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Cell Migration: Cooperation between Myosin II Isoforms in Durotaxis

A new study reveals that non-muscle myosin II plays a central role in the durotaxis of mesenchymal stem cells, with the two major isoforms, II-A and II-B, being cooperatively required for this cell movement, and serine phosphorylation of the II-A isoform playing a negative role.

Miguel Vicente-Manzanares

Durotaxis is the tendency of most cells to move towards stiffer substrates when they are migrating on a compliance gradient. This type of movement is a cellular behavior based on the mechanical, rather than biochemical, properties of its microenvironment; thus, it can be classified as a process involving mechanotransduction. The physiological relevance of this poorly studied form of migration is beginning to be elucidated, and the ramifications are fascinating. Tissue stiffness favors tumorigenesis [1] as well as cell proliferation [2]. Furthermore, cells spread and migrate more easily on stiff than on compliant substrates [3].

What mediates durotaxis is not well characterized, but major players include integrins and focal adhesion kinase (FAK) [3,4]. Now, based on a new study from Raab and co-workers [5], we can add non-muscle myosin II (NMII) to the list of durotaxis mediators. While studied extensively in the 80s and 90s, NMII has come back into the spotlight more recently due to its pivotal roles in various crucial cellular phenomena, for example, cell migration, division, differentiation and apoptosis (reviewed in [6]). By controlling these processes, NMII is a major integrator of the mechanical properties of the cellular microenvironment, controlling stem cell differentiation and morphology [7,8], tumorigenesis [9], and cell migration [10].

The relatively simple vision of NMII as a contraction- or force-generating device was complicated by the identification of three major isoforms of the heavy chain and their splice variants, the elucidation of different regulatory sites within the light and heavy chains, and the description of several regulatory kinases and phosphatases that control the contractile, ATPase-based activity of NMII. This picture is even more complex taking into account the fact that, despite their apparent inability to heterodimerize, the different isoforms of NMII cooperate to mediate their biological roles.

In most mammalian cells, there are two major NMII isoforms, which are defined by the nature of the actin-binding, ATPase myosin heavy chain: NMII-A and NMII-B (a third isoform, NMII-C, does exist, but its expression is more restricted, hence its biological significance on a broad context is not yet clear). Both isoforms are implicated in cell migration, but their inhibition produces separable outcomes. NMII-A is implicated in cortical stability [11] and retraction of the cell rear [12], whereas NMII-B is required for cells to polarize and migrate directionally [12,13]. The role of the NMII isoforms in the control of cell shape reflects their subcellular positioning: whereas NMII-A is homogenously distributed and localizes everywhere in the cell but the lamellipodium, NMII-B is more confined to the central and rear portions of the cell [14], defining the rear by segregating protrusive signals away from these regions [15,16]. However, an interplay between these isoforms exists because in NMII-A-deficient cells NMII-B is not confined to the central and rear parts of the cell but appears homogeneously distributed and seldom assembles into mini-filaments [15].

Raab *et al.* [5] now show that NMII-A and NMII-B mediate durotaxis of mesenchymal stem cells. They first demonstrate that NMII-B localizes to the center and rear of primary mesenchymal stem cells on stiff substrates, defining a non-protrusive region. Conversely, on more compliant surfaces, NMII-B is not polarized. On either substrate, NMII-A remains evenly distributed, although its assembly into mini-filaments increases as substrates become stiffer. The authors then probed which of the isoforms played a more prominent role in the control of durotaxis. siRNA-induced inhibition proved that a small reduction in NMII-B was sufficient to impair durotaxis, whereas only a large knockdown of NMII-A produced the same effect. This led the authors to conclude that, although both isoforms are implicated in durotaxis, NMII-B is a more sensitive part of the molecular mechanism that controls it.

To try to explain the differential sensitivity to depletion of each NMII isoform in controlling durotaxis, the authors studied the dependence of the dynamics of both isoforms on the compliance of the substrate. They noticed that NMII-A was more dynamic (which is a proxy for decreased affinity of NMII-A for stable actomyosin filaments) in cells on soft compared to stiff substrates, and this correlated with

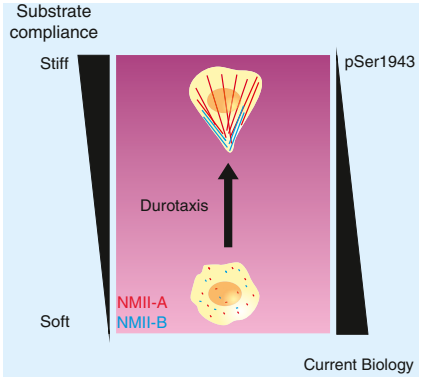

Figure 1. Rigidity correlates directly with NMII-A assembly, NMII-B rearward localization and cell polarization, and inversely with NMII-A phosphorylation on Ser1943.

Cells tend to move from compliant to stiff substrates via durotaxis. Stiff substrates promote the non-polarized assembly of NMII-A (red) into mini-filaments, which are necessary for NMII-B assembly and polarization. Conversely, soft substrates do not support NMII-A assembly nor NMII-B assembly/polarization. Soft substrates promote NMII-A phosphorylation on Ser1943, which may constitute a mechanism to inhibit the formation of large filaments.

increased phosphorylation of NMII-A on Ser1943: phosphorylation at this site has previously been reported to inhibit NMII-A assembly and stability [17]. The authors observed that there was an inverse correlation between substrate stiffness and Ser1943 phosphorylation, and that dephosphorylation of Ser1943 of NMII-A was required for proper NMII-B polarization on stiff substrates, suggesting that Ser1943 phosphorylation/dephosphorylation is a mechanosensitive signal that responds to changes in the pliability of the substrate.

These findings are summarized in Figure 1. Soft substrata prevent cell polarization and NMII-A assembly into large filaments and bundles, at least partially as a result of high levels of Ser1943 phosphorylation. Conversely, stiffer substrates decrease Ser1943 phosphorylation and promote NMII-A assembly and NMII-B assembly and polarization. The data presented in this study confirm that phosphorylation of NMII-A on Ser1943 negatively regulates NMII-A assembly. This work also postulates that the kinase that phosphorylates Ser1943 (possibly casein kinase II) responds to changes in the compliance of the substratum, i.e. is mechanoresponsive, and may be more active at low compliance than at high compliance. Another possibility is that a Ser1943 phosphatase is activated by stiff substrates. For example, protein phosphatase 2A (PP2A) is a broad-specificity serine phosphatase that interacts with p130CAS [18], an adaptor protein that is phosphorylated and activated by Src in response to molecular stretching [19]. A possible model is that activation of p130CAS by substrate stiffness recruits PP2A, which would in turn dephosphorylate NMII-A, promoting its assembly.

The data in this study clearly show that the assembly of NMII-A is required for the polarization of NMII-B. It has been suggested that NMII-A generates physical 'templates' that mediate the initial accumulation of NMII-B in discrete regions of the cell [15]. This study suggests that stiff substrates promote the assembly of NMII-A into myosin mini-filaments of sufficient size to nucleate NMII-B bundling. Conversely, more compliant substrates do not support assembly of NMII-A mini-filaments of sufficient size to serve as scaffolds for NMII-B.

The implications of this study for stem cell biology are also important. Most attempts to graft mesenchymal stem cells directly into tissues to promote regeneration *in situ* have failed, mainly because of the inability of these stem cells to migrate and/or differentiate into target cells. The study by Raab *et al.* [5] implies that these stem cells use NMII-A and NMII-B to adapt to the mechanical properties of their surroundings and promote migration towards stiffer microenvironments. The manipulation of the expression and/or activation of the non-muscle myosin II expressed by mesenchymal stem cells, or of the mechanical properties of the target tissue, might therefore be avenues worth exploring in the design of new strategies aimed at improving the migration of mesenchymal stem cells in therapy.

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