Acta Histochemica 116 (2014) 20–24

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Ultraviolet B irradiation induces the expression of hornerin in xenotransplanted human skin

Teruhiko Makino*, Takako Yamakoshi, Megumi Mizawa, Tadamichi Shimizu

Department of Dermatology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Sugitani 2630, Toyama 930-0194, Japan

ARTICLE INFO

Article history:
Received 21 February 2013
Received in revised form 23 April 2013
Accepted 25 April 2013

Keywords:
Hornerin
Profilaggrin
Ultraviolet B
Xenotransplant
Hyperproliferative epidermis
Skin

ABSTRACT

Ultraviolet (UV) irradiation exerts numerous effects on the skin. Exposure of human skin to UVB at doses that induce mild sunburn reactions causes epidermal hyperproliferation and alterations in the expression of several epidermal differentiation markers. This study investigated the effects of UVB irradiation on the expression of hornerin, a member of the S100 fused-type protein family, using the xenotransplantation of normal human skin onto nude mice. Hornerin mRNA was detected in the UVB-irradiated skin on day 2 using RT-PCR. In accordance with the results of the RT-PCR, the expression of hornerin was induced in the granular layers of the UVB-exposed skin beginning two days after UVB irradiation and occurred in parallel with the expressions of cytokeratin 6 and Ki67. This finding suggests that hornerin induction in UVB-irradiated skin might be associated with epidermal hyperproliferation. This study demonstrated that hornerin is a protein whose expression is changed by UVB irradiation and suggests that the expression of hornerin might be a useful marker of acute UV damage in skin.

Introduction

Skin is exposed to various types of environmental stressors such as noxious biological and physicochemical agents. Ultraviolet (UV) irradiation is one of the most serious sources of stress. UV irradiation exerts numerous effects on the skin, including suntan, sunburn, immunosuppression, photoaging and carcinogenesis (Gilchrest, 1989). Many studies assessing the biological effects of UV radiation on skin have been conducted. According to these studies, UVB irradiation alters the expression of differentiation markers, such as involucrin, loricrin and filaggrin, and induces the expression of keratin 6, 16, 17 and 19 in association with epidermal hyperproliferation (Moll et al., 1994; Smith and Rees, 1994; Bernaerd and Asselineau, 1997; Del Bino et al., 2004). However, the results of these studies vary. The differences in findings may be associated with the methods used in each study. For example, the skin of animals, such as mice differs from human skin in the structure of the epidermis and the expression of differentiation markers. Human keratinocyte monolayer cultures exhibit poor keratinocyte differentiation processes. Del Bino et al. (2004) demonstrated that exposure of human skin xenotransplanted onto nude mice to UVB results in epidermal hyperproliferation and alterations in the expression of several differentiation markers, including drastic modifications of the patterns of epidermal keratins. The authors concluded that human skin xenotransplanted onto nude mice provides a valuable tool for investigating the effects of UVB on early and late epidermal differentiation markers (Del Bino et al., 2004).

The terminal differentiation of a keratinocyte in the epidermis is a complex process that requires the regulated and sequential expression of a variety of genes. The epidermal differentiation complex (EDC) at chromosome band 1q21.3 contains a family of proteins described as "fused S100 proteins (profilaggrin, trichohyalin, repetin, cornulin, filaggrin-2 and trichohyalin-like 1)" that are involved in epidermal differentiation (Presland et al., 1992; Markova et al., 1993; Krieg et al., 1997; Makino et al., 2001; Contzler et al., 2005; Huber et al., 2005; Takaishi et al., 2005; Wu et al., 2009a,b, 2011; Yamakoshi et al., 2013). Hornerin is also a member of the S100 fused-type protein family (Makino et al., 2001; Takaishi et al., 2005). The structural feature of hornerin is similar to that of filaggrin, a protein essential for keratinization, and hornerin has been strongly detected in the granular cells of both regenerating and psoriatic skin (Takaishi et al., 2005). In this study, we examined the effects of UVB irradiation on the expression of hornerin using xenotransplantation of normal human skin onto nude mice.

Materials and methods

Clinical materials

Human skin samples measuring 10 mm × 10 mm in size were obtained from the abdominal skin of four Japanese healthy

* Corresponding author.
E-mail address: tmakino@med.u-toyama.ac.jp (T. Makino).

0065-1281/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.acthis.2013.05.001

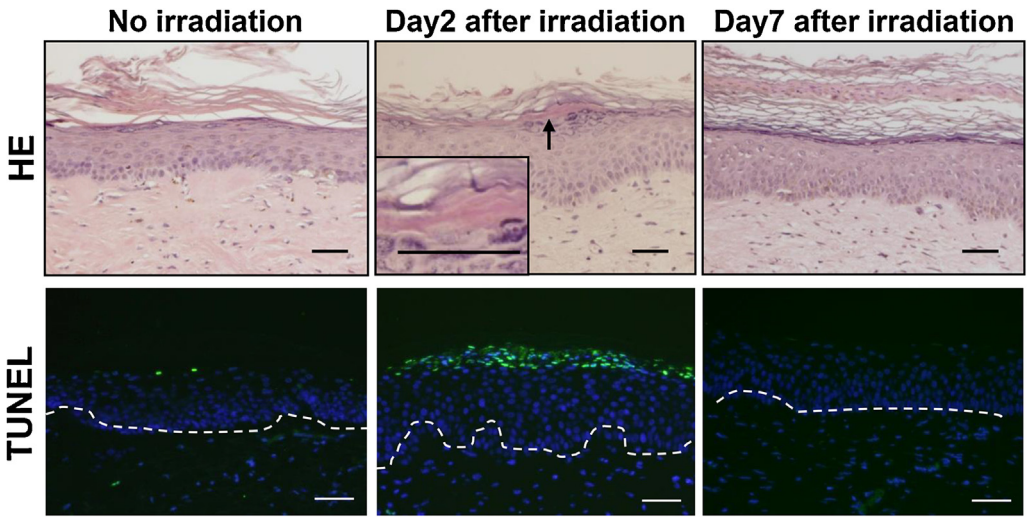

Fig. 1. Biological effects of UVB irradiation. Hematoxylin–eosin staining and the findings of TUNEL assays in the sham-irradiated skin, UVB-irradiated skin removed two days after exposure to 500 mJ/cm^2, and UVB-irradiated skin removed seven days after exposure to 500 mJ/cm^2. DNA staining by 4′,6-diamidine-2′-phenylindole dihydrochloride appears in blue. The arrow and higher magnification inset indicate the sun-burned cells induced by UVB exposure. The broken lines indicate the basement membrane. Scale bars = 100 μm.

volunteers with their informed consent. All of the volunteers had type III skin. The skin samples were transplanted on to 6 weeks old nude mice, KSN/Slc mice (Nippon SLC, Hamamatsu, Japan). A graft site was prepared on the back of each mouse. Briefly, a piece of skin obtained from the back of the mouse (left side) was surgically removed and replaced with the human skin sample. Each mouse received a single transplant. Twelve mice in total, with four mice in each experimental group, were used in this study. The skin tissues were excised two or seven days after UVB irradiation and analyzed using reverse transcription-polymerase chain reaction (RT-PCR) and immunofluorescence (IF). This study was performed under conditions that complied with the Principles of the Declaration of Helsinki and was approved by the Medical Ethics Committee of the University of Toyama.

Ultraviolet irradiation

The UVB light source was a fluorescent lamp (GL40SE; Sankyo Denki Co, Kanagawa, Japan) that emitted 0.1 mW/cm^2 of UV light between 280 and 315 nm (peak: 306 nm) at a distance of 40 cm, as measured with a UV radiometer (EKO Instruments Co., Tokyo, Japan). The grafted skin was exposed to 500 mJ/cm^2 of UVB two months after transplantation under conditions similar to those described by Del Bino et al. (2004). Four skin grafts were used as experimental sites in each of the experiments.

RT-PCR analysis

RNA samples prepared from the human skin tissues were pretreated with DNase I (Invitrogen, Carlsbad, CA, USA) and confirmed not to give positive signals without reverse transcription. Reverse transcription was performed using random hexamers and Superscript III polymerase (Invitrogen, Carlsbad, CA, USA) and subsequent amplification using Recombinant Taq DNA polymerase (Takara Bio Inc, Shiga, Japan). PCR was performed for 25–38 cycles with denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s and extension at 72 °C for 30 s using a thermal cycler (PE Applied Biosystems Gene Amp PCR system 9700). The exact PCR cycles were as follows: hornerin, 38; profilaggrin, 30; keratin 16, 28; keratin 5, 35; GAPDH, 25. The primers used for PCR were as follows:

Hornerin; sense 5′-ATGCCTAAACTCCTACAAGG-3′, antisense 5′-GTTATGGTCTCGATCCAGAC-3′:
Profilaggrin; sense 5′-GGGAAGTTATCTTTTCCTGTC-3′, antisense 5′-GATGTGCTAGCCCTGATGTTG-3′:
Cytokeratin 5; sense 5′-TGCTGCAAGTCACTGCCTTC-3′, antisense 5′-TTGAACACATTCTGGAGGTAG-3′:
Cytokeratin 16; sense 5′-GCTGAACAAAGAAGTGGCCTC-3′, antisense 5′-TGAAGCTGGATGAGCTCTGCT-3′:
GAPDH; sense 5′-CTTCACCACCATGGAGAAGGC-3′, antisense 5′-GGCATGGACTGTGGTCATGAG-3′.

The amplified DNA fragments were analyzed using 2% agarose gel electrophoresis.

Immunohistochemistry and TUNEL assays

Antibodies against human hornerin were generated as previously reported (Takaishi et al., 2005). The skin specimens were directly dipped into OCT compound and rapidly frozen in liquid nitrogen. Sections (6 μm) were blocked with Protein Block Serum-Free (Dako, Carpinteria, CA, USA) and incubated with primary antibodies, including anti-human hornerin antibodies, anti-human

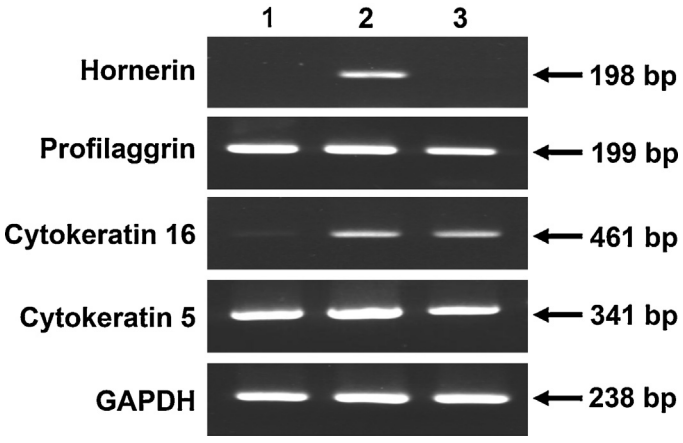

Fig. 2. RT-PCR analysis of the UVB-exposed skin. The RT-PCR analysis of the sham-irradiated skin (lane 1), UVB-exposed skin removed two days after exposure to 500 mJ/cm^2 (lane 2) and UVB-exposed skin removed seven days after exposure to 500 mJ/cm^2 (lane 3). The PCR cycles were adjusted to obtain an appropriate band thicknesses, *i.e.*, hornerin, 35; profilaggrin, 30; keratin 16, 28; keratin 5, 35; glyceraldehydes-3-phosphate dehydrogenase (GAPDH), 25.