Journal of Dermatological Science 85 (2017) 186–196

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Decreases in 15-lipoxygenase metabolites in Olmsted syndrome model rats

Masato Wakabayashi[a,b], Takeshi Yoshioka[a], Kenichi Higashino[a], Yoshito Numata[a], Yasuyuki Igarashi[c], Akio Kihara[b,d,*]

[a] Shionogi Innovation Center for Drug Discovery, Discovery Research Laboratory for Innovative Frontier Medicines, Shionogi & Co., Ltd., Sapporo 001-0021, Japan
[b] Laboratory of Biochemistry, Graduate School of Life Science, Hokkaido University, Sapporo 060-0812, Japan
[c] Laboratory of Biomembrane and Biofunctional Chemistry, Faculty of Advanced Life Science, Hokkaido University, Sapporo 001-0021, Japan
[d] Laboratory of Biochemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

ARTICLE INFO

Article history:
Received 26 August 2016
Received in revised form 2 December 2016
Accepted 15 December 2016

Keywords:
Lipid
Lipidomics
15-Lipoxygenase
Olmsted syndrome
TRPV3

ABSTRACT

Background: Olmsted syndrome (OS) is a congenital dermatosis characterized by palmoplantar keratoderma and periorificial keratotic plaque. *TRPV3* (transient receptor potential vanilloid subtype 3) encodes a thermosensitive Ca^{2+} channel and is the causative gene of OS. However, the molecular mechanism that causes the pathological development of OS is unclear.
Objective: We aimed to investigate the molecular mechanisms underlying OS pathology from the perspective of lipid metabolism.
Methods: Comprehensive lipidomics and microarray analyses were conducted on tissue samples from a non-lesional skin area of OS model rats (*Ht* rats) and from wild type (WT) rats as the control.
Results: Infiltration of leukocytes such as eosinophils and neutrophils and an increase in the fibrotic region were detected in the unaffected skin area of *Ht* rats compared with the WT rats. Among about 600 lipid species examined, the levels of 15-lipoxygenase (LOX) metabolites, the precursors of anti-inflammatory and pro-resolving lipid mediators, and dihydroceramides decreased by ≥16-fold in *Ht* rats compared with WT rats. Consistent with the decreases in the 15-LOX metabolites, expression levels of the genes that encode the 15-LOXs, *Alox15* and *Alox15b*, were largely reduced. Conversely, increased expression levels were detected of *Il36b*, *Ccl20*, *Cxcl1*, and *Cxcl2*, which encode cytokines/chemokines, and *S100a8* and *S100a9*, which encode the Ca^{2+} binding proteins that are implicated in epidermal proliferation.
Conclusion: The pro-inflammatory state in the unaffected skin of *Ht* rats caused by decreases in 15-LOX metabolites and increases in cytokines/chemokines may contribute to the pathogenesis of OS.

1. Introduction

Olmsted syndrome (OS; Online Mendelian Inheritance in Man (OMIM) #614594) is a severe keratinization disorder characterized by the combination of palmoplantar and periorificial keratoderma and is often associated with hypotrichosis and intense itching [1]. OS is a very rare congenital disorder, and only 73 cases have been reported so far [1]. Most of the reported OS cases were sporadic; however, some familial cases with different hereditary patterns were also found. The hereditary patterns were either autosomal or X-linked and either dominant or recessive [1]. *TRPV3* (transient receptor potential vanilloid subtype 3) on chromosome 17 and *MBTPS2* (membrane-bound transcription factor protease, site 2) on chromosome X have been identified as the causative genes of OS [2,3]. TRPV3 is a thermosensitive Ca^{2+} channel [4–6]. Most of the *TRPV3* mutations (p.Gly573Ser, p.Gly573Cys, p.Gly573Ala, p.Gln580Pro, p.Leu673Phe, p.Trp692Gly, and p.Trp692Cys) found in OS patients were dominant [1], and expression of the mutant

Abbreviations: OS, Olmsted syndrome; OMIM, Online Mendelian Inheritance in Man; *Ht*, WBN/Kob-Ht; WT, wild type; LOX, lipoxygenase; HE, hematoxylin-eosin; LC, liquid chromatography; MS, mass spectrometry; HETrE, hydroxyeicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; 15-oxETE, 15-oxoeicosatetraenoic acid; HDoHE, hydroxydocosahexaenoic acid; COX, cyclooxygenase; HEPE, hydroxyeicosapentaenoic acid; EOS, esterified ω-hydroxyacyl-sphingosine.

* Corresponding author at: Laboratory of Biochemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, Kita 12-jo, Nishi 6-chome, Kita-ku, Sapporo 060-0812, Japan.
E-mail address: kihara@pharm.hokudai.ac.jp (A. Kihara).

http://dx.doi.org/10.1016/j.jdermsci.2016.12.013

proteins caused increases in intracellular Ca^{2+} concentrations [2,7]. Recessive mutations in *TRPV3* have also been identified for OS [1,8]. The reason why these diverse inheritance patterns exist for OS is currently unclear. The *MBTPS2* mutations found in OS patients were recessive [3,9]. MBTPS2 is a component of the regulated intramembrane proteolysis machinery that regulates cholesterol homeostasis and the unfolded protein response by cleaving membrane-spanning regulatory proteins [10].

TRPV3 belongs to the vanilloid family of transient receptor cation channels [11]. *TRPV3* was shown to be highly expressed in skin, primarily in hair follicles and in keratinocytes that are present in the basal layer of the epidermis, and its expression has also been detected in brain, spinal cord, and dorsal root ganglion [5,6]. TRPV3 is activated by temperature, with a threshold of 31–39 °C [4–6], as well as by ligands such as 2-aminoethoxydiphenyl borate and farnesyl pyrophosphate, which is an intermediate metabolite of the mevalonate pathway [12,13]. Furthermore, arachidonic acid and other unsaturated fatty acids were reported to potentiate TRPV3 activity [14], while the pro-resolving lipid mediator 17(*R*)-resolvin D1 was found to inhibit TRPV3 activity [15]. Thus, there appears to be a relationship between TRPV3 activity and lipids.

WBN/Kob-*Ht* rats (hereafter called *Ht* rats) and DS-*Nh* mice were selected as spontaneous hairless rodent strains, and both strains had dominant *Trpv3* mutations (p.Gly573Cys in *Ht* rats and p.Gly573Ser in DS-*Nh* mice), the same as two of the mutations found in OS patients [16]. These rodents developed dermatitis accompanied with hyperkeratosis and acanthosis under conventional conditions [17,18]. The p.Gly573Ser transgenic mice also showed dermatitis with severe itching and defects in hair growth [19]. However, OS-like phenotypes were not observed in *Trpv3* knockout mice; rather, they exhibited thin stratum corneum, wavy whiskers, and misaligned hair follicles [20,21]. These results suggest that OS is caused by gain-of-function of TRPV3.

The skin permeability barrier is important for the prevention of loss of water and electrolytes from inside the body and for protection against the invasion of external pathogens, allergens, and harmful compounds. Multi-layered lipids, called lipid lamellae, are present in the outermost cell layer of the epidermis (stratum corneum), where they have a pivotal function in skin permeability barrier formation [22]. The major constituents of lipid lamellae are ceramides (about 50%), cholesterol (about 25%), and free fatty acids (about 15%) [23,24]. Ceramides form the backbones of sphingolipids, and a variety of ceramide species are present in the epidermis [22,24] (Fig. S1). Acylceramide is the epidermis-specific ceramide and is especially important for skin permeability barrier formation [22]. Normal ceramide consists of a long-chain base and an amide-linked fatty acid, whereas acylceramide contains an extra linoleic acid attached to the ω-carbon of the amide-linked fatty acid [22,24] (Fig. S1). Mutations in genes involved in acylceramide synthesis cause autosomal recessive congenital ichthyosis, which is characterized by severe skin permeability barrier defects, scaly skin, and hyperkeratosis [24,25].

Considering the importance of lipids in skin permeability barrier formation and the regulatory roles of lipids on TRPV3 activity, it is likely that changes in lipid metabolism contribute to OS pathology. However, lipidomics analyses of skin tissues of OS patients or OS model animals have not been performed so far, although transcriptome and proteome analyses have already been reported [7]. Here, we performed comprehensive comparative lipidomics analyses of skin samples from non-lesional areas of wild type (WT) and *Ht* rats to reveal the lipid metabolism underlying the OS pathology.

2. Methods

2.1. Animals

WBN/Kob (WT) and WBN/Kob-*Ht* (*Ht*; *Trpv3* p.Gly573Cys) rats were purchased from the Ishikawa Laboratory Animal Company (Saitama, Japan) and housed under conventional conditions under a 12-h light/dark cycle with standard food and water *ad libitum*. All animal experiments were conducted according to the guidelines for animal experimentation at Shionogi & Co., Ltd. (Osaka, Japan). Skin and brain tissue samples were taken from WT and *Ht* rats at 25 weeks of age, frozen immediately, and stored at −80 °C until used for analyses.

2.2. Histological analyses

Skin samples were fixed with 10% neutral pH-buffered formaldehyde (Wako Pure Chemical Industries, Osaka, Japan) for 2 days and embedded in paraffin. Paraffin-embedded skins were cut into 3–4-μm sections, deparaffinized, and subjected to hematoxylin-eosin (HE), toluidine blue, Masson's trichrome, and Luna stainings [26,27].

2.3. Hydroxyproline measurement

Hydroxyproline levels were measured using a Hydroxyproline Assay Kit (Quickzyme, Leiden, Netherlands), according to the manufacturer's instructions.

2.4. Measurement of myeloperoxidase activity

Myeloperoxidase activity was measured using an OxiSelect Myeloperoxidase Chlorination Activity Assay Kit (Cell Biolabs, San Diego, CA), according to the manufacturer's instructions.

2.5. Transmission electron microscopy

Samples taken from the non-lesional dorsal skin of WT and *Ht* rats at 3 weeks of age were fixed with 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at 4 °C and then with 0.1% tannic acid in 0.1 M cacodylate buffer (pH 7.4) at 4 °C. Fixed samples were washed four times with 0.1 M cacodylate buffer (pH 7.4), followed by post-fixation with 2% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4) at 4 °C. The samples were then dehydrated in graded ethanol solutions (50%, 70%, 90%, and 100%), followed by infiltration with propylene oxide for 1 h twice, and finally with a propylene oxide/Quetol-812 resin (Nisshin EM, Tokyo, Japan) mixture (7:3, v/v) for 1 h. After the propylene oxide was volatilized overnight, the samples were embedded in fresh 100% resin and incubated at 60 °C for 48 h. Ultra-thin sections (80 nm) were prepared using the ultramicrotome Ultracut UCT (Leica Microsystems, Wetzlar, Germany). The sections were mounted on copper grids, stained with 2% uranyl acetate at room temperature for 15 min, washed with distilled water, and secondary-stained with Lead stain solution (Sigma-Aldrich, St. Louis, MO) at room temperature for 3 min. Microscopy was performed using a JEM-1400Plus transmission electron microscope (JEOL, Tokyo, Japan) at an acceleration voltage of 80 kV. Digital images were taken using a VELETA charge-coupled device camera (Olympus, Tokyo, Japan).

2.6. Lipid analyses by liquid chromatography (LC) coupled with mass spectrometry (MS)

Details of the lipid extraction and lipid analyses by LC/MS are described in the Supplementary information and Tables S1–S4.