Biochemical and Biophysical Research Communications xxx (2015) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

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

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ARTICLE INFO

Article history:
Received 23 February 2015
Available online xxx

Keywords:
Directional migration
Neonatal human fibroblasts
Electrotaxis
Golgi polarization
Galvanotaxis

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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http://dx.doi.org/10.1016/j.bbrc.2015.03.021
0006-291X/

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

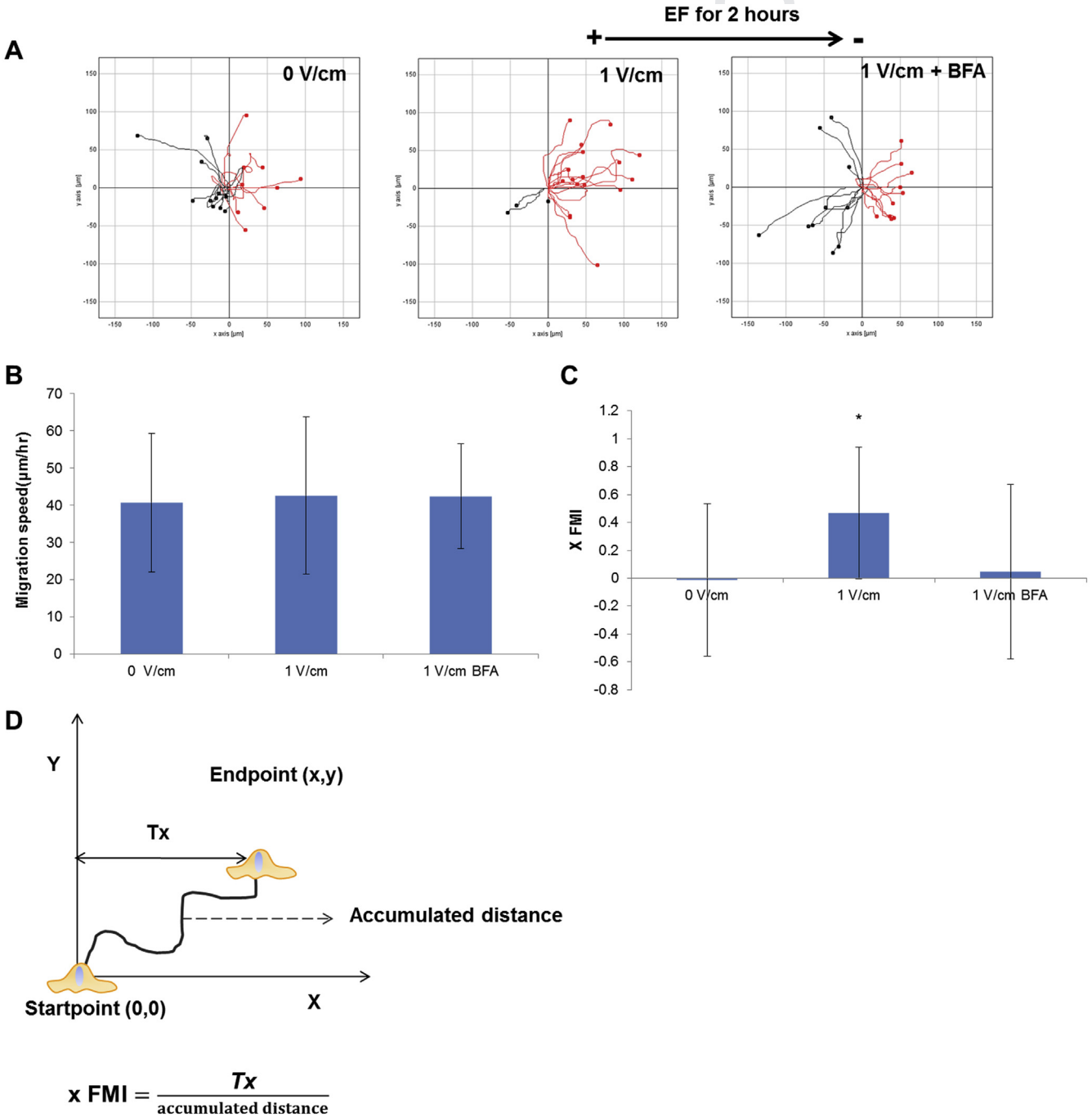

Fig. 1. The migration assay of nHDFs under dc EF condition (0, 1 V/cm) for 2 h with or without 1 μM of BFA. (A) 20 Cells of each condition were tracked, (B) migration speed was measured, and (C) x FMI was determined for nHDFs under dc EF conditions for 2 h (0, 1 V/cm), $^*p < 0.05$ compared to controls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

Please cite this article in press as: M.S. Kim, et al., Golgi polarization plays a role in the directional migration of neonatal dermal fibroblasts induced by the direct current electric fields, Biochemical and Biophysical Research Communications (2015), http://dx.doi.org/10.1016/j.bbrc.2015.03.021