ERK-mediated mechanochemical waves direct collective cell polarization

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Summary

During collective migration of epithelial cells, the migration direction is aligned over a tissue-scale expanse. Although the collective cell migration is known to be directed by mechanical forces transmitted via cell-cell junctions, it remains elusive how the intercellular force transmission is coordinated with intracellular biochemical signaling to achieve collective movements. Here we show that intercellular coupling of ERK-mediated mechanochemical feedback yields long-distance transmission of guidance cues. Mechanical stretch activates ERK through EGFR activation, and ERK activation triggers cell contraction. The contraction of the activated cell pulls neighboring cells, evoking another round of ERK activation and contraction in the neighbors. Furthermore, anisotropic contraction based on front-rear polarization guarantees unidirectional propagation of ERK activation, and in turn, the ERK activation waves direct multicellular alignment of the polarity, leading to long-range ordered migration. Our findings reveal that mechanical forces mediate intercellular signaling underlying sustained transmission of guidance cues for collective cell migration.

Keywords

Collective cell migration; ERK/MAPK; EGFR; FRET; front-rear polarity; intercellular signal transfer; mathematical model; mechanochemical feedback; mechanotransduction; wave propagation.

Introduction

Collective cell migration underpins various fundamental biological processes, including embryonic development and tissue repair (Friedl and Gilmour, 2009; Mayor and Etienne-Manneville, 2016). A migrating cell cluster exhibits multicellular coordination of cellular parameters, such as cytoskeleton organization (Farooqui and Fenteany, 2005), organelle positioning (Carvalho et al., 2019; Farooqui and Fenteany, 2005; Reffay et al., 2011), and cell velocity (Petitjean et al., 2010; Tlili et al., 2018). Directionality of these parameters in each cell can be provided by two mechanisms. First, cells sense a direction from global external cues, such as a gradient of chemoattractant or substrate stiffness (Haeger et al., 2015; Shellard and Mayor, 2019). Second, directional cues are transmitted from a leading edge of a cell cluster to the bulk; cells located at the edge, referred to as leader cells, sense the microenvironment to spread out and dictate the direction of follower cells located behind the leader cells (Omelchenko et al., 2003; Reffay et al., 2014; Yamaguchi et al., 2015). In the latter case, all follower cells as well as the leader cells generate mechanical forces to actively migrate, and those forces are orchestrated as cooperative intercellular forces over the cell cluster (Serra-Picamal et al., 2012; Tambe et al., 2011; Trepat et al., 2009). It has also been shown that the mechanical forces transmitted via cell-cell junctions provide local cues to direct an ordered cell migration in a cluster (Das et al., 2015; Tambe et al., 2011); however, our understanding of the signaling molecules responsible for the long-range transmission of the mechanical forces is far from complete.

Extracellular signal-regulated kinase (ERK), a serine/threonine kinase, plays critical roles in mechanotransduction that regulates differentiation (Tanabe et al., 2004), epithelial cell division (Gudipaty et al., 2017), and tissue homeostasis (Moreno et al., 2019). Earlier studies have shown that ERK activation propagates as multiple waves from leader cells to the follower cells during collective cell migration using *in vitro* cultured cells (Handly et al., 2015; Matsubayashi et al., 2004; Nikolic et al., 2006) and *in vivo* mouse ear skin (Hiratsuka et al., 2015). Recently, we have demonstrated that the ERK activation waves orient the directed migration of a cell cluster against the wave direction, indicating a critical role of the ERK activation waves in coordinated cell migration (Aoki et al., 2017). However, it remains largely unknown how cells harness the mechanotransduction and the ERK activation waves to coordinate collective cell migration.

In this study, by combining Förster resonance energy transfer (FRET)-based biosensors (Harvey et al., 2008; Komatsu et al., 2011), an optogenetic tool (Aoki et al., 2013), traction

force microscopy (Trepat et al., 2009), and mathematical modeling, we show that each follower cell possesses a mechanochemical feedback system, in which stretch-induced ERK activation triggers cell contraction. Intercellular coupling of the ERK-mediated mechanochemical feedback enables sustained propagation of ERK activation and contractile force generation, leading to multicellular alignment of front-rear cell polarity over long distances. Thus, our study clarifies a mechanism of intercellular communication underlying long-range sustained transmission of directional cues for collective cell migration.

Results

Cell deformation waves precede ERK activation waves

To investigate the relationship between ERK activation and cell deformation during collective cell migration, Madin-Darby canine kidney (MDCK) cells confluently seeded within compartments of a silicone confinement were released for collective cell migration. ERK activity and cell deformation were evaluated by FRET imaging with EKAREV-NLS, by which ERK activation is quantified as an increase in the FRET/CFP ratio (Komatsu et al., 2011), and particle image velocimetry (PIV)-based image processing, respectively. As the cells migrated toward free spaces, sustained ERK activation waves were propagated from the leader cells to the follower cells (Figures 1A and 1B; Movie S1), in agreement with previous reports (Aoki et al., 2017; Matsubayashi et al., 2004). The cell deformation, i.e., extension and shrinkage, was quantified by the cell strain rate in the direction of collective cell migration (x-strain rate). Similar to the ERK activity, the x-strain rate exhibited repeated positive and negative values, namely extension and shrinkage, which propagated from the leader cells to the follower cells (Figures 1A and 1C; Movie S1), as described previously (Serra-Picamal et al., 2012; Tlili et al., 2018). Hereinafter, we refer to the propagation of the x-strain rate as cell deformation waves (Rodriguez-Franco et al., 2017).

To analyze the correlation between the ERK activation and the cell deformation, time-series data of the ERK activity and those of the x-strain rate were collected at the single cell level. We then calculated ERK activation rate, i.e., the ERK activity change per min, to compare not ERK activity itself but a time derivative of ERK activity and x-strain rate. We noticed that the ERK activation rate and the x-strain rate oscillated almost synchronously with an approximately 90 min period (Figure 1D). Furthermore, temporal cross-correlation analysis revealed that the ERK activation rate lagged 3 min behind the x-strain rate (Figure 1E),

indicating that the cell deformation waves precede the ERK activation waves. In other words, cells are first extended, followed by ERK activation, and then the cells start contracting.

Cell extension triggers ERK activation via EGFR signaling

Because the cell extension precedes ERK activation, we reasoned that mechanical stretch activates ERK during collective cell migration. To demonstrate this, we stretched MDCK cells plated on an elastic silicone plate and compared the ERK activity before and after the mechanical stretch. As anticipated, uniaxial 50% stretch of the MDCK cells resulted in ERK activation (Figures 2A and 2B), indicating that passive extension of cells activates ERK. Furthermore, the ERK activation almost linearly responded to different degrees of stretch, and 30% stretch was sufficient for triggering significant ERK activation, indicating that ERK activation scales with degrees of stretch.

Previously, we reported that EGFR and a disintegrin and metalloprotease 17 (ADAM17), which catalyzes the shedding of membrane-tethered EGFR ligands (Maretzky et al., 2011a), affect ERK activation waves (Aoki et al., 2017; Aoki et al., 2013). Consistent with those reports, either treatment with an inhibitor of EGFR, PD153035, or that of matrix metalloproteinases (MMPs) and ADAMs, marimastat, suppressed the ERK activation waves during collective cell migration (Figure S1). These observations strongly suggest that the stretch-induced ERK activation requires EGFR and ADAM17 activity. In fact, both PD153035 and marimastat suppressed the stretch-induced ERK activation (Figure 2C). Remarkably, immunoblotting showed that cell stretch transiently increased auto-phosphorylated EGFR, followed by phosphorylation of the downstream kinases including RAF1, MEK1/2, and ERK1/2 (Figures 2D-2H). Together, these results indicate that cell stretch activates EGFR and its downstream signaling molecules, including ERK.

ERK activation induces cell contraction via Rho-associated kinase activation

Because the ERK activation precedes cell shrinkage (Figure 1E), we speculated that ERK activation would induce cell contraction. To prove this hypothesis, we used 2paRAF, a light-inducible ERK activation system based on cryptochrome 2 (CRY2)–CIBN dimerization (Aoki et al., 2013; Kennedy et al., 2010; Kinjo et al., 2019). In this system, RAF1 fused with CRY2 is recruited to the plasma membrane by heterodimerization of CRY2 and membrane-anchored CIBN upon blue light exposure, culminating in ERK activation. We seeded cells with and without 2paRAF (2paCRY2-RAF1/CIBN-mScarlet-I-CAAX) expression into two separated

compartments of a silicone confinement, respectively, and formed an interface of the two cell populations by the removal of the confinement (Figure 3A). Upon ERK activation by blue light exposure, the interface shifted toward the side of the cells expressing 2paRAF (Figures 3B and 3C; Movie S2). Moreover, inhibiting the ERK activation with a MAPK/ERK kinase (MEK) inhibitor, trametinib, treatment suppressed the interface shift (Figures 3B and 3C; Movie S2). Furthermore, even with the inhibition of the ERK activity propagation in the cells without 2paRAF expression by treating an inhibitor of EGFR PD153035, a degree of the light-induced contraction of the cells expressing 2paRAF was not significantly different from a case without the inhibitor (Figures S2A and S2B), indicating that the cell contraction is due to the optogenetic ERK activation but not to the ERK activation in the neighbors. Thus, these results clearly indicate that the ERK activation induces cell contraction of confluent MDCK cells. To confirm ERK activation also triggers cell contraction during collective cell migration, we tested the effect of ERK inhibition either with an inhibitor of EGFR, PD153035, or that of MAPK/ERK kinase (MEK), trametinib, on deformation of migrating cells. Both PD153035 and trametinib treatment damped the oscillation of the x-strain rate (Figure S2C-S2F). Thus, ERK activation is required for cell deformation during collective cell migration.

Cell contraction is driven by actomyosin, which involves Rho-associated kinase (ROCK)-mediated regulation (Amano et al., 1996; Kimura et al., 1996; Totsukawa et al., 2000). Thus, we examined the effects of a ROCK inhibitor, Y-27632, on the ERK-induced cell contraction. We found that Y-27632 suppressed the ERK-induced cell contraction (Figure 3B and 3C; Movie S2). This observation prompted us to examine whether ERK induces ROCK activation. To this end, we used a cytosolic FRET biosensor for ROCK activity (Li et al., 2017). After optogenetic ERK activation, ROCK activity increased, then plateaued within 12 min after the blue light exposure (Figures 3D and 3E). We next examined the ROCK activity dynamics during collective cell migration. Interestingly, ROCK activity exhibited repeated unidirectional wave propagation from leader cells to the follower cells, as did the ERK activity (Figures 3F and 3G; Movie S3). Inhibition of ERK with trametinib abolished the propagation of ROCK activation waves (Figure 3H; Movie S3) as well as temporal oscillatory activation in each cell (Figure 3I). In stark contrast, inhibition of ROCK with Y-27632 abolished the oscillatory activity in each cell and also decreased the basal activity of ROCK drastically (Figures 3J and 3K; Movie S3), indicating that ERK activity is responsible only for the oscillatory component of ROCK activity. Thus, we conclude that ROCK is activated downstream of ERK and is integral to ERK-induced cell contraction.

ERK activation decreases traction forces and accumulates F-actin at the cell-cell interface

How does ERK activation alter mechanical force generation to induce contraction? To answer this question, we combined light-inducible ERK activation with traction force microscopy (Trepat et al., 2009), which allows the measurement of traction forces loaded by cells on the substrate. On a substrate of polyacrylamide gel embedded with fluorescent beads, the interface between the cells with and without 2paRAF expression was formed, as already described (Figure 3A). The traction force exerted by the 2paRAF-expressing cells markedly decreased within 40 min after the start of blue light exposure (Figures 3L and 3M; Movie S4). By contrast, inhibiting ERK with trametinib restored the traction force generation (Figure 3N), confirming that ERK activation suppresses force loading on the substrate. The gradual increase in traction force by the cells without 2paRAF expression in Figure 3M and 3N is independent of the optogenetic ERK activation in the neighboring 2paRAF-expressing cell cluster because the traction force increased over time even without blue light exposure (Figure S2G and S2H). This is likely because increasing cell density by proliferation downregulates ERK activity (Aoki et al., 2013). Moreover, we found that subcellular localization of F-actin in migrating cells is altered depending on the ERK activity level (Figures 3O and 3P). ERK activation by epidermal growth factor (EGF) promoted F-actin localization at the lateral side of the cells. By contrast, ERK inhibition by trametinib treatment produced stress fibers at the basal side of the cells. Therefore, these results suggest that ERK activation triggers cell contraction by accumulating F-actin at the cell-cell interface and triggering predominant force loading on the interface while suppressing traction force generation.

Zonal cell contraction initiates sustained unidirectional ERK activation waves at the interface

We have shown that each cell possesses an ERK-mediated mechanochemical feedback system coupling cell deformation and ERK activation: ERK is activated by cell extension, and the activated ERK induces cell contraction. Considering the tight physical connection between epithelial cells, contraction of a cell cluster should stretch cells in the adjacent cluster, mainly at its border, thereby generating the ERK activity propagation. To test this hypothesis, we used a rapamycin-activatable (RA) Rho guanine nucleotide exchange factor (GEF) system to induce contraction of a cell cluster (Inoue et al., 2005; van Unen et al., 2015). We first seeded cells

carrying the RA Rho GEF expression system into a confinement (Figure 4A), and then seeded EKAREV-NLS cells after the removal of the confinement, resulting in the formation of an interface between cells expressing RA Rho GEF and cells expressing EKAREV-NLS. Before rapamycin treatment, ERK activity was randomly propagated without any preferential direction (Figure 4B; Movie S5). Upon rapamycin treatment, the cells with RA Rho GEF began to contract, resulting in the extension of the adjacent EKAREV-NLS–expressing cell cluster toward the RA Rho GEF-expressing cell clusters (Figures 4C and 4D; Movie S5). With this trigger, ERK activation waves emerged at the interface and were unidirectionally propagated toward the extended EKAREV-NLS–expressing cell cluster (Figures 4C-4E). In addition, the displacement of the EKAREV-NLS–expressing cells was oriented toward the RA Rho GEF-expressing cell clusters and was directed opposite the ERK activation waves (Figure 4F). Thus, these results suggest that cell contraction generates ERK activation waves in the adjacent cell cluster.

We then asked whether a cell-cell tight connection is required for intercellular propagation of ERK activation waves. To test this, we knocked-out α-catenin, a major cell-cell junction component (Nagafuchi et al., 1994), in MDCK cells (Figure 4G). As expected, E-cadherin at the cell-cell junction was reduced in the α-catenin KO cells in comparison with wild-type (WT) cells (Figure 4H). Importantly, there was no significant difference in the sensitivity of the WT and α-catenin KO cells to EGFR stimulation by EGF (Figure 4I). Thus, the cells possess equal sensitivity to EGFR stimulation even in α-catenin KO. Nevertheless, coordinated ERK activity propagation was severely disrupted in the α-catenin KO cells (Figures 4J and 4K; Movie S6), indicating that intercellular mechanical linkage is required for ERK activity propagation. Thus, we conclude that intercellular force transmission mediates ERK activation waves.

Front-rear cell polarization is required for the unidirectional propagation of ERK activation waves

We next investigated how unidirectional propagation of ERK activation waves is achieved during collective cell migration. If cells contract isotropically, the loss of directional information would impede the unidirectional propagation of ERK activation waves. Therefore, there must be a mechanism by which the directional information is retained during the wave propagation. We found that the cell deformed preferentially along the direction of migration (Figures 5A-5C), to an extent sufficient for activating ERK (Fig. 2B), indicating that cells deform anisotropically during collective cell migration. Furthermore, phosphorylated myosin

light chain, a marker of contractile myosin, and F-actin accumulated at the basal rear side of migrating cells (Figures 5D, S3A, and S3B), which are indicative of polarized rear contraction. Some cells showing the dense actomyosin fibers elongate almost perpendicular to the direction of cell migration. This suggests that cell contraction toward the migration direction would cause slight extension perpendicular to the direction of migration because the cell volume does not largely change during the cell contraction. The presence of a front-rear cell polarity was also confirmed by the localization of GM130, a Golgi apparatus marker, known to be located in front of the nucleus in some types of migrating cells (Magdalena et al., 2003; Nobes and Hall, 1999). When cells were examined immediately after release from confinement (0 h migration), the Golgi orientation relative to the nucleus was not biased toward the leading edge (Figures S3C and S3D). By contrast, 21 h after the initiation of collective cell migration, the Golgi orientation was biased toward the direction of the migration over more than a 1 mm range from the leader cells (Figures 5E and 5F). To quantify the degree of alignment, we defined the directedness of the Golgi orientation (Figure 5G), and found that the Golgi directedness first increased around the leader cells, and then the increase spread through the follower cells over time (Figure 5H). In addition, the directedness of ERK activation waves and that of migration showed similar dynamics to the Golgi directedness (Figures 5I and 5J). Thus, these results clarify that alignment of front-rear cell polarity in follower cells positively correlates with unidirectionality of ERK activation waves.

To investigate the requirement of front-rear cell polarization for the unidirectional ERK activation waves, we knocked down Rac1 or Cdc42, which are required for the establishment of front-rear polarization (Nobes and Hall, 1999). Expression of short hairpin RNAs reduced Rac1 and Cdc42 expression to 50% and 30%, respectively (Figures S3E and S3F). In the knocked-down cells, the front-rear polarization of Golgi in the leader cells was still directed in the direction of the migration; however, that of the follower cells was impaired after 21 h migration (Figures 5K and 5L). Moreover, the unidirectionality of the ERK activation waves and the alignment of migration direction were also impaired in the follower cells of the knocked-down cells (Figures 5M and 5N; Movie S7). Taken together, these results demonstrate that the alignment of front-rear polarization toward the direction of the migration is required for the unidirectional ERK activation waves and the ordered cell migration over long distances from the leading edge.

ERK activation waves orient front-rear polarity in follower cells

We further addressed the relationship between the ERK activation waves and the front-rear polarization. Previously, it has been proposed that force loading on cell-cell junctions directs front-rear polarization (Das et al., 2015). Consistent with this, disruption of cell-cell junctions by α-catenin KO abolished the alignment of the front-rear polarization toward the direction of migration (Figures S4A-S4C). From this observation, we speculated that ERK-induced contractile force generation would direct the front-rear polarization in neighboring follower cells. As expected, inhibition of ERK activation with trametinib suppressed the alignment of the front-rear polarization and migration directionality in the follower cells at 21 h after migration (Figures 6A-6C and S4D; Movie S8). Interestingly, constitutive ERK activation by 12-*O*-tetradecanoylphorbol 13-acetate (TPA) also inhibited the alignment of the front-rear polarization and migration directionality in the follower cells (Figures 6A-6C and S4E; Movie S8), suggesting that periodic ERK activation in the form of waves is important for polarizing follower cells. We then tested whether synthetic ERK activation waves can enhance the directedness of the Golgi polarization in the follower cells. To this end, we used MDCK cells expressing 2paRAF (2paCRY2-RAF1/CIBN-CAAX) and Golgi-7-mCherry as a Golgi apparatus marker. Those cells were confluently seeded in a confinement, and then released for migration with an EGFR inhibitor PD153035 to suppress autonomous ERK activation waves (Figure 6D). Of note, inhibition of EGFR signaling suppresses ERK activation waves in follower cells, but ERK activation in the cells near the leading edge remains (Figure S1B and S1C). Thus, the leader cells still migrate, even with EGFR signaling inhibition, while follower cell migration is abrogated (Aoki et al., 2017). Under this condition, we created optogenetic ERK activation waves by shifting patterned blue light-illumination to mimic spontaneous ERK activation waves; i.e., ~35 μm width and 400 μm intervals at 3 μm min^{-1} velocity (Figure 6D). With the synthetic ERK activation waves, the front-rear cell polarizations were significantly aligned even in follower cells more than 600 μm distant from the leader cells, compared with the samples in the absence of ERK activation waves (Figures 6E and 6F; Movie S9). Thus, these results clearly demonstrate that ERK activation waves orient the front-rear polarity in cells against the direction of the waves. Collectively, we conclude that unidirectional ERK activation waves and multicellular alignment of the front-rear polarization propagate cooperatively, enabling long-distance transmission of directional cues for collective cell migration.

Modeling cellular mechanochemical feedback with polarity demonstrates long-range

unidirectional ERK activation waves

Finally, we developed a mathematical model to understand the role of ERK-mediated mechanochemical waves in collective cell migration. We employed a two-dimensional cellular Potts model (CPM) to represent behavior of the epithelial monolayer at a single cell scale (Glazier and Graner, 1993; Hirashima et al., 2017; Merks and Glazier, 2005). In this model, each cell morphology is represented as a cluster of regular lattices, and a state of lattices determine an energy of the system. The dynamics of a multicellular system proceeds stochastically on the basis of an energy minimization using a Monte Carlo simulation algorithm. We here regard a unit of trials in Monte Carlo simulation (Monte Carlo steps: MCS) as 1 h in experiments. The energy in the model includes essential properties in multicellular mechanics, i.e., intercellular adhesion, cell elasticity, and active cellular contraction (Guillot and Lecuit, 2013; Heisenberg and Bellaiche, 2013). In addition, the cells possess an inherent orientation regarding the polarized rear contraction, equivalent to the front-rear polarity defined as the Golgi orientation in our experiments (Figure 7A). In our model, orientations of the cell polarity in individual cells tend to be aligned as a result of the physical interaction with neighboring cells (Supplementary notes) (Hirashima et al., 2013; Peyret et al., 2019; Szabo et al., 2010). The anisotropy along the front-rear polarity and the strength of cell contraction are governed by the cell polarity parameter ω and the ERK activity, respectively. That is, the cell contraction is isotropic when ω=0 and polarized for the rear side of the cell with increasing ω (Figure 7B); we set ω=1 as suggested in experiments (Figures 5A-5J), unless otherwise noted. In simulations, we assumed that the follower cells obey the stretch-induced contraction, while the leader cells keep migrating towards the free space.

We performed simulation analysis under a similar condition to the confinement release assay, and confirmed that the unidirectional ERK activation waves from the leader cells to the follower cells were reproduced (Figures 7C and 7D; Movie S10). Moreover, the simulation results well mimicked experimental measurements in terms of spatio-temporal profiles of front-rear polarity directedness, ERK wave directedness, and migration directedness (Figure 7E). We then examined the effect of the cell polarity level ω on the directedness. For any type of directedness, the spatial range with high values becomes more limited around the leading cells, with the decrease of ω (Figure 7F; Movie S10) as observed with disruption of polarity proteins (Figure 5K-5N). This indicates that the cell anisotropy in contraction along the front-rear polarity is essential for long-range multicellular guidance. In addition, we investigated the effect of ERK activation waves on the polarity directedness (Figures 7G and 7H). Even without the

ERK activation waves, the front-rear polarity in the follower cells around the leading edges is relatively directed toward the front because the migrating leader cells pull near-follower cells; however, the polarity directedness gradually decreases with distance from the leader cells. By contrast, the polarity directedness is maintained long-range from the leader cells via the ERK activation waves (Figure 7H). Hence, ERK activation waves direct the front-rear polarity of the follower cells, consistent with the experiments with optogenetic ERK activation waves (Figure 6F).

Further in silico analysis led to the finding that the strength of cell-cell adhesion γ controls synchronization of the ERK activity along a perpendicular axis to its propagation direction (y-axis in Figure 7I). We introduced a spatial correlation length of the ERK activity along the y-axis as a measure of its synchronization, and examined a dependency of γ on the spatial correlation length. Our numerical investigation clearly shows that the spatial correlation length increases with increasing cell-cell adhesion strength γ (Figure 7J), indicating that multicellular integrity via intercellular mechanical linkages plays a key role in the ERK activity synchronization. Taken together, tight connections between cells, each of which experiences mechanochemical feedback with front-rear polarity, effect well-ordered long-range ERK activation waves.

Discussion

During collective cell migration of epithelial cells, not only leader cells but also follower cells exert traction forces on their substrates to drive cell movement, resulting in long-distance coordinated cell migration (Trepat et al., 2009). Thus, the follower cells can sense the direction of the leader cell migration. Previous studies have revealed that the directional cues are transmitted to the follower cells by intercellular mechanical forces (Serra-Picamal et al., 2012). The mechanical tension varies dynamically and spreads over long distances, leading to establishment of local anisotropic stress, along which the cells tend to migrate (Das et al., 2015; Tambe et al., 2011). However, the molecular mechanism by which the mechanical forces are sustainably transmitted over long distances to direct collective cell migration remains to be revealed. Here we identified that ERK is a key molecule regulating cellular mechanochemical feedback, which governs long-distance sustained propagation of the directional cues. We propose a mechanism of ERK and mechanical force-mediated intercellular communication underlying the collective cell migration (Figure S4G). In the initial process of collective cell

migration, advancement of the leader cells toward the free space exerts pulling forces on the adjacent follower cells, directing front-rear cell polarization. The pulling force then stretches the follower cells and activates the EGFR-ERK signaling cascade, which in turn generates contractile forces at the rear side of the follower cells through the ROCK pathway. The contraction of the follower cells exerts pulling forces on the next follower cells, directing front-rear polarization in the next follower cells and likely providing space for cryptic lamellipodia to protrude for active migration (Das et al., 2015; Farooqui and Fenteany, 2005). At the same time, the pulling force evokes another round of cell stretch, ERK activation, and following ERK-mediated rear contraction in the next follower cells. Thus, intercellular coupling of the ERK-mediated mechanochemical feedback via cell-cell junctions enables sustained propagation of pulling forces, front-rear cell polarity, and ERK activation over a large tissue scale, leading to collective cell migration.

The combination of optogenetic ERK activation and traction force measurement revealed that ERK plays a critical role in translocating cellular force generators. Our experiments demonstrate that the ERK activation triggers cell contraction through ROCK activation (Figure 3B-3E) while it decreases the traction forces (Figures 3L and 3M). The effect of ERK activation on the force generation can be explained by the translocation of dense F-actin fibers from cell bases to cell-cell junctions (Figures 3O and 3P), which should lead to an efficient tug on adjacent cells. It is plausible that the ERK-induced cell contraction through ROCK involves Rho GEF activation localized at cell-cell junctions. Along this line of reasoning, a previous study demonstrated that mechanical stretching of cells caused localization of Rho GEF at cell-cell junctions, giving rise to local activation of RhoA, and thereby the junction-associated actomyosin (Acharya et al., 2018). On the other hand, the decrease in traction forces by ERK activation could be attributed to disruption of focal adhesion-associated actin stress fibers due to promotion of focal adhesion turnover (Colo et al., 2012; Webb et al., 2004). The focal adhesion turnover should increase deformability of cells to efficiently contract upon contractile force generation.

Remarkably, our results imply that ERK activation triggers polarized rear contraction in each cell. As the intracellular ERK activation may not be significantly polarized due to rapid ERK diffusion within the cytoplasm (Fujioka et al., 2006), the effect of ERK activation on the polarized rear contraction is explained by an antagonistic activation of Rac1 and RhoA (Guilluy et al., 2011). Force loading on a cell-cell junction activates Rac1 via dissociation of merlin from the junction, which directs the cell front (Das et al., 2015). The preoccupied Rac1 activation at

the cell front should restrict the activation of RhoA-regulated contractile machineries to the rear side of the cells, leading to polarized rear contraction upon ERK activation. Therefore, ERK should convey the signal to stimulate the contractile machineries within a cell at the back in response to pulling forces at the front.

An important unsolved question is how the mechanical stimuli are converted into signal transduction to activate ERK. We have previously demonstrated that ADAM17 plays a role for the propagation of the ERK activation in MDCK cells (Aoki et al., 2017). It has been proposed that ADAM17 catalyzes the ectodomain shedding of EGFR ligands (Sahin et al., 2004), and the released ligands can bind and activate EGFR in an autocrine or paracrine manner. In this study, we found that stretch of MDCK cells activates EGFR-ERK pathway (Figures 2D and 2E), as reported in other cell types (Kippenberger et al., 2005; Zhang et al., 2007), and the stretch-induced ERK activation requires ADAM proteinase activity (Figure 2C). Therefore, we suppose that the stretch-induced EGFR activation involves the ADAM17-mediated shedding of EGFR ligands. It has been shown that ERK activates ADAM 17 by means of phosphorylation of Thr735 (Diaz-Rodriguez et al., 2002; Li et al., 2018). However, EGFR activation occurs as early as 1 min after stretching and precedes ERK activation (Figures 2D-2H), which is at odds with the involvement of ERK activation in the stretch-induced EGFR activation. It has also been reported that src, a protein tyrosine kinase, is involved in ADAM17 activation (Maretzky et al., 2011b; Niu et al., 2015) and is required for the stretch-induced EGFR activation (Zhang et al., 2007). However, we do not know whether ADAM17 is activated upon stretch to cause the ligand shedding. Another possible mechanism is that EGFR ligands are constitutively shed by ADAM proteinases and the sensitivity of EGFR to the ligands may be increased upon stretch. Thus, future studies will be necessary to explore how the mechanical stimuli activate EGFR in a manner dependent on the ligands in the ERK activation waves. Also, we have recently obtained preliminary data showing that ADAM17 is involved in ERK-induced cell contraction as well as stretch-induced ERK activation. Therefore, further investigations should focus on the regulation of ERK activation waves by ADAM17 through multi-tiered system.

In conclusion, we have revealed that mechanical force transmission functions as a mediator of the intercellular ERK signal transduction underlying collective cell migration. Current understanding of the intercellular signal transduction mostly emphasizes the importance of intercellular transfer of biochemical molecules including growth factors, hormones, and neurotransmitters. Thus, our present study raises another consideration, which is the critical role of cellular response to mechanical stimuli in intercellular signal transduction.

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Author contributions

Conceptualization, N.H., M.M., T.H.; Methodology, N.H., K.A., X.T., M.M., T.H.; Software, N.H., X.T., T.H.; Validation, N.H., L.R., A.M.L., T.H.; Formal analysis, N.H., T.H.; Investigation, N.H., L.R., A.M.L.; Resources, N.H., K.A., X.T., T.H.; Data curation, N.H., T.H.; Writing - original draft, N.H., M.M., T.H.; Writing - review & editing, N.H., K.A., M.M., T.H.; Visualization, N.H., T.H.; Supervision, M.M., T.H.; Project administration, M.M., T.H.; Funding acquisition, N.H., X.T., M.M., T.H.

Declaration of interests

The authors declare no competing interests.

Main figure titles and legends

Figure 1. Cell deformation waves precede ERK activation waves during collective cell migration

(A) Phase contrast images (upper), FRET/CFP ratio indicating ERK activity (middle), and strain rate in the direction of collective cell migration (x-strain rate; lower) are represented at 1h (left), 8 h (center), and 14 h (right) after start of time-lapse imaging. In the x-strain rate images, extending and shrinking regions are shown in red and blue, respectively. Scale bar, 200 μm. The imaging interval is 1 min.

(B and C) x-t kymographs of ERK activity (B) and x-strain rate (C), from (A). White arrows indicate the rightward propagation of ERK activation waves (B) and cell deformation waves (C).

(D) Temporal change of ERK activation rate and x-strain rate in a representative cell.

(E) Temporal cross-correlations between x-strain rate and ERK activation rate. The blue line indicates the average temporal cross-correlation coefficients with standard deviations (SDs). n = 212 cells from three independent experiments. See also Movie S1.

Figure 2. Passive cell extension triggers ERK activation via EGFR signaling

(A and B) ERK activity change in MDCK cells on an elastic chamber. (A) Representative images before (left) and 20 min after uniaxial 50% stretch (right). The black double-headed arrow indicates the axis of the uniaxial stretch. White and yellow arrowheads each correspond to the same cells in the left and right images. Scale bar, 30 μm. (B) Violin plots of ERK activity 20 min after 20-50% stretch or that without stretch (0% stretch). Red and gray lines indicate median and mean values, respectively. n = 3612 cells (0% stretch), 4771 cells (20% stretch), 3856 cells (30% stretch), 4070 cells (40% stretch), and 3178 cells (50% stretch) from three independent experiments. Dunnett's test; $P = 0.0969$ (0 versus 20% stretch), ****$P < 0.0001$ (0 versus 30, 40, or 50% stretch).

(C) ERK activity changes in cells treated with DMSO, 1 μM PD153035, and 10 μM marimastat, from immediately after to 20 min after the stretch are shown as means with SDs. n = 3634 cells (DMSO), 3237 cells (PD153035), and 4000 cells (marimastat) from three independent experiments. Dunnett's test, ****$P < 0.0001$.

(D) MDCK cells were stretched and then lysed at the indicated time points. The cell lysates were analyzed by immunoblotting with the indicated antibodies.

(E-H) Normalized phosphorylation levels of EGFR (E), RAF1 (F), MEK1/2 (G), and ERK1/2

(H) are represented as means with SDs (n = 3).
See also Figure S1.

Figure 3. ERK activation triggers cell contraction via Rho-associated kinase activation
(A) Schematics of an experiment with the light-inducible system. The boundary between the cell populations with and without 2paRAF expression was imaged.
(B) Representative images of CFP (EKAREV-NLS; upper) and CIBN-mScarlet-I-CAAX (2paRAF; middle) treated with DMSO (left), 200 nM trametinib (center), and 10 μM Y-27632 (right) were obtained at the boundary between the cells with and without 2paRAF. Lower panels indicate kymographs of the CIBN-mScarlet-I-CAAX (2paRAF) images. Blue lines indicate the blue light illumination. Scale bar, 30 μm. The imaging interval is 5 min.
(C) Displacement of the boundary is plotted over time after blue light exposure. The lines represent the average with SDs. n = 9 from three independent experiments.
(D) ROCK activity images of MDCK cells expressing 2paRAF and a ROCK biosensor are represented at 0 min (left) and 15 min (right) after the start of blue light exposure. Scale bar, 20 μm. The imaging interval is 1 min.
(E) Quantification of ROCK activity in each cell in (D) after the start of blue light exposure. The line represents the average with SDs. n = 15 from three independent experiments. Unpaired t-test, P = 0.0065.
(F) ROCK activity images are represented at 0 h (upper) and 12 h (lower) after removal of confinement. Scale bar, 200 μm. The imaging interval is 2 min.
(G) A kymograph of the ROCK activity in (F). White arrows indicate the rightward propagation of ROCK activity.
(H and J) A kymograph of ROCK activity is shown before and after 200 nM trametinib (H) and 100 μM Y-27632 (J) treatment. The imaging interval is 2 min.
(I and K) ROCK activity in 5 representative cells was plotted over time after trametinib (I) and Y-27632 (K) treatment.
(L) Traction force microscopy of the cell seeded on polyacrylamide gels with optogenetic ERK activation. Differential interference contrast (DIC) images (upper), CIBN-mScarlet-I-CAAX fluorescence (2paRAF; middle), and traction force (lower) are represented at −1 h (left), 1 h (center), and 4 h after the start of blue light exposure. Scale bar, 50 μm. The imaging interval is 5 min.
(M) Mean traction force in cells with and without 2paRAF, as shown in (L), with SDs (n = 3).

(N) Mean traction force in cells with 2paRAF with DMSO or 200 nM trametinib treatment at 2 h after the start of blue light exposure (n = 3).
(O) Fluorescence images of F-actin (phalloidin) in migrating MDCK cells on collagen-coated glass substrates 45 min after treatment with DMSO, 100 ng mL^{-1} EGF, 200 nM trametinib, or both EGF and trametinib. Scale bar, 10 μm.
(P) Intensity profile of F-actin along the orange lines in (O). Orange arrowheads indicate edges of cells.
See also Figure S2, Movie S3 and S4.

Figure 4. ERK activation waves are mediated by intercellular mechanical force
(A) Schematics of an experiment using the rapamycin-inducible system. The boundary between the cell population with RA Rho GEF and that with EKAREV-NLS expression was imaged.
(B) DIC images (upper), ERK activity (middle), and ERK wave directionality (lower) are represented at 0 h after DMSO (left) and 50 nM rapamycin (right) treatment. Broken lines indicate the cell population boundary. Arrows represent the direction of ERK activity propagation. For the analysis of ERK wave directionality, the EKAREV-NLS–expressing cells located to the left of the cell population boundary were omitted from the analysis. Scale bar, 100 μm. The imaging interval is 5 min.
(C) ERK activity (upper) and ERK wave directionality (lower) are represented at 13 h after the drug treatment.
(D) Kymographs of ERK activity with DMSO (left) or rapamycin (right) treatment. The EKAREV-NLS–expressing cells located on the left side of the cell population boundary were excluded from the kymographs for visibility.
(E and F) Polar histograms showing the distribution of ERK wave (E) and cell displacement (F) direction over 13-13.5 h after treatment with DMSO (left) and rapamycin (right). For ERK wave direction, n = 1078 (DMSO) and 1127 (rapamycin) from three independent experiments. For cell displacement direction, n = 475 (DMSO) and 1167 (rapamycin) from three independent experiments.
(G) Analysis of the expression level of α-catenin and β-actin in WT and α-catenin KO MDCK cells by immunoblotting.
(H) DIC images (upper) and immunofluorescence images of E-cadherin (lower) in the confluent WT and α-catenin KO MDCK cells.
(I) The ERK activity 16 min after the addition of EGF was plotted as log(dose) response. Orange

circles (WT cells) and blue squares (α-catenin KO cells) represent the average of ERK activity with SDs. The data were obtained from three independent experiments.

(J and K) Kymographs of ERK activity during collective cell migration in WT (J) and α-catenin KO (K) MDCK cells. The imaging interval is 5 min.

See also Movie S5 and S6.

Figure 5. Multicellular alignment of front-rear polarization underpins unidirectional ERK activation waves

(A) A representative cell expressing mCherry in the cytosol is marked by a broken line. The left column shows composite images of phase contrast and mCherry fluorescence (green), and the right column shows ERK activity. Cells showed low ERK activity in the contracted state (upper and lower) and high ERK activity in the extended state (middle).

(B) Changes in lengths of cells along the x axis and y axis from contracted (minimum ERK activity) to extended (maximum ERK activity) states are plotted. Each line indicates an individual cell. $n = 30$ cells from three independent experiments. Paired t-test, ****$P < 0.0001$.

(C) Changes in lengths of cells along the x axis and y axis from extended (maximum ERK activity) to contracted (minimum ERK activity) states are plotted. Each line indicates an individual cell. $n = 30$ cells from three independent experiments. Paired t-test, ****$P < 0.0001$.

(D) Immunofluorescence images of F-actin (left) and di-phosphorylated MLC (ppMLC; center) at the basal plane in MDCK cells at 17 h after migration. The right column indicates the composite images of F-actin and ppMLC.

(E) Immunofluorescence images of the Golgi apparatus (GM130) and the nucleus (EKAREV-NLS) in MDCK cells at 21 h after migration. The upper image shows a wide-view field, and the lower images are magnified images of the regions corresponding to the numbered windows in the upper image.

(F) Polar histograms showing the distribution of Golgi orientation relative to the nucleus in the cells at 0-0.5 mm (left), 0.5-1.0 (center), and greater than 1.0 mm (right) distant from the leader cells. $n = 1297$ cells (0-0.5 mm), 2096 cells (0.5-1.0 mm), and 1452 cells (>1.0 mm) from three independent experiments.

(G) θ is the angle between the reference direction and sample direction. Directedness was calculated by the indicated equation. The reference direction is set to the left for analysis of Golgi orientation and cell migration direction, and to the right for ERK wave direction, in order to match the signs of their directedness.

(H-J) Mean directedness of Golgi orientation (H), ERK wave direction (I), and cell migration (J) binned every 300 μm from the leader cells after 0 h, 6 h, and 21 h migration is plotted over the distance from the leader cells, with SDs. The data were obtained from three independent experiments. The first bin of the ERK wave directedness was excluded from the result because of outliers due to boundary effects.

(K) Immunofluorescence images of Golgi (GM130) and nuclei (EKAREV-NLS) in shRac1- and shCdc42-expressing MDCK cells at regions greater than 1 mm from the leader cells after 21 h migration.

(L-N) Directedness of Golgi orientation (Dunnett's test, ***P = 0.0001 (0.6-0.9 μm, WT versus shCdc42), P = 0.0508 (0.9-1.2 mm, WT versus shCdc42), ***P = 0.0009 (0.6-0.9 μm, WT versus shRac1), P = 0.2023 (0.9-1.2 μm, WT versus shRac1)) (L), ERK wave direction (Dunnett's test, **P = 0.0067 (0.9-1.2 mm, WT versus shCdc42), *P = 0.0290 (1.2-1.5 mm, WT versus shCdc42), **P = 0.0019 (0.9-1.2 mm, WT versus shRac1), *P = 0.0248 (1.2-1.5 mm, WT versus shRac1)) (M), and cell migration direction (Dunnett's test, *P = 0.0238 (1.2-1.5 mm, WT versus shRac1), P = 0.0655 (1.2-1.5 mm, WT versus shCdc42)) (N) binned every 300 μm from the leader cells after 21 h migration were plotted over the distance from the leader cells with SDs. The data of WT cells is the same as that of 21 h migration in (H-J). The data were obtained from three independent experiments.

See also Figure S3 and Movie S7.

Figure 6. Synthetic ERK activation waves orient front-rear polarity in follower cells

(A) Immunofluorescence images of Golgi (GM130) and nuclei (EKAREV-NLS) in MDCK cells treated with DMSO (left), 200 nM trametinib (center), and 10 nM TPA (right) at a region more than 1.0 mm distant from the leader cells at 21 h after migration. Scale bar, 100 μm.

(B and C) Mean directedness of Golgi orientation (B) and cell migration direction (C) binned every 300 μm from the leader cells after 21 h migration were plotted over distance from the leader cells, with SDs. The data were obtained from three independent experiments.

(D) Flow diagram of an experiment on synthetic ERK activation waves.

(E) Fluorescence images of Golgi apparatus (Golgi-7-mCherry) and nuclei (EKAREV-NLS) in MDCK cells with or without optogenetic ERK activation waves for 18 h. The upper images are wide-view fields, and the lower images are magnified views of the regions outlined by white windows in the upper images.

(F) The mean directedness of Golgi orientation binned every 100 μm from the leader cells in

(E) was plotted over distance from the leader cells, with SDs. The data were obtained from three independent experiments. Unpaired *t*-test; ***P* = 0.0011 (0.6-0.7 μm, plus versus minus), **P* = 0.0267 (0.7-0.8 mm, plus versus minus).
See also Figure S4, Movie S8 and S9.

Figure 7. Modeling cellular mechanochemical feedback with polarity demonstrates long-range unidirectional ERK activation waves

(A) Schematics of the mathematical model.
(B) Contraction mode depending on the parameter ω, defined as the polarity level.
(C) Snapshot of ERK activity in the CPM simulation. Scale bar, 200 μm. (D) Kymograph of ERK activity along the x-axis and simulation step. The color corresponds to values of the color bar in (C).
(E) Spatial distribution of mean directedness, with SDs. The color shows different simulation steps. *n*=5.
(F) The effect of polarity level ω on the directedness. The color represents mean values of the directedness. *n*=5.
(G and H) Cell polarity directedness with (+) or without (–) ERK wave. The color in (G) represents the angle of cell polarity. (H) Mean polarity directedness from the leading edge, with SDs. *n*=5.
(I) Snapshot of ERK activity distribution in weak (γ=0.5) and strong (γ=1.5) multicellular integrity.
(J) Mean y-axis spatial correlation length of the ERK activity on the intercellular integrity γ, with SDs. *n*=5. See also Movie S10.

STAR Methods

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Tsuyoshi Hirashima (hirashima.tsuyoshi.2m@kyoto-u.ac.jp).

Materials Availability

Materials developed for this study are available on request to the corresponding authors.

Data and Code Availability

Data collected and computer codes developed for this study are available on request to the corresponding authors.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cell culture

MDCK cells were from the RIKEN BioResource Center (no. RCB0995). Lenti-X 293T cells were purchased from Clontech (no. 632180, Mountain View, CA). These cells were maintained in D-MEM (no. 044-29765, Wako, Osaka, Japan) supplemented with 10% FBS (no. 172012-500ML, SIGMA, St. Louis, MO), 100 unit mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin (no. 26253-84, Nacalai Tesque, Kyoto, Japan) in a 5% CO_2 humidified incubator at 37°C.

Establishment of stable cell lines

For the establishment of EKAREV-NLS–expressing MDCK cells, a Tol2 transposon system was used. MDCK cells were co-transfected with pT2A-EKAREV-NLS and pCAGGS-T2TP encoding Tol2 transposase, and sorted by FACS as previously described (Kawabata and Matsuda, 2016; Sakurai et al., 2012). To establish MDCK cells stably expressing 2paRAF, pT2ADWpuro_2paRAF or pT2ADWpuro_2paRAFΔmScarlet-I were used for the Tol2 transposon system. For the generation of cells stably expressing Eevee-ROCK-NES and the other ectopic proteins, a lentiviral or retroviral system was employed. To prepare the lentivirus, pCSII-based lentiviral vector (Miyoshi et al., 1998) or lentiCRISPRv2 (Addgene Plasmid: no. 52961), psPAX2 (Addgene Plasmid: no. 12260), and pCMV-VSV-G-RSV-Rev were co-transfected into Lenti-X 293T cells using polyethylenimine (no. 24765-1, Polyscience Inc.,

693 Warrington, PA). To prepare the retrovirus, pCX4-based retroviral vector (Akagi et al., 2003)
694 or pSUPER (Oligoengine, Seattle, WA), pGP, and pCMV-VSV-G-RSV-Rev were co-
695 transfected into Lenti-X 293T cells. Stable cell lines of MDCK cells were selected and
696 maintained in media containing the following antibiotics: MDCK/EKAREV-NLS/2paRAF, 4
697 μg mL^{-1} puromycin (no. P-8833, SIGMA); MDCK/EKAREV-NLS/2paRAF/Golgi-7-mCherry,
698 4 μg mL^{-1} puromycin and 10 μg mL^{-1} blasticidin S (no. 029-18701, Wako); MDCK/Eevee-
699 ROCK-NES, 10 μg mL^{-1} blasticidin S; MDCK/Eevee-ROCK-NES/2paRAF, 10 μg mL^{-1}
700 blasticidin S and 4 μg mL^{-1} puromycin; MDCK/EKAREV-NLS/mCherry, 4 μg mL^{-1}
701 puromycin; MDCK/RA Rho GEF, 4 μg mL^{-1} puromycin and 10 μg mL^{-1} blasticidin S.
702
703 **CRISPR/Cas9-mediated KO cell lines**
704 For CRISPR/Cas9-mediated KO of dog *CTNNA1* (α-catenin), single guide RNAs (sgRNA)
705 targeting the exon were designed using the CRISPRdirect (Naito et al., 2015). The following
706 sequence was used for the sgRNA sequence: GTAGAAGATGTTCGAAAACA. Oligo DNAs
707 for the sgRNA were cloned into the lentiCRISPRv2 vector, and the sgRNA and Cas9 were
708 introduced into MDCK cells by lentiviral infection. The infected cells were selected with 4.0
709 μg mL^{-1} puromycin. After the selection, reduction in expression levels of the proteins was
710 confirmed by immunoblotting. Bulk cells were used for the experiments.
711
712 **shRNA-mediated KD cell line**
713 For shRNA-mediated KD of dog *RAC1* and *CDC42*, the DNA oligomers corresponding to the
714 shRNAs targeting the genes were subcloned into pSUPER vector. The following sequences
715 were used for shRNA target sequences: *RAC1*, GCCTTCGCACTCAATGCCAAG; *CDC42*,
716 GAACAAACAGAAGCCTATC. The shRNAs were introduced into MDCK cells by retroviral
717 infection. The infected cells were selected with 4.0 μg mL^{-1} puromycin. After the selection,
718 reduction in expression levels of the target proteins was confirmed by immunoblotting.
719
720 **METHOD DETAILS**
721 **Plasmids**
722 pT2A-EKAREV-NLS and plasmids for RA Rho GEF (pCX4puro-LDR, and pCX4bsr-3HA-
723 FKBP-p63RhoGEF-DH) were described previously (Kawabata and Matsuda, 2016; Komatsu
724 et al., 2011; Li et al., 2017; Urasaki et al., 2006; van Unen et al., 2015). pT2ADWpuro_2paRAF
725 (Addgene plasmid: no. 129653) encoding 2paCRY2-RAF1-P2A-CIBN-mScarlet-I-CAAX was

described previously (Kinjo et al., 2019). pT2ADWpuro_2paRAFΔmScarlet-I encoding 2paCRY2-RAF1-P2A-CIBN-CAAX was generated by PCR and subcloned into the pT2ADWpuro vector. The FRET biosensor for ROCK activity, Eevee-ROCK-NES, was described previously (Li et al., 2017) and subcloned into a pCSII vector. pCSII and pCMV-VSVG-RSV-Rev were kindly gifted from H. Miyoshi (RIKEN BioResource Center, Ibaraki, Japan). pGP was gifted from T.Akagi. psPAX2 was the kind gift of Didier Trono (Addgene plasmid: no. 12260). mCherry-Golgi-7 was a gift from Michael Davidson (Addgene plasmid: no. 55052) and the cassette was subcloned into a pCSIIbsr vector. pCAGGS-T2TP was a gift from Koichi Kawakami (National Institute of Genetics, Shizuoka, Japan).The cDNA of mCherry was subcloned into a pCSIIpuro vector to generate pCSIIpuro-mCherry.

Reagents and antibodies

The following reagents were used: trametinib (no. T-8123, LC Laboratories, Woburn, MA), Y-27632 (no. 253-00513, Wako, Osaka, Japan), PD153035, marimastat (no. SC-202223, Santa Cruz Biotechnology, Dallas, TX), rapamycin (no. R-5000, LC Laboratories), TPA (no. P-1680, LC Laboratories), Rhodamine Phalloidin (no. R415, Invitrogen, Carlsbad, CA, 1:40 dilution for immunofluorescence), EGF (no. E9644, SIGMA, St. Louis, MO).

The following primary and secondary antibodies were used for immunoblotting: Anti-EGFR rabbit antibody (no. 4267, Cell Signaling Technology, Danvers, MA, 1:1,000 dilution); anti-phospho-EGFR (Tyr1068) rabbit antibody (no. 3777, Cell Signaling Technology, 1:1,000 dilution); anti-RAF1 mouse antibody (no. 610152, BD Biosciences, Franklin Lakes, NJ, 1:1,000 dilution); anti-phospho-RAF1 (Ser338) rabbit antibody (no. 9427, Cell Signaling Technology, 1:1,000 dilution); anti-MEK1/2 rabbit antibody (no. 9122, Cell Signaling Technology, 1:1,000 dilution); anti-phospho-MEK1/2 (Ser217/221) rabbit antibody (no. 9121, Cell Signaling Technology, 1:1,000 dilution); anti-ERK1/2 mouse antibody (no. 610123, BD Biosciences, 1:2,000 dilution); anti-phospho-p44/42 MAPK (Erk1/2; Thr202/Tyr204) rabbit antibody (no. 9101, Cell Signaling Technology, 1:2,000 dilution); anti-β-Actin rabbit antibody (no. 4970, Cell Signaling Technology, 1:1,000 dilution); anti-Cdc42 mouse antibody (no. 610929, BD Biosciences, 1:1,000 dilution); anti-Rac1 mouse antibody (no. 610650, BD Biosciences, 1:1,000 dilution); anti-α-catenin mouse antibody (no. 610194, BD Biosciences, 1:1,000 dilution); IRDye 680-conjugated goat anti-mouse IgG antibody (no. 926-32220, LI-COR Biosciences, Lincoln, NE, 1:10,000 dilution); and IRDye 800CW goat anti-rabbit IgG antibody (no. 926-32211, LI-COR Biosciences, 1:10,000 dilution).

The following primary and secondary antibodies were used for immunofluorescence: anti-phospho-Myosin Light Chain 2 (Thr18/Ser19) rabbit antibody (no. 3674, Cell Signaling Technology, 1:50 dilution); anti-E-cadherin rabbit antibody (no. 3195, Cell Signaling Technology, 1:300 dilution); Alexa 647-conjugated goat anti-mouse IgG (H+L) antibody (no. A-21235, Thermo Fisher Scientific, Waltham, MA, 1:1,000 dilution); alexa 647-conjugated goat anti-rabbit IgG (H+L) antibody (no. A-21245, Thermo Fisher Scientific, 1:1,000 dilution); and alexa 568-conjugated goat anti-rabbit IgG (H+L) antibody (no. A-11036, Thermo Fisher Scientific, 1:1,000 dilution).

Confinement release assay

To confine the MDCK cell monolayer, a Culture-Insert 2 Well (no. 81176, ibidi, Martinsried, Germany) was placed on a glass-bottom dish coated with 0.3 mg mL^{-1} type I collagen (Nitta Gelatin, Osaka, Japan). MDCK cells (7×10^3 cell) were seeded in the Culture-Insert. 24 h after seeding, the Culture-Insert was removed, and the medium was replaced with Medium 199 (11043023; Life Technologies, Carlsbad, CA) supplemented with 10% FBS, 100 unit mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin. 30 min after the removal of the Culture-Insert, the cells were imaged with an epifluorescence microscope every 1 to 10 min.

Boundary assay

For the light-induced ERK activation experiment, MDCK/EKAREV-NLS/2paRAF cells were seeded in a well of a Culture-Insert 2 well placed on a 24 well glass bottom plate, and MDCK/EKAREV-NLS cells were seeded in the other well of the insert and outside of the insert. After 15 h incubation, the insert was removed, followed by further incubation for 36h, allowing the cells to fill the gap between the cell populations. The interface between cells with and without 2paRAF expression was imaged, and the cells were exposed to 438 nm blue LED light every 5 min for CRY2 activation.

For the drug-induced cell contraction experiment, a well of the insert was removed by cutting, and the remained insert with a well was placed on a 24 well glass-bottom plate. MDCK cells expressing RA Rho GEF were seeded in the well. After 6 h incubation, the insert was removed, and MDCK/EKAREV-NLS cells were plated in the 24 well glass-bottom plate. The interface between cells with and without RA Rho GEF expression was imaged, and dimethyl sulfoxide (DMSO; final 0.1%) or rapamycin (final 50 nM) was added into the medium for the Rho GEF activation. To determine the ERK wave directionality, heat maps of ERK activity

were obtained by interpolating the signals in regions between the nuclei of MDCK/EKAREV-NLS cells in the FRET/CFP ratio images. The heat maps of ERK activity were analyzed by particle image velocimetry (PIV) using a free Matlab-toolbox, MatPIV (Sveen, 2004), to calculate the ERK wave directionality. The size and overlap of the interrogation window was set to 349 μm and 75%, respectively. To determine the directionality of cell displacement, the Fiji TrackMate plugin (Jaqaman et al., 2008; Schindelin et al., 2012) was applied to the CFP fluorescence images for tracking each cell over 13-13.5 h after treatment with DMSO or rapamycin.

Cell stretch assay

For the cell stretch assay, MDCK cells (2×10^5 cell) were seeded on an elastic silicone chamber (no. STB-CH-04, STREX, Osaka, Japan) coated with 0.3 mg mL^{-1} type I collagen. After 24 h incubation, the MDCK cells on the stretch chamber were uniaxially stretched by 20-50% with a manual cell-stretching system (no. STB-100-04, STREX) on an epifluorescence microscope. The ERK activity 20 min after 20-50% stretch or that without stretch (0% stretch) was shown as violin plots by using a MATLAB code, Violin Plot (Hoffmann, 2020). Red and gray lines indicate median and mean values. For immunoblotting, stretched MDCK cells were lysed with SDS sample buffer containing 62.5 mM Tris-HCl (pH6.8), 12% glycerol, 2% SDS, 40 ng mL^{-1} bromophenol blue, and 5% 2-mercaptoethanol, followed by sonication with a Bioruptor UCD-200 (Cosmo Bio, Tokyo, Japan). After boiling at 95°C for 5 min, the samples were resolved by SDS-PAGE on SuperSep Ace 5-20% precast gels (Wako), and transferred to PVDF membranes (Merck Millipore, Billerica, MA). All antibodies were diluted in Odyssey blocking buffer (LI-COR Biosciences). Proteins were detected by an Odyssey Infrared Imaging System (LI-COR Biosciences).

Time-lapse imaging

FRET images were obtained and processed under essentially the same conditions and procedures as previously described (Aoki and Matsuda, 2009). Briefly, cells were imaged with an IX83 inverted microscope (Olympus, Tokyo, Japan) equipped with a UPlanFL-PH 10x/0.3 (Olympus), a UPlanSApo 20x/0.75 (Olympus), or a UPlanSApo 40x/0.95 objective lens (Olympus), a DOC CAM-HR CCD camera (Molecular Devices, Sunnyvale, CA), a Spectra-X light engine (Lumencor Inc., Beaverton, OR), an IX3-ZDC laser-based autofocusing system (Olympus), an electric XY stage (Sigma Koki, Tokyo, Japan), and a stage top incubator (Tokai

Hit, Fujinomiya, Japan). The filters and dichromatic mirrors used for time-lapse imaging were as follows: for FRET imaging, a 438/24 excitation filter (incorporated in the Spectra-X light engine), a FF458-Di02-25x36 (Semrock, Rochester, NY) dichromatic mirror, and FF01-483/32-25 (Semrock) and FF01-542/27-25 (Semrock) emission filter for CFP and FRET, respectively. For mCherry and mScarlet-I imaging, a 575/25 excitation filter, a glass dichromatic mirror (Olympus), and FF01-624/40-25 (Semrock) emission filters were used.

Immunofluorescence and confocal microscopy

MDCK cells were fixed with 4% paraformaldehyde in PBS for 15 min at room temperature, followed by permeabilization with 0.2% Triton X-100 in PBS for 5 min. The samples were then incubated with 1% BSA in PBS for 1 h at room temperature, followed by sequential incubation with primary and secondary antibodies diluted with 1% BSA in PBS overnight at 4°C (primary antibodies) or for 1 h at room temperature (secondary antibodies). Images were collected using a Fluoview FV1000 confocal microscope (Olympus) equipped with a UPlanSApo 60x/1.35 or a UPlanSApo 100x/1.40 objective lens (Olympus).

Traction force microscopy

Polyacrylamide gel substrates were prepared as previously described, with slight modifications (Aoki et al., 2017; Rodriguez-Franco et al., 2017). Briefly, glass-bottom dishes (IWAKI, Shizuoka, Japan) were treated with 2% acetic acid (WAKO) and 0.2% 3-(Trimethoxysilyl)propyl methacrylate (SIGMA) in 80% ethanol for 2 min. After the removal of the solution, the dishes were dried for 15 min. For 3 kPa gels, 5.5% acrylamide, 0.09% bisacrylamide, 0.05% ammonium persulfate, 0.05% N,N,N',N'-tetramethyl ethylenediamine, and 0.01% deep red fluorescent carboxylate-modified beads (0.2 μm diameter; F8810, Thermo Fisher Scientific) in PBS were prepared. 18 μL of the solution was put on the glass-bottom dishes and 18 mm glass cover slips (Matsunami, Osaka, Japan) were placed on top of them. After polymerization, the gels were covered with 2 mg mL^{-1} Sulfo-SANPAH (no. ab145610, abcam, Cambridge, UK) and activated by ultraviolet light for 5 min with an Ultraviolet Crosslinker CL-1000 apparatus (UVP, Upland, CA). The procedure was repeated again with 7 min UV irradiation. The gels were coated with 100 μm mL^{-1} type I collagen overnight at 4°C. Then, the gels were washed three times with PBS and incubated with culture medium for 1h. For traction force microscopy, the bead fluorescence was imaged with an IX83 inverted microscope equipped with a 632/22 excitation filter (incorporated in the Spectra-X light engine),

a glass dichromatic mirror (Olympus), and FF01-692/40-25 (Semrock) emission filter. A reference image was obtained after the removal of cells by trypsinization. Traction forces were computed by Fourier-transform traction microscopy as described previously (Trepat et al., 2009).

EGF stimulation assay

To evaluate the sensitivity of cells to EGFR stimulation, WT or α-catenin KO MDCK cells (2.5 × 10^3 cell) were seeded on a 96 well glass bottom plate coated with 0.3 mg mL^{-1} type I collagen. After 24 h incubation, the MDCK cells were treated with different dose of EGF (0.01, 0.1, 1, 3, 10, 100 ng mL^{-1}). The ERK activity 16 min after the addition of EGF was plotted as log(dose) response and the plots were fitted to a variable slope model by least squares fit method with GraphPad Prism 7 software.

Optogenetic ERK activation wave assay

To illuminate a defined rectangular region with blue light, transillumination light of a 100-W halogen lamp filtered with BA420-460 (Olympus) was covered by a homemade aperture mask containing a slit. The image of the slit was focused on the sample plane by the condenser lens. The width of the focused image of the illumination light was approximately 35 μm. The illuminated region was moved at 3 μm min^{-1} velocity, and the illumination was patterned with 400 μm spatial intervals at each time point. The procedure was applied to the migrating MDCK/EKAREV-NLS/2paRAF/Golgi-7-mCherry cells treated with 1 μM PD153035, an EGFR inhibitor, to suppress autonomous ERK activation waves.

Kymography

To obtain the kymographs of FRET/CFP ratios and x-strain rate, these values were averaged along the y-axis in a defined region of the images, providing an intensity line along the x-axis. The operation was repeated for the respective time points, and the intensity lines were stacked along the y-axis for all time points.

Mathematical modeling

A cellular Potts model for cell migration

We used a two-dimensional cellular Potts model (CPM), also known as a Glazier-Graner-Hogeweg model, widely used for multicellular dynamics (Balter et al., 2007; Krieg et al., 2008;

te Boekhorst et al., 2016; van Helvert et al., 2018). In the CPM, each cell morphology is represented as a cluster of square lattices identified with the identical index σ, denoting a cell; the lattice distribution mainly determines an energy of the system *H*, such that cell behavior depends on a balance of forces defined by the energy. The energy in our model is composed of minimal factors necessary to capture the two-dimensional multicellular dynamics, such as interfacial energy, cell area constraint, and active cell contraction as follows:

$$H = \sum_{\boldsymbol{r},\boldsymbol{r}'} J_{\tau(\sigma_{\boldsymbol{r}})\tau(\sigma_{\boldsymbol{r}'})}\left(1 - \delta_{\sigma_{\boldsymbol{r}}\sigma_{\boldsymbol{r}'}}\right) + \lambda_A \sum_{\sigma}(A_\sigma - A_0)^2 + H_{contraction}, \quad (1)$$

where each of $\boldsymbol{r}$ and $\boldsymbol{r}'$ represents a position of lattice site, τ is an attribute of the lattice, i.e., cell or medium, *J* is the interfacial energy between cell-cell or cell-medium, δ is the Kronecker delta, λ_A is the magnitude of resistance to cell deformation, A_σ is the current cell area, A_0 is the ideal cell area, and $H_{contraction}$ is a term for cell contraction.

The first term in Eq. (1) describes a strength of cell-cell adhesion. The energy to maintain cell-cell adhesion is determined by an energy of cell-cell adhesion J_{cc} relative to that of cell-medium adhesion J_{cm} and is expressed as $\gamma = J_{cm} - J_{cc}/2$ (Davies and Rideal, 1963; Glazier and Graner, 1993). We determined $J_{cm} = J_{cc} = 3$ in light of a balance with other parameters for imitating MDCK behaviors, and changed the value in J_{cm} for the change in γ. The second term represents cell elasticity, meaning that the cells attempt to retain the ideal area. We set λ_A as 0.02 and 1 when cells extend beyond and shrink below the ideal area, respectively, because of the unique material property of cells (Latorre et al., 2018; Trepat and Sahai, 2018). The ideal cell area was determined as A_0= 400 μm^2, by the use of actual cell images. The third term relates to the active cell contraction. In this model, individual cells are assigned unit vectors $\boldsymbol{p_\sigma}$ corresponding to the front-rear cell polarity, and the cell contraction occurs depending on the polarity level ω with the contraction strength λ_σ, a variable related to the ERK activity as shall be explained later. We define the contraction term as follows:

$$H_{contraction} = \sum_{\sigma} \sum_{l_\sigma} \lambda_\sigma \Phi, \quad (2)$$

and

$$\Phi = \begin{cases} 1 + 2\omega\pi^{-1}(\varphi_{l_\sigma} - \pi) & \text{if} \quad \varphi_{l_\sigma} > \pi(1 - (2\omega)^{-1}) \\ 0 & \text{if} \quad \varphi_{l_\sigma} \le \pi(1 - (2\omega)^{-1}) \end{cases}, \quad (3)$$

where l_σ represents the index of lattices composing the cell periphery, and $\varphi_{l_\sigma} \in [0, \pi]$ is an angle between $\boldsymbol{p_\sigma}$ and the vector connecting from the centroid of a cell to a peripheral lattice. Note that $H_{contraction} = \sum_\sigma \lambda_\sigma P_\sigma$, where P_σ is the perimeter of a cell when $\omega = 0$, meaning that cells shrink independent of the direction of polarity. By contrast, cell contraction is biased to the rear of the cell when ω becomes larger.

In the CPM, the system transition occurs stochastically by a lattice-based Monte Carlo method; that is, the labeled value of a randomly chosen lattice site $\sigma_{\boldsymbol{r}}$ is attempted to be replaced by a different labeled value of randomly-chosen adjacent lattice site $\sigma_{\boldsymbol{r}'}$. The transition occurs by evaluating a change in energy ΔH associated with its replacement. In the case of energy increase, i.e., $\Delta H > 0$, the index replacement occurs stochastically according to a Boltzmann acceptance function $exp(-\Delta H)$, while it deterministically occurs in the case of energy decrease, i.e., $\Delta H \leq 0$. For the details of CPM, see (Glazier and Graner, 1993; Graner and Glazier, 1992; Hirashima et al., 2017; Marée et al., 2007; Merks and Glazier, 2005; Scianna, 2015). We regard the number of trials for lattice replacement as a total number of pixel domains in simulations as a unit simulation step (USS); an update at each lattice site is attempted once per 1 USS on average. We then defined 100 USS as 1 Monte Carlo step, corresponding to 1 hour.

Dynamics of polarity orientation

Explicit rules that govern the dynamics of front-rear cell polarity have not been clear. Yet, earlier experimental studies have proposed that the front-rear polarity is oriented according to the tensile force on cell-cell junctions (Das et al., 2015; Hayer et al., 2016). In particular, it has been shown that Merlin localized at a cell-cell junction inhibits the activation of Rac1 when the tension is low. In contrast, with high tension by strong contraction of neighbor cells, Merlin is released into the cytoplasm, and Rac1 is locally activated (Das et al., 2015). Thus, orientation of the front-rear polarity in individual cells changes through physical interaction with neighboring cells over time. With this fact, we model the dynamics of cell polarity orientation ϑ_σ as a phenomenological coupling with cell displacement according to earlier theoretical studies (Hirashima et al., 2013; Notbohm et al., 2016; Peyret et al., 2019; Szabó et al., 2010a; Szabó et al., 2010b; Tlili et al., 2018):

$$\frac{d\vartheta_\sigma}{dt} = \mu \frac{v_\sigma}{\sqrt{A_\sigma}} \Delta\phi_\sigma, \tag{4}$$

where μ is the degree of alignment to polarity in neighbor cells, v_σ is the cell speed, and

$\Delta\phi_\sigma$ is the angle between cell velocity $\boldsymbol{v}_\sigma$ and the polarity vector $\boldsymbol{p}_\sigma$. $v_\sigma/\sqrt{A_\sigma}$ contributes to weighting of how much the neighboring cells affect reorientation of cell polarity and/or self-reinforcement for a persistent polarization.

The behavior of cell polarity according to Eq. (4) can vary. For example, consider a situation in which a cell A pulls an adjacent cell B in a direction opposite to the polarity within cell B, and the center position of cell B is moved toward cell A. When μ is larger, the polarity in cell B will re-orient to the direction in which cell B is pulled. Conversely, when μ is smaller, the polarity orientation in cell B will not change, and it tends to be persistent over time. In addition to values of μ, the magnitude of the cell displacement resulting from interactions between multiple neighboring cells affects multicellular alignment of polarity. We chose $\mu = 1$ for reproducing a proper polarity alignment.

ERK-mediated mechanochemical feedback

In the main text, we have shown experimentally that cell extension activates ERK, and that the ERK activation induces cell contraction. Here we incorporated this observation into the framework of CPM.

We define the cell areal strain as $\varepsilon_\sigma = A_\sigma/A_0 - 1$, and the dynamics of a normalized ERK activity level $[ERK]_\sigma$, bounded from -1 to 1, is represented as

$$\frac{d[ERK]_\sigma}{dt} = (\tanh(\alpha\varepsilon_\sigma) - [ERK]_\sigma)/\eta_E, \quad (5)$$

where α is a sensitivity parameter and η_E denotes a timescale of this dynamics. We set $\alpha = 3$ for a proper response to cell areal strains in simulations, and $\eta_E = 3$ min with measured data in Figure 1.

The dynamics of cell contraction strength λ_σ is defined as

$$\frac{d\lambda_\sigma}{dt} = (\lambda U([ERK]_\sigma - [ERK]^*) - \lambda_\sigma)/\eta_c, \quad (6)$$

where λ is a controlling parameter for conversion of ERK activation to contraction, $[ERK]^*$ denotes a threshold of ERK activation-induced contraction, and η_c denotes a timescale of this dynamics. $U(x)$ is a step function: $U(x) = 1$ for $x \geq 0$ and $U(x) = 0$ for $x < 0$. We chose $\lambda = 10$ and $[ERK]^* = 0.5$ since the ERK activation waves are reproduced with them.

We set $\eta_c = 30$ min, reflecting the response time delay observed in Figure 3C.

Simulations

Matching time

All dynamics on polarity orientation, ERK activity, and contraction (Eq. (4)-(6)) were calculated in their discretized form and were updated every USS. The cell velocity $\boldsymbol{v}_\sigma$ was also calculated by the change in the center of mass of cells per 1 USS. We ran the simulation until steps equivalent to 23 hours after a start of cell migration in the confinement release assay.

Confinement release assay, Boundary conditions, and Initial conditions.

In reference to a size of imaging windows, we set a pixel length of the simulation space as 2.5 μm × 2.5 μm, and the computer simulation was performed in a space with 1200 (horizontal) × 400 (vertical) pixels, corresponding to 3 mm × 1 mm. We placed a cluster of cells, arranged with 120 cells in the x-axis direction by 50 cells in the y-axis direction, in a simulation field with a reflecting boundary condition. There is a free space only along the left side of the cell cluster, but no free space along the top, bottom, or right side. We assumed that cells facing the free space were leader cells, so that leaders could be changeable throughout the simulation. At initiation, each cell area was set to the ideal area value, and the orientation of front-rear cell polarity in each cell was random except for the leader cells. Cell proliferation was not included in simulations because it seems to minimally affect the ERK activation wave propagation (Tlili et al., 2018).

Leader cells and follower cells

We defined the leader cells as cells facing a free space, corresponding to medium in the model, and follower cells as the others. The leader cells sense free spaces to migrate and communicate to the follower cells located behind the leader cells (Omelchenko et al., 2003; Yamaguchi et al., 2015). Thereby, we set the following two rules for the leader cells in polarity orientation and cell contraction. First, the cell polarity in the leader cells is persistently oriented towards the free space with a white Gaussian noise regardless of Eq. (4). Second, the leader cells keep generating polarized contractile force ($\omega = 1$) independent of cell extension-induced ERK activity (λ_σ =100) in Eq. (2) and (3) throughout the simulations.

Parameters

The default parameter values are shown in the column "Values" in Table S1. In the rightmost column, the range of the respective parameters used in Figure 7 is summarized (Table S1). Each parameter value was determined based on either measured data, observation, or a balance of other parameter values. Under an energy minimization framework, relative values of a parameter to values in other parameters should be significant if the system is not far from its equilibrium.

Additional comments on modeling and simulations

Wave propagation of cell velocities and/or mechanical stress during collective cell migration have been modeled in some earlier studies, and most of them adopt a continuum approach (Alert and Trepat, 2019; Banerjee et al., 2015; Notbohm et al., 2016; Tlili et al., 2018; Yabunaka and Marcq, 2017). They are constructed with a relatively simple assumption and include just a few parameters, which makes the analysis easier. In contrast, we chose a cell-based approach to express the collective cell behavior and modeled a system on a cellular mechanochemical feedback at tissue scale. Despite being complex and limited to numerical simulations, our model can recapitulate multiple unidirectional ERK activation waves in collective cell migration. Moreover, it simulates conditions under which the waves are not generated, and those are consistent with our experimental results. We believe that this approach contributes to further understanding of unique systems within and between cells, as well as refining our theoretical modeling of such systems. Additional numerical investigations on multicellular dynamics and ERK activation waves were beyond the scope of this study.

Repeatability of experiments

All experiments were performed on at least three independent cell culture preparations.

QUANTIFICATION AND STATISTICAL ANALYSIS

Quantification of ERK activity and cell deformation

For the analysis of the cell strain rate along an axis of collective cell migration (x-strain rate), PIV using MatPIV (Sveen, 2004) was applied to phase contrast time-lapse images to calculate velocity fields of cells. Velocity fields at time T were computed by the displacement between the images at $T-\Delta t$ and $T+\Delta t$ by using ‘single’ option for the method of pattern matching. The

size of the interrogation window was set to 29.1 μm, approximately corresponding to the typical cell size, and the window overlap was set to 50%. Here, we defined the x-strain rate as spatial derivative of velocity field along x-axis divided by the distance between the centers of two adjacent interrogation windows. Thus, the x-strain rate $\dot{\varepsilon}_{i,j}$ was calculated according to

$$\dot{\varepsilon}_{i,j} = \frac{v^x_{i,j} - v^x_{i+1,j}}{L}, \tag{7}$$

where $v^x_{i,j}$ is the *x* component of the velocity at spatial indices of resultant velocity vectors obtained by PIV analysis (*i, j*), and *L* is the distance between the windows (14.5μm). The value of x-strain rate is assigned at the center between the x-coordinate of two source velocity vectors. The obtained discrete data were processed by a cubic interpolation method to fill the gap.

To represent the FRET efficiency, FRET/CFP ratio images were generated after the background intensity was subtracted from the original fluorescence images in the CFP and FRET channel, respectively, using Metamorph software (Molecular Devices, Sunnyvale, CA). Then, the Fiji TrackMate plugin was applied to the CFP fluorescence images for tracking each cell. The time derivative of ERK activity (ERK activation rate) at time *t* for each cell was computed by the change in FRET/CFP ratio between *t*-Δ *t* and *t*+*Δt*. The obtained time-series data of the x-strain rate and the ERK activation rate were processed with a Savitzky-Golay filter to reduce the noise. The cross-correlation coefficient $r(\tau)$ between time-series data of x-strain rate $f(t)$ and ERK activation rate $g(t)$ was calculated as follows:

$$r(\tau) = \frac{\sum_t f(t+\tau)g(t)}{\sqrt{\sum_t f(t)^2}\sqrt{\sum_t g(t)^2}}, \tag{8}$$

where τ is the lag time.

To quantify the amplitude of x-strain rate oscillation, the time-series data were fitted to a multi-peak function as described previously (Muta et al., 2018).

Quantification of directedness

For quantification of directionality of Golgi positioning, ERK activation waves, and cell migration, we defined directedness as shown in Figure 5D. The reference direction is set to the forward direction for analysis of Golgi orientation and cell migration direction, and to the backward direction for that of ERK wave direction to match the signs of their directedness. To obtain the sample directions of the Golgi apparatus, the center of mass of the nucleus in each cell was determined with Fiji. Fluorescence images of Golgi apparatus markers were processed with a mean filter. The position that showed the highest intensity in the region within 6.8 μm

from the center of mass of the nucleus was defined as the position of the Golgi apparatus. The sample directions were determined by the direction of the Golgi apparatus relative to the center of mass of the nucleus. To determine the sample directions of ERK activation waves, heat maps of ERK activity were obtained by interpolating the signals in regions between the nuclei of MDCK/EKAREV-NLS cells in the FRET/CFP ratio images. The heat maps of ERK activity were analyzed by MatPIV with a 174 μm window size and a 75% window overlap. The directions of the calculated velocity vectors were obtained as the sample directions. For the determination of the sample directions of cell migration, each cell was tracked with a Fiji TrackMate plugin. The direction of cell displacement for 20 min was defined as the sample direction of cell migration for that individual cell.

Statistical analysis

Statistical analyses were performed with GraphPad Prism 7 software (GraphPad Software, San Diego, CA). No statistical analysis was used to predetermine the sample size. The sample sizes, statistical tests, and p-values are indicated in the figures and the figure legends. To compare two sets of data, paired or unpaired *t*-tests were used. To compare multiple sets of data, the samples were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. P-values of less than 0.05 were considered to be statistically significant in two-tailed tests, and were classified as follows: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, and n.s. (not significant, i.e., P ≥0.05).

Supplemental video titles and legends

Movie S1. ERK activation waves and cell deformation waves during collective cell migration, related to Figure 1

Time-lapse video of collectively migrating MDCK cells expressing EKAREV-NLS. Phase contrast images are shown at the top. The golden pseudo-color represents the FRET/CFP ratio indicating ERK activity (middle). Red and blue indicate positive and negative x-strain rate, respectively (bottom). The color scales correspond to those in Figure 1A. Time in hr:min.

Movie S2. Cell contraction upon optogenetic ERK activation, related to Figure 3

Time-lapse video of the boundary between confluent MDCK cells with and without 2paRAF expression. The upper frames represent the fluorescence of CIBN-mScarlet-I-CAAX, a component of 2paRAF. The lower images show differential interference contrast (DIC).
The blue light illumination started at 0 min and was repeated every 5 min. The cells were treated with DMSO (left), 200 nM trametinib (center), 10 μM Y-27632 (right) 30 min before the start of the imaging. Time in hr:min.

Movie S3. ROCK activity propagation during collective cell migration, related to Figure 3

First part: Time-lapse video of collectively migrating MDCK cells expressing a FRET biosensor for ROCK activity. The color represents the FRET/CFP ratio indicating ROCK activity and its scale corresponds to the one in Figure 3F. Second part shows migrating cells that were treated with 200 nM trametinib (left) and 100 μM Y-27632 (right) at 0 min. Time in hr:min.

Movie S4. Traction force microscopy with optogenetic ERK activation, related to Figure 3

Time-lapse traction force microscopy at the boundary between confluent MDCK cells with and without 2paRAF expression. Upper frames show differential interference contrast (DIC). Middle frames represent the fluorescence of CIBN-mScarlet-I-CAAX, a component of 2paRAF. Lower frames show traction forces. The color scale corresponds to the one in Figure 3L. The blue light illumination started at 0 min and was repeated every 5 min. Time in hr:min.

Movie S5. Cell contraction triggers ERK activation waves, related to Figure 4

Time-lapse videos of the boundary between confluent MDCK cells with and without

rapamycin-activatable Rho GEF (RA Rho GEF). The approximate boundary is indicated as dotted lines at the initial time frame. The color represents the FRET/CFP ratio indicating ERK activity, and the scale corresponds to the one in Figure 4B. The cells were treated with DMSO (left) or 50 μM rapamycin (right) at 0 min to induce contraction of the RA Rho GEF-expressing cells. Time in hr:min.

Movie S6. Intercellular mechanical linkage is required for ERK activation waves, related to Figure 4

Time-lapse videos of collectively migrating WT (left) and α-catenin KO (right) MDCK cells. The color represents the FRET/CFP ratio indicating ERK activity, as shown in Figure 4, and its upper and lower value is 1.8 and 0.85. Time in hr:min.

Movie S7. Requirement of Cdc42 and Rac1 for unidirectional ERK activation waves, related to Figure 5

Time-lapse videos of collectively migrating WT (upper), shCdc42 (middle), and shRac1 (lower)-expressing MDCK cells. The color represents the FRET/CFP ratio indicating ERK activity, as shown in Figure 1, and its upper and lower value is 1.8 and 0.85 for each of the videos. Time in hr:min.

Movie S8. Effect of constitutive ERK activation and inhibition on collective cell migration, related to Figure 6

Time-lapse videos of collectively migrating MDCK cells. The color represents the FRET/CFP ratio indicating ERK activity, and the scale corresponds to the one in Figure S4D and S4E. The cells were treated with DMSO (upper), 200 nM trametinib (middle), and 10 nM TPA (bottom) at 0 min. Time in hr:min.

Movie S9. Optogenetic ERK activation waves and front-rear cell polarization, related to Figure 6

Time-lapse videos of migrating MDCK cells without (upper) and with (lower) Optogenetic ERK activation waves. Green and red indicate nuclei (EKAREV-NLS) and Golgi apparatus (Golgi-7-mCherry), respectively. Cyan represents regions illuminated with blue light. The cells were pre-treated with 1 μM PD153035, an EGFR inhibitor, to suppress autonomous ERK activation waves. Time in hr:min.

Movie S10. in silico ERK activation waves, related to Figure 7

Simulations for ERK activation waves in migrating cells with (ω=1, upper frame) and without (ω=0, lower frame) cell polarity. The color represents the ERK activity and its scale corresponds to the one in Figure 7C.

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