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Real-time imaging of human epidermal calcium dynamics in response to point laser stimulation

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ABSTRACT

Background: Changes of epidermal calcium ion concentration are involved in regulation of barrier homeostasis and keratinocyte differentiation. Moreover, intracellular calcium dynamics might play a role in skin sensation. But, although calcium dynamics of cultured keratinocytes in response to mechanical stresses has been well studied, calcium propagation in stimulated human epidermis is still poorly understood.
Objective: The aim of this study was to demonstrate a novel method for real-time measurement of calcium dynamics in response to point stimulation of human epidermis at the single-cell level.
Methods: We examined calcium propagation in cross-sectional samples of living human epidermis ex vivo, as well as in cultured human keratinocytes, by means of two-photon microscopy after stimulating cells in stratum granulosum with the emission laser of a two-photon microscope.
Results: Cells in different epidermal layers showed different responses, and those in stratum basale showed the greatest elevation of intracellular calcium. Calcium propagation in epidermis was inhibited in the presence of apyrase (which degrades adenosine triphosphate; ATP) or gap-junction blockers. In cultured keratinocytes, on the other hand, calcium propagated in a simple concentric wave-like manner from the stimulation site, and propagation was strongly suppressed by apyrase.
Conclusion: Our results suggested that ATP and gap junctions play important roles in calcium propagation induced by point laser stimulation of the uppermost layer of epidermis. Our method should be broadly useful to study calcium dynamics, epidermal physiological mechanisms, and mechanisms of skin sensation at the single-cell level.

1. Introduction

Calcium ions are involved in epidermal barrier homeostasis and keratinocyte differentiation [1,2], as well as in various aspects of epidermal physiology, including keratinocyte proliferation [3]. Moreover, intracellular calcium dynamics is thought to play an important role in skin sensation. We previously reported the dynamics of intracellular calcium in cultured keratinocytes in response to air exposure and mechanical stimulation [4,5]. It is well established that epidermal keratinocytes express multiple sensory receptors that are also found in peripheral nerve fibers [6,7], and we have suggested that keratinocytes might function as a skin sensory system [8]. For example, activation of the receptor TRPV1 in keratinocytes induced nociception [9]. As regards the signaling mechanism, keratinocytes contain adenosine triphosphate (ATP) receptors [10] and stimulation of keratinocytes results in release of ATP, which in turn induces elevation of intracellular calcium [4,11]. In addition, intercellular communication might occur in epidermis via gap junctions; for example, some connexin family members have been found in keratinocytes, and gap-junction blockers suppressed calcium propagation in response to mechanical force or air exposure [5,12,13]. Thus, it is plausible that calcium dynamics in human skin has a central role in mediating responses to external stimuli.

The aim of this study was to demonstrate a novel method for real-time measurement of calcium dynamics in response to point

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stimulation of ex vivo human epidermis and cultured keratinocytes in the presence of an intracellular calcium indicator. Using the emission laser of a two-photon microscope for point stimulation of cells in stratum granulosum, we were able to observe real-time calcium propagation throughout thick (30–100 μm) cross-sections of living epidermis by means of two-photon microscopy. Our method should be valuable for studies of epidermal physiological responses to various external stimulations, at the single-cell level.

2. Materials and methods

2.1. Preparation of human skin tissues

Human abdominal skin tissues were purchased from Biopredic International (Rennes, France) via KAC Co., Ltd. (Kyoto, Japan). The tissues had been collected during plastic surgery after informed consent had been obtained. The donors were 8 healthy, independent Caucasian women, aged 31–44 years. The excised skin was

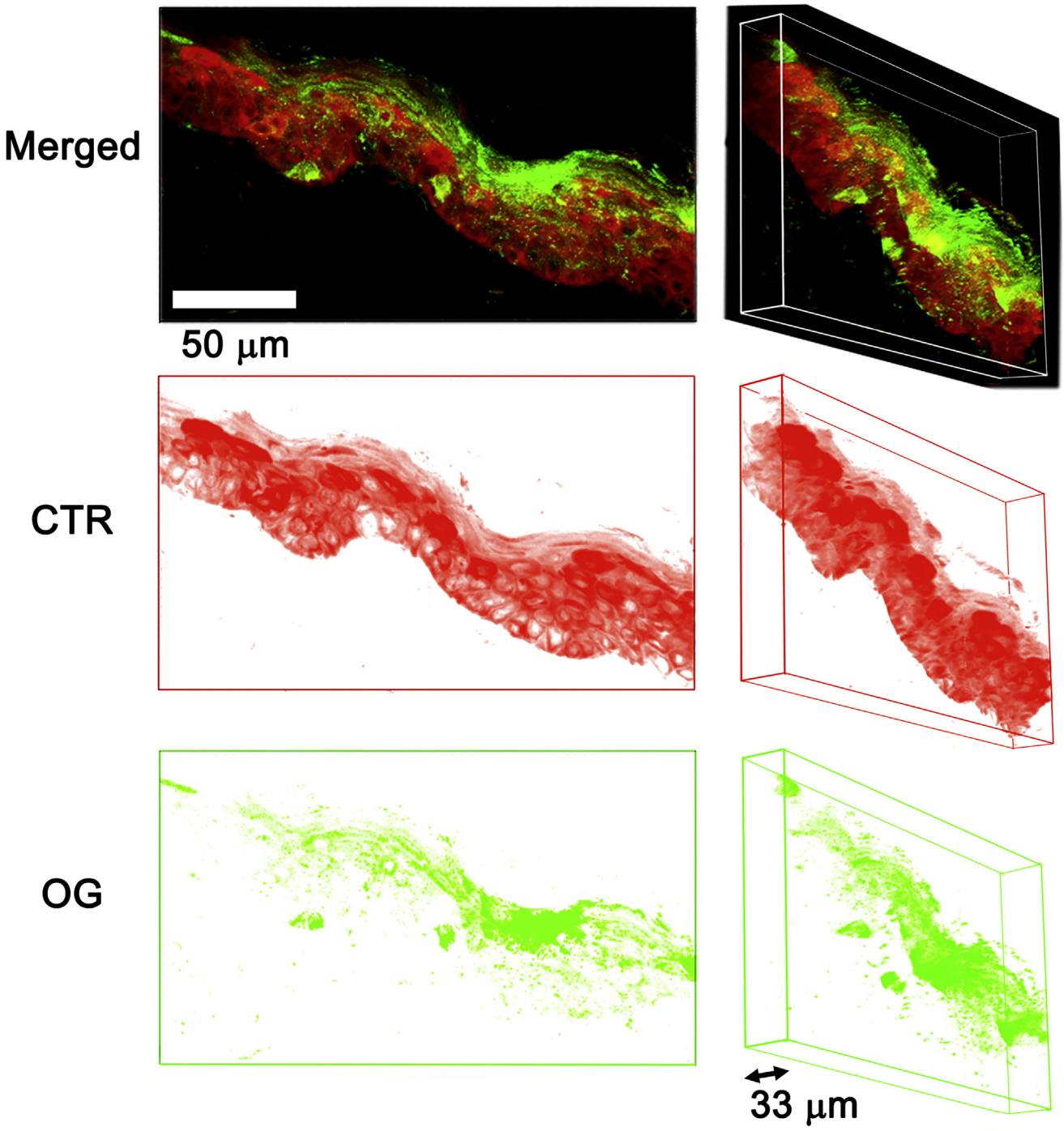

Fig. 1. Three-dimensional images of human epidermis labeled with Oregon Green (OG) and CellTracker™ Red (CTR), obtained by two-photon microscopy. Upper images are merged images, middle: CTR, and bottom: OG. Bar = 50 μm.

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