Journal of Dermatological Science 72 (2013) 54–60

Contents lists available at SciVerse ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Aberrant distribution patterns of corneodesmosomal components of tape-stripped corneocytes in atopic dermatitis and related skin conditions (ichthyosis vulgaris, Netherton syndrome and peeling skin syndrome type B)

Satomi Igawa [a,*], Mari Kishibe [a], Masaru Honma [a], Masamoto Murakami [b], Yuki Mizuno [c], Yasushi Suga [c], Mariko Seishima [d], Yuka Ohguchi [e], Masashi Akiyama [f], Kenji Hirose [g], Akemi Ishida-Yamamoto [a,*], Hajime Iizuka [a]

[a] Department of Dermatology, Asahikawa Medical University, Asahikawa, Japan
[b] Department of Dermatology, Ehime University Graduate School of Medicine, Toon, Japan
[c] Department of Dermatology, Juntendo University Urayasu Hospital, Urayasu, Japan
[d] Department of Dermatology, Gifu University Graduate School of Medicine, Gifu, Japan
[e] Department of Dermatology, Hokkaido University School of Medicine, Sapporo, Japan
[f] Department of Dermatology, Nagoya University Graduate School of Medicine, Nagoya, Japan
[g] Department of Dermatology, Shikoku Medical Center for Children and Adults, Zentsuji, Japan

ARTICLE INFO

Article history:
Received 22 August 2012
Received in revised form 2 May 2013
Accepted 22 May 2013

Keywords:
Corneodesmosome
Skin barrier
Atopic dermatitis
Ichthyosis
Peeling skin syndrome
Netherton syndrome

ABSTRACT

Background: Atopic dermatitis (AD), Netherton syndrome (NS) and peeling skin syndrome type B (PSS) may show some clinical phenotypic overlap. Corneodesmosomes are crucial for maintaining stratum corneum integrity and the components' localization can be visualized by immunostaining tape-stripped corneocytes. In normal skin, they are detected at the cell periphery.
Objective: To determine whether AD, NS, PSS and ichthyosis vulgaris (IV) have differences in the corneodesmosomal components' distribution and corneocytes surface areas.
Methods: Corneocytes were tape-stripped from a control group ($n = 12$) and a disease group (37 AD cases, 3 IV cases, 4 NS cases, and 3 PSS cases), and analyzed with immunofluorescent microscopy. The distribution patterns of corneodesmosomal components: desmoglein 1, corneodesmosin, and desmocollin 1 were classified into four types: peripheral, sparse diffuse, dense diffuse and partial diffuse. Corneocyte surface areas were also measured.
Results: The corneodesmosome staining patterns were abnormal in the disease group. Other than in the 3 PSS cases, all three components showed similar patterns in each category. In lesional AD skin, the dense diffuse pattern was prominent. A high rate of the partial diffuse pattern, loss of linear cell–cell contacts, and irregular stripping manners were unique to NS. Only in PSS was corneodesmosin staining virtually absent. The corneocyte surface areas correlated significantly with the rate of combined sparse and dense diffuse patterns of desmoglein 1.
Conclusion: This method may be used to assess abnormally differentiated corneocytes in AD and other diseases tested. In PSS samples, tape stripping analysis may serve as a non-invasive diagnostic test.

1. Introduction

In vertically cut histology sections, the stratum corneum (SC) of the epidermis shows a basket-weave-like appearance. These corneocytes are attached only at the cell periphery by corneodesmosomes, which are modified desmosomes [1–3]. The intercellular components of corneodesmosomes are desmoglein 1 (Dsg1), corneodesmosin (Cdsn) and desmocollin 1 (Dsc1) [4]. Distributions of these molecules can easily and clearly be

* Corresponding authors at: Department of Dermatology, Asahikawa Medical University, Midorigaoka-Higashi 2-1-1-1, Asahikawa 078-8510, Japan. Tel.: +81 0166 68 2523; fax: +81 0166 68 2529.
E-mail addresses: igasato@asahikawa-med.ac.jp (S. Igawa), akemi@asahikawa-med.ac.jp (A. Ishida-Yamamoto).

0923-1811/$36.00
http://dx.doi.org/10.1016/j.jdermsci.2013.05.004

visualized by immunostaining of tape-stripped corneocytes [5,6]. In normal skin, these molecules show the same pattern detected only at the periphery of the cells [5]. Morphology of SC changes drastically in skin diseases with abnormal keratinocyte differentiation. Oyama et al. [7] reported that tape-stripped corneocytes from lesional skin of psoriasis vulgaris and lichen planus showed unique diffuse distribution patterns of Dsg1, but other skin diseases such as atopic dermatitis (AD) have not been tested. Distribution patterns of corneodesmosomal components other than Dsg1 have not been examined either.

Corneocyte surface areas vary not only by anatomic sites [8,9], but also by inflammation in contact dermatitis [10] and AD [11]. The classical method for the measuring of corneocyte surface areas is to first separate individual corneocytes by using ethanol [10] or hexan [8], and then measure the surface area with a light microscope. By tape-stripping, we could easily obtain horizontal images of corneocytes, and measure corneocyte surface areas directly from immunofluorescence images as performed by Mohammed et al. [12].

Atopic dermatitis (AD) [13], Netherton syndrome (NS) [14,15] and peeling skin syndrome type B (PSS) [16] may show some overlap in clinical manifestations, especially in childhood. Patients with these diseases have red scaly skin lesions with severe pruritus. Laboratory tests often show increased serum IgE levels. Defective epidermal barrier function and recurrent skin infections are common complications. Diagnostic hair shaft abnormalities of NS may not always be apparent, particularly in childhood. The suggestion that PSS and NS are the same disease had been proposed [16–18], until the defective genes were identified in both diseases, namely *SPINK5* and *CDSN* for NS [19] and PSS [20–23], respectively. However, such causative gene analysis cannot be performed as a routine clinical diagnostic test. Immunohistochemical staining of LEKTI and Cdsn using patient skin could be a useful diagnostic test for NS [24–26] and PSS [20], but invasive skin biopsy is required.

In this study, we analyzed distribution patterns of corneodesmosome components and cell surface area in AD. We found that AD corneocytes showed different results depending on disease conditions: lesional or non-lesional. We also compared these results with those of NS, PSS and ichthyosis vulgaris (IV) which is a major predisposing condition for AD [27], in order to determine if there are any differences as compared with AD.

2. Materials and methods

All participants provided informed consent and the protocol was approved by the medical ethics committee of the Asahikawa Medical University. The study was conducted according to the principles of the Helsinki declaration.

2.1. SC samples

SC samples were obtained from 22 adult and 15 pediatric cases of AD, three adult cases of IV (two heterozygote for the filaggrin mutations (S2889X in both cases), one compound heterozygote (3321delA, S2554X)), two adult cases of NS (a heterozygote for the *SPINK5* mutations R790X [28] and a compound heterozygote for the *SPINK5* mutations 377delAT and R790X [29]), two pediatric cases of NS (one is Case 3 in our previous report [30], the other was reported previously [31]) and another three cases of PSS (including Patient B in our previous study [21], and Patient M in our previous study [32]) with tape stripping. Among the AD samples collected, 14 obtained from adults and 6 from children were collected from pretreated lesional skin of the forearms; the remaining samples (8 samples from adults and 6 from children) were collected from non-lesional skin of the arms and the lower legs. No AD cases showed ichthyosis clinically, and the filaggrin gene mutation analysis was not performed. In all IV cases, we collected samples from ichthyotic skin of the lower legs. In NS cases, samples were collected from the lichenified area of the upper arm. In PSS cases, we obtained them from both non-erosive and erosive areas. For normal controls, we obtained samples from the forearms of 7 adults and 5 children with no history of skin disease. We used an 18 mm wide double-sided plastic adhesive tape (Nichiban No TW-18SD; Nichiban, Tokyo, Japan). According to the methods described by Oyama et al. [7], we cut the tape into 18 mm × 5 mm pieces, and then fixed them onto a glass slide. The slide was pressed on the skin once for about 10 s and then stripped away slowly.

2.2. Antibodies

The following were used as primary antibodies: polyclonal rabbit antibodies raised against the central part of human Cdsn [33], monoclonal mouse antibodies against the extracellular part of human Dsg1 (Dsg1-P23, Progen Biotechnik GmbH, Heidelberg, Germany), and polyclonal goat antibody raised against extracellular domain of human Dsc1 (L-15, Santa Cruz). The following secondary reagents were used for immunofluorescence analysis: Alexa-Fluor 488 goat anti-mouse IgG highly cross-absorbed (Molecular Probes, Eugene, OR), Alexa-Fluor 546 donkey anti-goat IgG (Molecular Probes), and Cy3-labeled goat anti-rabbit IgG (Amersham Bioscience, Buckinghamshire, UK).

2.3. Immunofluorescence and microscopy

The tape-stripped corneocytes were rinsed with phosphate-buffered saline (PBS) for 10 min at room temperature and incubated with primary antibodies diluted in PBS overnight at 4 °C. After washing in PBS three times, fluorescent antibodies diluted in PBS were applied for 30 min at 37 °C. For double-labeling with antibodies raised in different animals, a mixture of primary antibodies (Dsg1 and Cdsn/Dsg1 and Dsc1) was applied and this was followed by incubation with a mixture of secondary antibodies-conjugated with different fluorescent dyes. The samples were covered with an aqueous-based mounting medium (PermaFluor, Thermo Fisher Scientific, Waltham, MA) and a cover glass. Fluorescence images were obtained using an Olympus BX50 microscope (Olympus, Tokyo, Japan) with a digital camera (DP71, Olympus). Imaging was performed using Lumina Vision software ver. 2.4.4 (Mitani Corporation, Fukui, Japan). Frequency of different staining patterns of corneodesmosome components in different diseases was compared using the Mann–Whitney *U* test.

2.4. Measurement of corneocyte surface area

Corneocyte surface area was measured on immunofluorescence images of Dsg1 stained corneocytes using Image-Pro Plus ver. 4.0 software (Media Cybernetics, USA). Data were expressed as median [range] of 10 corneocytes in each sample. Statistical significance of the differences between different samples was evaluated using Kruskal–Wallis test and Steel–Dwass test. We also tested whether the corneocyte surface areas were correlated with the dot distribution patterns of Dsg1 with Spearman's correlation.

3. Results

3.1. Classification of the distribution patterns of corneodesmosomal components

All three corneodesmosomal components were localized in discrete dots on the cell surface in immunofluorescent microscopy (Fig. 1). We classified the distribution patterns into four types and