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Human epidermal basal cell responses to ultraviolet-B differ according to their location in the undulating epidermis

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Summary

Background: Exposure of skin to excessive ultraviolet-B (UVB) radiation causes epidermal hyperproliferation that leads to epidermal hyperplasia, however, it is not yet clear exactly how these responses progress.
Objectives: We attempted to clarify the response patterns involved with epidermal hyperproliferation following UVB radiation.
Methods: UVB was irradiated at 2 minimal erythema doses (MED) to human back skin and epidermal morphologic changes were evaluated using in vivo confocal laser microscopy. Skin biopsy specimens were collected from exposed and from non-exposed regions, and were subjected to histochemical and immunohistochemical analysis.
Results: The in vivo confocal laser microscopic analysis showed that UVB-induced epidermal hyperplasia was prominent at the epidermal rete ridges. Further, 3 days after UVB exposure, numerous Ki67-positive epidermal cells were observed in the epidermal rete ridges, but not in the epidermis at the top of the dermal papilla. These results suggest that cells highly responsive to UVB exist in the epidermal rete ridges and that their hyperproliferation leads to elongation of the epidermal rete ridges. In contrast, the number of keratin 10-positive basal cells, known as transitional cells, was increased throughout the epidermis, suggesting that an upward migration of keratinocytes from the epidermal basal layer occurred regardless of their location. However, diffusion of melanin to the suprabasal layers was markedly observed in epidermal regions above the dermal papillae, suggesting the occurrence of strong upper cell movement at this position.

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Conclusion: Based on our results, we conclude that differences in keratinocyte responses to UVB radiation exist in cells located in the undulating epidermal basal layer.

1. Introduction

Human epidermis is made up of multiple layers of keratinocytes, which are in a continual process of cell replacement. Within the epidermis, proliferation takes place in the basal layer, whereas desquamation occurs at the skin surface, and this balance between cell production and loss coordinates tissue homeostasis and epidermal architecture. Normally, the quantity of cell production is precisely controlled by two distinct subpopulations of basal epidermal cells, stem cells, which have an unlimited capacity for self-renewal, and transit cells, which are the progeny of stem cells with a limited proliferative capacity [1—3]. Any disruption of this regulation implicates a skin disease pathogenesis. Psoriasis is a typical example of hyper-proliferative epidermis with a greatly increased germinative compartment, in which the epidermis falls into an abnormal state of structure, with epidermal projections reaching down into the dermis [4,5]. In addition, exposure of skin to excessive ultraviolet-B (UVB) radiation also easily causes epidermal hyperplasia because the speed of epidermal cell production temporarily becomes faster than that of normal epidermis [6—8]. Thus, UVB-irradiated epidermis is a good model to study hyperproliferative conditions of the epidermis. Many reports have shown a variety of cutaneous responses to UVB, however, it is not yet clear exactly how the epidermal hyper-proliferative response progresses.

The aim of the present study was to elucidate the details of hyperproliferation and movement of epidermal cells following UVB irradiation with 2 minimal erythema doses (MED) to human back skin. To assess these events, we performed histological and immunohistochemical staining, while the sequential structural changes of the epidermis following irradiation were analyzed using a confocal laser microscope.

2. Materials and methods

2.1. Volunteers

Ten healthy male volunteers (Japanese; age range, 26—45 years old) participated in this study, following approval from the Ethical Committee of the Kanebo Basic Research Laboratory to harvest human skin. All subjects consented to the study, after being informed of its nature and aims.

2.2. UVB irradiation and skin biopsies

Irradiation with 2 MED of UVB (FL20 SE30 lamp, Toshiba, Tokyo, Japan) was performed on the upper-back (2.0 cm $\times$ 2.0 cm area) of each volunteer at 10 and at 3 days prior to a skin biopsy, which was performed by taking 5-mm-punch biopsy specimens from each exposed region. MED is defined as the minimum dose required to yield sharply demarcated erythema 24 h after irradiation. As a control, non-exposed areas of skin in each subject were also sampled.

2.3. Light microscopy

The skin biopsy samples were frozen in tissue mount with liquid nitrogen and 5-μm cross-sections were used for each type of staining. Sections were fixed in 4% paraformaldehyde (PFA) in 0.01 M phosphate buffered saline (PBS), and were routinely processed for Hematoxylin and eosin (HE) staining. In addition, Fontana—Masson staining was used to detect melanin.

2.4. Immunohistochemistry

For single immuno-staining of cryostat sections, sections were fixed with PFA and ethanol. After preincubation for 20 min with 10% normal horse serum (NHS) in PBS, sections were incubated for 1 h with polyclonal rabbit anti-Ki67 (NeoMarkers, NY, USA). Immunolocalization of the primary antibody was performed using the avidin-biotin peroxidase complex method (ABC-Elite kit, Vector Laboratories, CA, USA) with nickel-diamino-benzidine (DAB) as the substrate. For double-labeling with keratin 10 and either Ki67 or laminin, sections were fixed as described above or in acetone. After preincubation with NHS in PBS, sections were incubated for 1 h with a primary antibody cocktail comprised of monoclonal mouse anti-keratin 10 (RKSE60, CosmoBio, Tokyo, Japan) and either anti-Ki67 or polyclonal rabbit anti-laminin (ICN Biomedicals Inc., CA, USA). Sections were then washed in PBS and incubated for 30 min with a secondary antibody cocktail comprised of Oregon green-labeled anti-mouse IgG and Texas red-labeled anti-rabbit IgG (Molecular Probes Inc., OR, USA). After further washes in PBS, the sections were mounted using fluorescent mounting medium (DAKO, CA, USA).