Biochemical and Biophysical Research Communications xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Expression of filaggrin-2 protein in the epidermis of human skin diseases: A comparative analysis with filaggrin

Teruhiko Makino [a,1,*], Megumi Mizawa [a,1], Takako Yamakoshi [a], Mikiro Takaishi [b], Tadamichi Shimizu [a]

[a] Department of Dermatology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Sugitani, Toyama 930-0194, Japan
[b] Department of Dermatology, Kochi Medical School, Kochi University, Nankoku, Japan

ARTICLE INFO

Article history:
Received 24 April 2014
Available online xxxx

Keywords:
Filaggrin-2
Filaggrin
S100 fused-protein family
Atopic dermatitis
Ichthyosis vulgaris
Psoriasis vulgaris

ABSTRACT

Filaggrin-2 is a member of the S100 fused-type protein family, and the structural features and expression of filaggrin-2 are similar to those of profilaggrin, a protein essential for keratinization. In the present study, we investigated the expression profile of filaggrin-2 in patients with skin diseases using antibodies against the repetitive region of filaggrin-2. In tissue samples from patients with skin diseases which are associated with a decrease in filaggrin, including ichthyosis vulgaris, atopic dermatitis and psoriasis vulgaris, the expression level of filaggrin-2 was markedly decreased compared to that in normal skin samples. In contrast, the expression of filaggrin-2 increased in parallel with that of filaggrin in samples of tissue from patients with skin diseases associated with hyperkeratosis, such as lichen planus and epidermolytic ichthyosis. Interestingly, filaggrin-2 signals were observed in slightly higher layers of the epidermis in comparison to those of filaggrin. Similarly, the expression of filaggrin-2 proteins was induced slightly later than filaggrin in the cultured keratinocytes. These findings suggest that filaggrin-2 may play an overlapping role with filaggrin in epithelial cornification; however, it may also have a partially distinct role in the molecular processes of cornification.

1. Introduction

Cornification is an essential process to confer a barrier function upon the skin in terrestrial mammals, which help resist against noxious biological and physicochemical insults from the environments and prevent the loss of body fluid [1]. A family of proteins described as "S100 fused-type proteins" are found within a 2 Mb region at chromosome band 1q21.3 [2]. The S100-fused type proteins include profilaggrin, trichohyalin, hornerin, trichohyalin-like 1, repetin, cornulin and filaggrin-2 [3–10]. Among these proteins, filaggrin plays an important role in the cornification process. Filaggrin is produced by the post-translational proteolysis of a precursor protein, profilaggrin and it promotes the aggregation of keratin filaments [2,11]. Filaggrin-2 is another member of the S100 fused-type protein family and was recently identified [5]. The deduced amino acid sequence of 2391 residues shows typical structural features of the "fused-type" S100 protein family members, which consists of an EF-hand domain at the N-terminus followed by a large repetitive domain. This repetitive domain of filaggrin-2 contains two types of tandem repeats, each 75–77 amino acids in length. The A-type repeats (A1–A9) are similar to the repeats of hornerin (50–77% identity) and the B-type repeats (B1–B14) are similar to the repeats of filaggrin (28–39% identity). Filaggrin-2 protein was detected in the granular and horny layers of normal stratified epithelium, which is the same pattern of distribution as filaggrin. Recently, the expression of filaggrin-2 was shown to be decreased in patients with atopic dermatitis, as has been observed for filaggrin [12]. Furthermore, genetic variation of filaggrin-2 was reported to be associated with more persistent atopic dermatitis in African American subjects [13]. However, the precise biological role of filaggrin-2 is still unknown. In the present study, to clarify the differences in the functions between filaggrin-2 and filaggrin, the expression profile of filaggrin-2 was examined in both skin samples from patients with diseases associated with decreased filaggrin and from patients with hyperkeratotic skin diseases, as well as healthy subjects.

Abbreviations: EDC, the epidermal differentiation complex; RT-PCR, reverse transcription polymerase chain reaction; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; GST, anti-glutathione S-transferase; DAPI, 6-diamidine-2′-phenylindole dihydrochloride; FLG, filaggrin.

* Corresponding author. Address: Department of Dermatology, Faculty of Medicine, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Sugitani, Toyama 930-0194, Japan. Fax: +81 76 434 5028.
E-mail address: tmakino@med.u-toyama.ac.jp (T. Makino).

[1] These authors equally contributed to this study.

http://dx.doi.org/10.1016/j.bbrc.2014.04.165
0006-291X/

2. Materials and methods

2.1. Clinical materials

Normal human skin tissue samples and tissue samples from patients with skin diseases were obtained from Toyama University Hospital. The skin tissue samples from subjects with diseases included samples from subjects with ichthyosis vulgaris, atopic dermatitis, psoriasis vulgaris, epidermolytic ichthyosis, lichen planus and actinic keratosis. The diagnosis of atopic dermatitis was performed using the AD diagnostic criteria proposed by Hannifin and Rajka [14]. Other diseases were diagnosed based on the clinical and histological findings by experienced dermatologists and pathologists. All patients gave their written informed consent and the study protocol complied with all of the Principles of the Declaration of Helsinki. This study was approved by the Medical Ethics Committees of the University of Toyama, Toyama.

2.2. Cell culture

Normal human epidermal keratinocytes (Kurabo Industries Ltd., Osaka, Japan) were cultured in Humedia-KG2 (Kurabo Industries Ltd., Osaka, Japan) in a humidified atmosphere with 5% CO_2. To induce the differentiation of keratinocytes, 1.5 mM Ca^{2+} was added to the medium of semi-confluent cultures, and the cells were harvested two, five and seven days after the addition of Ca^{2+}.

2.3. Preparation of specific antibodies against filaggrin-2 proteins

Antibodies against filaggrin-2 were prepared as reported previously [7,10]. Briefly, to prepare antibodies, an oligopeptide TQTGSRSSRASHFQSH corresponding to a part of the type-B repetitive units of filaggrin-2 was synthesized, conjugated with keyhole limpet hemocyanine and injected with adjuvant (TyterMax Gold, CytRx) into rabbits. The resulting antibodies were affinity-purified using a Hitrap NHS-activated column (GE healthcare UK Ltd., Buckinghamshire, England) conjugated with the peptide.

2.4. Preparation of recombinant filaggrin-2 and filaggrin proteins

To prepare recombinant proteins covering a part of repetitive domains of filaggrin-2 and filaggrin, cDNA fragments were amplified using reverse transcription-polymerase chain reaction (RT-PCR), and were subcloned into the pDEST15 gateway vectors (glutathione S-transferase gene fusion vector; Invitrogen, Carlsbad, CA). The primers used for PCR were: filaggrin-2-sense 5′-CACCA CAACTGGAAGAAGGGGATCTAGACT-3′, filaggrin-2-antisense 5′-TC AATGTCTAGACAGTTGCTTGTTTGC-3′, filaggrin-sense 5′-CACCCAGG AGTCCAGGACAAGAAAGCGT-3′ and, filaggrin-antisense 5′-CTAGTC

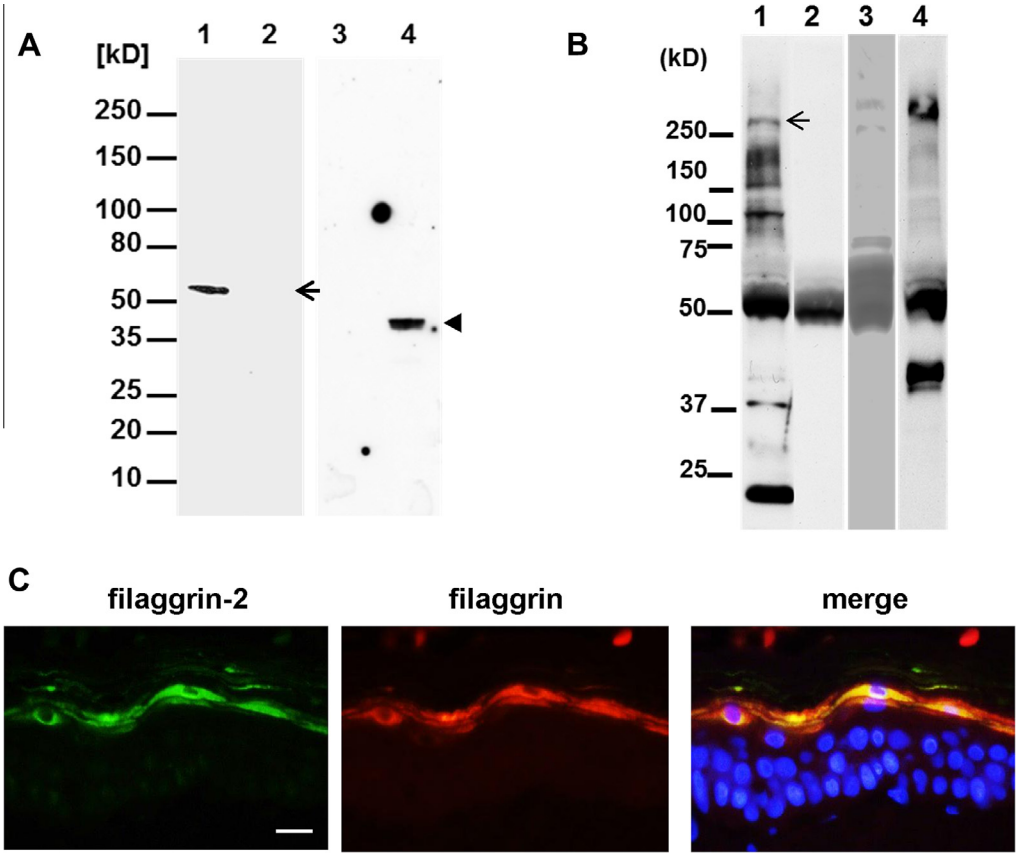

Fig. 1. (A) The specific recognition of the recombinant filaggrin-2 proteins by the antibodies. Purified recombinant filaggrin proteins were applied onto a 10% SDS gel and blotted with anti-filaggrin antibodies (lane 1) and anti-filaggrin-2 antibodies (lane 2). Purified recombinant filaggrin-2 was applied and blotted with anti-filaggrin antibodies (lane 3) and anti-filaggrin-2 antibodies (lane 4). The arrow indicates the size of the recombinant filaggrin protein. The arrowhead indicates the size of the recombinant filaggrin-2 protein. (B) The results of a Western blot analysis of normal human skin. Twenty micrograms of the protein preparation was blotted with an anti-filaggrin-2 antibody (lane 1), preimmune serum from the respective rabbits (lane 2), antiserum pre-absorbed with the peptide of the immunogen (lane 3), or an anti-filaggrin antibody (lane 4). The arrow indicates the expected size of the intact filaggrin-2 protein. (C) Immunostaining for filaggrin-2 proteins in normal human skin tissue samples. The tissue sections of normal human skin were doubly immunostained for filaggrin-2 and filaggrin. The tissue sections were also stained with 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI) to visualize the nuclei. The scale bar, 50 μm, is the same for all panels.

Please cite this article in press as: T. Makino et al., Expression of filaggrin-2 protein in the epidermis of human skin diseases: A comparative analysis with filaggrin, Biochem. Biophys. Res. Commun. (2014), http://dx.doi.org/10.1016/j.bbrc.2014.04.165