Dissecting cell adhesion cross-talk with micropatterns

Kaelyn D. Sumigray and Terry Lechler[1]
Department of Cell Biology, Duke University, Durham, NC 27710

A hallmark of epithelial cells is their attachment to both neighboring cells and an underlying substratum. Much research has been devoted to integrin-based substratum adhesion and cadherin-based cell–cell adhesion, both of which coordinate the underlying actin cytoskeleton. Although advances in our understanding of these dynamic structures have been rapid, what has lagged is our knowledge of how signaling and cytoskeletal changes coordinated by these systems are integrated. In PNAS, Borghi et al. (1) describe a unique approach for studying the interactions between cell–cell and cell–substratum adhesion. Using a micropatterning approach, they have accomplished the study of distinct adhesion systems in single cells.

To study both integrin and cadherin-based adhesion in single cells, the authors generated surfaces with alternating stripes of exposed collagen IV (an integrin ligand) and E-cadherin (which homodimerizes on the cell surface). Collagen stripes are the predominant site for formation of focal adhesions, whereas cadherin/catenin complexes are formed over the E-cadherin stripes. Individual cells spanned a number of stripes and therefore had distinct domains of "cell–cell" and cell–substratum adhesion adjacent to one another (Fig. 1). Not only are complexes localized to the proper stripes, but the dynamics of the cadherin/catenin proteins are very similar to what is observed in normal cell–cell adhesions (2).

Borghi et al. (1) focus on characterizing the effects of increasing E-cadherin ligation on cell migration activities. Cell–cell adhesion has often been hypothesized to inhibit cell migration, and probably does in some tissue contexts. This hypothesis is supported by observations that loss of E-cadherin is both associated with and, in some cases, causative for increased invasion and metastasis (3, 4). Physiologically, however, there are many examples of cells migrating with intact cell–cell adhesion, such as intestinal epithelial cells migrating up the villus axis and *Drosophila* border cells that crawl as a group in an E-cadherin–dependent manner (5, 6). In this study, although cells can readily crawl over collagen-covered surfaces, they are immobile on E-cadherin–treated surfaces, demonstrating that the E-cadherin–actin linkages are not productive for migration. Understanding how this differs from cells that do use E-cadherin for migration, such as *Drosophila* border cells (6), may shed light on whether and how cadherin complexes alone can productively engage the actin cytoskeleton. Although E-cadherin alone cannot support migration, it does not inhibit migration on collagen surfaces either (1). This is presumably because the turnover of complexes within the cadherin adhesions is fast enough not to limit forward movement. An alternative explanation is that in this assay, the actin cytoskeleton is not engaging with the cadherin adhesions in a way that allows force generation. In either regard, the data presented here clearly demonstrate that there is no diffusible signal from E-cadherin ligation to decrease the rate of cell migration by integrin-based methods.

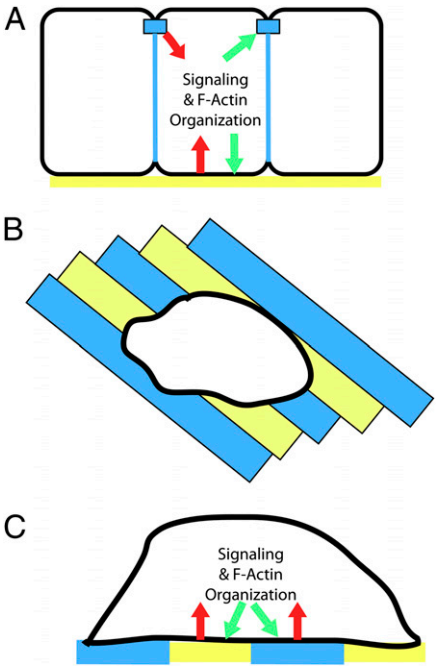

Fig. 1. Micropatterned surfaces to study cell adhesion. (*A*) Epithelial cells have spatially segregated cell–cell adhesions [mediated by E-cadherin (blue)] and cell–substratum adhesions [mediated by collagen and other components binding to integrins (yellow)]. Both of these induce soluble cytoplasmic signals and control actin organization, which feed back to the cell-adhesion structures. To study cross-talk between adherens junctions and integrins, alternating stripes of collagen IV (yellow) and E-cadherin (blue) were micropatterned. (*B*) Cell sitting on the stripes as viewed from above. (*C*) Cell sitting on the stripes as viewed from the side. Not only does this allow analysis of "cell–cell" and cell–substratum adhesion in single cells, it puts them in the same focal plane for image analysis.

Although E-cadherin ligation does not affect cell migration rates, it does dampen lamellipodial dynamics in the cell (1). Initial cell–cell contacts are made by lamellipodial interactions between two adjacent cells. Upon contact, a burst of lamellipodial activity is induced by Rac, facilitating an increase in the surface area of nascent contacts between two cells (7). Along these contacts, E-cadherin localizes and forms adherens junctions. As cadherins interact between two cells, Rac1 levels decrease at the membrane, and lamellipodia become less active, allowing junction maturation (7). After initial cell–cell contact, a second stage of adhesion is necessary to expand and strengthen junctions. This stage is driven by RhoA activation and actomyosin contractility (8).

Loss of α-catenin, which binds to the β-catenin/E-cadherin complex at adherens junctions, causes an increase in lamellipodia activity (1). Without α-catenin, recruitment of E-cadherin is impaired; therefore, these cells phenocopy those grown on stripes with low levels of E-cadherin. Surprisingly, loss of α-catenin also leads to an increase in cell migration rate. Because E-cadherin ligation has no effect on cell migration, this appears to be a cadherin-independent function for α-catenin.

To examine this in more detail, the authors specifically deplete the cytoplasmic pool of α-catenin but leave the adherens junction-bound pool intact (9). Cytoplasmic α-catenin is largely dimeric, can bind directly to actin, and can inhibit the Arp2/3 complex (2, 9, 10). Depleting this pool did not cause defects in lamellipodial activity but did increase the rate of cell migration. Therefore, α-catenin functions at the adherens junction to regulate E-cadherin stability and lamellipodia formation, and functions within the cytoplasm to restrict cell migration. Whether this decrease in cell migration rate is due to actin-binding activity, inhibition of Arp2/3, or other means remains to be determined.

An outstanding question raised by these studies is the role of force generation at the adherens junction, and its regulation of adherens junction components. Although tension sensing and force

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[1]To whom correspondence should be addressed. E-mail: lechler@cellbio.duke.edu.

transduction at focal adhesions has received much attention, we know comparatively little about this at intercellular contacts (11, 12). A number of studies have implicated the adherens junction in force generation and sensing (13–16). In the current study, little force generation was detected on E-cadherin stripes. Whether this is due to the physical presentation of the E-cadherin in this system, the presence of adjacent focal adhesion complexes, or is a property of the cell type is not yet clear. Evidence that cadherin complexes can exist in distinct forms depending on whether they are under tension has recently emerged. Yonemura et al. (16) suggested that the application of force on α-catenin results in recruitment of vinculin, an actin-binding protein that is also found at focal adhesions. Whether competition for vinculin between these two sites occurs, and how this might mediate cross-talk between force-inducing and -sensing systems, remains to be addressed. This has significant implications for how we think about coordination between adhesion systems. If the cadherin complexes can sense and transduce force, are they more productive for cell migration? Is the physical separation of force generation at the zonula adherens and focal adhesions required for proper cytoskeletal organization and migration? Do the pools of actin associated with the focal adhesions or adherens junction interact and integrate their forces?

The approach of using spatially segregated micropatterns on a surface will have many additional uses in both cell-adhesion and cell-signaling studies. It could be used to study the effect of spatial localization of different integrin ligand/integrin pairs. In addition, if extended to other cell–cell adhesion systems, it could be used to unravel the spatial and signaling requirements for the formation of distinct cell–cell adhesion structures, which show interdependence in many cell types.

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