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Rotation is the primary motion of paired human epidermal keratinocytes

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ABSTRACT

Background: Collective motion of keratinocytes is involved in morphogenesis, homeostasis, and wound healing of the epidermis. Yet how the collective motion of keratinocytes emerges from the behavior of individual cells is still largely unknown.
Objective: The aim of this study was to find the cellular behavior that links single and collective motion of keratinocytes.
Methods: We investigated the behavior of two-cell colonies of HaCaT keratinocytes by a combination of time-lapse imaging and image processing.
Results: The two-cell colonies of HaCaT cells were formed as a contacted pair of keratinocyte clones. Image analysis and cell culture experiments revealed that the rotational speed of two-cell colonies was positively associated with their proliferative capacity. α6 integrin was required for the rotational motion of two-cell keratinocyte colonies. We also confirmed that two-cell colonies of keratinocytes predominantly exhibited the rotational, but not translational, motion, two modes of motion in a contact pair of rotating objects.
Conclusion: The rotational motion is the primary motion of two-cell keratinocyte colonies and its speed is positively associated with their proliferative capacity. This study suggests that the assembly of rotating keratinocytes generates the collective motion of proliferative keratinocytes during morphogenesis and wound healing of the epidermis.

1. Introduction

The epidermis, the outermost layer of the skin, is a stratified squamous epithelium that functions as a bidirectional barrier against external environmental insults and loss of internal bodily fluids. The basal layer of the epidermis contains keratinocyte stem cells, and the terminally differentiating progeny leaves this layer to move toward the skin surface and functions as the structural barrier of the skin [1]. Whereas keratinocyte stem cells in the interfollicular epidermis mainly contribute to epidermal homeostasis [2–4], the stem cells located in the bulge region of hair follicles also participate in regeneration of interfollicular epidermis after wounding [2,5–7]. These processes require the migration of keratinocytes [8,9], and understanding its underlying mechanisms advances keratinocyte biology and regenerative medicine of the skin [1,10].

The motion dynamics of single keratinocytes is well investigated; however, it is still largely unknown how the collective motion of keratinocytes emerges from the motion of individual cells. Recently, we have reported that the two-cell colony derived from a single keratinocyte stem cell exhibits a rotational motion [11]. In appropriate culture conditions, seeded keratinocytes adhere to the surface of culture dishes and subsequently start to proliferate. The first mitosis generates two daughter cells that remains adhered to each other and form two-cell colonies. This is the first time that the single keratinocytes can have a neighbor, so that the behavior of two-cell colonies is fundamental for collective motion. Although the motion dynamics of two-cell colonies are not well investigated, the motion of paired cells had been observed in

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human mammary epithelial cell lines and normal epidermal keratinocytes [12–14].

In keratinocyte cultures, the mean value of rotational speed of paired cells decreases with the passages of the culture [13]. We have also demonstrated that the rotational speed of two-cell colonies is positively correlated with the clonal growth of normal human epidermal keratinocytes [11]. Importantly, two-cell colonies of keratinocytes were only formed in serum-containing medium with 3T3 feeder layers, but not in serum-free media including CnT-PR (Cell n Tec), EpiLife (Life technologies), and MCDB153 [15], when keratinocytes were seeded at clonal density [11]. The feeder cells, however, make it difficult to analyze the behavior of keratinocyte colonies. Therefore, alternative experimental models to investigate the motion dynamics of two-cell colonies of keratinocytes are needed.

Here we develop a model system to investigate the dynamics of keratinocytes using a HaCaT cell line, a spontaneously immortalized human epidermal keratinocyte line [16]. HaCaT cells can be maintained with serum-containing medium, but do not require feeder cells, which facilitates the observation and analysis of two-cell colonies of keratinocytes. In this study, we first confirm that the rotational speed of two-cell colonies of HaCaT keratinocytes positively correlates with their proliferative capacity. We then demonstrate that the two-cell colonies of HaCaT keratinocytes predominantly exhibit the rotational motion accomplished by rotation of two keratinocytes in the colony in the same direction. This study suggests that the rotational motion of two keratinocytes around one another in the same direction is the first step to generate the collective motion of keratinocytes.

2. Materials and methods

2.1. Cell culture and time-lapse imaging

HaCaT cells were maintained in Dulbecco's modified Eagle medium (DMEM) (Invitrogen) containing 10% fetal bovine serum (FBS) (SAFC Biosciences) at 37 °C and 10% CO_2. HaCaT cells were also cultivated with CnT-PR™ medium (CELLnTEC), EpiLife™ medium containing supplement S7 (Life Technologies), and MCDB153 medium containing bovine pituitary extract (BPE). To visualize HaCaT colonies, cultures were fixed with 3.7% buffered formaldehyde and stained with 1% rhodamine B. For time-lapse imaging, HaCaT cells were maintained at 37 °C and 10% CO_2 in a chamber mounted on an Axiovert 200 M microscope (Zeiss). Images were obtained at 5 min intervals for 60 min.

2.2. Image processing

Time-lapse images of two-cell colonies were collected and obtained images were analyzed with Volocity (PerkinElmer) on a graphic tablet (Wacom). The area and perimeter of a two-cell colony were measured by surrounding the periphery of a colony. The angle of contact surface between cells in a two-cell colony was given by an upper right angle of contact surface against perpendicular axis. The center position in a two-cell colony was

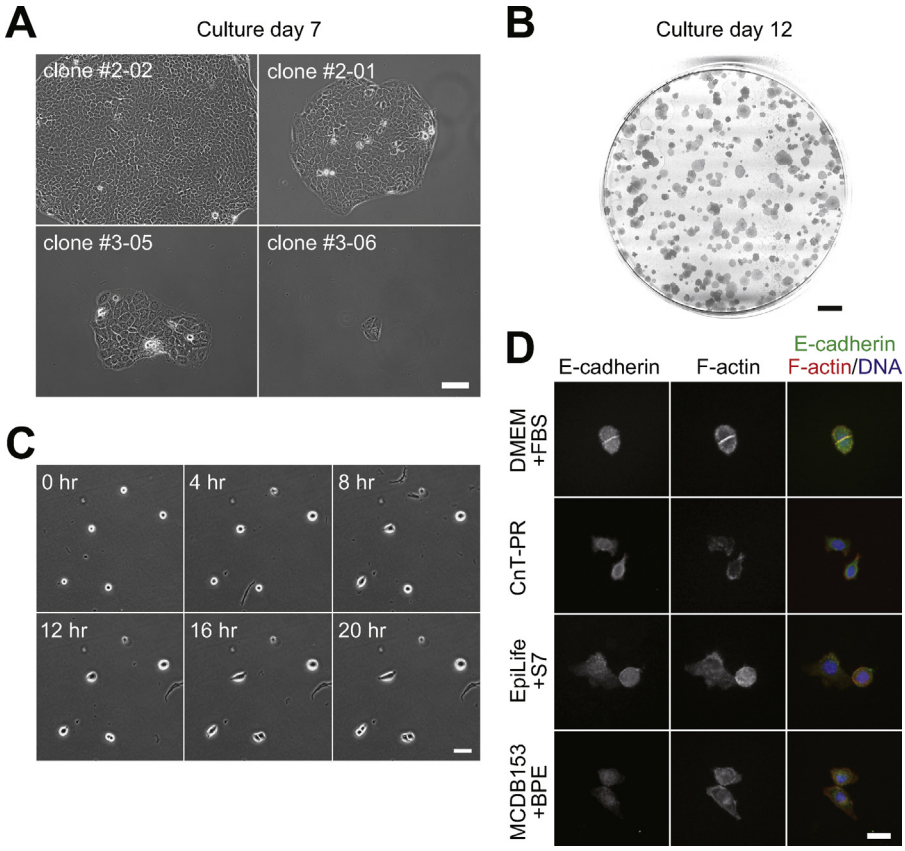

Fig. 1. Heterogeneity in proliferative capacity of HaCaT keratinocytes.
(A) HaCaT cells were seeded at clonal density and photographed at day 7 in culture. Bar 100 μm. (B) Five hundred HaCaT cells were seeded in a 10 cm culture dish and cultured for 12 days, and then fixed and stained with rhodamine B. Bar 10 mm. Note that heterogeneity in proliferative capacity of each HaCaT clone was observed. (C) Formation of HaCaT two-cell colonies. HaCaT cells gave rise to two-cell colonies within 16–20 h after inoculation. Bar 50 μm. (D) The generation of two-cell colonies of HaCaT cells and the expression of E-cadherin in various culture conditions. The cells were also treated with rhodamine-phalloidin and Hoechst 33,258 to visualize actin filaments and nuclei. Bar 20 μm.

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