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RESEARCH ARTICLE

E-cadherin plays an essential role in collective directional migration of large epithelial sheets

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Abstract In wound healing and development, large epithelial sheets migrate collectively, in defined directions, and maintain tight cell–cell adhesion. This type of movement ensures an essential function of epithelia, a barrier, which is lost when cells lose connection and move in isolation. Unless wounded, epithelial sheets in cultures normally do not have overall directional migration. Cell migration is mostly studied when cells are in isolation and in the absence of mature cell–cell adhesion; the mechanisms of the migration of epithelial sheets are less well understood. We used small electric fields (EFs) as a directional cue to instigate and guide migration of epithelial sheets. Significantly, cells in monolayer migrated far more efficiently and directionally than cells in isolation or smaller cell clusters. We demonstrated for the first time the group size-dependent directional migratory response in several types of epithelial cells. Gap junctions made a minimal contribution to the directional collective migration. Breaking down calcium-dependent cell–cell adhesion significantly reduced directional sheet migration. Furthermore, E-cadherin blocking antibodies abolished migration of cell sheets. Traction force analysis revealed an important role of forces that cells in the leading rows exert on the substratum. With EF, the traction forces of the leading edge cells coordinated in directional re-orientation. Our study thus identifies a novel mechanism—E-cadherin dependence and coordinated traction forces of leading cells in collective directional migration of large epithelial sheets.

Keywords Collective cell migration · Electric fields · Galvanotaxis/electrotaxis · E-cadherin · Cell–cell adhesion · Traction force

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Introduction

Epithelial cells migrate collectively as a coherent sheet to heal wounds. In multilayered corneal epithelium and rat skin, the stratified epithelia migrate en masse following injury [1–3]. This collective migration, maintaining intercellular connection and relative positions, is conserved in wound healing of many types of epithelia, such as cornea, skin, respiratory and digestive epithelia, and endothelium [4–6]. Collective migration has also been recognized as an important mode during embryonic development and cancer [7–10]. Clusters of epithelial cells in culture do not have an

overall directional migration unless wounded. Wounding an epithelial monolayer induces directional migration of a cell sheet, which may involve many directional signals [4, 10].

How epithelial sheets migrate directionally and change their migration direction is not fully understood. In vertebrate epithelia, individual cells form sheets by four types of cell junctions: gap junctions, adherent junctions, tight junctions, and desmosomes. For adjacent epithelial cells, adherens junctions provide strong mechanical attachments through E-cadherin molecules, which are transmembrane proteins and interact with catenins. Catenins regulate actin organization at the sites of cell–cell adhesion. Both actin filaments and catenins play critical roles in wound healing and mechanotransduction [11, 12]. For a single cell, traction forces were generated by a gripping of the actin network to the substrate at the leading edge of a cell, while at the trailing edge the forces were a result of the actin network's slipping over the substrate [13, 14]. For epithelial sheets, traction forces are mainly generated at the edges and cell–cell junctions [15]. This different pattern of traction force distribution may be a result of the mechanical communication between cells and play a role in collective cell migration of epithelial sheets [16, 17]. However, it is unknown whether E-cadherin-dependent cell–cell adhesion plays a role or whether traction forces become coordinated in a defined direction during directional collective cell migration in an electric field (EF).

In the current study, we instigated directional migration of epithelial sheets in a uniform and well-controlled way by application of low level EFs, which mimic the endogenous EFs at wounds [18–23]. We found that, consistent in five different types of epithelial cells, cell sheets, in a size-dependent manner, respond significantly better than the same type of cells in isolation. E-cadherin-mediated adherens junctions are essential for the directional migration of cell sheets, while gap junctions only made a minimal contribution. The leading edges of cell sheets in an EF displayed significant orientated traction forces, which re-orientated when the field polarity was reversed and the cell sheets migrated in a new direction.

Materials and methods

Cell cultures

Madin-Darby canine kidney (MDCK I) and normal rat kidney (NRK) cells were obtained from the German Cell Bank DSMZ. MDCK cells were cultured in minimal essential medium (MEM, Gibco) with Earle's salts supplemented with 10% fetal bovine serum (FBS; Gibco), 2 mM L-glutamine solution (Gibco), 100 U/ml penicillin (Gibco) and 100 μg/ml streptomycin (Gibco) at 37°C, 5% CO_2, and 90% humidity. NRK cells were kept in DMEM (4.5 g/l glucose) supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. Primary corneal epithelial cells and tracheal epithelial cells were cultured as described [24, 25]. Fresh bovine eyes were obtained from a local abattoir and fresh trachea from the California National Primate Research Center. The use of necropsy samples was approved by the University of California, Davis, Institutional Animal Use and Care Committee. Cells were seeded at different concentrations to yield cultures where cells are in isolation, or a monolayer of cells, or sheets (groups) of cells of different sizes.

Electric field stimulation

Methods of applying EFs have been described previously [26]. Cells in the electrotaxis chamber were maintained in a CO_2-independent culture medium (Gibco) plus 10% FBS during field exposure. Steady EFs were applied for indicated period of time. Time-lapse videos were acquired and analyzed using MetaMorph 7.7 software (Molecular Devices) and Image J (NIH imaging software).

Quantification of cell behavior

Directional cell migration was quantified as published [27]. Directedness (cos θ) is used to quantify how directionally cells moved, where θ is the angle between the field vector and the cell migration direction. Cell migration speed was quantified as trajectory speed, which is the total length of the migration trajectory of a cell (T_t) divided by the given period of time. The coefficient of movement efficiency (CME) was quantified as T_d/T_t, where T_d refers to the displacement of a cell (the straight-line distance between the start and end positions). The CME would be equal to 1 when cells move persistently along a straight line in a given direction. The MDCK II cell monolayer migration was also analyzed by the particle image velocimetry (PIV) method (URAPIV). The PIV analysis quantifies the movement of objects (cells) and the analysis settings (the interrogation window size, the spacing, etc.) were chosen based on the cell size and speed to optimize the computation accuracy.

Gap junction blockade, cell–cell adhesion disruption, and E-cadherin blockade

Gap junctions in the MDCK I monolayer were blocked by pretreating with 50 μM Oleamide for 50 min. Successful

blockade was confirmed by scrape dye loading [28]. After the drug pretreatment, Lucifer yellow CH (0.05% dye in PBS; Sigma) was loaded by scraping cells in the monolayer with a sharp knife. The dye solution was left in the dish for 90 s and discarded, and the dish was washed with PBS. Dye transfer was examined under an inverted epifluorescence microscope (Zeiss Observer Z1).

Phorbol myristate acetate (PMA; 100 nM) was used to disrupt cell–cell adhesion in monolayer. Monolayer cultures were treated for 30 min, then exposed to an EF of 200 mV/mm for 6 h in the presence of the inhibitor. Calcium chelation was used to disrupt cell–cell adhesion of monolayers. Calcium chelator ethylene glycol tetraacetic acid (EGTA, 4 mM) was added to the culture medium for 10 min. The cells were then exposed to an EF for 6 h in calcium-free medium (Sigma) with 10% FBS and 1 mM EGTA. E-cadherin was blocked with anti-E-cadherin antibody DECMA-1 (Sigma). Cells were first treated with 4 mM EGTA for 10 min, then returned to the normal growth medium containing 50 μg/ml DECMA-1 for 2 h, and subsequentially exposed to an EF in the presence of the antibody.

Traction force measurement

Cellular traction forces were quantified using a force sensing substrate as described previously (Supplemental Figure S1a) [29]. Briefly, the silicon master containing micron-sized pillars (2 μm diameter, 6 μm height, and 4 μm pitch; Advanced MEMS, Berkeley, CA, USA) was used as a mold to fabricate PDMS-based pillars (Supplemental Figure S1a and 1b). The pillar deflection was measured using bright-field images and a custom macro written in ImageJ. Traction force (F) exerted by migrating cells is proportional to the pillar deflection and pillar stiffness (23.2 nN/μm). To facilitate cell adhesion onto the pillar array, the surface of pillars was coated with fibronectin (Supplemental Figure S1c), and GFP-tagged integrin α5 expressing MDCK cells were seeded onto the substrates. The pillar substrate-containing Petri dish was assembled into an EF chamber (Supplemental Figure S1d, e). The average traction force was calculated based on the forces at boundary of cell cluster (leading and trailing edges) or under cell clusters (middle), and the directionality of traction force was calculated relative to the direction of EF.

Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was done using the unpaired, two-tailed Student's t test.

Results

Monolayer epithelial cells responded to weak applied EFs, while cells in isolation did not

We first examined electrotaxis of epithelial monolayer (epithelial sheets of at least 1 cm × 0.8 cm in size) in MDCK II cells. In an EF of 200 mV/mm, monolayer cells migrated almost perfectly directionally, as demonstrated by single cell tracking analysis and the PIV analysis (Fig. 1a–c; Supplemental Figure S2). In contrast, cells in isolation in a field of the same strength did not show any directional migration (Fig. 1d–f; Supplemental Video S1). In an EF, cells in the monolayer migrated more efficiently with a significantly higher coefficient of movement efficiency (CME) (Supplemental Figure S3a).

We then tested the voltage dependence of cell sheet migration. Significant directional migration of cells in the monolayer was observed in EFs of 50 and 100 mV/mm (Fig. 1j). In an EF of 50 mV/mm which is likely to exist in vivo [19], cells in the monolayer showed significant electrotaxis towards the anode. In contrast, isolated cells in a field of the same strength displayed random migration.

We next determined how the group size of epithelial sheets affected collective electrotaxis. When no EF was applied, cells within sheets of different sizes migrated randomly and, for each cell sheet, there was no overall migration (Supplemental Video S2). In an EF, the bigger the cell group, the more directionally the cells migrated towards the anode (Fig. 1g–i; Supplemental Video S2). Quantitative analysis showed that epithelial sheets consisting of more than 10 cells started to show a significantly higher directedness value compared to the no EF control. The directedness rose with the increase of group size (Fig. 1k). Therefore, EF-guided directional collective cell migration depended on the size of the cell group or sheet.

Better collective electrotaxis is a fundamental characteristic of various epithelial cells

Different cell types migrate in opposite directions in an EF. We next tested collective electrotaxis of cell types that have anode- or cathode-directed cell migration. MDCK I cells in a monolayer culture displayed robust electrotaxis to the anode. As with MDCKII, MDCK I cells in isolation did not respond to a field of the same strength (Fig. 2a, b, g; Supplemental Video S3). NRK cells collectively or in isolation both showed directional migration to the anode in an EF. Cells in monolayer culture demonstrated significantly stronger electrotaxis with a higher directedness value than cells in isolation (Fig. 2c, d, g; Supplemental Video S3). For both types of cells, significantly higher

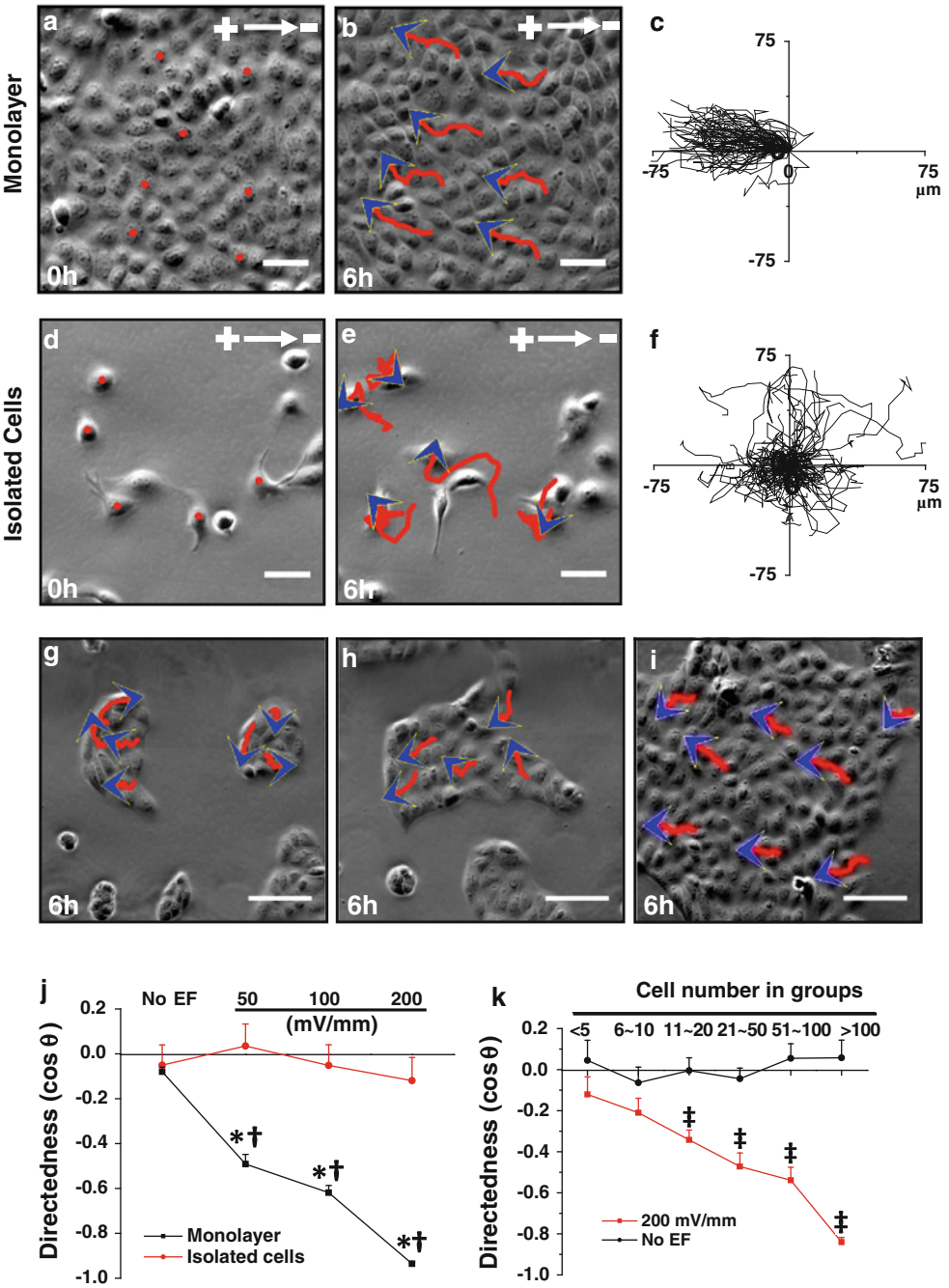


Fig. 1 Robust electrotaxis in monolayer, not in isolation. **a–c** MDCK II cells in a sheet showed robust collective electrotaxis in an EF of 200 mV/mm for 6 h. *Red lines* with *blue arrowheads* represent migration paths and direction. **c** Cell migration trajectories with starting positions placed at the origin. **d–f** In a field of the same strength, isolated MDCK II cells did not show electrotaxis. **g–i** Collective electrotaxis depends on the size of cell sheets. **g** MDCK II cells in small sheets (~20 cells) showed little directional migration in an EF. **h** Cells in a medium-sized sheet (~50 cells) migrated to the anode. **i** Cells in a large sheet (>100 cells) displayed much more obvious electrotaxis. **j** MDCK II cells showed electrotaxis when in monolayer, but not in isolation in EFs of different voltages. **k** Migration directedness versus the size of cell sheets (EF = 200 mV/mm for 6 h). Data are from at least 100 cells from 3 independent experiments and shown as mean ± SEM. $^{*}p < 0.01$ compared with no EF control of cells in monolayer; $^{\dagger}p < 0.01$ compared with isolated cells; $^{\ddagger}p < 0.01$ compared with no EF control of cell sheets of the same size. *Scale bars* (**a**, **b**, **d**, **e**) 50 μm, (**g–i**) 100 μm. See Supplementary Video S1 and S2

CME was found for the cells in monolayer (Supplemental Figure S3b and S3c).

We further confirmed enhanced collective electrotaxis in two types of primary cultures of epithelial cells which migrated to the cathode in an EF. The first type of cells was derived from monkey trachea. Exposed to an EF of 200 mV/mm for only 5 min, cells in monolayer culture displayed a directedness value nearly two times higher than the value measured for cells in isolation (Fig. 2g). After application of the EF for 30 min, both isolated cells and cells in a monolayer culture displayed very high directedness values, while cells in monolayer had a faster speed and higher migration efficiency (Fig. 2e, f; Supplemental Figure S3d and Video S3).

The second type of cells was primary cultured bovine corneal epithelial cells (BCECs). In an EF of 50 or 100 mV/mm, cells in monolayer migrated more directionally to the cathode, with higher directedness values

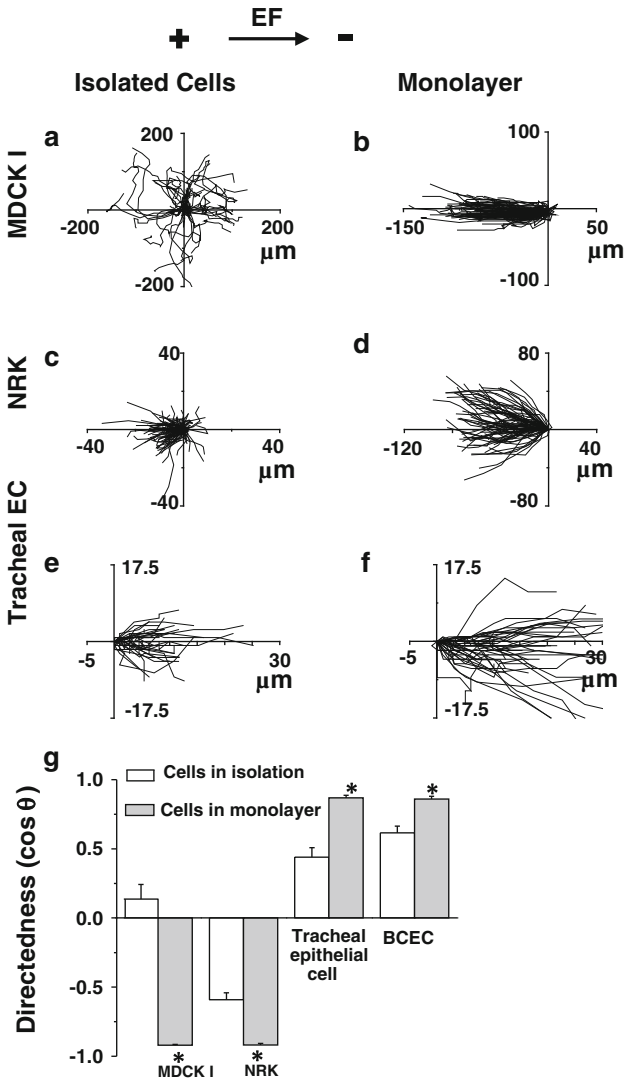


Fig. 2 Enhanced collective electrotaxis in epithelial sheets in both cathode and anode migrating cells. **a**, **b** Migration tracks of MDCK I cells in isolation and in monolayer in an EF of 200 mV/mm for 6 h. **c**, **d** Migration tracks of NRK cells in isolation and in monolayer in an EF of 200 mV/mm for 2 h. **e**, **f** Migration tracks of tracheal epithelial cells in isolation and in monolayer in an EF of 200 mV/mm for 30 min. **g** Consistently significantly enhanced electrotaxis of cells in monolayer over cells in isolation. MDCK I cells were exposed to 200 mV/mm for 6 h; NRK cells were exposed to 200 mV/mm for 2 h; tracheal epithelial cells were exposed to 200 mV/mm for 5 min; and BCECs were exposed to 50 mV/mm for 20 min. Data are from at least 100 cells from 3 independent experiments and shown as mean ± SEM. $*p < 0.01$ compared with isolated cells. See Supplementary Video S3

than cells in isolation. Cells in monolayer also displayed higher migration efficiency than cells in isolation (Fig. 2g; Supplemental Figure S4 and Video S3). Thus, significantly better electrotaxis of cell sheet compared to isolated cells was found in both cathode- and anode-directed cell types.

Gap junction contributed weakly to the collective electrotaxis

We then blocked gap junctions to test its role in electrotaxis. It was suggested that cells coupled by gap junctions might have increased sensitivity to respond to an applied EF [30]. Functional gap junctions in MDCK I monolayer were confirmed with dye scrape loading assay. Oleamide, a gap junction blocker (50 µM for 50 min) effectively prevented transfer of Lucifer yellow dye compared to the control group (Supplemental Figure S5a, b). Cells treated with oleamide still showed significant directional migration. The directedness value was decreased by 6.4% ($p < 0.001$) (Supplemental Figure S5c–e) and the migration speed was decreased by 16.9% ($p < 0.001$) (Supplemental Figure S5f). Thus, gap junction communication only contributes to a limited extent to the directional collective electrotaxis.

Breakdown of cell–cell junction significantly decreased collective electrotaxis

To test whether cell–cell adhesion was involved in EF-guiding collective cell migration, we used phorbol myristate acetate (PMA) to cause disintegration of adhesion junctions in monolayer cultures [31, 32]. Treatment with 100 nM PMA for 30 min induced loosening of the cell–cell adhesion. Both cell migration directionality and rate were significantly reduced (Fig. 3a, b). The directedness value dropped significantly ($p < 0.001$) (Fig. 3c) and the migration speed was decreased by 32% ($p < 0.001$) (Fig. 3d).

E-cadherin was required for collective electrotaxis

To further confirm the requirement of cell–cell adhesion in collective electrotaxis, we depleted extracellular Ca^{2+}. We replaced the normal culture medium with Ca^{2+}-free medium with EGTA (4 mM). Within 10 min, the cell–cell junctions became phase bright, and gaps between cells appeared and enlarged (compare Fig. 4a, b with Fig. 4d, e).

Depletion of extracellular Ca^{2+} resulted in significant decrease of electrotaxis (Fig. 4c, f and m), while showing little influence on cell motility and migration rate (Fig. 4n; Supplemental Video S4). The electrotaxis was completely rescued if cells were returned to normal culture medium for 2 h and then exposed to EFs (Fig. 4g–i, m; Supplemental Video S4).

To identify adhesion molecules that underlie the collective electrotaxis, we chose a specific E-cadherin antibody DECMA-1 [33] to block the E-cadherin. DECMA-1 was added to the rescuing medium (culture medium with Ca^{2+}). Addition of DECMA-1 prevented recovery of

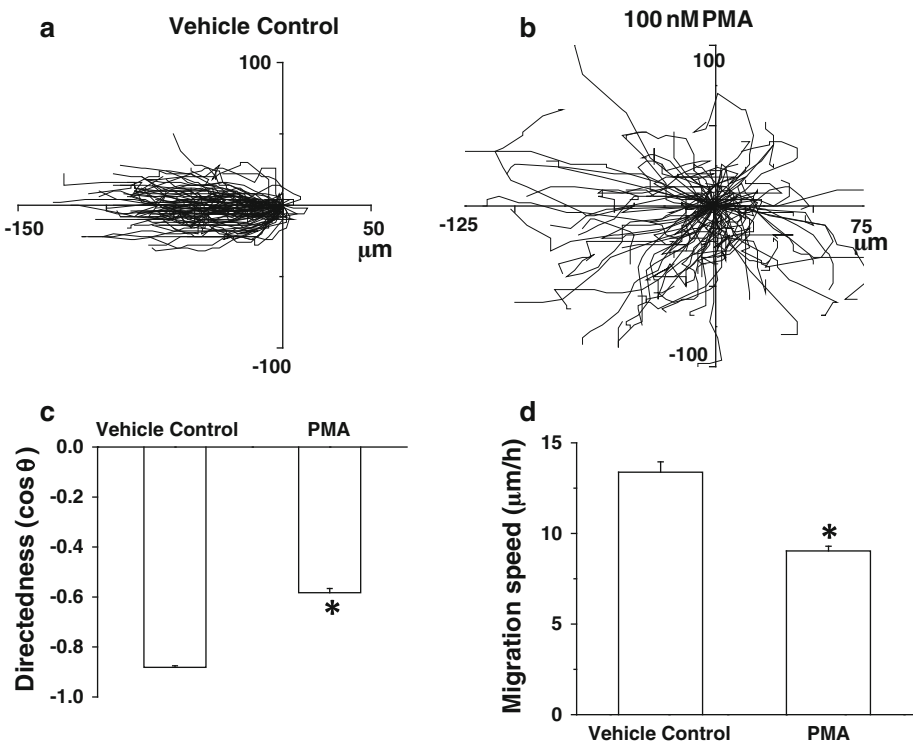


Fig. 3 Breakdown of cell–cell junction significantly decreases collective electrotaxis. **a** MDCK I cells migrated directionally to the anode. **b** Electrotaxis of the cells was significantly decreased in the presence of 100 nM PMA. **c**, **d** Effects of PMA on migration directedness and migration speed. EF = 200 mV/mm for 6 h. Data are from at least 100 cells from 3 independent experiments and shown as mean ± SEM. $^*p < 0.01$ compared with vehicle control. *Scale bars* 50 μm

the electrotaxis, and almost completely abolished the electrotaxis (Fig. 4j–m; Supplemental Video S4). The migration speed showed a significant increase ($p = 0.013$) (Fig. 4n). Taken together, these observations suggested that extracellular Ca^{2+} and cell–cell adhesion are required for the collective migration, and that the E-cadherin function mediates the response.

Re-orientation of traction force with respect to an applied EF

Finally, we focused on analysis of traction forces of isolated cells versus cell groups in an applied EF, because adherens junctions form a strong cell–cell mechanical connection and directly regulate the actin cytoskeleton in the epithelial sheets. Similar to migrating cells on a culture dish, cells in isolation on force-sensitive pillar arrays did not show significant electrotaxis. In both the absence and presence of an EF, the traction forces of an isolated single cell were mainly concentrated on the cell peripheral, with force vectors pointing toward the center of the cell (Figure S6a). Average directionality values of traction force were close to 0 (Figure S6b), which indicated that the traction forces of isolated cells had no specific directionality. The average force magnitude of isolated cells did not change regardless of EF stimulation (Figure S6c).

In an EF, traction force analysis of cell colonies revealed significant "leading" cell forces. In the absence of an EF, the traction forces at colony edges generally oriented toward the center of colonies (Fig. 5a). In the presence of an EF (2–4 h), cells migrated directionally towards the anode, which was consistent with electrotaxis of MDCK cells on a solid surface (see Fig. 1). At the cell colony edge facing the anode (+ in lower edge of cell sheet in Fig. 5a), the direction of traction forces aligned towards the cathode, as indicated by the cosine values approaching −1 ($p < 0.05$ when compared with no EF control; Fig. 5b). While, at the cathode-facing cell edge or the middle part of the cell colony, the force directionality showed little change (Fig. 5b). Significantly, when the EF vector was reversed (at hour 4), traction forces at the upper edge, which became the new leading edge, showed significant orientation towards the new cathode, while the force directionality at the lower edge returned to the baseline level (similar to that of the no EF condition) (Fig. 5a, b; Supplemental Video S5). The average forces at both colony edges were significantly higher than that of the middle area of a group (Fig. 5c). Those results indicate that orientation of traction forces at the leading edge of a cell sheet in an EF "lead" the "sheet" and play an important role in collective electrotaxis of the whole group.

Discussion

In the present study, we found that epithelial cells collectively respond to an EF significantly better—more directionally and efficiently—than cells in isolation. We

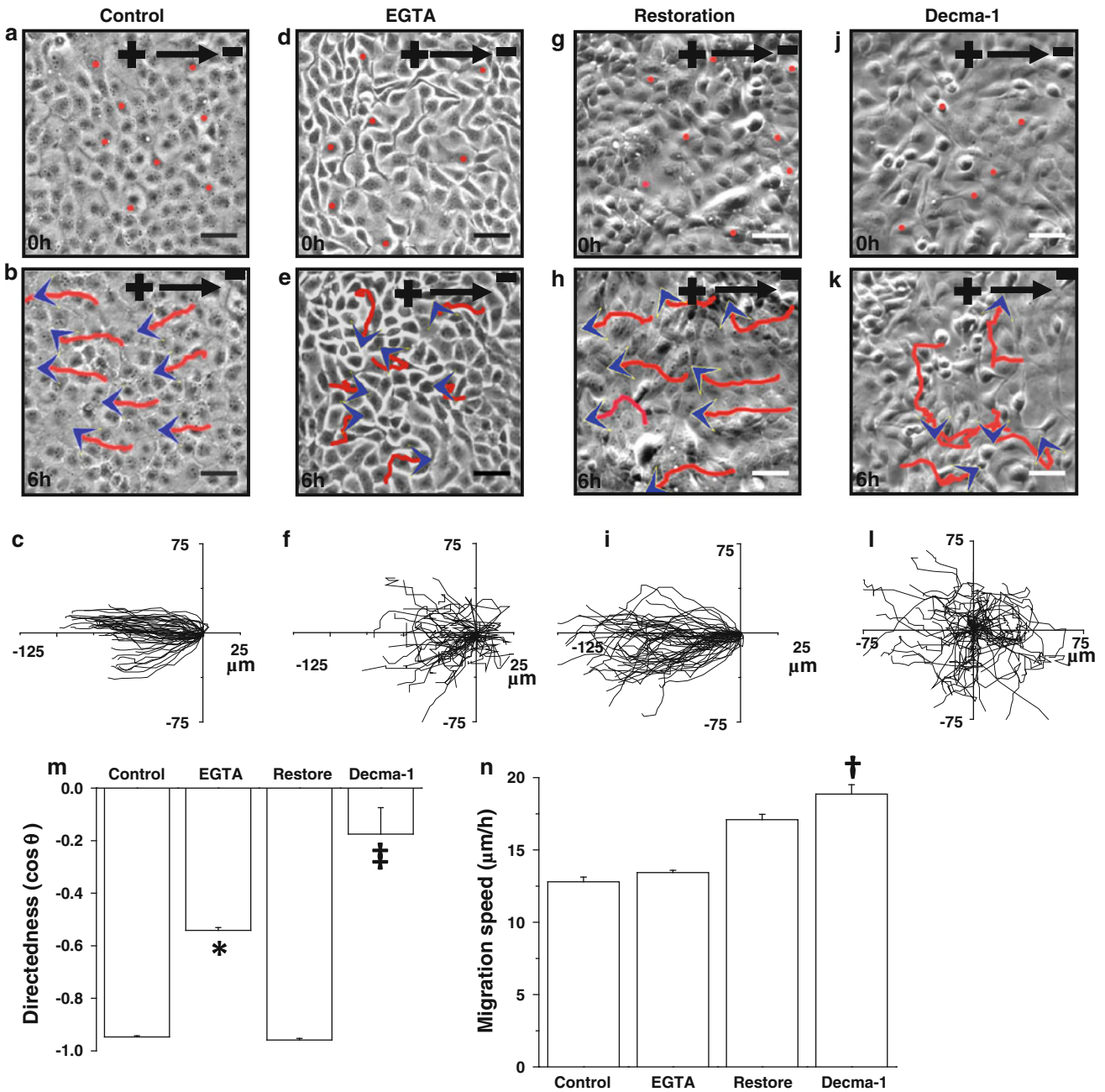
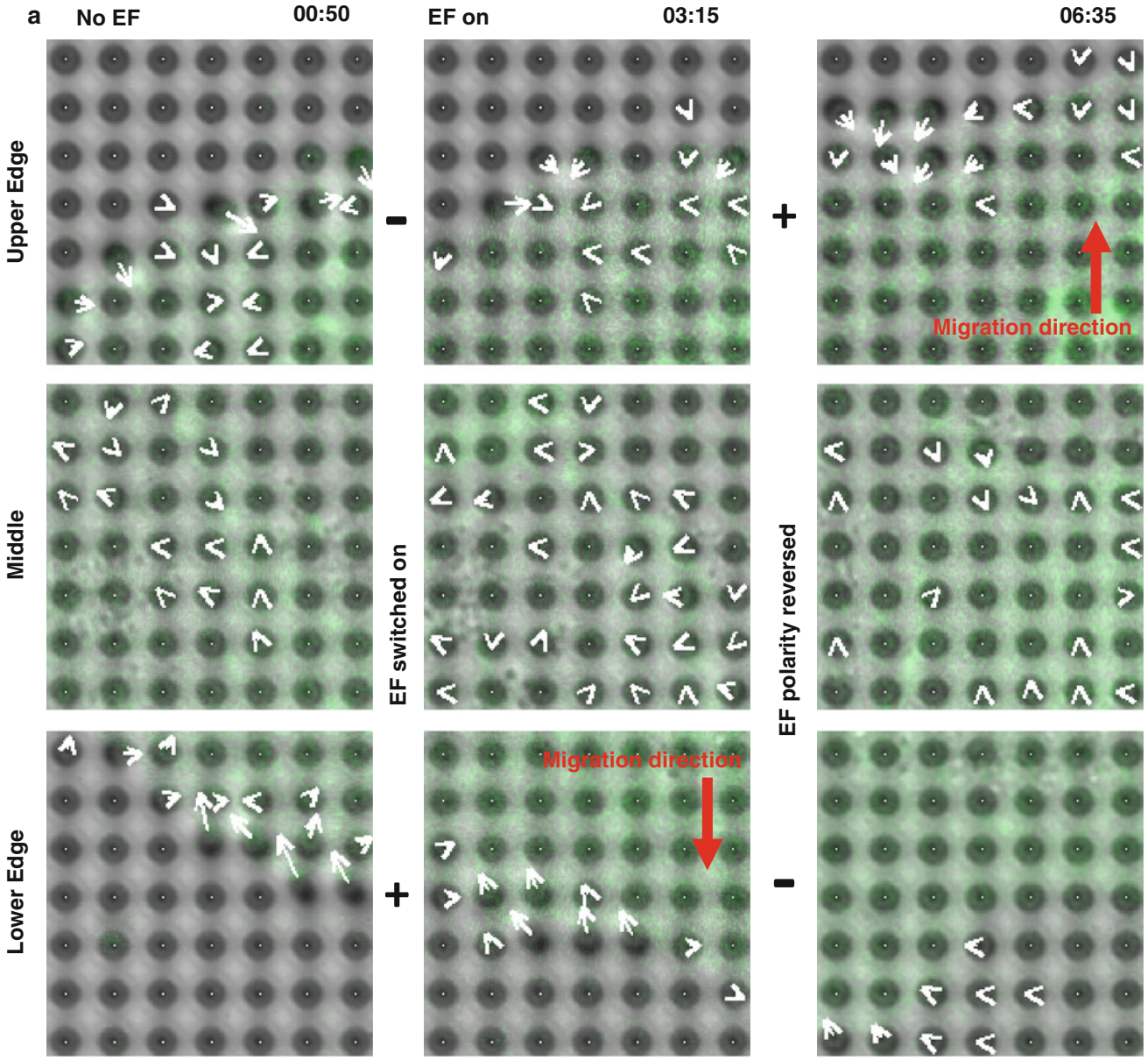
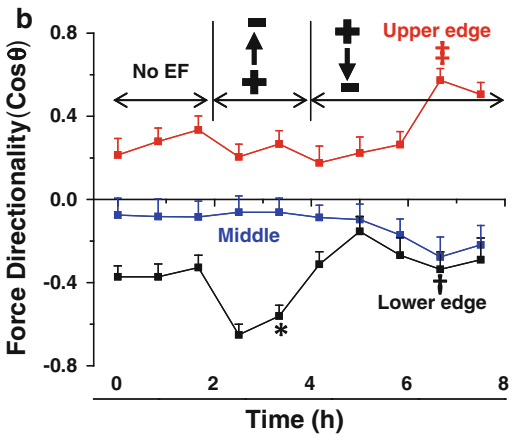


Fig. 4 E-cadherin-mediated cell–cell adhesion is essential for collective electrotaxis. **a–c** MDCK I cells in a monolayer culture migrated directionally to the anode. **d–f** After pretreatment with 4 mM EGTA for 10 min, directedness values decreased significantly in an EF in the continuous presence of 1 mM EGTA in calcium-free medium. **g–i** Restoration of the extracellular Ca^{2+} level completely restored the electrotaxis response. The extracellular Ca^{2+} level was restored by replacing Ca^{2+} free medium with normal growth medium containing calcium for 2 h following pretreatment in 4 mM EGTA for 10 min. **j–l** Blocking the E-cadherin with DECMA-1 (E-cadherin antibody; 50 µg/ml) completed blocked the recovery of the directedness. **m**, **n** Migratory directedness and speed of MDCK I cells in monolayer culture under different treatments as indicated. EF = 200 mV/mm for 6 h. The data are shown as mean ± SEM. $^{*}p < 0.01$ compared with control group; $^{\dagger}p < 0.05$, $^{\ddagger}p < 0.01$, compared with restoration group, respectively. *Scale bars* 50 µm. See Supplementary Video S4

showed for the first time that the larger the cell sheets, the more efficient the electrotaxis, especially at low physiological field strength. The E-cadherin-mediated adherent junction is essential for the collective electrotaxis, while gap junction communication appears to contribute less than 10% to the migration directionality of big cell sheets. Traction forces at the leading edge cells respond and re-orientate, thus "lead" the sheet collective migration.


a
No EF
00:50
EF on
03:15
06:35
Upper Edge
Middle
Lower Edge
EF switched on
EF polarity reversed
Migration direction
Migration direction


b
Force Directionality (Cosθ)
0.8
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-0.4
-0.8
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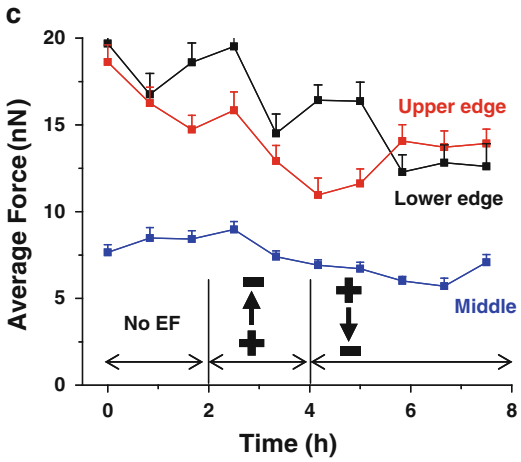
Time (h)


c
Average Force (nN)
20
15
10
5
0
Upper edge
Lower edge
Middle
No EF
0
2
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6
8
Time (h)

◀ **Fig. 5** The leading edge cells re-orientate and increase traction forces to "lead" the cell sheet. **a** Traction forces of cell groups at the upper edge, middle area and lower edge. *White arrowhead* and its length indicate the direction and magnitude of traction forces, respectively. Time in hh:mm. EF = 200 mV/mm. Anode is on the *lower side* from 2 to 4 h, and on the *upper side* from 4 to 8 h. **b** Traction forces displayed significant orientation at both the upper edge and lower edge when it acted as the leading edge of the cell group, while the forces were randomly orientated in the middle area of the group during the experiment. $^{*}p < 0.05$ when compared with that of lower edge at 1 h; $^{\dagger}p < 0.05$ when compared with that of lower edge at 3 h; $^{\ddagger}p < 0.01$ when compared with that of upper edge at 1 or 3 h. **c** The average forces at both edges were significantly higher than that of the middle area. The data are expressed as mean ± SEM. See Supplementary Video S5

EFs induce migration of epithelial sheets

Many signals are able to stimulate cell migration. Epithelial cell sheets, however, in culture do not normally migrate, unless wounded. Applied EFs induce directional migration of group of epithelial cells, those including keratinocytes, and corneal epithelial cells in culture [34]. We have demonstrated before that large epithelial sheets migrate in an applied EF and maintain tight intercellular junctions at the same time. This feature appears to be different from some biochemical stimulations. Epidermal growth factors (EGFs), for example, induce barrier function decrease and dispersal of cells [35]. Some growth factors and cytokines stimulate cell migration and at the same time break down cell–cell junctions and thereby disperse epithelial sheets. They are sometime named "scatter factors" [36, 37]. Importantly, in this study, we showed that the bigger the epithelial groups (or sheets), the better response to an EF. Being together thus modulates important cellular responses.

E-cadherin-mediated cell–cell adhesion determines electrotaxis of cell sheets

How cells sense weak EFs is not well understood. The striking difference in electrotaxis found for MDCK cells in sheets and in isolation is intriguing and may offer clues for possible mechanisms.

Phorbol myristate acetate treatment or extracellular Ca^{2+} depletion disrupted cell–cell adhesion and significantly decreased electrotaxis of cell sheets. However, PMA and EGTA have other effects on cells. They affect signaling pathways and cell adhesion to the substratum. In neural crest cells, inhibition of protein kinase C (PKC) abolished galvanotaxis. PMA treatment reversed the inhibition of electrotaxis following inhibition of PKC. This may be due to activation of PKC by PMA [38]. Extracellular Ca^{2+} depletion affects Ca^{2+} signaling, which has been suggested to be important for electrotaxis [39]. The essential role of E-cadherin-dependent cell–cell adhesion in electrotaxis of cell sheets is further confirmed by restoring the Ca^{2+} level which recovered the electrotaxis, while addition of E-cadherin antibodies to the restoring medium abolished electrotaxis.

E-cadherin forms calcium-dependent homophilic intercellular adhesions between epithelial cells. Lumenal epithelial cells retain E-cadherin along cell–cell interfaces while elongating collectively within elongating ducts during branching morphogenesis in the mammary gland [40]. Blocking of E-cadherin with specific antibodies caused disruption of coordinated cell movement in epithelial wounds, which resulted in a ragged uneven epithelial wound margin [8, 41]. Chemotaxis of neural crest cells has recently been shown to require N-cadherin [42]. E-cadherin mechanically co-ordinates individual cells in cell sheets and thus promotes collective electrotaxis.

Based on the assumption that cells sense extracellular EFs through mechanisms related to membrane polarization, Cooper [30] suggested that cells coupled by gap junctions would react as a single unit, and have an increased sensitivity to an applied EF by a factor of 10–100 times over single, uncoupled cells. While the involvement of membrane potential is supported by some experimental evidence [43], our present study suggests that gap junction in epithelial sheets contribute only minimally to collective electrotaxis.

The leading edge cells generate directional traction force in collective electrotaxis

Individual cells mechanically couple together to form cell sheets and exert traction forces on the substratum. Traction forces in a cell group are mainly localized to the colony edges (Fig. 5a) [15, 44, 45]. We have shown that, consistent with directional migration, the traction forces at the leading edge of a group are oriented with an EF. However, the force directionality at the trailing edge or the middle did not change significantly. After the EF vector was reversed, the new leading edge cells collectively re-orientate the direction of traction force. It appears that the leading edge re-orientates and increases traction forces to "lead" the cell sheet, similar to the "leading edge" in single cell migration.

Physiological significance of migration of epithelial sheets in weak EFs

Electric fields occur naturally at wounds and may provide a directional signal for migration of epithelial sheets [46–48]. Injury that breaks the barrier instantly short-circuits the transepithelial potential and generates wound EFs. The transepithelial potential difference, which collapses to almost zero at the wound, is normal a couple of 100 μm

back from the wound edge [18, 19]. Groups of mammalian corneal epithelial cells, human keratinocytes, and fish keratocytes have been shown to respond to applied EFs [34, 49, 50]. In addition, stratified corneal epithelial cells in organ culture can also be guided by applied EFs [21, 51, 52].

In wound healing, the primary goal for epithelial cells is to restore the epithelial barrier. It is therefore important that, while the epithelial cells migrate over the wound bed, proper cell–cell adhesion is maintained so that the epithelial barrier is not further compromised. Electrical cues therefore may offer and be developed as a preferred mechanism to promote skin wound healing, because EFs stimulate and guide migration of epithelial sheets without compromising cell–cell adhesion. Application of most growth factors or other migration-stimulating chemicals would usually have a potential detrimental effect to "scatter" cells in epithelial sheets, thus compromising the epithelial barrier function. Epithelial sheets with good cell–cell adhesion respond significantly better to the electrical cue. When cells become isolated, they lose or decrease the response, which helps to maintain the integrity of the epithelial sheets.

E-cadherin in collective migration suggests the importance of "coordinating" factors in addition to intracellular signaling pathways in collective cell migration versus that in individual cells. When connected by E-cadherin, epithelial sheets generate mechanical forces as a single unit. Compromised coordination, especially leading edge force generation, may affect migration of epithelial sheets in non-healing wounds or chronic wounds. Further investigation on migration of epithelial sheets, in addition to that on cells in isolation, will be necessary for development of effective approaches to enhance epithelialization in non-healing and chronic wounds.

In conclusion, physiological EFs induce significantly more robust directional collective migration of epithelial cell sheets than cells in isolation. E-cadherin-mediated cell–cell adhesion is essential for this robust EF-guided directional collective migration. The leading edge cells exert directional traction forces on the substratum to "lead" the following cells. In addition to the implication of electrical guidance in migration of epithelial sheets in vivo in wound healing, application of EFs offers a useful tool for studying migration mechanisms of epithelial sheets.

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