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SPOTLIGHT

Hands and feet: Closer than you think in epithelial migration

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Epithelial migration requires that substrate-based motility be coordinated with cell–cell adhesion. In this issue, Ozawa et al. (2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.202006196) identify a central role for actin assembly at adherens junctions that contributes to both of these processes.

Epithelia move. Their ability to migrate critically supports morphogenesis, tissue homeostasis, and wound healing. For example, differentiated cells that are born in the crypts of the small intestine constantly migrate up the villus until they are shed at its tips (1). In this and similar cases, cells are thought to move by exerting locomotor forces on their underlying ECM. But, at the same time, they retain physical contacts with one another to preserve tissue integrity (2). If we think of ECM adhesions as the feet of cells, then cell–cell contacts are their hands. How these different adhesion systems are regulated has been a long-standing challenge for understanding epithelial migration. Do the feet and hands operate independently, or is there some cross-talk that coordinates their activity? In this issue of *JCB*, Ozawa, Takeichi, and their collaborators (3) reveal that the links are much closer than we previously suspected.

Ozawa et al. began by asking how migration of cultured epithelia was affected when they disrupted E-cadherin cell–cell adhesions (key elements of adherens junctions [AJs]). They did this by deleting α-catenin, a scaffolding protein that couples classical cadherins to the F-actin cytoskeleton. Then they tested how the monolayers migrated into the open space of an artificial "wound." In this system, migration begins when the cells that immediately abut the wound (a.k.a. "leader" cells) spread and locomote; but it soon comes to also involve the movement of "follower" cells behind the leaders (Fig. 1 a). However, the speed of wound closure reflects many things, including the intrinsic capacity of cells to move (locomotility) and to hold their direction into the wound. Ozawa et al. found that α-catenin knockout (KO) cells closed wounds more slowly than controls, but this wasn't because the speed of the individual, constituent cells was altered (3). Instead, wound closure was slowed because α-catenin KO cells failed to hold their direction as effectively as controls. Similarly, earlier studies reported that coordination of cell movement within monolayers requires α-catenin and, by implication, cadherin adhesions (4).

More surprising was what they found when they looked at the motility of isolated epithelial cells. Several different cell types moved much less when grown in isolation compared with when they were in groups, something that was not affected by deletion of α-catenin. Cell–cell contact is often understood to inhibit motility (2), but these observations indicated that multicellularity might be an important precondition to get epithelial cells moving effectively in the first place.

To understand how this might come about, the team then focused on lamellipodia: dynamic, veil-like cellular protrusions that are key components when cells migrate on substrates (5). In epithelial wound assays, lamellipodia are most readily seen at the free edges of leader cells. However, Ozawa et al. also saw many lamellipodia in the follower cells; these were less apparent because they extend underneath neighboring cells. Indeed, cryptic lamellipodia are increasingly being recognized in epithelial migration (1, 6, 7), but their precise functional contribution has been harder to dissect.

Importantly, cryptic lamellipodia seemed to involve the same molecular machinery as lamellipodia elsewhere. Specifically, Ozawa et al. found that the leading edges of cryptic lamellipodia were enriched in the Arp2/3 actin nucleator and its activator, the WAVE regulatory complex (WRC); together, these are responsible for assembly of the branched actin networks essential for lamellipodia to form (5).

This provided an opportunity for Ozawa et al. to test the role of cryptic lamellipodia in epithelia migration (3). In a clever, simple experiment, they depleted cells of either p34 (an Arp2/3 component) or Nap1 (a WRC component) and mixed these with wild-type cells to generate mosaic cultures. Strikingly, they saw that disabling the WAVE-Arp2/3 apparatus caused follower cells to be left behind as the mixed monolayers migrated into the wounds. Therefore, cryptic lamellipodia were necessary for follower cells to move with the population. This finding also

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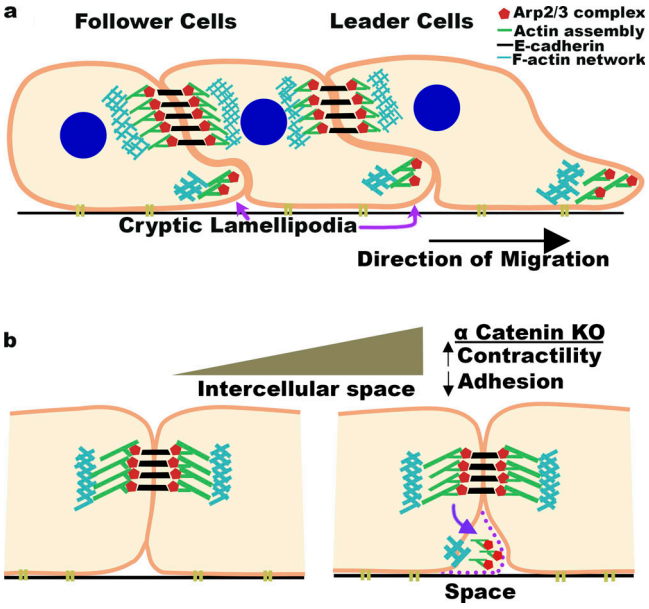

Figure 1. **The several uses for junctional actin assembly in epithelial migration. (a)** A model of epithelial collective migration into open spaces (a.k.a. "wounds"). Migration is initiated by leader cells at the border with the wound but also involves follower cells behind the leader cells. Cells are linked by cadherin junctions and migrate with lamellipodia; both are sites for Arp2/3 and WAVE-mediated actin assembly. **(b)** A model for biogenesis of cryptic lamellipodia. Actin assembly is nucleated by Arp2/3-WAVE at cadherin junctions. Where cells retain close apposition, this remains the main site of branched actin nucleation. But when intercellular space is increased, Arp2/3 can flow into the free surface to form a cryptic lamellipodium.

has a more general implication. In some circumstances, leader cells have been thought to be the principal drivers of epithelial migration. But Ozawa et al.'s new observation indicates that follower cells move actively during epithelial sheet migration and are not simply pulled along by their leaders. Interestingly, cryptic lamellipodia were more frequent in α-catenin KO cells. As lamellipodia have been implicated in guiding migration (5), this could explain why these cells changed direction more frequently than controls.

So, where do cryptic lamellipodia come from? Here was the second surprise. Videos showed that cryptic lamellipodia actually seemed to originate from pools of F-actin found at AJs. This is interesting because AJs are prominent sites of actin assembly in confluent epithelia, and they concentrate some of the same actin regulators as are used in lamellipodia, namely Arp2/3 and the WRC (8). Indeed, imaging showed Nap1 moving from its pool at AJs into the leading edges of cryptic lamellipodia. This indicates that branched actin assembly at AJs can be used for multiple distinct purposes: to support the junctional actin cortex, but also to make cryptic lamellipodia (Fig. 1 b).

There must then be mechanisms that control how actin nucleated by WAVE-Arp2/3 at AJs is used. Here, an important clue comes from the observation that cryptic lamellipodia often appeared first at the vertices between multiple junctions. As these are points where tensile stress is often highest between cells (9), the team wondered whether mechanical force might be involved. Indeed, they found that myosin II, the principal contractile force generator, was activated more in the α-catenin KO cells, where cryptic lamellipodia are prevalent, than in controls. And inhibiting myosin II suppressed the generation of cryptic lamellipodia and also corrected wound healing in α-catenin KO cells. Together, this indicated that enhanced contractility was responsible for promoting cryptic lamellipodial assembly when α-catenin was deleted.

How might this occur? One simple explanation is that contractility provides space between cells for actin assembly to create a cell protrusion (Fig. 1 b). Where cell surfaces are apposed by adhesion, space may be limited and newly formed actin instead flows to form the junctional cortex. But when contractility creates space—at a multicellular vertex, and even more so in α-catenin KO cells, where adhesion would also be compromised—protrusions may form to create cryptic lamellipodia. Consistent with this, the authors noted that cryptic lamellipodia tended not to form when cell surfaces remained closely apposed, even in α-catenin KO cells. Whether this simple scenario is enough to explain the authors' findings will be an important question for future research.

Irrespective of the precise underlying mechanism, the work of Ozawa et al. indicates that we should not think of cell–cell adhesion and the locomotor apparatus as independent processes in epithelial migration (3). Instead, since cadherin adhesions can recruit the Arp2/3-WAVE apparatus (10), the dynamic actin cytoskeleton at the junctions might be a nexus that coordinates cell–cell interactions and locomotility. Takeichi and his collaborators have played an instrumental role in how we understand the contribution of cadherins to morphogenesis since their first discovery. This latest paper continues that tradition, providing a rich new perspective for us to investigate the cellular basis of epithelial migration.

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