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Skin-derived precursor cells undergo substrate-dependent galvanotaxis that can be modified by neighbouring cells

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ABSTRACT

Many cell types respond to electric fields (EFs) through cell migration, a process termed galvanotaxis. The galvanotactic response is critical for development and wound healing. Here we investigate whether skin-derived precursor cells (SKPs), which have the potential to differentiate into mesodermal and peripheral neural cell types, undergo directed migration in the presence of an EF. We found that EF application promotes SKP migration towards the anode. The migratory response is substrate-dependent as SKPs undergo directed migration on laminin and Matrigel, but not collagen. The majority of SKPs express the undifferentiated cell markers nestin, fibronectin and Sox2, after both EF application and in sister cultures with no EF application, suggesting that EFs do not promote cell differentiation. Co-cultures of SKPs and brain-derived neural precursor cells (NPCs), a population of cells that undergo rapid, cathode-directed migration, reveal that in the presence of NPCs an increased percentage of SKPs undergo galvanotaxis, providing evidence that cells can provide cues to modify the galvanotactic response. We propose that a better understanding of SKP migration in the presence of EFs may provide insight into improved strategies for wound repair.

1. Introduction

Endogenous EFs are important throughout development and in wound healing. Physiologically, EFs and electric potentials are created through the separation of ions. These EFs and electric potential patterns control an organism's anatomy and morphology (Levin et al., 2017; Levin and Martyniuk, 2018). EFs affect cellular processes such as migration, alignment, proliferation and differentiation (Thrivikraman et al., 2018). Galvanotaxis, which is the migration due to EFs, has been shown to override other migration cues (Funk, 2015; Zhao, 2009). Cells can sense EFs through the electrophoresis of charged membrane channels and the polarization of charged molecules triggering localized signalling pathways and cytoskeletal changes for cell migration (Allen et al., 2013; Huang et al., 2017; Nakajima et al., 2015). Further, the galvanotactic response is cell-type dependent. Cells are differentially electro-sensitive and migrate at different speeds and directions, *i.e.* towards the positive or negative EF terminal called the anode and cathode, respectively (Ozkucur et al., 2011; Sillman et al., 2003) and field strengths of 3 mV/mm to over 1000 mV/mm can elicit a galvanotactic response depending on the cell type (Iwasa et al., 2017). One of the most well-studied stem cell types that undergo galvanotaxis are NPCs which demonstrate a robust, rapid and directed cathodal migration in response to EFs (Babona-Pilipos et al., 2011, 2015; Cao et al., 2013; Meng et al., 2011).

When an injury occurs, an EF is created which directs cell migration to the injury site. Understanding how EFs affect the migration of stem cells is important to understanding how wound repair occurs. In the skin, cell migratory responses to EFs are varied. Mature cell phenotypes such as human epidermal keratinocytes migrate towards the cathode in an EF (Nishimura et al., 1996), human dermal fibroblasts migrate towards the anode (Guo et al., 2010), and melanocytes have not been shown to undergo galvanotaxis (Grahn et al., 2003). With regard to dermal skin-derived stem cells, the galvanotactic response has not been examined. SKPs are found in the dermal papillae and are multipotent, self-renewing cells (Toma et al., 2001). These stem and progenitor cells give rise to adipocytes, dermal fibroblasts, skeletogenic cells, smooth muscle cells, Schwann cells and neuronal precursors (Biernaskie et al., 2009; Fernandes et al., 2008; Lavoie et al., 2009; McKenzie et al., 2006;

Abbreviations: SKPs, Skin-derived precursor cells; NPCs, Neural precursor cells; EFs, Electric fields

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Steinbach et al., 2011; Toma et al., 2001). These cells contribute to wound healing through migration and differentiation in response to injury. Indeed, SKPs migrate in response to biopsy punch wounds and their transplantation can promote wound repair in diabetic ulcers (Biernaskie et al., 2009; Ke et al., 2015). Further, factors that promote their proliferation *in vitro* can improve wound healing *in vivo* (Naska et al., 2016). Given the promising therapeutic potential of SKPs for enhancing skin repair, we sought to determine their responsiveness to EF application.

Herein, we examine the effects of EFs on SKP galvanotaxis. We demonstrate that SKPs undergo anodal galvanotaxis. SKP galvanotaxis is substrate-dependent and the presence of NPCs and their conditioned media modify their galvanotactic response.

2. Materials and methods

2.1. Ethics statement

All animal work was approved by the University of Toronto Animal Care Committee in accordance with institutional guidelines (protocol no. 20011515). All SKP dissections were performed on CD1 post-natal day 0 to 6 murine pups (Charles River). Neurosphere dissections were performed from adult C57/BL6 or transgenic mouse R26R-EYFP bred in house.

2.2. Cell culture

SKPs were obtained as previously described (Biernaskie et al., 2006; Toma et al., 2001). Briefly, back skin tissue from wild-type CD1 pups (postnatal day 0–6) was dissected free of other tissue, and was dissociated both mechanically and enzymatically with collagenase (1 mg/mL, Sigma-Aldrich). Cells were plated at most 50 cells/μL in T25 or T75 culture flasks (BD Falcon) in SKP media: (DMEM + Glutamax:F12 + Glutamax, 3:1) with 1% penicillin/streptomycin (Invitrogen) supplemented with epidermal growth factor (EGF, 20 ng/mL; Sigma-Aldrich), basic fibroblast growth factor (bFGF, 40 ng/mL; Sigma-Aldrich) and 2% B27 supplement. SKPs were fed with the addition of 2–5 mL of fresh medium containing all growth factors and supplements every 2–5 days to replenish the entirety of the culture media. Every 7–21 days SKPs were passaged. As previously described, media and cells were transferred to new flasks to avoid SKP sphere contact with adherent cells. To passage, SKP-derived spheres were collected, mechanically and enzymatically dissociated into single cells and re-plated in SKP media. For all experiments, SKP spheres were used between passage numbers 1–3.

For co-culture experiments, NPCs were derived from a transgenic mouse which ubiquitously expressed yellow fluorescent protein. Neurospheres were isolated from adult mouse brains as previously described (Babona-Pilipos et al., 2011). Briefly, brains were removed and the periventricular regions surrounding the lateral ventricles were isolated. These were enzymatically and mechanically dissociated. The cells were plated in serum free media with EGF, (20 ng/mL; Sigma-Aldrich), bFGF, (10 ng/mL; Sigma-Alderich) and heparin (2 μg/mL, Sigma-Aldrich). The NPCs were passaged every 7 days and were used between passages 1–4 for the galvanotaxis assay.

For conditioned media (CM) collection, C57/BL6-derived neurosphere cells were plated at 40 cells/μL. The following day the flask was spun down at 1500 rpm and CM was collected and stored at −20 °C or − 80 °C prior to use.

2.3. Galvanotaxis chambers and assay

Galvanotaxis chambers were constructed as described (Babona-Pilipos et al., 2011, 2012). Briefly, acid-treated glass slides were coated with poly-L-lysine (Sigma-Aldrich) plated with laminin from Engelbreth-Holm-Swarm murine sarcoma basement membrane (50 μg/mL, Sigma), collagen I rat tail (40 μg/mL, Gibco) and 4% (*v*/v) Corning Matrigel Basement Membrane Matrix (BD Biosciences, Canada). Following coating, SKP spheres were triturated with a P200 and incubated for 17–22 h in the presence of EGF, bFGF and B27 at 37 °C, 5% CO_2 in 100% humidity. Prior to being placed in the presence of the direct current EF, the chambers received a glass slide as a roof and were placed in the microscope for live-cell imaging. Silver wire (Alfa Aesar) chlorinated in bleach was used as electrodes and 1.5% agarose gel bridges were used to separate the toxic electrode-media interface from the cells.

For co-culture experiments, chamber construction was the same as above. Both SKP and NPC spheres were removed from their respective cultures, triturated and plated together on chambers coated with laminin. For CM experiments, on the day of chamber construction the CM was thawed and supplemented with EGF, bFGF and B27, and sterile filtered. The CM was added to the chambers and triturated SKP spheres were plated onto the galvanotaxis chamber and placed in the presence or absence of the EF.

2.4. Quantification of cell migration

Cells were live-imaged and tracked in the presence and absence of the EF. For SKP migration experiments, images were taken every minute for 150 min. At least 15 cells per experiment were tracked using AxioVision Cell Tracking Software and at least 3 chambers were run per experimental condition. For co-culture experiments, a microscope field of view, which included at least 15 cells that were non-fluorescent (SKPs) and 15 cells that were fluorescent (yellow fluorescent NPCs), was chosen. Images were taken every 5 or 10 min for 150 min. Cells were tracked with automatic tracking software or with the manual tracking option at 10 min intervals. X and Y-displacement shifts in the chamber itself were subtracted from the displacement of the tracked cells as appropriate in the following analyses.

For each individual cell the following parameters were calculated using the measurements as depicted in the diagram in Fig. 1A. The magnitude of the velocity (|velocity|) was calculated by dividing the cell displacement by the elapsed time. The directedness of the migration was calculated by dividing the cell displacement in the direction of the EF (x-displacement) by the cell displacement. This gives the cosine of the angle (θ) between the cell displacement vector and the EF vector. A directedness value of 1 is a cell migrating directly towards the cathode and a value of −1 is a cell migrating directly towards the anode. These values were averaged over the total number of cells tracked in each experimental condition.

Cell migration path graphs were plotted with each cell migration path starting at the origin (0,0) as seen in Fig. 1H-M and Fig. 3C and D. To quantify cell migration directional preference on laminin, NPCs or SKPs were considered to have migrated towards a terminal (cathode or anode), if its displacement towards a terminal was greater than the average of all cell displacements in the absence of an EF plus the standard error of the means for their respective cell type. Otherwise, the cell was considered undirected.

2.5. Immunocytochemistry

Immunocytochemistry was performed as previously described (Babona-Pilipos et al., 2011). Cells were plated overnight (17–22 h) in the absence of an EF. Cells were placed in the EF for 150 min and then fixed with 4% paraformaldehyde (Sigma) for 10–20 min at room temperature for 3 chambers. Control cells were plated overnight (17–24 h) in the absence of an EF for 3 chambers and then fixed. The cells were washed 3 times with PBS for 5 min and permeabilized with 0.3% Triton X-100 (Sigma) for 20 min at room temperature. The cells were exposed to 10% NGS (Jackson Immunoresearch Laboratories) in PBS for 1 h at room temperature and then washed and incubated overnight in primary antibody cocktail at 4 °C. The cells were then triple washed for 5 min