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Review

The important role of epidermal triacylglycerol metabolism for maintenance of the skin permeability barrier function☆

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ABSTRACT

Survival in a terrestrial, dry environment necessitates a permeability barrier for regulated permeation of water and electrolytes in the cornified layer of the skin (the stratum corneum) to minimize desiccation of the body. This barrier is formed during cornification and involves a cross-linking of corneocyte proteins as well as an extensive remodeling of lipids. The cleavage of precursor lipids from lamellar bodies by various hydrolytic enzymes generates ceramides, cholesterol, and non-esterified fatty acids for the extracellular lipid lamellae in the stratum corneum. However, the important role of epidermal triacylglycerol (TAG) metabolism during formation of a functional permeability barrier in the skin was only recently discovered. Humans with mutations in the *ABHD5/CGI-58* (α/β hydrolase domain containing protein 5, also known as comparative gene identification-58, CGI-58) gene suffer from a defect in TAG catabolism that causes neutral lipid storage disease with ichthyosis. In addition, mice with deficiencies in genes involved in TAG catabolism (*Abhd5/Cgi-58* knock-out mice) or TAG synthesis (acyl-CoA:diacylglycerol acyltransferase-2, *Dgat2* knock-out mice) also develop severe skin permeability barrier dysfunctions and die soon after birth due to increased dehydration. As a result of these defects in epidermal TAG metabolism, humans and mice lack ω-(O)-acylceramides, which leads to malformation of the cornified lipid envelope of the skin. In healthy skin, this epidermal structure provides an interface for the linkage of lamellar membranes with corneocyte proteins to maintain permeability barrier homeostasis. This review focuses on recent advances in the understanding of biochemical mechanisms involved in epidermal neutral lipid metabolism and the generation of a functional skin permeability barrier. This article is part of a Special Issue entitled The Important Role of Lipids in the Epidermis and their Role in the Formation and Maintenance of the Cutaneous Barrier. Guest Editors: Kenneth R. Feingold and Peter Elias.

1. Introduction

The skin is the largest organ of the human body. Its main function consists of forming a barrier between the external environment and the individual's internal milieu [1,2]. In this way the skin protects the individual from environmental influences such as mechanical impacts, ultraviolet light, chemicals, or pathogenic microorganisms. However, to survive in a terrestrial, dry environment the skin also provides a barrier that prevents the loss of water and electrolytes through the body surface to minimize dehydration [3,4]. This so-called skin permeability barrier resides in the stratum corneum, the outermost layer of the epidermis, and consists of large, flattened, enucleated and therefore metabolically inactive corneocytes. Highly cross-linking of structural corneocyte proteins such as involucrin, loricrin, small proline-rich proteins, keratins, and filaggrin produces a rigid structure, the cornified envelope (CE), that contributes to the mechanical strength of the skin [5,6]. To mediate permeability barrier function, the corneocytes are embedded in a lipid-enriched extracellular matrix, which forms lamellar membranes with a unique composition of ceramides, cholesterol, and non-esterified fatty acids (FAs) [7,8]. Thus, the stratum corneum consists of rigid building blocks that are stuck together with space-filling extracellular lipids, similar to a wall that is built of bricks and mortar [9].

The lipids of the extracellular matrix originate from polar precursor lipids that are secreted by lamellar bodies into interstices of the stratum corneum, where various co-secreted lipid processing enzymes and proteases metabolize them to the more hydrophobic barrier constituents [10,11]. Triacylglycerol (TAG) is a minor component of lamellar bodies and the extracellular matrix in the stratum corneum of healthy skin [12,13] and thus the epidermal TAG metabolism did not attract much

Abbreviations: ABHD, α/β hydrolase fold domain-containing protein; AcylCer, ω-(O)-acylceramide; ATGL, adipose triglyceride lipase; CE, cornified envelope; CGI-58, comparative gene identification-58; CLE, cornified lipid envelope; DAG, diacylglycerol; DGAT-2, acyl-CoA: diacylglycerol acyltransferase-2; DCS, Dorfman–Chanarin syndrome; FA, fatty acid; HSL, hormone-sensitive lipase; LPAAT, acyl-CoA:lysophosphatidic acid acyltransferase; LXR, liver X receptor; MAG, monoacylglycerol; MGL, monoacylglycerol lipase; NLSD, neutral lipid storage disease; NLSDI, NLSD with ichthyosis; NLSDM, NLSD with myopathy; PNPLA, patatin-like phospholipase domain-containing protein; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; TAG, triacylglycerol; VLC, very long-chain

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attention in the past. However, severe skin defects in humans and mice that originate from dysfunctions of enzymes and activators of TAG synthesis and degradation suggested a critical function of the epidermal neutral lipid metabolism for maintenance of a proper permeability barrier in the skin. This review summarizes the recent discoveries relating to neutral lipid metabolism in keratinocytes and its role in the development of neutral lipid storage disease (NLSD) in humans.

2. Neutral lipid storage disease in humans — a disease with two different phenotypes

The first indication that epidermal TAG metabolism may be linked to skin barrier function was provided in 1966 when Rozenszajn and colleagues reported on two sisters, who both suffer from NLSD with severe ichthyosis [14]. Later, Dorfman et al. and Chanarin et al. presented additional cases of NLSD patients with ichthyosis and the condition was subsequently termed Dorfman–Chanarin syndrome (DCS, or also known as Chanarin–Dorfman syndrome) [15,16]. NLSD is a very rare autosomal recessive, non-lysosomal lipid storage disorder with an estimated prevalence of less than 1:1,000,000 in humans. Affected individuals accumulate a large number of neutral lipid-containing droplets in peripheral blood granulocytes and granulocyte precursors in the bone marrow (Jordans' anomaly) [17] as well as in other tissues and organs of the body, including the skin, cardiac and skeletal muscle, liver, and brain. The clinical manifestation of the disorder is very heterogeneous and NLSD patients were classified in two groups depending on whether the patient suffers from ichthyosis or instead develops a severe form of skeletal and/or cardiac myopathy, a condition that is not described in NLSD patients with ichthyosis [18]. In addition, in both groups of NLSD patients, liver steatosis with hepatosplenomegaly as well as other clinical symptoms with inconsistent manifestations that include neurosensory hearing loss, ataxia, nystagmus, or mental and developmental retardation have been reported [14–19].

Using homozygosity mapping, linkage-disequilibrium analysis, and candidate gene sequencing, Fischer and colleagues analyzed the genome of patients from both groups and identified disease-causing mutations in *ABHD5/CGI-58* (α/β hydrolase fold domain-containing protein 5, also known as comparative gene identification-58, CGI-58) in NLSD patients with ichthyosis and *ATGL* (adipose triglyceride lipase, also referred to as patatin-like phospholipase domain-containing protein 2 — PNPLA2, desnutrin or transport secretion protein-2.2 — TTS-2.2) mutations in the subgroup of NLSD patients, who mainly suffer from myopathy [20,21]. Based on these results, the condition caused by mutations in *ABHD5/CGI-58* is now called NLSD with ichthyosis (NLSDI, formerly known as DCS, OMIM #275630) and the NLSD variant associated with *ATGL* mutations is referred to as NLSD with myopathy (NLSDM, OMIM #610717). To date, a total of more than one hundred NLSD cases have been reported that include the usual spectrum of mutations [19,22]. Moreover, large genomic deletions that affect one or two exons of *ABHD5/CGI-58* as well as a genomic insertion, which results in an aberrant *ABHD5/CGI-58* mRNA splicing, were also recently reported in affected patients [23,24].

3. ABHD5/CGI-58 and ATGL — two players in cellular neutral lipid metabolism

The *ABHD5/CGI-58* gene was originally identified in a comparative-gene-identification approach, where the human and the *Caenorhabditis elegans* proteomes were aligned to detect novel human genes [25]. According to this experiment, ABHD5 is also designated as CGI-58. The *ABHD5/CGI-58* gene encodes 7 exons and spans a region of about 31.8 kb on chromosome 3. Translation of the 5.37 kb sized mRNA generates a protein that consists of 349 amino acids with a molecular mass of approximately 39 kDa. The amino acid sequence of ABHD5/CGI-58 is evolutionary highly conserved among vertebras, e.g. the human protein shares 94% amino acid homology with its murine orthologue. The protein is predominantly expressed in mature adipocytes and testes, and lower levels are detected in liver, skeletal and cardiac muscle, skin, and brain [26,27].

ABHD5/CGI-58 is a member of the esterase/thioesterase/lipase subfamily of proteins that are structurally defined by the presence of an α/β hydrolase fold (ABHD1-15) and an active site that comprises a catalytic triad as found in enzymes with hydrolytic activity against lipid esters [28]. This catalytic triad usually consists of a serine residue within an esterase/lipase GXSXG sequence motif, an aspartate, and a histidine residue [29]. However, in the corresponding sequence region of ABHD5/CGI-58 (amino acid residues 151–155 of the human protein), an asparagine residue replaces the putative active serine in the nucleophile position of the catalytic triad [20]. As a result of this amino acid substitution, ABHD5/CGI-58 most likely does not exhibit lipase/esterase activity itself [26,30].

To explore the enzymatic function of ABHD5/CGI-58 and the corresponding biochemical defect associated with NLSDI, several groups have performed studies with skin fibroblasts, myocytes, and lymphocytes derived from affected patients. These early biochemical experiments revealed that the total cellular TAG content in the analyzed patient cells was 2- to 20-fold higher than in controls even though these cells exhibit unchanged uptake, transport, and oxidation of FAs [31–33]. With regard to this lipid storage phenotype, Douste-Blazy and colleagues as well as Hilaire et al. and Williams et al. [34–40] concluded from their experiments that the defect in NLSD cells is due to a functional deficiency of a TAG lipase that impairs cellular TAG catabolism. Igal and Coleman, however, reported results that were contradictory to this finding. They found normal TAG hydrolase activity in patient cells but demonstrated instead that acylglycerol recycling to phospholipids was defective and caused increased diacylglycerol (DAG) reesterification and TAG formation [41,42].

It was the finding of the causal relationship between *ABHD5/CGI-58* mutations and NLSDI that led to the identification of an important biochemical function of the protein in lipid metabolism and provided novel insights into disease development. In 2006, Lass et al. discovered that efficient TAG hydrolysis by ATGL requires the presence of ABHD5/CGI-58 [26]. ATGL is an essential enzyme for the hydrolysis of cellular, non-lysosomal TAG stores, a process that is commonly referred to as lipolysis [43–46]. The complete degradation of TAG to FAs and glycerol requires the hydrolytic activities of three enzymes that act in a sequential manner: (i) ATGL, which exhibits high substrate specificity for TAG and catalyzes the initial and rate-limiting step in lipolysis by specifically removing the first FA from TAG molecules that generates DAG substrate [47–49]; (ii) hormone-sensitive lipase (HSL), the major lipase in DAG hydrolysis, which leads to the formation of monoacylglycerol (MAG) and another FA [50,51]; (iii) in the final step of lipolysis, monoglyceride lipase (MGL) is needed to efficiently hydrolyze MAG to glycerol and FA [52,53]. In this process, ABHD5/CGI-58 interacts with ATGL and stimulates its enzymatic activity several fold [26]. In line with this biochemical function, mutant variants of ABHD5/CGI-58 that possess amino acid substitutions, which are known to be associated with NLSDI, completely lost their ability to activate ATGL-mediated lipolysis [26,54]. Conversely, expression of functional ABHD5/CGI-58 in dermal NLSDI fibroblasts restored TAG hydrolysis and reversed the lipid storage phenotype in patient cells [26]. These important findings demonstrated that ABHD5/CGI-58 is an essential regulatory factor for efficient hydrolysis of cellular TAG stores by ATGL, and moreover, they provided a plausible biochemical explanation for the multi-systemic TAG accumulation in NLSD patients.

4. ABHD5/CGI-58 possesses an ATGL-independent function in epidermal TAG catabolism

Since ichthyosis or other skin abnormalities were never reported in patients with *ATGL* mutations nor observed in ATGL-deficient mice [21,48,55], the finding of the causal relationship between *ABHD5/CGI-58* mutations and defective ATGL-mediated TAG hydrolysis did not explain