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SPOTLIGHT

EPHecting cell contact by increasing cortical tension

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EPH/EPHRIN signaling is crucial to the segregation of cell populations during the morphogenesis of many tissues. In this issue, Kindberg et al. (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202005216) show that EPH activation can drive both heterotypic cell repulsion and homotypic aggregation by triggering increased cortical tension.

Heightened awareness of the ability of cells to sense and generate mechanical force has enhanced our appreciation of the sophisticated ways that cells self-organize to create architecturally patterned tissues (1). It is now clear that well-known patterns of cell fate gene expression are coordinated with biophysical patterns to segregate and organize cell populations. Central to understanding the design principles underlying tissue self-organization are studies of EPH receptors and their membrane-associated EPHRIN ligands, which are important drivers of morphogenesis across many tissues (2, 3). Both ligand and receptor are membrane bound, and signaling, which can be bidirectional, requires cell–cell contact, enabling the study of proximal influences of EPH/EPHRIN signaling on individual cells.

The major consequence of EPH/EPHRIN signaling is to impair cell contact between ligand and receptor-expressing cells, thereby contributing to cell segregation and boundary formation in developing tissues (2, 3). Critical roles for EPH/EPHRIN signaling in neuronal pathfinding have uncovered a key role in repulsive migration, but this mechanism may not explain how EPH/EPHRIN signaling drives cell segregation in dense developing tissues where cells continuously contact other cells (4). Differential adhesion is also thought to contribute to EPH/EPHRIN-driven cell segregation, for example via EPH-stimulated E-cadherin cleavage (5). However, forces from adhesion tension are fundamentally integrated with those imparted by cortical tension, which govern many aspects of cell behavior and tissue morphogenesis (6). Indeed, the differential interfacial tension hypothesis holds that increased cortical tension can reduce the ability of cells to make stable cell contacts (7). Actomyosin accumulation occurs at EPH/EPHRIN interfaces, suggesting that interfacial tension driven by increased cortical actomyosin contractility may be an important driver of EPH/EPHRIN-mediated cell segregation (2, 3). In this issue, Kindberg et al. set out to test this directly by systematically stripping away the complexity of other inputs (8).

First, the authors eliminated cell-matrix adhesion and therefore the contribution of cell migration by examining cell doublets cultured in engineered agarose-coated wells. In contrast to homotypic pairs of EPHB2 or EPHRIN-B1–expressing cells that formed an extended contact face with large contact angles, heterotypic EPHB2- and EPHRIN-B1–expressing cell pairs exhibited a signaling-dependent reduction in contact face and angle of contact, consistent with an increase in interfacial tension. Importantly, when EPHB2- and EPHRIN-B1–expressing cells were plated in 3D aggregates in the absence of extracellular matrix attachment, they segregated completely, suggesting that increased interfacial tension may be the key driver of cell segregation.

Given the established interdependence of cortical tension and cadherin-based cell contact, Kindberg et al. investigated if the EPHB2/EPHRIN-B1–driven increase in interfacial tension required cadherin-mediated adhesion (6, 7). Surprisingly, the authors found that elimination of cadherin function in low calcium medium did not affect cell segregation, suggesting that EPH/EPHRIN may drive a more general increase in cortical tension. To test this, they pharmacologically interfered with actomyosin contractility, which restored large cell contact areas and angles to heterotypic EPHB2- and EPHRIN-B1–expressing cell pairs and reversibly impaired their ability to segregate in 3D aggregates. Direct measurement of cortical stiffness by atomic force microscopy confirmed an increase in cellular stiffness in both EPHB2- and EPHRIN-B1–expressing cells at early times after mixing and before the onset of segregation, consistent with a general increase in cortical tension.

The authors noticed that EPHB2-expressing cells themselves tended to aggregate at a particularly high density in EPHB2/EPHRIN-B1 segregation assays. Importantly, when mixed with both wild-type and EPHRIN-B1–expressing cells, EPHB2-expressing cells segregated into clusters that excluded both cell types. Examination of doublets revealed close contact between EPHB2 homotypic cell pairs that was not influenced by calcium depletion, but was eliminated by inhibition of actomyosin contractility. These data strongly suggest that, in addition to increasing interfacial tension at heterotypic contacts, increased

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actomyosin contractility in EPHB2 cells elevates cortical tension at the cell–medium interface. This in turn favors the establishment of homotypic EPHB2 cell interactions to minimize tension with the medium. The authors then explored the physiological relevance of these findings and showed that EPHB2- and EPHRIN-B1–expressing cells segregate into more complex structures in free-form hanging drop culture in an actomyosin-dependent manner. Likewise, they demonstrated through elegant genetic experiments that myosin II is required for EPH/EPHRIN-driven cell segregation in a mouse model of X-linked craniofrontonasal syndrome.

It seems clear that repulsive migration, differential adhesion, and increased interfacial tension can all contribute to EPH/EPHRIN-driven segregation, depending on the context. While cortical tension may play a more minor role in other contexts (9), the study by Kindberg et al. clearly shows that increased cortical tension can govern boundary formation, highlighting cortical tension modulation as a key driver of tissue self-organization. Exciting follow-up studies will identify the mechanisms by which EPH-driven changes in cortical tension are achieved and determine whether the cortex is organized differently at heterotypic and cell–medium interfaces. Possibilities include direct modulation of myosin II activity, which is thought to dominate cortical tension, alteration of the composition or configuration of the cortical actin network, plasma membrane-to-cortex attachment, and/or the organization of the plasma membrane itself (10). Clues may come from live imaging, which revealed strikingly dynamic cell–cell contacts among EPHB2/EPHRIN-B1 cell doublets, which could reflect pulsed cortical contractions that are now thought to be an inherent property of the cortical cytoskeleton that is stabilized in a regulated manner (10).

Beyond aspects of morphogenesis, an appreciation that EPH-triggered changes in cortical tension can promote both heterotypic and homotypic cellular interactions could provide important insight into the heavily studied but poorly understood role of EPH receptors in cancer development and progression, particularly given the growing recognition of spatially important aspects of tumor heterogeneity. More broadly, these studies should prompt us to consider whether altering cortical tension is an important component of the signaling output of other membrane receptors. Many receptor tyrosine kinases are known to elicit changes in cell contact, surface topology, and cytoskeletal organization, but most studies focus on downstream signaling, leaving a largely unexplored chasm between receptor activation and cellular and tissue architecture.

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