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Electric field as a potential directional cue in homing of bone marrow-derived mesenchymal stem cells to cutaneous wounds

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ABSTRACT

Bone marrow-derived cells are thought to participate and enhance the healing process contributing to skin cells or releasing regulatory cytokines. Directional cell migration in a weak direct current electric field (DC-EF), known as electrotaxis, may be a way of cell recruitment to the wound site. Here we examined the influence of electric field on bone marrow adherent cells (BMACs) and its potential role as a factor attracting mesenchymal stem cells to cutaneous wounds. We observed that in an external EF, BMAC movement was accelerated and highly directed with distinction of two cell populations migrating toward opposite poles: mesenchymal stem cells migrated toward the cathode, whereas macrophages toward the anode. Analysis of intracellular pathways revealed that macrophage electrotaxis mostly depended on Rho family small GTPases and calcium ions, but interruption of PI3K and Arp2/3 had the most pronounced effect on electrotaxis of MSCs. However, in all cases we observed only a partial decrease in directionality of cell movement after inhibition of certain proteins. Additionally, although we noticed the accumulation of EGFR at the cathodal side of MSCs, it was not involved in electrotaxis. Moreover, the cell reaction to EF was very dynamic with first symptoms occurring within <1 min. In conclusion, the physiological DC-EF may act as a factor positioning bone marrow cells within a wound bed and the opposite direction of MSC and macrophage movement did not result either from utilizing different signalling or redistribution of investigated cell surface receptors.

1. Introduction

Cutaneous wound healing is a multistage process that employs many types of cells, including skin cells, inflammatory cells and endothelial cells [1]. The appropriate coordination of cell migration and release of cytokines or growth factors are necessary to orchestrate this process. However, in the case of extensive and deep skin injuries, the resident progenitor cells are unable to reconstitute a fully functional tissue and may be supported by cells that are primarily absent at the wound site, thus recruited from distant niches. Mounting evidence suggests that bone marrow-derived cells, including mesenchymal stem cells (BM-MSC) may be attracted to the skin lesion [2–5]. BM-MSC, also referred to as stromal progenitor cells, are self-renewing and expandable stem cells that were found to differentiate into adipocytes, osteoblasts and chondrocytes [6]. Recently, it was shown that BM-MSC play an active role in the healing process contributing to different types of skin cells, i.e. keratinocytes and fibroblasts [2,4]. Moreover, data indicate that BM-MSC also enhance healing by producing and releasing proangiogenic factors such as VEGF and angiopoietin-1 (Ang-1) or extracellular matrix proteins, i.e. collagen III [4].

Cells participating in wound healing have to be guided specifically to the site of action in order to perform their functions. Cell migration through an increasing gradient of soluble chemoattractant created in the healing tissue is a common way of cell recruitment to the wound site. However, cells may also respond by directed migration to other factors such as adhesion site gradient, matrix topography or matrix stiffness [7]. Moreover, one of the first directional cues that appears in the skin immediately after wounding is a DC-EF. A weak DC-EF is created as a result of local transepithelial potential collapse at the breached epithelium. This gives rise to a steady voltage gradient of 40–200 mV/mm directed toward the wound edge and parallel to the epithelial layer with the wound negative with respect to intact tissue [8–10]. DC-EF lasts during healing and may guide cell migration by a process known as

Abbreviations: A-BMAC, anode-migrating bone marrow adherent cells; BMAC, bone marrow adherent cells; C-BMAC, cathode-migrating bone marrow adherent cells; DC-EF, direct current electric field; MSC, mesenchymal stem cells.

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electrotaxis or galvanotaxis. It is well documented that endogenous EFs guide fibroblasts and keratinocytes and thus accelerate the wound healing process. However, many other types of cells were shown to respond to physiological levels of EF with directed migration toward one of the electrodes, mostly to the cathode.

Although the process of electrotaxis was firstly described in the XIX century, the mechanism of electric field detection by cells is still poorly understood. One hypothesis proposed by Jaffe and then Poo and McLaughlin, assumes that the electrostatic or electroosmotic forces redistribute charged components of the cell membrane including receptors of chemoattractants, increasing their density at one site of the cell [11–13]. As a result signal propagation within a cell polarizes and leads to directional cell migration in EF. Over the last 20 years a number of membrane receptors such as epidermal growth factor receptor (EGFR), concanavalin A receptor (ConA), sodium-hydrogen exchanger 3 (pNHE3), N-methyl-D-aspartate receptor (NMDAR), acetylocholine receptor (AchR) or integrins were shown to be redistributed under EF influence and involved in cell electrotaxis [14–21]. Moreover, several downstream signalling mechanisms, including Rho GTPases, phosphatidylinositol 3-kinase (PI3K), extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (p38 MAPK), Src kinase, Akt kinase or calcium ions were proposed as essential in cell electrotaxis [22–24]. However, the 'sensor' responsible for the initial detection of the presence of an applied EF still remains elusive.

In this study, we examined the influence of EF on migration of mouse bone marrow-derived cells and its potential role as a factor attracting mesenchymal stem cells to the wound site. We also addressed the question which factor is responsible for cathodal or anodal direction of cell movement. Finally, we analyzed the kinetics of electric field detection by cells.

2. Materials and methods

2.1. Cell isolation and culture

4- to 6-week-old C57Bl/6 mice were sacrificed by cervical dislocation and tibias and femurs were harvested immediately after animal euthanasia. Bone marrow adherent cells (BMACs) were isolated by flushing cavities of femurs and tibias with DMEM/F12 medium (Sigma-Aldrich). Cells were centrifuged, re-suspended in complete medium (DMEM/F12 with 10% FBS, Sigma-Aldrich; and 1% penicillin/streptomycin, P/S, Sigma-Aldrich) and seeded into a Primaria culture flask (BD Falcon) at a density of 3×10^5 nucleated cells/cm^2. The nonadherent cell population was removed after 72 h and the adherent layer was washed once with fresh medium. Cells were passaged with 0.25% trypsin/EDTA (Gibco, Life Technologies) when confluence of cells reached close to 90% and were typically diluted 1:2 at each passage. All procedures were performed in accordance with the approval of the Ethical Committee on Animal Testing at the Jagiellonian University (JU) in Krakow (approval number: 56/2009).

2.2. Electric field application

2 to 5 passage BMACs were exposed to EF at a strength of 50–300 mV/mm in the plexiglass apparatus described in detail by Korohoda et al. [25]. Briefly, EF was applied for 4 h through Ag/AgCl reversible electrodes of 6 cm^2 immersed in saline-filled wells connected by agar bridges (2% agar in 0.5 n KCl, 8 cm long) to neighbouring wells, to which the observation chambers were attached. The observation chambers were made of cover glasses measuring 60 × 35 × 0.2 mm. The investigated cells were plated for 2 h onto one of the cover glasses at a density of 2×10^4 cells/cm^2 and incubated in DMEM/F12 supplemented with 10% FBS in a humidified atmosphere with 5% CO_2 at 37 °C. Then the chamber was mounted with silicone grease in the plexiglass apparatus.

2.3. Signalling pathway analysis

In some experiments BMACs were pre-incubated for 1 h with 50 μM NSC23766 (Rac1 inhibitor, Calbiochem), 50 μM ZCL278 (Cdc42 inhibitor III, Calbiochem), 30 μM Rhosin (Rho inhibitor, Calbiochem) or for 30 min with 10 μMY-27632 (ROCK inhibitor, Calbiochem), 100 μM CK-666 (Arp2/3 inhibitor, Sigma-Aldrich), 10 μM LY-294002 (PI3K inhibitor, Sigma-Aldrich), 50 μM U0126 (MEK1/2 inhibitor, Sigma-Aldrich), 5 or 20 μM DMPQ-2HCl (PDGFRβ inhibitor, Abcam), 20 μM PQ401 (IGF1R inhibitor, Abcam), 10 or 50 μM NSC668036 (Dishevelled inhibitor, Sigma-Aldrich), 10 μM AG1478 (EGFR inhibitor, Sigma-Aldrich), 5, 20 or 50 μM PD158780 (EGFR inhibitors, Abcam) in DMEM/F12 with 10% FBS. In all cases inhibitors were present in medium throughout the 4 h of experiment.

To investigate the role of Ca^{2+} in electrotaxis, intra- or extracellular calcium ions were eliminated in different approaches. The intracellular Ca^{2+} were complexed with cell-permeant chelator BAPTA-AM (Calbiochem). In these experiments cells were loaded by 30 min preincubation with 1 μM BAPTA-AM in DMEM/F12 supplemented with 0.5% BSA (Sigma-Aldrich). The extracellular calcium ions were eliminated either by changing experimental medium to serum-free and calcium-free DMEM/F12 supplemented with 1% P/S or by addition of 0.5 mM EGTA (Sigma-Aldrich) to calcium-free DMEM/F12 with 10% FBS. Ion influx through mechanosensitive channels was prevented by 30 min cell pre-incubation with 3 μM GsMTx-4 (Smartox) - the inhibitor of stretch-activated channels.

2.4. Cell movement analysis

The movement of BMACs was time-lapse recorded for 4 h at 5 min time intervals, both in isotropic conditions and in EFs. The tracks of individual cells were determined from the series of changes in cell centroid positions, pooled and analyzed as previously described [26]. The following parameters were estimated: (i) the displacement length (μm), i.e. the distance from the starting point directly to the cell's final position, (ii) the cell speed (μm/h), i.e. trajectory length/time of recording, (iii) the coefficient of movement efficiency (CME) corresponding to the ratio of cell displacement to cell trajectory length, (iv) average directional cosines γ; γ is defined as the directional angle between the *x*-axis (parallel to the field direction) and the vector AB. A and B are the first and subsequent positions of the cell, respectively. Trajectories of cells from no less than two independent isolations (number of cells >20) were taken for the estimation of statistical significance.

2.5. Cell shape analysis

The parameters characterizing cell morphology were calculated as described by Dunn and Brown [27]. The following parameters were estimated: (i) cell area (μm^2) and (ii) cell elongation, a measure of how much the shape must be compressed along its long axis to minimize its extension. The minimum elongation of zero is only achieved if the shape is a circle and increases without limit as the shape becomes more elongated.

2.6. Cell sorting

For cell sorting, BMACs between 2 and 5 passage were harvested by 0.25% trypsin-EDTA and re-suspended in sorting medium containing DMEM/F12 supplemented with 2% FBS and 2% P/S. For cell sorting based on CD45 expression, cells were additionally stained with 1.5 μg/10^{6}cells of rat-anti-CD45-FITC monoclonal antibody (BD Biosciences). Then cells were incubated for 30 min on ice in the dark and washed with DMEM/F12 containing 2% FBS. Next, cells were re-suspended in sorting medium, filtered through a 70 μm filter (BD Falcon) and sorted with FACSAria (Becton Dickinson). After separation, cells were seeded into Primaria flasks in complete medium (DMEM/F12