Effects of Physiological Electric Fields on Migration of Human Dermal Fibroblasts

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Abstract

Endogenous electric currents generated instantly at skin wounds direct migration of epithelial cells and are likely to be important in wound healing. Migration of fibroblasts is critical in wound healing. It remains unclear how wound electric fields guide migration of dermal fibroblasts. We report here that mouse skin wounds generated endogenous electric currents for many hours. Human dermal fibroblasts of both primary and cell-line cultures migrated directionally but slowly toward the anode in an electric field of 50–100 mV mm^{-1}. This is different from keratinocytes, which migrate quickly to the cathode. It took more than 1 hour for dermal fibroblasts to manifest detectable directional migration. Larger field strength (400 mV mm^{-1}) was required to induce directional migration within 1 hour after onset of the field. Phosphatidylinositol-3-OH kinase (PI3 kinase) mediates cathode-directed migration of keratinocytes. We tested the role of PI3 kinase in anode-directed migration of fibroblasts. An applied electric field activated PI3 kinase/Akt in dermal fibroblasts. Dermal fibroblasts from *p110γ* (a PI3 kinase catalytic subunit) null mice showed significantly decreased directional migration. These results suggest that physiological electric fields may regulate motility of dermal fibroblasts and keratinocytes differently, albeit using similar PI3 kinase-dependent mechanisms.

INTRODUCTION

Wound healing involves multiple cell types undergoing regulated sequential events: inflammation, reepithelialization, and tissue remodeling (Martin, 1997; Singer and Clark, 1999). Migration of immune cells, keratinocytes, and fibroblasts are essential elements of these events. Fibroblasts migrate into the wound, deposit extracellular matrix, organize the substratum, and contract the wound. Extracellular matrix, growth factors, cytokines, and other

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CONFLICT OF INTEREST
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SUPPLEMENTARY MATERIAL
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molecules at wounds have important chemokinetic and chemotactic effects on dermal fibroblasts (DFs; Diegelmann and Evans, 2004; Falanga, 2005; Gurtner *et al.*, 2008).

Studies suggest the importance of biophysical factors in cell migration. Mechanical forces such as stiffness of substratum and cell mechanical stress, for example, direct migration of fibroblasts (Lo *et al.*, 2000; Parker *et al.*, 2002). Another biophysical factor at wounds, the naturally occurring electric field (EF), is less studied. Epithelia, typically corneal epithelium with tight junctions, actively pump ions to maintain a transepithelial electric potential difference (TEP; Klyce, 1972). The epidermis also maintains a TEP. Barker and colleagues have mapped the TEP across the human body surface and the glabrous epidermis of guinea pigs. TEPs in these studies measured 15–45 mV mm^{-1} with the basal side positive relative to the skin surface (Barker *et al.*, 1982; Foulds and Barker, 1983). Wounds that break down the epithelial barrier lead to flow of positive charge (electric current) from the surrounding tissues toward the wound center, establishing EFs orientated toward the wound center. The currents return between the stratum corneum and live layers of epidermis in the opposite direction (Nuccitelli, 2003). Live tissues are electrically conductive, so EFs are generated whenever electric current flow.

Keratinocytes and many other cell types respond to applied EFs by directional cell migration, a phenomenon termed galvanotaxis or electrotaxis (Robinson, 1985; Nuccitelli, 1988; Nishimura *et al.*, 1996; Fang *et al.*, 1998). Because there are endogenous wound EFs, and cells important to wound healing respond to applied EFs *in vitro*, endogenous EFs have long been proposed to regulate wound healing (Borgens *et al.*, 1979; Jaffe and Vanable, 1984; see also reviews by McCaig and Zhao, 1997; Nuccitelli, 2003; Robinson and Messerli, 2003; McCaig *et al.*, 2005). Remarkably, EFs of physiological strength may override other coexisting directional cues to guide migration of the corneal epithelial cells and keratinocytes (Zhao *et al.*, 2006; Zhao, 2009). *In vivo* application of EFs appears to modulate corneal epithelial wound healing (Song *et al.*, 2002, 2004; Reid *et al.*, 2005). Importantly, electric stimulation has been approved for the treatment of some refractory skin wounds (Olyaee Manesh *et al.*, 2006).

The effects of physiological EFs on human DFs, however, are not well understood. Embryonic quail somitic fibroblasts migrate to the cathode in weak EFs (~ 10 mV mm^{-1}; Erickson and Nuccitelli, 1984). Much stronger EFs (400 mV mm^{-1}) are needed to induce cathodal migration of cell line fibroblasts (CLFBs) of murine embryonic origin (3T3 and SV101) and ligament fibroblasts (Brown and Loew, 1994; Finkelstein *et al.*, 2004; Chao *et al.*, 2007). Fibroblasts from bovine pulmonary artery and corneal stroma migrate to the anode in an EF of 200 mV mm^{-1} (Soong *et al.*, 1990; Bai *et al.*, 2004). Recently, Isseroff and colleagues elegantly compared electrotactic migration of human DFs with human keratinocytes. They showed that unlike keratinocytes, 1 hour exposure to a physiological EF of 100 mV mm^{-1} did not affect migration of human DFs (Sillman *et al.*, 2003). Endogenous wound EFs, however, may persist for many hours, even days and weeks, until wounds heal (Illingworth and Barker, 1980). We hypothesize that longer exposure to a physiological EF affects and guides migration of human DFs. We show here that mouse skin wounds maintained endogenous electric currents for at least several hours. Prolonged exposure to a physiological EF induces directional migration of human DFs to the anode through phosphatidylinositol-3-OH kinase (PI3 kinase) signaling.

RESULTS

Skin wounds maintained EFs for many hours

To characterize the endogenous EFs at skin wounds, we measured the endogenous electric currents at the surface of lancet wounds on the skin of recently killed mice using a vibrating probe noninvasively (Figure 1a). In moist tissues with certain resistance and conductance, the EFs are directly proportional to the electric currents as per Ohm's law. Initially, all wounds

showed transient inward currents (flow of positive charge into the wound) afterwards the current direction quickly reversed to an outward current (Figure 1b). Compared with endogenous currents at corneal wounds, the skin wound currents rose slowly, reaching a peak about 150 minutes after wounding. The electric currents were maintained at this level for up to 6 hours (the longest time point we carried out the measurements), at which time the wounds remained open with currents at ~ 3 μA cm^{-2}.

Delayed directional migration of human dermal fibroblasts in physiological EFs

We next tested the effects of EFs on the migration of human DFs. In an EF of 100 mV mm^{-1} for 1 hour, cells from primary cultures, as well as a cell line of human DFs, did not show directional migration. This is consistent with a previous report (Sillman *et al.*, 2003). Exposing the cells to the same field for longer periods, however, induced the cells to migrate directionally toward the anode. Shortly after 1 hour in an EF of 100 mV mm^{-1}, cells extended filopodia and lamellipodia and migrated toward the anode (Figure 2; see Supplementary Movies online).

To ensure that the cells continued to respond to the applied EFs, we reversed the field polarity after 5 hours. This caused both types of DFs to reverse their direction of migration so that they continued to migrate toward the anode (Figure 2; Supplementary Movies). Reversal of direction of migration took place 1–2 hours after EF reverses. These events were quantified as described previously using the value of directedness (see Materials and Methods). Quantitative analyses confirmed directional migration toward the anode and the latency in response in a large number of cells from several independent experiments (Figure 2i). If cells migrated toward the left (the anode side in the first 5-hour period; Figure 2), the directedness value would be negative. If cells migrated toward the right (the anode side after reversal of the polarity in the following 5-hour period; Figure 2), the directedness value would be positive. The more directional the cells migrated to the left, the closer the directedness value is to −1. The more directional the cells migrated to the right, the closer the directedness value is to 1. Quantitative analysis confirmed the directional migration of human DFs in an applied EF and reversal of migration direction following reversal of the field polarity (Figure 2i).

Voltage and time dependence of directional migration of dermal fibroblasts in EFs

We determined the effects of different voltages on the migration of DFs. An EF of 50 mV mm^{-1} for 5 hours tended to induce cell migration toward the anode, but without statistical significance. A field of 100 mV mm^{-1} or more induced significant cell migration directed toward the anode. The higher the voltage, the more directionally the cells migrated to the anode (Figures 3 and 4a). The migration rate in cells exposed to an EF of 50 mV mm^{-1} was significantly reduced compared with cells not exposed to an EF (Figure 4b).

To characterize the latency in directional cell migration, we analyzed the time course of migration directedness. In an EF of 100 mV mm^{-1} for 1 hour, neither primary DFs nor CLFBs showed directional migration (Figure 5). With continued exposure to an EF, the directionality of migration of both types of human DFs gradually increased and reached a peak by 4 hours. Therefore, EFs of a strength close to the *in vivo* value, if present for long enough (~ 4–5 hours), direct the migration of human DFs. The longer exposure time may compensate for the lower voltage.

PI3 kinase signaling mediates electrotaxis of dermal fibroblasts

PI3 kinase mediates cathode-directed migration of corneal epithelial cells and keratinocytes (Zhao *et al.*, 2006). We used genetic manipulation and pharmacological treatment to test if the anode-directed migration of DFs involves PI3 kinase signaling. Using western blot analysis, we found that an applied EF significantly increased the levels of phosphorylated Akt (p-Akt) in mouse and human DFs, indicating EF activation of PI3 kinase signaling (Figure 6a).

We then used a *p110γ* knockout mouse to confirm the role of PI3 kinases. *p110γ* is the catalytic subunit of PI3 kinase-γ, a major isoform of PI3 kinases. Absence of *p110γ* significantly decreased EF-induced PI3 kinase signaling, as indicated by significantly reduced p-Akt levels (Figure 6a). DFs from *p110γ* knockout mice also showed significantly reduced EF-directed cell migration, whereas the motility (trajectory speed) was largely unaffected (Figure 6b and c). To exclude developmental defects of *p110γ* knockout on EF-directed migration of DFs, we further confirmed the role for PI3 kinase signaling with pharmacological inhibition of PI3 kinases. Treatment of mouse DFs with LY294002 (a PI3 kinase inhibitor) caused significant decrease in EF-directed anodal migration (Figure 6b and c).

DISCUSSION

Our study results show that skin wounds generated endogenous electric currents for at least 5 hours, probably much longer, until the epithelial wound closes and the TEP is restored (Illingworth and Barker, 1980). The outward electric currents rise to a peak slower than that at corneal wounds (Figure 1b; Reid *et al.*, 2005). The ionic currents leave the wound and generate lateral fields on either side of the wound. DFs at wounds are therefore exposed to long-lasting endogenous currents and EFs. We mimicked the long-lasting endogenous EFs and exposed human DFs to physiological EFs for several hours. After a latent period > 1 hour, human DFs started migrating toward the anode, in a direction opposite to that of keratinocytes, which migrate toward the cathode.

Physiological EFs have different effects on migration of human DFs than they do on non-DFs and keratinocytes. In strong EFs (200–400 mV cm^{-1}), non-DFs derived from murine embryos (NIH3T3 and SV101), fibroblasts from bovine ligament, and embryonic quail somites migrate toward the cathode. In contrast, corneal stroma fibroblasts and pulmonary artery fibroblasts migrate toward the anode (Nuccitelli and Erickson, 1983; Soong *et al.*, 1990; Brown and Loew, 1994; Bai *et al.*, 2004; Finkelstein *et al.*, 2004; Chao *et al.*, 2007). The EFs inducing these responses are larger than endogenous EFs, while the strongest endogenous EFs and currents are measured at the wound edge (Barker *et al.*, 1982; Chiang *et al.*, 1992; Reid *et al.*, 2005; Mukerjee *et al.*, 2006). EFs of approximately 150 mV mm^{-1} are typical of the cornified layer of the epidermis (Nuccitelli *et al.*, 2008). In the dermis, where fibroblasts reside, the EFs are likely to be smaller because of relatively lower resistance and voltage dissipation in three-dimensional tissues. Human skin fibroblasts do not respond within 1 hour to an EF of 100 mV mm^{-1} (Sillman *et al.*, 2003). However after a latent period of 1–2 hours, the cells migrate directionally to the anode (Figure 2). In an EF of 50 mV mm^{-1}, primary cultured DFs migrated at lower rates compared with that of control cells cultured without EF (Figure 4b). The effects of physiological EFs on the direction of migration and rate are different from those on keratinocytes, which migrate to the cathode with increased speed (Nishimura *et al.*, 1996; Farboud *et al.*, 2000; Pullar *et al.*, 2006; Zhao *et al.*, 2006).

Sequential migration of different cell types into the wound has to be well choreographed to achieve proper wound healing. Physiological EFs have different effects on different cell types (for example, the effect on migration speed and direction of DFs and keratinocytes). This observation supports a recent intriguing theory of "traffic control" of sequential cell migration in wound healing (Henry *et al.*, 2003; Bandyopadhyay *et al.*, 2006). The authors propose that a "plasma-to-serum-to-plasma" transition during wound healing orchestrates the orderly migration of epidermal and dermal cells. Transforming growth factor-β3 (TGF-β3) binds to the type II TGFβ receptor and inhibits cell motility. Wounding exposes skin cells to serum, which contains high level of TGF-β3. Bandyopadhyay *et al.* (2006) showed that DFs expressed higher levels of type II TGFβ receptor than keratinocytes. Addition of TGF-β3 in a medium with plasma significantly decreased migration of DFs in a dose-dependent manner. Neutralizing TGF-β3 with antibodies abolished this effect of TGF-β3. This inhibitory effect

was not present when neutralizing antibodies against TGF-β1 or TGF-β2 were added. The authors therefore suggest that high TGF-β3 level in serum may selectively inhibit motility of DFs and favor reepithelialization at an early stage after wounding (Bandyopadhyay *et al*., 2006). As the wound heals, the cells experience a transition of serum back to plasma with less inhibitory effects on the migration of DFs, which then migrate into the wound. Differential effects of wound EFs on the motility of keratinocytes and DFs may represent a similar regulatory mechanism that participates in the "traffic control" for the orderly and organized migration of keratinocytes and DFs during wound healing. The *in vivo* control of the "traffic" is likely to involve multiple factors. For example, serum and wound fluid contain many mitogens and motogens. A major one of these is platelet-derived growth factor (Werner and Grose, 2003; Barrientos *et al*. 2008). Platelet-derived growth factor significantly stimulates both growth and migration of DFs (Katz *et al*., 1991; Li *et al*., 2004). Biochemical factors thus may collectively regulate cell motility and migration direction, and control the traffic with biophysical cues. Coordination of these controllers will be an important topic for future research.

In addition to the endogenous wound EFs, exogenously applied EFs of higher voltage have been used in the treatment of some chronic and refractive wounds and have shown some beneficial effects (reviewed by Weiss *et al*., 1990; Davis and Ovington, 1993; Lee *et al*., 1993; Kloth and McCulloch, 1996; Poltawski and Watson, 2009). For example, a frequency of 105 Hz, an intraphase interval of 50 μ seconds, and voltages of 100–175 V over 15cm significantly accelerated healing of stage IV decubitis ulcers in humans. The percentage of healed wound increased several-fold with this scheme of electric stimulation (Kloth and Feedar, 1989). These EFs are relatively large, about 10 times the physiological field strength (~ 100 mV mm^{-1}). In addition to the possible effects on cell migration, such EFs might have other effects, such as activating intracellular signaling pathways or stimulating blood circulation (Zhao, 2009). We showed in this study that strong EFs (400 mV mm^{-1}) induced responses of DFs within 1 hour (Figure 5a and b), supporting the effectiveness of high voltage stimulation in regulating migration of human DFs. We speculate that exposure duration and voltage strength may compensate each other in regulating cell migration, suggesting that lower voltages for more prolonged periods of time might have an equally beneficial effect (Figure 4b).

Genetic disruption of the *p110γ* gene, the catalytic subunit of an isoform of PI3 kinase, significantly reduced EF-induced PI3 kinase signaling and inhibited electrotaxis of mouse DFs. This was corroborated with a PI3 kinase inhibitor (Figure 6). PI3 kinase, the same molecule that regulates cathode-directed migration in keratinocytes, also mediates anode-directed migration in fibroblasts. Considerable amount of information is also available regarding ligand–receptor interactions at the cell surface in epithelial cells in an EF, but little is presently known about fibroblasts (Forrester *et al*., 2007). The activation of PI3 kinase may not define the directionality; some upstream molecules are likely to do this. Further investigation to identify molecules upstream of PI3 kinase may yield direction "sensor(s)."

In conclusion, physiological EF stimulation lasting for more than 1 hour induces human DFs to migrate toward the anode, mediated by the PI3 kinase signaling pathway. Thus, endogenous wound EFs may contribute to cell "traffic control" during wound healing through differentially regulated motility of different cell types. Long exposure (> 1 hour) to physiological EFs, or short exposure (< 1 hour) to strong EFs, may modulate wound healing by regulating migration of DFs.

MATERIALS AND METHODS

Materials

Falcon tissue culture dishes (100 mm) were from BD Bioscience (Franklin Lakes, NJ). Eagle's minimum essential medium and PI3 kinase inhibitor (LY294002) were from Sigma-Aldrich (St Louis, MO). CO_2-independent medium, penicillin, and streptomycin were from Gibco Life Technology (Paisley, Scotland, UK). Antibodies against total and phosphorylated forms of Akt (Ser473) were from Cell Signaling (Beverly, MA).

Measurement of EFs at skin wounds

C57BL/6 mice were killed by cervical dislocation, which was approved by the Ethical Committee of University of Aberdeen and the Home Office. Hair was removed from the back and a lancet wound (~ 3 mm in length) was made by cutting into the full thickness skin, one wound on each of the three mice. Endogenous wound electric currents were measured using a vibrating probe (Vibrating Probe Company, Davis, CA) as described earlier (Reid *et al.*, 2007). Briefly, an insulated stainless steel electrode was electroplated with gold and platinum. When the probe is vibrated in a fluid through which a steady current flows, it detects a voltage gradient. Measurements were carried out at room temperature with the probe approximately 50 µm away from the wound edge surface (see Figure 1a). Measurements were carried out in phosphate-buffered saline solution (pH 7.4; Sigma-Aldrich, catalog no. P4417).

Cultures of human and mouse dermal fibroblasts

A human DF cell line (161BR, ECACC No. 90011809; CLFB) and primary cultures of human DFs (PMFB) were maintained in Eagle's minimum essential medium (10% fetal bovine serum, 100 U ml^{-1} penicillin, and 100 U ml^{-1} streptomycin), at 37 °C in a 5% CO_2 incubator, and the medium was changed every 2–3 days.

Primary cultured human DFs were derived from the eyelids of adult donors (59–66 years old) who underwent surgical blepharoplasty and did not have preexisting pathological conditions. The dermal tissue was washed with Ca^{++}-Mg^{++}-free PBS, minced with sterile scissors, and allowed to adhere to tissue culture flasks for 30 minutes in a CO_2 incubator at 37 °C. Eagle's minimum essential medium was then added to the flasks. Fibroblasts were allowed to grow out of the tissue pieces attached to the tissue culture flask. Cells were subcultured when they reached 50–70% confluence. The fourth passage of cells from four donors was used for all experiments. Use of human donor tissues was approved by the National Health Service Grampian Ethical Committee and adhered to the Declaration of Helsinki Principles. Informed consent was obtained from each donor.

Wild-type C57BL/B6 mice and *p110γ* null mice were as described previously (Zhao *et al.*, 2006). Genotypes were confirmed with PCR. All mice were kept according to institutional guidelines. Killed mice were shaved and their back skin was excised. After trimming off the interstitial tissues, DF cells were cultured using procedures as described earlier.

EF exposure and quantification of cell responses

The electrotaxis experiments were carried out as described (Zhao *et al.*, 1996). The electrotaxis chamber was coated with calf collagen I (Sigma-Aldrich, catalog no. C 8919) at 10 mg cm^{-2} and incubated at 37 °C for 24 hours. Excess solution was then removed by washing with PBS and dishes were air-dried before experiments. Cells were seeded in the chamber and incubated up to 24 hours (37 °C; 5% CO_2), allowing them to settle and adhere to the dish, before a roof of no. 1 cover glass was applied as a lid and sealed with silicone grease to create a thin, wafer-like chamber of ~ 200 µm deep. The small cross-sectional area provided a high resistance to

the current flow and minimized Joule heating. CO_2-independent culture medium was used in electrotaxis experiments to maintain stable pH.

The cells were exposed to an EF (at 50, 100, and 400 mV mm^{-1}) for 5 hours at 37 °C. In some experiments, the field polarity was reversed for another 5 hours. To inhibit PI3 kinases, we pretreated cells with 30 μM LY294002 (a PI3 kinase inhibitor) for 2 hours before EF application. Time-lapse images of cell migration were acquired at 10 minute intervals and analyzed using the MetaMorph 6.0 imaging system (Universal Imaging Corporation, Downingtown, PA). To exclude the possible influence of cell–cell interactions on cell behavior, we analyzed only isolated cells that were not in contact with other cells.

Two components of cell migration were quantified: (1) trajectory speed, which is used to define the migration rate, is the distance (in μm) traveled by the cell divided by time (5 hours); and (2) directedness, as a parameter to define how directionally cells migrated in response to EFs (Pu and Zhao, 2005). The angle that each cell moved with respect to the EF vector was measured and its cosine value was calculated as directedness (Gruler and Nuccitelli, 1991). The cosine of this angle would be equal to 1 if the cell moved perfectly along the field vector toward the right, 0 if the cell moved perpendicularly to the field vector, and −1 if the cell moved directly toward the left. The average of all the cosine values ($\Sigma i \cos\theta/n$) gives the mean migration directedness of a population of cells, where θ is the angle between the field vector and the cellular translocation direction and n the total number of cells analyzed in each experimental condition. In the case of experiments where cells were treated with an EF and then the polarity reversed, the second half of the EF with the polarity reversed was treated as a new experiment for the calculation. Unpaired, two-tailed Student's t-test was used for statistical analysis.

Western blotting

Primary cultured DFs from humans or mice were starved in serum-free culture medium for 8 hours before EF stimulation at 100 mV mm^{-1}. After application of the EF for different time periods, cells ($\sim 5 \times 10^6$) were lysed in 500 μl SDS sample buffer. Cell extracts were resolved on 4–12% Bis-Tris gels and transferred onto polyvinylidene difluoride membranes. The membranes were blocked with 5% non-fat milk in Tris-buffered saline, and incubated with specific antibodies. Antibodies against total and phosphorylated forms of Akt (Ser473) were used at 1:1,000 dilution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

CLFB	cell line fibroblast
DF	dermal fibroblast
EF	electric field
PI3 kinases	phosphatidylinositol-3-OH kinase
TEP	transepithelial electric potential difference
TGF-β3	transforming growth factor-β3

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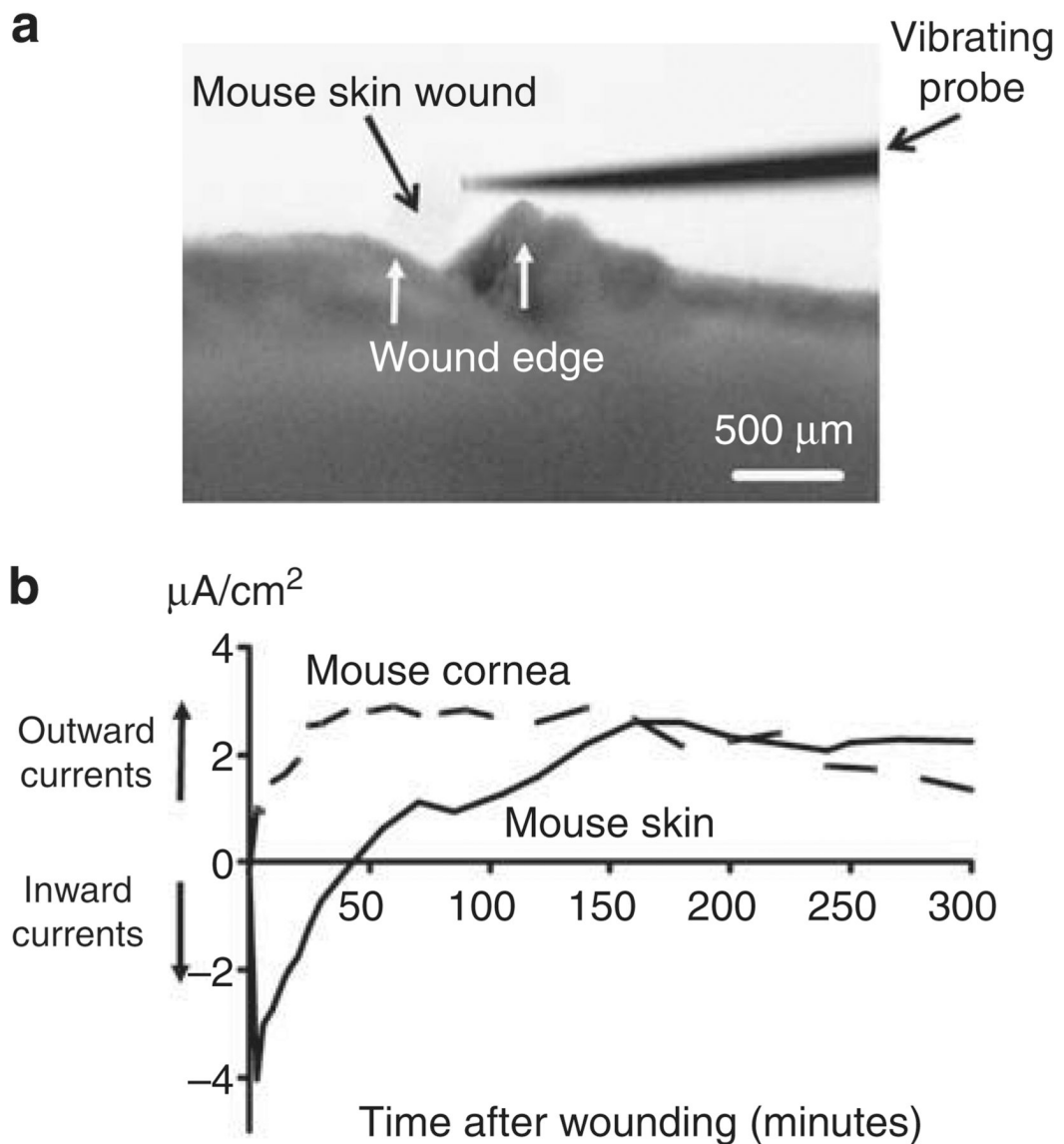
a

b

Figure 1. Endogenous electric currents at skin wounds
(**a**) View down dissecting microscope showing a vibrating probe in measuring position at a shallow lancet wound on the skin of a mouse. Bar = 500 µm. (**b**) Time courses of endogenous electric currents. Note the slow rise and long-lasting nature of the skin wound electric currents. Measurements were carried out immediately after cervical dislocation and wounding. Positive value: outward current; negative value: inward current. The data are grouped from three independent experiments with three individual mice.
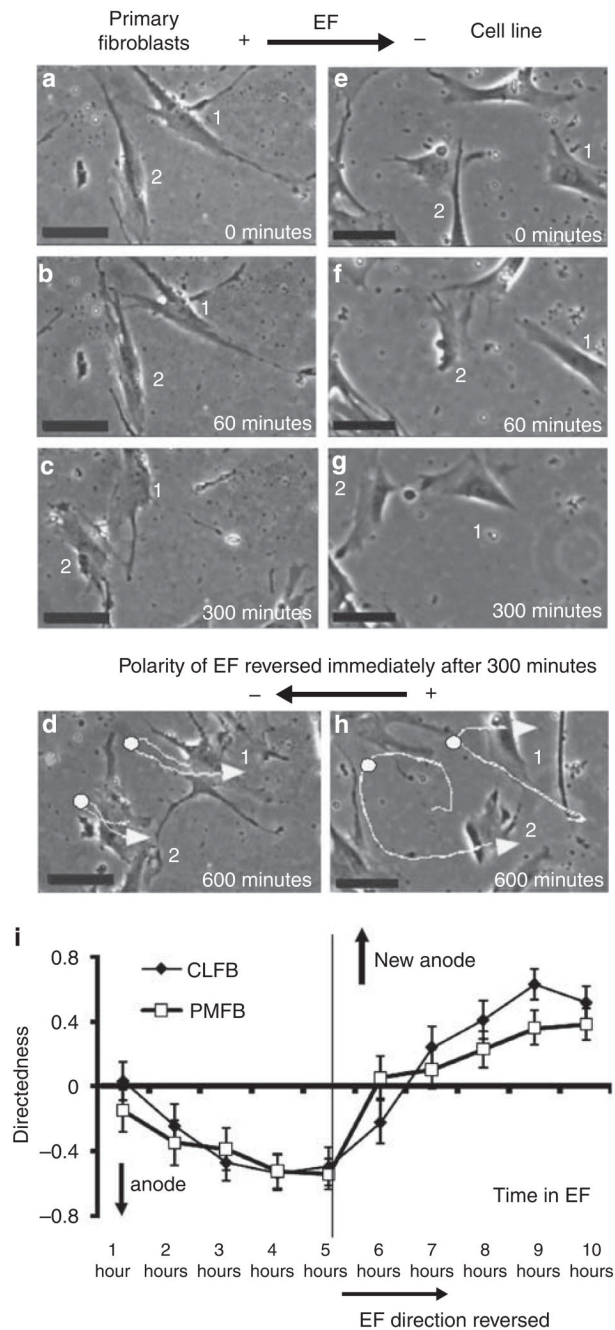
Figure 2. Human dermal fibroblasts migrate directionally toward the anode in an electric field (EF) of 100 mV mm^{-1} after prolonged exposure

(**a**–**d**) Primary DFs, (**e**–**h**) fibroblast cell line. Exposure to an EF of 100 mV mm^{-1} for more than 60minutes started to induce cell migration to the anode. When the field polarity was reversed, the cells reversed the migration direction. Trajectories of cells are highlighted with white lines in **d** and **h**. Arrows indicate end points of the cells, and the white dots on the trajectories the point the field polarity was reversed at 5 hours (immediately after **c** and **g**). (**i**) Quantification of migration direction shows the reversal of cell migration following reversal of the field polarity. Data are from four independent experiments (86 cells from primary cultures and 55 cells from cell line cells). Bar = 50 µm. See Supplementary Movies online.
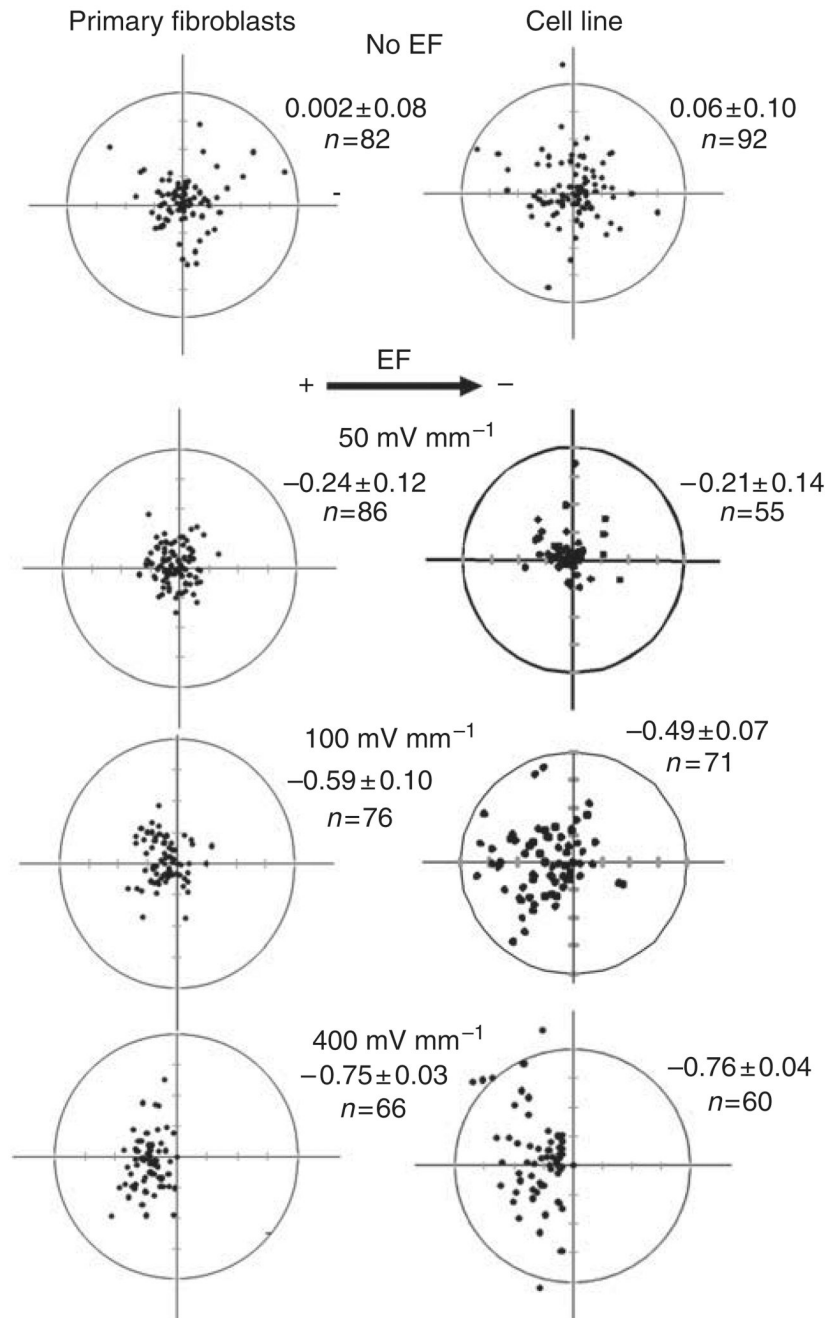
Figure 3. Scatter plots show cell migration for 5 hours in the presence or absence of an electric field (EF)

The start position of each cell was set as the origin in the center of the scatter plots (0,0). Black dots represent positions of individual cells after 5 hours. Primary culture and cell line fibroblasts migrated directionally toward the anode (left) in an EF of 100 and 400 mV mm^{-1}. The radius of each circle represents 150 µm of translocation distance. "n" represents the total number of cells in each condition. The directedness of migration is shown on the upper right corner of each plot (mean±SEM). The cathode is to the right of each graph. The data shown are from at least four independent experiments.
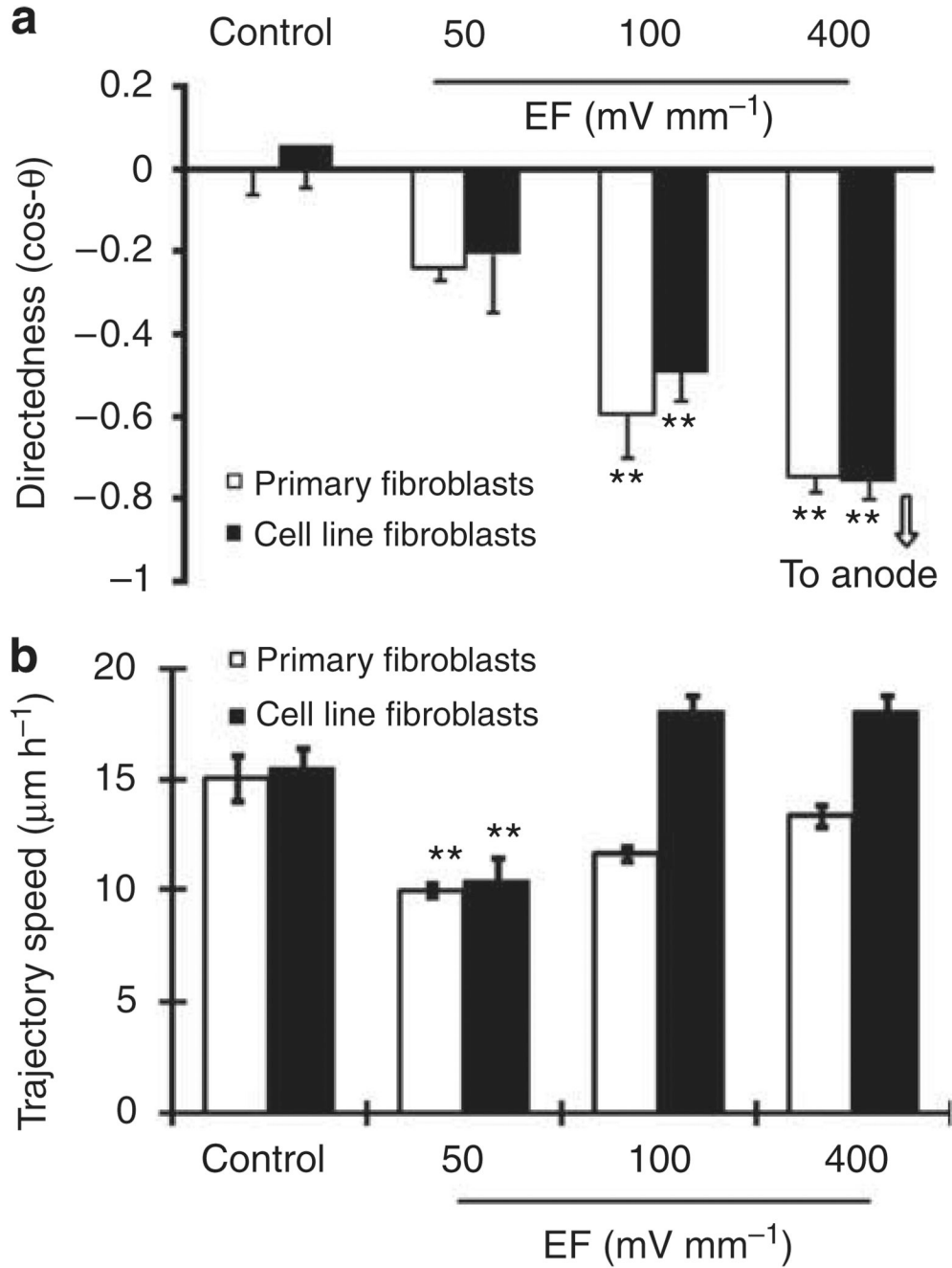
Figure 4. Voltage dependence of migration directedness and migration rate of human dermal fibroblasts (DFs)
(**a**) Both primary cultures and a cell line of human DFs migrated directionally toward the anode. Increasing the field strength increased the migration directedness. (**b**) Migration rate of human DFs depended on strength of applied EF. Cell numbers 60–92 from at least three individual experiments. **P<0.01 when compared with controls with no EF.
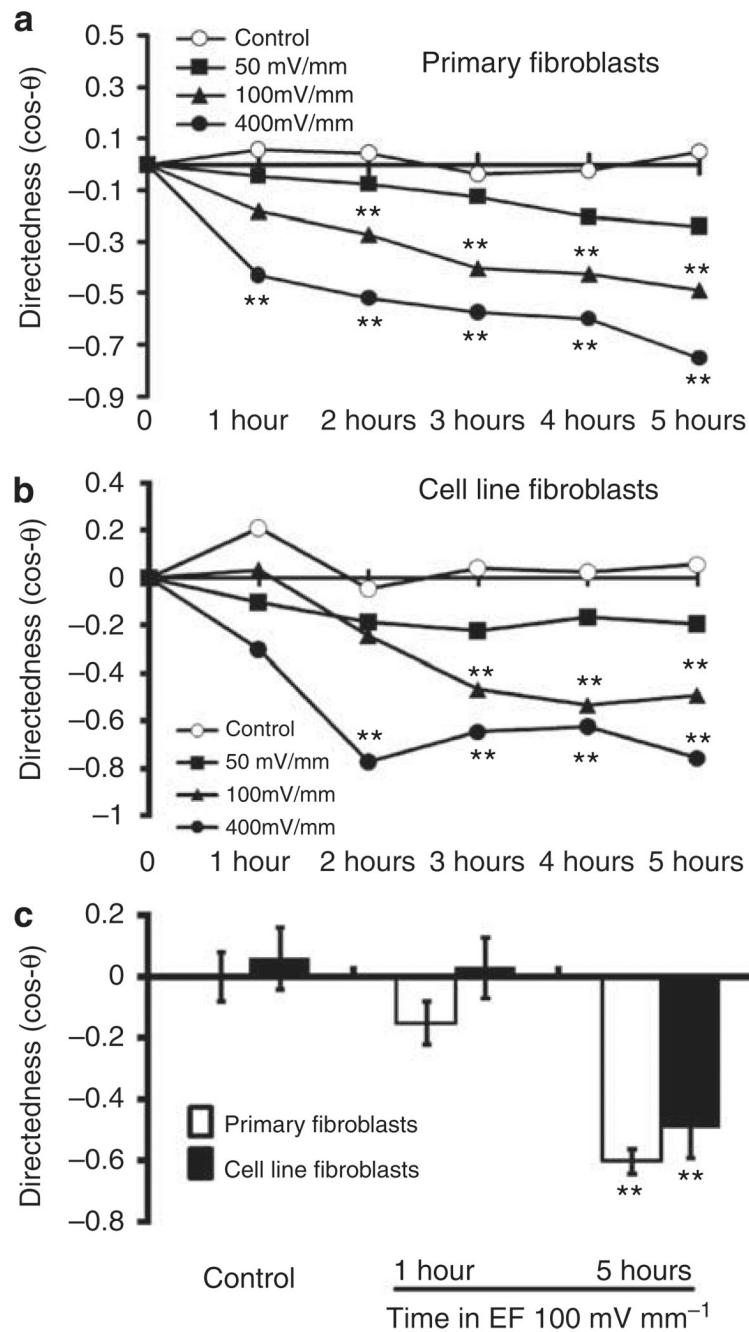
Figure 5. Directional migration of human dermal fibroblasts (DFs) was time dependent
(**a**, **b**) Time dependence of directional migration of both types of human DFs at different voltages. **P<0.01 when compared with the control values at the same time points without an electric field (EF). (**c**) In an EF of 100 mV mm^{-1} for 1 hour, cells migrated randomly, whereas in the same EF for 5 hours, cells showed significantly directional migration. **P<0.01 comparing with 1 hour groups.
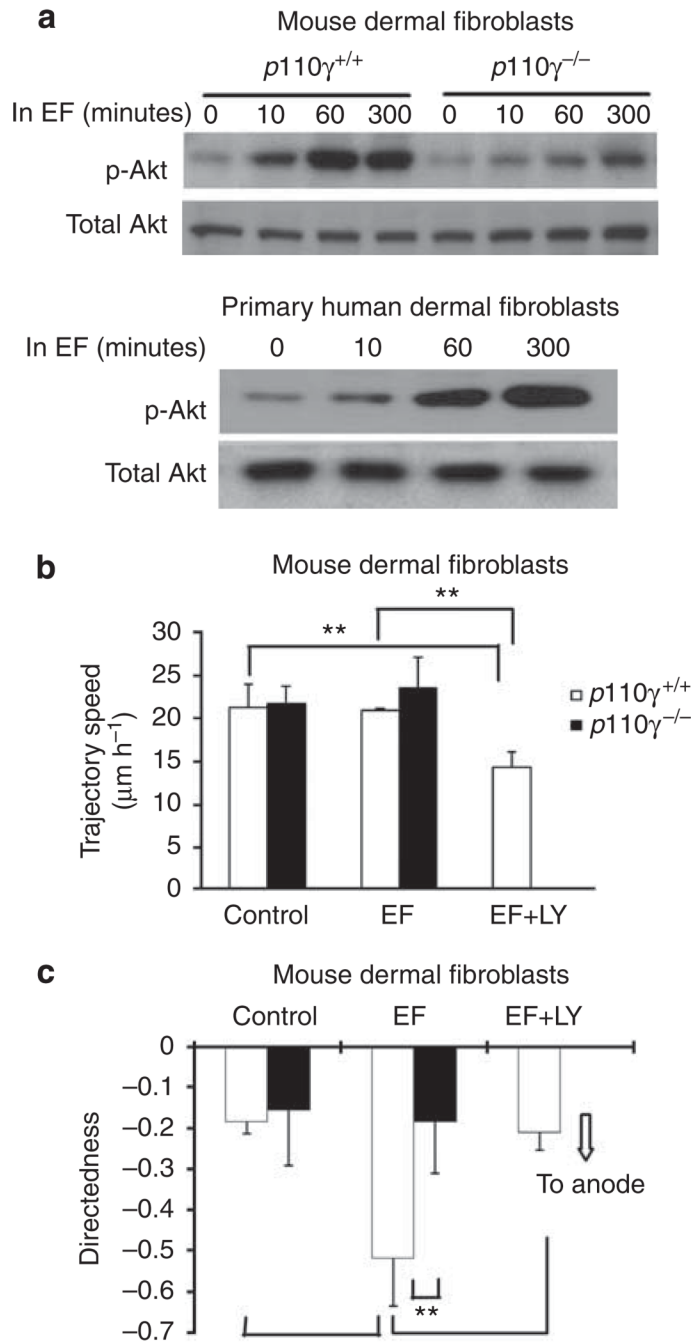
Figure 6. Role of phosphatidylinositol-3-OH kinase (PI3 kinase) signaling in electric field (EF)-directed migration of dermal fibroblast (DF) cells
(**a**) Western blot analysis shows that exposure to an EF increased cell levels of phosphorylated Akt (p-Akt), indicating activation of PI3 kinase signaling. Knockout of PI3 kinase catalytic subunit (*p110γ*) significantly reduced PI3 kinase signaling. (**b, c**) Knockout of *p110γ* abolished directional migration, whereas the migration speed did not change significantly. Pharmacological inhibition of PI3 kinase also resulted in loss of directional migration in an EF. Cell numbers were 59–69, and 60–92 for *p110γ*^{+/+} and *p110γ*^{−/−} cells, respectively, from three independent experiments. EF = 100 mV mm^{−1}. **P<0.01.