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Golgi polarization plays a role in the directional migration of neonatal dermal fibroblasts induced by the direct current electric fields

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ABSTRACT

Directional cell migration requires cell polarization. The reorganization of the Golgi apparatus is an important phenomenon in the polarization and migration of many types of cells. Direct current electric fields (dc (EF) induced directional cell migration in a wide variety of cells. Here nHDFs migrated toward cathode under 1 V/cm dc EF, however 1 μM of brefeldin A (BFA) inhibited the dc EF induced directional migration. BFA (1 μM) did not cause the complete Golgi dispersal for 2 h. When the Golgi polarization maintained their direction of polarity, the direction of cell migration also kept toward the same direction of the Golgi polarization even though the dc EF was reversed. In this study, the importance of the Golgi polarization in the directional migration of nHDf under dc EF was identified.

1. Introduction

Cell polarization is one of the most important phenomenon for the directional cell migration. It is explained by asymmetric cytoskeletal arrangement, intracellular organelle localization, membrane domain segregation and the polarized cell morphology. These characteristics are connected with the membrane protrusion or elongation and directional cell migration in cell migration [1–8]. In cell polarization, Golgi apparatus (GA) polarization plays an important role and this is also critically involved in directional cell migration, because the Golgi apparatus is very important in supplying the membrane components to the leading edge for membrane protrusion when the cell is moving [7,9–11]. Many studies were accomplished to figure out the molecular mechanisms of Golgi polarization, however it is not fully understood how Golgi polarization regulates directional cell migration.

Applied direct current electric fields (dc EFs) guide the directional migration of many cell types, including endothelial cells, bone marrow mesenchymal stem cells and human dermal fibroblasts [12–14]. The dc EFs also induced the directional cell migration in wound healing and development in vivo [15–21]. We refer to this as “electrotaxis/galvanotaxis.” During electrotaxis, cells move toward the anode or cathode under direct current electric fields (dc EFs); these dc EFs can be used to control the directional cell migration. In addition, the dc EFs may have in vivo or in vitro application of the technique to induce the predictable cell polarization and directional cell migration. Our previous research already confirmed that the change of Golgi apparatus polarization and cytoskeleton reorganization during the directional migration by the dc EFs [22]. This study was designed to figure out the role of Golgi polarization as a predominant guidance cue in directed cell migration by dc EFs.

2. Methods

2.1. Chemical agents and cell culture

Brefeldin A (BFA) was from Sigma (St. Louis, MO, USA). Neonatal human dermal fibroblasts (nHDFs) were purchased from Lonza Group, Ltd. (Walkersville, MD, USA) and maintained in fibroblast basal medium-2 (FBM-2) supplemented with a growth kit containing 10 ml of fetal bovine serum, 0.5 ml of insulin, 0.5 ml of gentamicin sulfate amphotericin-B (GA-1000), and 0.5 ml of r-human fibroblast growth factor-B (Lonza, USA). The cells were

Abbreviations: GA, Golgi apparatus; dc EFs, direct current electric fields; BFA, Brefendin A; nHDFs, neonatal human dermal fibroblasts.

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incubated at 37 °C in a 5% CO_2 atmosphere. nHDFs between passages 7 and 9 were used in all experiments.

2.2. Electric field stimulation and drug treatment

To apply a direct current electric field to the nHDFs, we used a customized electrotaxis incubator and chamber system [22]. Briefly, the electrotaxis chamber and incubator system consisted of the incubator system and electrotaxis chamber. The incubator system is installed with a microscope to observe live cells and the electrotaxis chamber applies a direct current electric field to the cells. The incubator which maintains the proper growth environment (CO_2 5%, 37 °C) is regulated by a temperature and gas composition-controlling program (CCP ver. 3.8, Live Cell Instrument, Seoul, Korea). A gold patterned glass slide was mounted on the chamber bottom, and the chamber top and silicon gasket were placed on top of the slide. The electrotaxis chamber top with the electric wires connects the gold patterned. To sterilize the chamber, 70% ethanol (700 μl) was added to each electrotaxis chamber and removed after 30 min followed by three washes with distilled water (DW). The nHDFs were seeded at 1×10^4 cells density in the electrotaxis chamber and incubated for 16–24 h in the CO_2 incubator. Immediately before electrotaxis experiments, media was changed. Cells were exposed to a dc EF at 37 °C in the electrotaxis

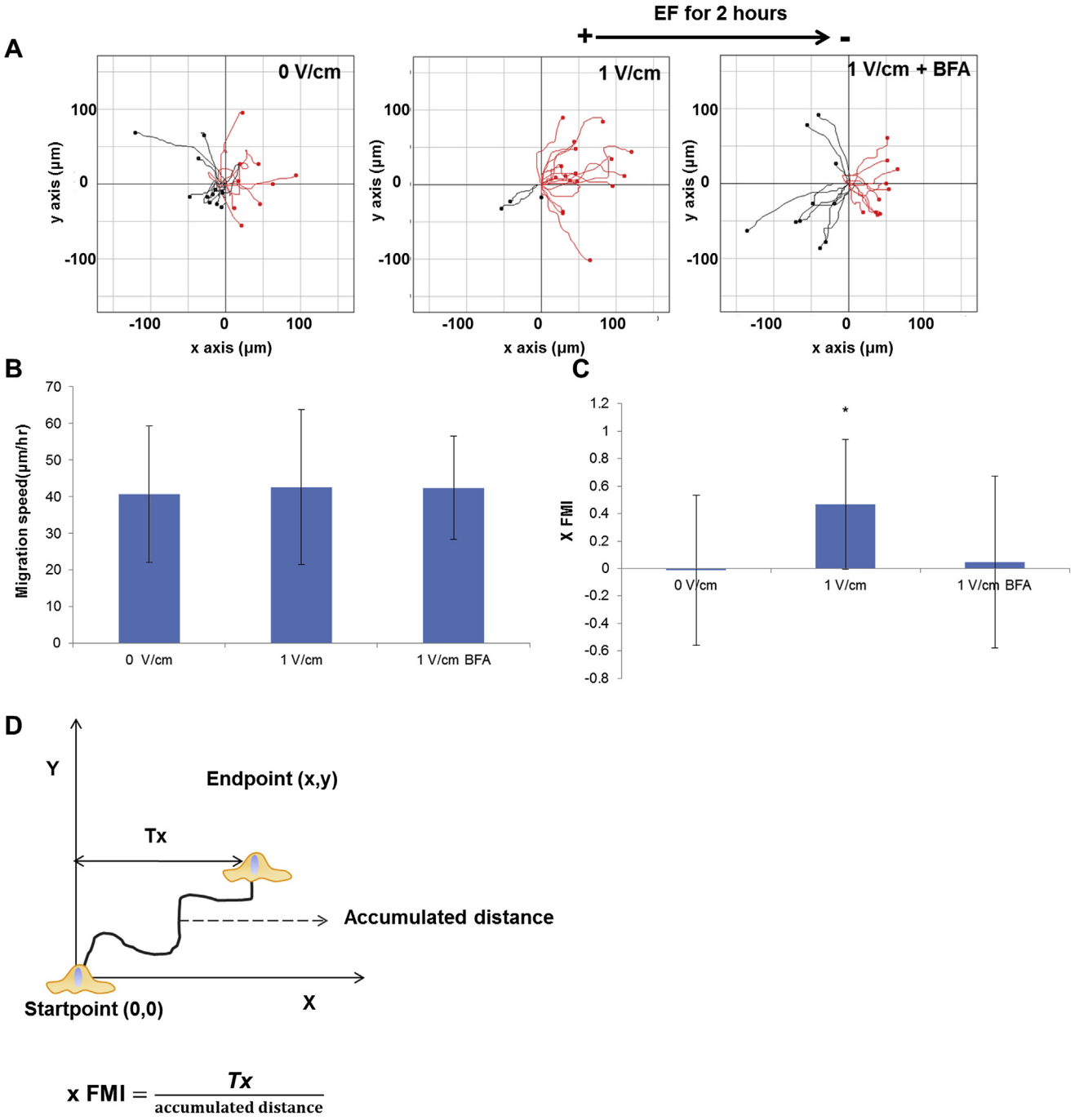

Fig. 1. The migration assay of nHDFs under dc EF condition (0, 1 V/cm) for 2 h with or without 1 μM of BFA. (A) 20 Cells of each condition were tracked, (B) migration speed was measured, and (C) x FMI was determined for nHDFs under dc EF conditions for 2 h (0, 1 V/cm), $^*p < 0.05$ compared to controls grown with no EF. (D) A schematic diagram shows the x Forward Migration Index.

incubator and chamber system. 1 μM of BFA was treated to interrupt the Golgi polarization before or during the dc EF stimulation.

2.3. Time-lapse phase contrast microscopy and analysis of cell migration

The electrotaxis incubator was placed on the microscope stage. The cell seeded electrotaxis chamber was inserted into the electrotaxis incubator. The cell images were recorded every 5 min (until the electric treatment ended) using a charge-coupled device (CCD) camera (Electric Biomedical Co. Ltd., Osaka, Japan) attached to an inverted microscope (Olympus Optical Co. Ktd., Tokyo, Japan). The images were saved in the computer using the tomoro image capture program; images were saved as JPEG files. Captured images were analyzed by ImageJ (ImageJ 1.37v by W. Rusband, National Institutes of Health, Bethesda, MD, USA). The image analyses were performed using the manual tracking and chemotaxis tool plug-in (v. 1.01, distributed by ibidi GmbH, Munchen, Germany). The XY coordinates were obtained using manual tracking. The data were then imported into the chemotaxis plug-in. This tool was used to compute cell migration speed and the x Forward Migration Index (x FMI), both of which were used to plot the cell migration pathway. The migration speed, referred to as the migration speed, indicates how fast cells move in response to the stimulation, calculated using the total length of the migration path divided by the total observation time [22]. The x FMI of the cell (Fig. 1D) was defined as the straight-line distance along the *x*-axis (Tx) between the start position and the end position of the cell divided by the accumulated distance. For each experiment, 20 cells on the gold patterned glass were randomly selected and cells undergoing division, death, or migration outside of the field of view were excluded from the analysis.

2.4. Immunofluorescence microscopy

After electric current treatments, nuclei, GA and actin cytoskeletons were visualized by immunofluorescence staining. The dc EF treated cells were fixed with 70% ethanol for 30 min at 4 °C and were then washed 3 times with PBS. Cells were permeabilized with 0.1% Triton X-100 in PBS for 5 min at room temperature and rinsed 3 times with PBS. 1% Bovine serum albumin (BSA) was treated for 30 min at room temperature to block the nonspecific binding with, followed by incubation with a purified mouse anti-GM130 primary antibody (dilution 1:100, BD Transduction Laboratories TM, BD Biosciences, CA, USA) overnight at 4 °C. Cells were then washed at least 3 times with PBS and treated with the secondary antibody Alexa (488)-conjugated phalloidin (5 U/ml, Invitrogen, Carlsbad, CA) for the actin cytoskeleton and goat anti-mouse IgG conjugated with Texas Red (dilution 1:100, Santa Cruz, CA, USA) for the GA, for 1 h at room temperature in the dark. After PBS washing, cells were treated with Hoechst #33258 (Sigma, St. Louis, MO, USA) for 5 min at room temperature in the dark. The cell seeded slide glass was then mounted on the fluorescence-inverted microscope (LSM700, Carl Zeiss, New York, USA) and observed.

2.5. Golgi polarization analysis

The nHDF cells in a dc EF of 1 V/cm for 2–5 h showed distinct polarized morphology and Golgi apparatus. The cells with the

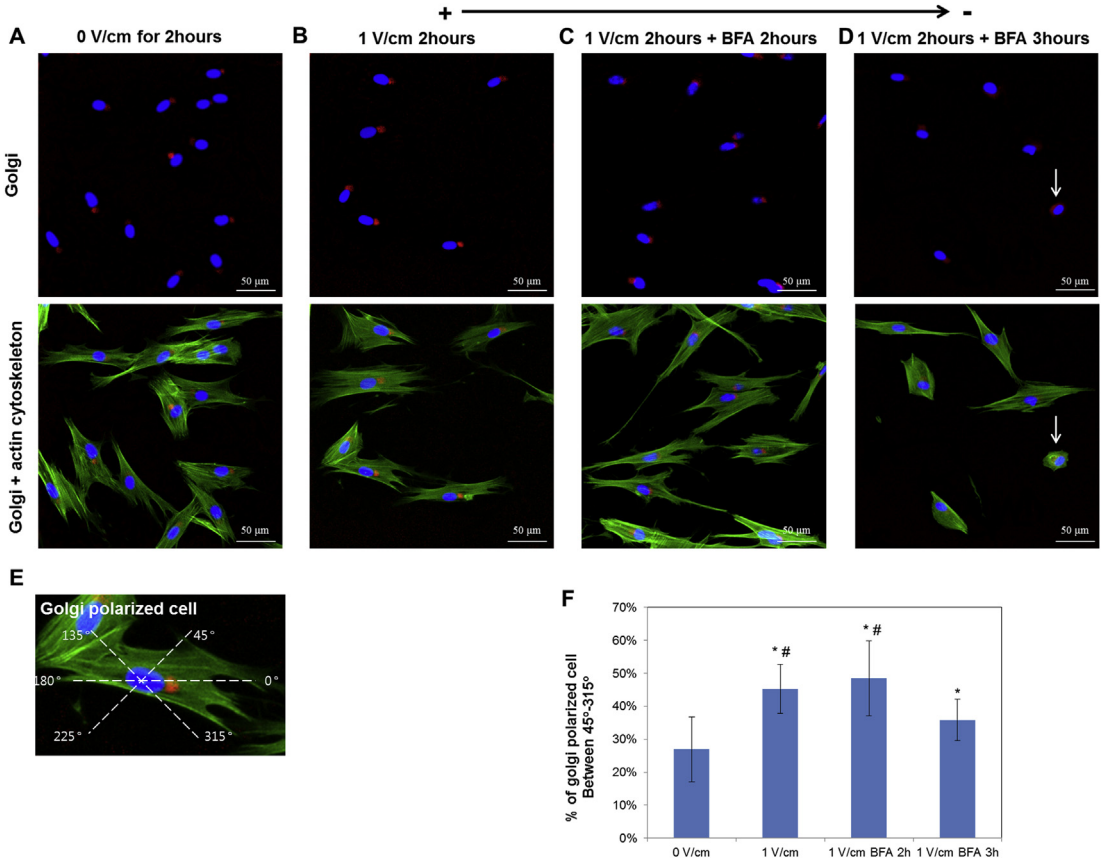

Fig. 2. Immunostaining images and Golgi polarization data of dc EF and BFA treated nHDFs. (A) Immunostaining of the nucleus, the actin cytoskeleton, and the GA under 0 V/cm, (B) 1 V/cm for 2 h, (C) 1 V/cm for 2 h then 1 μM of BFA treated for 2 h, (D) 1 V/cm for 2 h then 1 μM of BFA treated for 3 h. The nuclei were stained with Hoechst #33258, the actin cytoskeleton was stained with Alexa (488)-conjugated phalloidin (green), and GA were stained with Texas Red conjugated antibody (red). Scale bar = 50 μm. (E) GA polarization quantification is shown. (F) The percentage of cells with Golgi polarized between 45° and 315°. *$p < 0.05$ compared to the 0 V/cm and #$p < 0.05$ compared to the 1 V/cm BFA 3 h. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

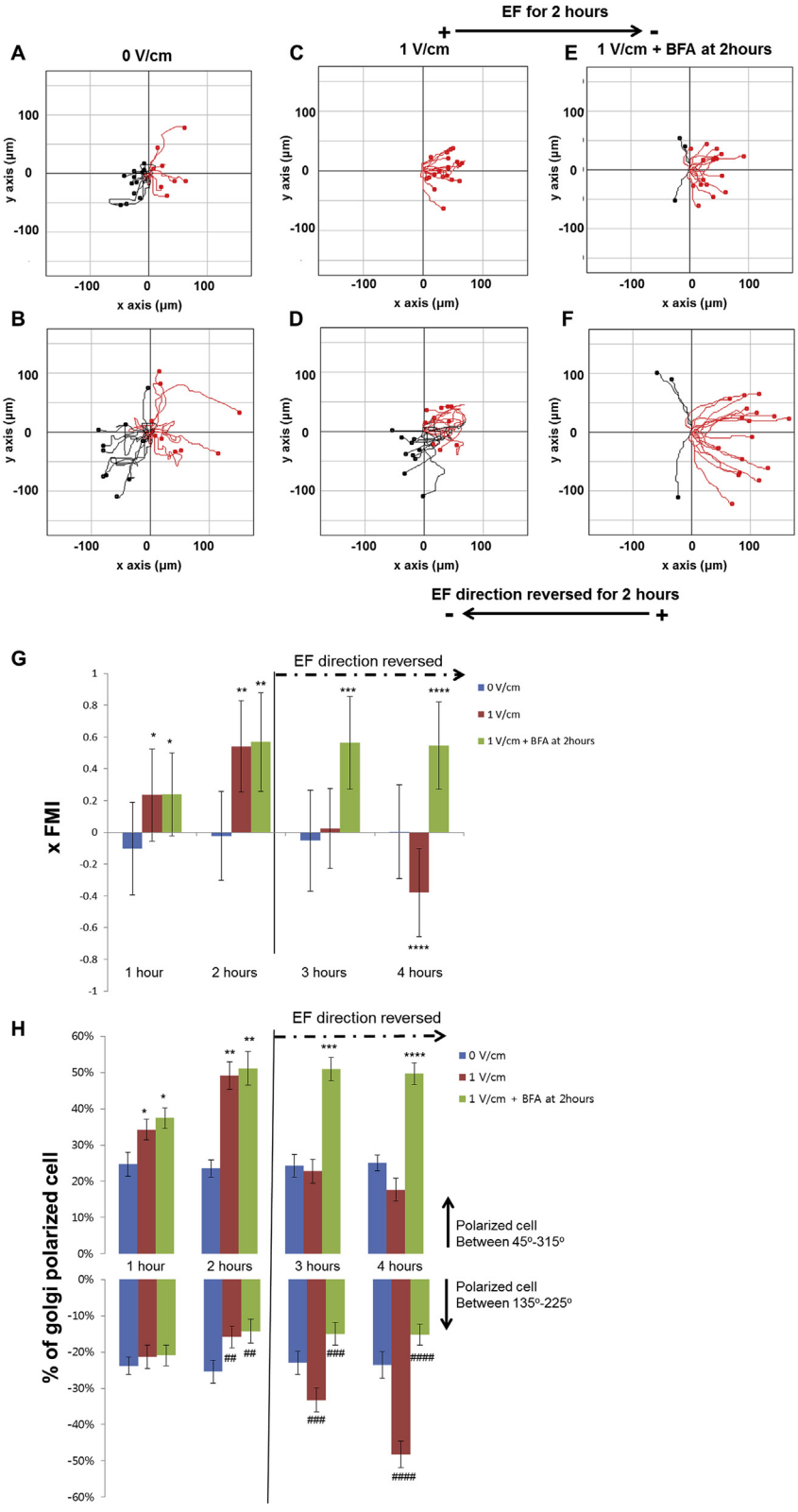

Fig. 3. The cell tracking data, x FMI and Golgi polarization data of nHDFs in an EF. The movement of cells for 2 h (A) and 4 h (B) in no dc EF. The direction of dc EF was right (cathode right) for 2 h (C) then reversed the direction for 2 h (D). (E) Cell tracking data that the direction of dc EF was right for 2 h. (F) 1 μM of BFA treated immediately before reverse the direction for 2 h. (G) x FMI was determined for nHDFs under dc EF conditions for 4 h (0, 1 V/cm). $^{*}p < 0.05$ compared to x FMI of EF 0 V/cm at 1 h, $^{**}p < 0.05$ (0 V/cm at 2 h), $^{***}p < 0.05$ (0 V/cm at 3 h), $^{****}p < 0.05$ (0 V/cm at 4 h). (H) The percentage of cells with Golgi polarized between 45° and 315°, between 135° and 225°. $^{*}p < 0.05$ compared to the polarized cell between 45° and 315° EF 0 V/cm at 1 h, $^{**}p < 0.05$ (1 V/cm at 2 h), $^{***}p < 0.05$ (1 V/cm), $^{****}p < 0.05$ (1 V/cm at 4 h). $\#\#p < 0.05$ compared to the polarized cell between 135° and 225° EF 1 V/cm at 2 h, $\#\#\#p < 0.05$ (1 V/cm at 3 h), $\#\#\#\#p < 0.05$ (1 V/cm at 4 h).

polarized Golgi between 45° and 315° (cathode right) or between 135° and 225° (cathode left) of the dc EF direction were scored as polarized in the EF direction (Fig. 2E).

2.6. Statistical analyses

Data are reported as means ± standard error of the mean (SEM). The letter *n* denotes the number of tests, except in the migration assay where *n* denotes the number of cells. Means were compared using one-way analyses of variance (ANOVA). Two-tailed Student's *t*-tests were used for unpaired data. A value of $p < 0.05$ is considered statistically significant.

3. Results and discussion

3.1. Blocking of Golgi polarization inhibited the directional migration induced by the dc EF

In our previous study, the optimal condition of nHDFs directional migration by the electrotaxis was identified and the Golgi polarization was observed during the electrotaxis [22]. To identify the effect of electrotaxis on the nHDF migration when the Golgi polarization was blocked, 1 μM of BFA was treated before the dc EF treatment to the cells. Brefeldin A (BFA) prevents the assembly of cytosolic coat proteins onto Golgi membranes, resulting in the formation of Golgi tubules and prevents tubule detachment from the Golgi structure which then fuses with the endoplasmic reticulum (ER). This leads to rapid diffusion of Golgi membrane into the ER and disruption of Golgi. BFA at high concentrations of 2–5 μg/ml (~18 μM) causes this rapid dispersal of the Golgi within 5–8 min after addition of the [23–25]. We used a concentration of BFA (5 μM) and found the same quick dispersal of the Golgi apparatus. This treatment resulted in discoid cell morphology. It is impossible to analyze Golgi polarization and cell migration as there is no visible Golgi staining and the cells do not move. So, 1 μM of BFA was chosen for this experiment. The cell moved randomly without EF, and cell migrated toward the cathode under the dc EF 1 V/cm for 2 h (Fig. 1A). However, there was no directional migration when the BFA was treated before the 1 V/cm EF stimulation. The migration speed of nHDFs was not affected by both dc EF and BFA treatment (Fig. 1B). The x FMIs of 0 V/cm and 1 V/cm BFA were closed to “0”, however that of 1 V/cm was significantly increased (Fig. 1C). The x forward migration index (x FMI) value of 1 means the cell has migrated perfectly toward the right, −1 means the cell has migrated perfectly toward the left and 0 indicates the cell has migrated perpendicular to the stimulation direction. These results indicated that the interruption of Golgi polarization inhibits the directional migration of nHDF induced by the dc EF.

3.2. Golgi polarization versus Golgi dispersal in dc EF

To figure out the optimal condition of BFA and dc EF treatment, first the cells were polarized toward the cathode by the 1 V/cm EF for 2 h. Then the 1 μM of BFA was treated immediately and dc EF stimulation was stopped. The immunofluorescence images of Golgi apparatus and actin cytoskeleton were observed (Fig. 2A–D). In no dc EF condition, the Golgi and actin cytoskeleton polarization were random (Fig. 2A). However, the Golgi and actin cytoskeleton were polarized toward the cathode under 1 V/cm EF for 2 h without BFA (Fig. 2B). There were no significant changes of polarized Golgi after BFA treatment for 2 h. The Golgi polarization was still toward cathode and deformation of actin cytoskeleton was not observed (Fig. 2C). The Golgi dispersal and actin cytoskeleton deformations were detected at 3 h after BFA treatment (Fig. 2D). The white arrows indicated the Golgi dispersal and round shaped actin cytoskeleton. The quantified Golgi polarization data showed that the percent of Golgi polarization in 1 V/cm 2 h and 1 V/cm 2 h + BFA 2 h were increased significantly, but slightly decreased in 1 V/cm 2 h + BFA 3 h (Fig. 2F). The drop of Golgi polarization in 1 V/cm 2 h + BFA 3 h was due to the dispersal of Golgi apparatus by BFA effect. According to these results, 2 h after 1 μM of BFA treatment is optimal condition for the experiment that BFA could cause the derangement of Golgi complex reorientation, although did not cause complete dispersal of Golgi.

3.3. Golgi polarization is more important to the directional migration than the dc EF

The nHDFs moved towards the cathode and also altered direction in response to a switch in the direction of the applied dc EF. The Golgi polarization of nHDFs was also changed toward the new cathode when the direction of cell migration is altered by dc EF [22]. To identify the effect of Golgi polarization on the dc EF induced directional migration, 1 μM of BFA was treated immediately after the cells were exposed to 1 V/cm dc EF for 2 h. The direction of dc EF was reversed right after the BFA treatment and maintained the direction of reversed dc EF for another 2 h. The cells in no dc EF showed random migration for 2 h and 4 h (Fig. 3A and B). When the cathode was right, the cells also moved toward right (Fig. 3C and E). The cells moved toward right for first 2 h and the direction of cell migration was changed toward left response to the switch in the direction of the dc EF toward left (Fig. 3D). However, the cells treated with BFA after dc EF induced directional migration toward right maintained the direction of migration toward right even though the dc EF was reversed (Fig. 3F). The x FMIs of 0 V/cm were closed to “0” from 1 h to 4 h. The x FMI of 1 V/cm significantly increased until 2 h, after that it considerably decreased because the direction of dc EF was reversed. However, that of 1 V/cm + BFA at 2 h increased remarkably and kept the high value of x FMI (Fig. 3G). The Golgi polarization of each condition assumed similar aspect with the x FMI results. There is no significant difference that the Golgi polarization of 0 V/cm for 4 h between 45° and 315° or between 135° and 225°. Golgi polarization between 45° and 315° means right, 135° and 225° indicates left. In 1 V/cm, the Golgi polarization was increased toward right for first 2 h, then significantly increased toward left for rest 2 h. However, in 1 V/cm + BFA at 2 h maintained the high percent of Golgi polarization toward right for 4ours even though the direction of dc EF was reversed. These results indicated that Golgi polarization is more important to the directional migration of the nHDFs than the dc EF. Also, the induction of Golgi polarization is necessary to induce the directional migration using the dc EF.

Conflict of interest

The authors have declared that no conflict of interest exists.

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References

[1] A. Kupfer, D. Louvard, S.J. Singer, Polarization of the Golgi apparatus and the microtubule-organizing center in cultured fibroblasts at the edge of an experimental wound, Proc. Natl. Acad. Sci. U. S. A. 79 (1982) 2603–2607.

[2] A. Kupfer, G. Dennert, S.J. Singer, Polarization of the Golgi apparatus and the microtubule-organizing center within cloned natural killer cells bound to their targets, Proc. Natl. Acad. Sci. U. S. A. 80 (1983) 7224–7228.

[3] S. Etienne-Manneville, A. Hall, Integrin-Mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCζ, Cell 106 (2001) 489–498.

[4] S. Etienne-Manneville, A. Hall, Cdc42 regulates GSK-3beta and adenomatous polyposis coli to control cell polarity, Nature 421 (2003) 753–756.

[5] C.D. Nobes, A. Hall, Rho GTPases control polarity, protrusion, and adhesion during cell movement, J. Cell Biol. 144 (1999) 1235–1244.

[6] A.J. Ridley, M.A. Schwartz, K. Burridge, R.A. Firtel, M.H. Ginsberg, G. Borisy, J.T. Parsons, A.R. Horwitz, Cell migration: integrating signals from front to back, Science 302 (2003) 1704–1709.

[7] M. Raftopoulou, A. Hall, Cell migration: Rho GTPases lead the way, Dev. Biol. 265 (2004) 23–32.

[8] C. Gomez-Mouton, R.A. Lacalle, E. Mira, S. Jimenez-Baranda, D.F. Barber, A.C. Carrera, C. Martinez-A, S. Manes, Dynamic redistribution of raft domains as an organizing platform for signaling during cell chemotaxis, J. Cell Biol. 164 (2004) 759–768.

[9] A.D. Bershadsky, A.H. Futerman, Disruption of the Golgi apparatus by brefeldin A blocks cell polarization and inhibits directed cell migration, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 5686–5689.

[10] I.R. Nabi, The polarization of the motile cell, J. Cell Sci. 112 (1999) 1803–1811.

[11] N.L. Prigozhina, C.M. Waterman-Storer, Protein kinase D mediated anterograde membrane trafficking is required for fibroblast motility, Curr. Biol. 14 (2004) 88–98.

[12] Z. Zhao, L. Qin, B. Reid, J. Pu, T. Hara, M. Zhao, Directing migration of endothelial progenitor cells with applied DC electric fields, Stem Cell Res. 8 (2012) 38–48.

[13] Z. Zhao, C. Watt, A. Karystinou, A.J. Roelofs, M.C. Caig, I.R. Gibson, Directed migration of human bone marrow mesenchymal stem cells in a physiological direct current electric field, Eur. Cell Mater. 22 (2011) 344–358.

[14] Aihua Guo, Bing Song, Brian Reid, Yu Gu1, John V. Forrester, Colin A.B. Jahoda, Min Zhao, Effects of physiological electric fields on migration of human dermal fibroblasts, J. Invest. Dermatol. 130 (2010) 2320–2327.

[15] L.F. Jaffe, J.W. Vanable, Electric fields and wound healing, Clin. Dermatol. 2 (1984) 34–44.

[16] C.D. McCaig, M. Zhao, Physiological electrical fields modify cell behavior, Bioessays 19 (1997) 819–826.

[17] C.D. McCaig, A.M. Rajnicek, B. Song, M. Zhao, Has electrical growth cone guidance found its potential, Trends. Neurosci. 25 (2002) 354–359.

[18] R. Nuccitelli, A role for endogenous electric fields in wound healing, Curr. Top. Dev. Biol. 58 (2003) 1–26.

[19] R. Nuccitelli, Endogenous electric fields in embryos during development, regeneration and wound healing, Radiat. Prot. Dosim. 106 (2003) 375–383.

[20] J.C. Ojingwa, R.R. Isseroff, Electrical stimulation of wound healing, J. Invest. Dermatol. 121 (2003) 1–12.

[21] K.R. Robinson, M.A. Messerli, Left/right, up/down: the role of endogenous electrical fields as directional signals in development, repair and invasion, Bioessays 25 (2003) 759–766.

[22] M.S. Kim, J.C. Park, Control of neonatal human dermal fibroblast migration on poly(lactic-co-glycolic acid)-coated surfaces by electrotaxis, J. Tissue Eng. Regen. Med. (2015) (in progress).

[23] J.F. Presley, C. Smith, K. Hirschberg, C. Miller, N.B. Cole, K.J. Zaal, J. Lippincott-Schwartz, Golgi membrane dynamics, Mol. Biol. Cell 9 (1998) 1617–1626.

[24] N. Sciaky, J. Presley, C. Smith, K.J.M. Zaal, N. Cole, J.E. Moreira, M. Terasaki, E. Siggia, J. Lippincott-Schwartz, Golgi tubule traffic and the effects of brefeldin A visualized in living cells, J. Cell Biol. 139 (1997) 1137–1155.

[25] K. Hirschberg, C.M. Miller, J. Ellenberg, J.F. Presley, E.D. Siggia, R.D. Phair, J. Lippincott-Schwartz, Kinetic analysis of secretory protein traffic and characterization of Golgi to plasma membrane transport intermediates in living cells, J. Cell Biol. 143 (1998) 1485–1503.