Lipid rafts sense and direct electric field-induced migration

Bo-jian Lin, Shun-hao Tsao, Alex Chen, Shu-Kai Hu, Ling Chao, and Pen-hsiu Grace Chao

Endogenous electric fields (EFs) are involved in developmental regulation and wound healing. Although the phenomenon is known for more than a century, it is not clear how cells perceive the external EF. Membrane proteins, responding to electrophoretic and electroosmotic forces, have long been proposed as the sensing molecules. However, specific charge modification of surface proteins does not change cell migration motility nor directionality in EFs. Moreover, symmetric alternating current (AC) EF directly affects migration in a frequency-dependent manner. Due to their charge and ability to coalesce, glycolipids are therefore the likely primary EF sensor driving polarization of membrane proteins and intracellular signaling. We demonstrate that detergent-resistant membrane nanodomains, also known as lipid rafts, are the primary response element in EF sensing. The clustering and activation of caveolin and signaling proteins further stabilize raft structure and feed-forward downstream signaling events, such as rho and PI3K activation. Theoretical modeling supports the experimental results and predicts AC frequency-dependent cell and raft migration. Our results establish a fundamental mechanism for cell electrosensing and provide a role in lipid raft mechanotransduction.

During development and wound healing, cells experience electrical currents (1–3). The electric field (EF) results in polarized cell organization and induces directional cell migration (galvanotaxis or electrotaxis), morphological changes (galvanotropism), and alterations in gene expression (4, 5). In development, the electrical currents arise from regulation of ion channels that lead to ion flux and establish polarization and morphogenesis (1, 6). EF is also generated from the disruption of membrane potentials at wound sites and promotes oriented cell division and migration, facilitating wound healing (7, 8). Suppression of the electrical currents can lead to impaired healing and failed development (9, 10).

How do cells perceive the external EFs? As the plasma membrane consists of mostly negatively charged molecules that move in the plane of the membrane, the external field induces electrophoresis and electroosmosis of these molecules (11–13). A number of membrane proteins have been found to polarize in direct current (DC) EF, including acetylcholine receptors, VEGF/EGF receptors, and integrins (14–17). In addition, electrical stimulations are found to regulate ion channel activities with higher activation toward the cathode (18). Through these polarized surface receptors, the external EFs activate intracellular signaling, such as src kinase (src), small GTPases, and phosphoinositol kinase pathways, which are polarized in the EF-induced migration direction (7, 17).

Although the preferential distribution and activation of cell membrane proteins in EF support the notion that charged cell surface molecules are influenced by the electrophoretic and electroosmotic forces, Finkelstein et al. (19) report that modification of membrane protein charges with avidin conjugation does not change cell migration motility nor directionality in EF. Interestingly, the classical neurominidase treatment, which removes sialic acids from both glycoproteins and glycolipids, indeed inhibits directional migration. These results suggest that glycolipid redistribution in EF can be an alternative candidate as the primary EF sensor in cell membrane. Moreover, we previously reported that symmetric alternating current (AC) at 50 Hz drives directional cell migration (17). Glycolipids are capable of congregating into structures such as lipid rafts that can increase in size by recruiting proteins and lipids. EFs may induce glycolipid movement and density increase due to preferential distribution in the field, leading to increases in lipid raft size (20). If raft size increases during movement in EF, the concomitant decrease in raft motility will result in polarization of the raft structures and lead to directional migration.

Lipid rafts, detergent-resistant membrane nanodomains, are highly dynamic and heterogeneous in composition and interaction (21). They are essential in many cell membrane processes and modulate activation of integrin and many of the aforementioned growth factor receptors that polarize in EF (22–24). Rich in gangliosides, lipid rafts are linked to sialic acids and negatively charged. In addition to cholesterol, lipid raft proteins, such as caveolin (Cav), further stabilize lipid raft structure and control lipid raft dynamics (25, 26). In the current study, we hypothesize that lipid rafts are the primary sensor to EF stimulation due to their charge and ability to coalesce. Preferential distribution of lipid rafts in EF polarizes membrane proteins such as integrin and Cav, and the clustering and activation of these proteins further stabilize raft structure and feed-forward raft polarization, leading to directional cell migration.

Results and Discussion

We quantified the distribution of lipid rafts upon field exposure with fluorescent cholera toxin B (CTxB) to confirm the


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To whom correspondence should be addressed. Email: pgchao@ntu.edu.tw.

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and mesenchymal stem cells (MSCs) (Fig. 1 and SI Appendix, Fig. S1). In the anodally migrating CL1-5 adenocarcinoma cells, rafts were polarized toward the anode (27) (Fig. 1C). In the randomly migrating CL1-0 cells, no preferential raft distribution was found. Superresolution microscopy revealed a significant increase of raft sizes with DC EF exposure in MSCs, indicative of raft clustering (control = 0.028 ± 0.005 μm², EF = 0.032 ± 0.007 μm², n = 19–24, P = 0.014; Fig. 1D). When lipid rafts were disrupted by cholesterol depletion with methyl β-cyclodextrin (MβCD) or saturation, migration directionality was suppressed, whereas the influence on motility, as quantified by migration speed, was minor (Fig. 2). These data support our hypothesis that EF-induced migration directionality corresponds with membrane raft clustering and polarization.

We previously reported that integrin mediates directional cell migration in applied EFs (17). The polarized distribution of integrin in response to EF was abolished with raft disruption, whereas raft polarization in EF was less influenced by functional blocking of integrin (Fig. 3A). To further determine if lipid rafts indeed act upstream of integrin or other active cellular mechanisms, we disrupted the actin cytoskeleton with cytochalasin D to monitor raft distribution. Although cytochalasin treatment polarization of lipid rafts in applied DC and AC EFs. In DC fields, lipid rafts indeed polarized toward the cathode with time, corresponding with the migration directionality of fibroblasts

![Fig. 1. Applied EF directs cell migration and lipid raft polarization.](https://example.com/image1)

![Fig. 2. Lipid raft polarization in applied AC and DC fields.](https://example.com/image2)

![Fig. 3. Lipid rafts act upstream of intracellular structure and signaling mechanisms.](https://example.com/image3)
significantly suppressed cell motility and directionality (SI Appendix, Fig. S1B), actin disruption enhanced raft and integrin polarization (Fig. 3B). In addition, no preferential lamellipodia extension toward the cathode (SI Appendix, Fig. S1A) or microtubule organizing center polarization (28) was found with EF exposure. As cytoskeleton structures, especially actin, regulate membrane domains and protein organization (29, 30), the increase of raft polarization with cytochalasin treatment supports the restrictive role of submembranous cytoskeleton structures in the diffusion of membrane proteins (31).

To understand the interaction between integrin and lipid raft, dual labeling with CTxB and antibody against active β1 integrin (clone 12G10, Abcam) found no changes in raft and integrin colocalization after EF stimulation (P = 0.775; SI Appendix, Fig. S1C). Due to the pentavalent nature of CTxB, we tested the effects of CTxB on raft size and integrin interactions by treating the control cells with CTxB before formalin fixation. Indeed, CTxB treatment increased raft size by 15% (CTxB incubation after fixation, 0.028 ± 0.005 μm²; CTxB incubation before fixation, 0.032 ± 0.007 μm²; P = 0.039) and reduced integrin and raft colocalization by 36% (CTxB incubation after fixation, 0.242 ± 0.090; CTxB incubation before fixation, 0.156 ± 0.103, P = 0.019). As both CTxB and formalin fixation can artificially induce raft clustering (32), the reported raft size and colocalization may not reflect the actual values. Nonetheless, our results demonstrate that both CTxB and electrical stimulation increase raft clustering. Furthermore, EF-induced clustering has a different effect on integrin partitioning from the chemically induced clustering. These data suggest that EF may play an additional role in integrin and raft interaction, and integrin is not merely a passive passenger on the raft during EF-induced raft clustering. Clustering and activation of other molecules may also participate in the dynamics. Future studies should determine if inactive integrin association with raft, the ratio of active/inactive integrins, or recruitment of different integrin species change with EF-induced raft clustering. Our results demonstrate that exogenous EF alters raft and integrin interactions.

Polarization of intracellular signaling molecules, including RhoA, src, and PI3K, mediates EF-induced directionality (7, 17). To understand the role of raft in the polarization of these downstream factors, we examined their distribution in applied EF after raft disruption and found polarization of these signaling proteins was abolished (Fig. 3C). Pharmacological inhibition of these molecules, on the other hand, had no effect on lipid raft polarization in EF (Fig. 3D and SI Appendix, Fig. S2), further demonstrating that lipid raft polarization acted upstream of these intracellular signaling events.

An integral inner membrane protein, Cav stabilizes lipid raft structures and interacts with β1 integrin to activate RhoA through inactivation of p190RhoGTPase (33). In applied DC EF, Cav-1 polarized toward the cathode, similar to the gangliosides of lipid raft (Fig. 4A). EF stimulation for 1 h significantly increased Cav oligomerization (Fig. 4B), indicating a clustering effect in response to the applied EF. Cholesterol depletion with MjCD inhibited Cav-1 polarization (Fig. 4C). Knockdown of Cav-1 abolished migration directionality in response to applied EF (Fig. 4C). As Cav regulates membrane cholesterol content (34), we replenished membrane cholesterol in the Cav-1 knockdown cells and found a similar suppression of directionality, indicating that Cav indeed participated in EF-induced raft re-distribution (SI Appendix, Fig. S3). Cav-1 knockdown also inhibited RhoA and PI3K polarization in EF, demonstrating the key role of Cav-1 in EF-induced directional signaling (Fig. 4E). Interestingly, although inhibitors for PI3K and src did not
suppress Cav polarization in EF, functional blocking of integrin reduced Cav polarization (Fig. 4D and SI Appendix, Fig. S2). Inhibition of raft and integrin polarization from Cav-1 knockdown cells suggested reciprocal interactions among raft, integrin, and Cav-1, and the integrity of all three components was necessary for EF-directed migration. Activation of integrin by extracellular matrix proteins has been shown to change integrin partitioning (35–37), stabilize lipid rafts (38), and modulate EF-induced motility and directionality (4, 39). Integrin activation may also induce src signaling that phosphorylates Cav (40). However, as the src-family inhibitor used in this study (PP2) acts on all known Cav kinases (src, lyn, and abl) (41, 42), Cav phosphorylation is unlikely to be involved in integrin and Cav association in EF. Furthermore, as PP2 has no effect on raft or Cav polarization (Fig. 3D and SI Appendix, Fig. S2), src does not mediate Cav interactions to stabilize lipid raft.

Stemming from the experimental observations, we built a theoretical framework based on previous models to describe the qualitative electrodynamic behavior of lipid rafts in membranes. The model calculated lipid raft displacement in DC and AC EFs based on parameters extracted in fibroblasts. Detailed analyses can be found in SI Appendix. The applied EF can induce three forces acting on lipid rafts (12, 43, 44): the electrical force due to an external EF ($F_E$) (45), the hydrodynamic force resulting from the aqueous medium ($F_{HA}$) (46), and the drag force in membrane ($F_{DM}$) (47). Drift velocity of lipid rafts was obtained by expressing $F_E$, $F_{HA}$, and $F_{DM}$. As illustrated in Fig. 5A, raft velocity decreased exponentially with increasing radius. Equilibrium location of lipid rafts was therefore determined by the rate of lipid raft size increases and EF-driven drifts. The direction of the field when lipid raft reached critical size (where its velocity approximates zero) governed the equilibrium location of the raft, predicting a frequency-dependent behavior of lipid raft distribution in DC and AC fields. The model also predicted that while in AC fields, rafts would be located toward the cathode at low frequency (10 Hz), toward the anode at intermediate frequency (50 Hz), and exhibit low directionality at high frequency (250 Hz). Indeed, experimental results matched the finding and demonstrated that raft distribution and migration directionality exhibited AC frequency dependence in fibroblasts (Fig. 5C). Previous studies also described frequency-dependent surface protein polarization in AC EF (48).

Lipid rafts have been shown to mediate mechanotransduction through spatial or allosteric regulation of protein functions (37, 49). However, it is not clear what initially leads to the changes in raft organization. In this study, we demonstrate that lipid rafts are the primary sensing mechanism to external EF and regulate

![Diagram](https://example.com/diagram.png)

**Fig. 5.** Theoretical model of lipid raft movement in applied EF. (A) Lipid raft velocity as a function of lipid raft radius (0–10 μm) in an EF. (B) Model prediction of lipid raft displacement when the EF is applied as DC and AC field at 10 Hz, 50 Hz, or 250 Hz. Blue line and the left y axis, displacement of a lipid raft (x). Green line and the right axis, lipid raft radius (r). Dashed line, original location of lipid raft (x = 0). (C) DC and AC frequency-dependent response of lipid rafts and migration directionality of fibroblasts ($n = 58$–$178$, *P < 0.0001 vs. 0 V, †P < 0.0001 vs. DC).
Migration Analysis. Images of cell location were captured every 15 min on an inverted microscope (Leica). Cell migration was measured by manually determining the centroid with time and calculating the displacement and direction (angle between the EF direction and the cell translocation vector). Migration speed was calculated as the net displacement per hour, and migration directionality was calculated as the cosine of the migration angle where a negative value indicates migration toward the cathode.

Image Analysis. A custom LabView program (National Instruments) allowed manual selection of cell area (via the bright-field channel) and automated partition of four quadrants (Fig. 1B). The mean fluorescent intensity was calculated for each quadrant and normalized to overall cell intensity. Asymmetry index (AI) was calculated by subtracting the normalized intensity of the anodal quadrant from the cathodal quadrant (17). A positive AI value indicates a preferential anodal distribution of the labeled molecules, and a negative value of AI indicates cathodal distribution. For stimulated emission depletion (STED) images, a custom Matlab program segmented and measured raft sizes.

Cav Oligomerization Assay. A modified galvanotaxis chamber was made by adapting the parallel plate geometry in a 10-cm culture dish with PDMS molding. Cells were cultured overnight and stimulated for 1 h. To determine the degree of Cav oligomerization, total cell lysates harvested with RIPA buffer were denatured in gel loading buffer at 70 °C for 10 min (54). Proteins were separated via standard SDS/PAGE procedures and blotted on PVDF membrane. The whole membrane was probed with Cav-1 antibody (Cell Signaling), and bands above 250 kDa (representing Cav oligomers) and at 22 kDa (Cav monomers) were detected (54).

Membrane Modeling. When an EF is applied, three forces act on lipid rafts (12, 43, 44): the electrical force due to an EF (45), the hydrodynamic force resulting from the aqueous medium (F_HA) (46), and the drag force in membrane (F_DM) (47). Drift velocity of the lipid rafts can be obtained by expressing F_E, F_HA, and F_DM in the forms with lipid raft velocity, as shown in Eq. 1:

$$\frac{d(r)}{d(t) + g(r)} = \frac{d(r)}{C_0}$$

where $d(r)$ and $g(r)$ are the drag coefficients associated with the hydrophilic portion in the aqueous phase and with the portion embedded in the membrane, respectively.

The drag coefficient $d(r)$ is related to the shape, size, and orientation of the hydrophilic portion with respect to the aqueous flow (45, 55) and was obtained by using COMSOL Multiphysics software. For the cylindrical hydrophilic portion with a height of 1 nm, the obtained $d(r)$ is $2 \times 10^{-11}$ (55). The hydrophobic portion-associated drag force coefficient, $g(r)$, can be obtained by using the Saffman-Delbrück approximation, and is expressed below (55–57):

$$g(r) = \frac{4\pi \rho_m}{\eta_m} \left[ 1 - \left( \frac{r}{\gamma} \right)^2 \ln \left( \frac{r}{\gamma} \right) + \frac{c_1}{\rho_m} \left( \frac{r}{\gamma} \right)^{2/3} \ln \left( \frac{r}{\gamma} \right) \right]$$

where $r$ is the lipid raft radius, $\gamma = 0.58$, $b_1 = 2.75$, $b_2 = 0.61$, $c_1 = 0.74$, and $c_2 = 0.52$. Detailed descriptions and definitions of additional symbols can be found in SI Appendix.

Statistical Analysis. SPSS 22 (IBM) was used to perform ANOVA with LSD post hoc tests ($\alpha = 0.05$). All results represent more than two separate cell preparations. Error bars represent SEMs.

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Methods and Materials

Detailed methods are described in SI Appendix.

Electrical Stimulation. The galvanotaxis chamber, as described previously (53), consisted of a modified, parallel-plate flow chamber where the medium inlet and outlet were connected to agarose salt bridges. Constant DC EF was applied at a field strength of 6 V/cm (3 mA) with a Keithley SourceMeter, and AC sinusoid waves were applied at a peak intensity of 1.2 V at 50 Hz using a custom stimulator (Dynaprog).


