observed in stationary cells and as cells experience stretching forces, suggesting that a universal mechanism is involved in detecting cell migration, substrate rigidity, and external mechanical forces [19].

IV. FUNCTIONAL IMPLICATIONS

Cell-substrate adhesions play a fundamental role in regulation. Strong evidence indicates that the responses involve not only receptor-ligand binding, but also mechanical events mediated by the bonding association as well as downstream events mediated by a still poor-defined mechanotransduction process. The so-called "traction forces" may in fact be an important component of this signal transduction mechanism.

There is increasing evidence that physical signals such as forces and rigidity are as important as chemical signals such as growth factors and cytokines in regulating physiological events. The potential impact on regenerative medicine is particularly noteworthy. For example, soft substrates favor cell-cell adhesions and tissue formation, while stiff surfaces favor cellular emigration and tissue dissociation [20]. Substrate rigidity also has a profound effect on the differentiation pathway of stem cells [21]. Thus a successful strategy of regenerative medicine must consider the control of parameters like scaffold rigidity and cellular contractility. Abnormal responses to mechanical signals may also represent a fundamental defect in metastatic invasion, where cancerous cells may dissociate from the home tissue due to either stiffening of the surrounding connective tissue or loss of the ability to read mechanical signals that keep them in the normal environment.

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