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Review

Role of cholesterol sulfate in epidermal structure and function: Lessons from X-linked ichthyosis☆

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ABSTRACT

X-linked ichthyosis is a relatively common syndromic form of ichthyosis most often due to deletions in the gene encoding the microsomal enzyme, steroid sulfatase, located on the short area of the X chromosome. Syndromic features are mild or unapparent unless contiguous genes are affected. In normal epidermis, cholesterol sulfate is generated by cholesterol sulfotransferase (SULT2B1b), but desulfated in the outer epidermis, together forming a 'cholesterol sulfate cycle' that potently regulates epidermal differentiation, barrier function and desquamation. In XLI, cholesterol sulfate levels my exceed 10% of total lipid mass (≈1% of total weight). Multiple cellular and biochemical processes contribute to the pathogenesis of the barrier abnormality and scaling phenotype in XLI. 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

X-linked ichthyosis (XLI) (OMIM #308100) is due to loss-of-function mutations in the gene that encodes the microsomal enzyme, steroid sulfatase (SSase; STS) [1–5]. Female carriers rarely exhibit a skin phenotype [6,7], probably because the region of the X chromosome where STS resides escapes X-inactivation. Affected males present at birth, or shortly thereafter, with generalized peeling or exaggerated neonatal desquamation, although some may exhibit a collodion membrane [8,9].

2. Clinical features

After the neonatal period, fine scaling persists on the trunk and extremities, but over time, scales often become coarser and darker. While scaling is generalized, it typically spares the anticubital and popliteal fossae, palms, soles, and the mid-face, but the lateral face, axillae and the neck always remain involved.

The clinical features of XLI bear some similarities to *ichthyosis vulgaris* (IV), a common, autosomal semi-dominant trait caused by mutations in the filaggrin gene. However, the darker color of the scale and its more 'centripetal' distribution, as well as the sparing of the palms and soles, point to a clinical diagnosis of XLI [9]. Yet, in the absence of an X-linked pedigree, phenotypic overlap with other mild-to-moderate ichthyosis requires further studies to definitively establish the diagnosis of XLI. Moreover, because IV and the xerosis associated with atopic dermatitis (AD) are both quite common, the two disorders may co-exist, producing a more severe phenotype in affected patients [10–12]. Indeed, both of these disorders are relatively-common (XLI occurs in 1:1,800; filaggrin mutations occur in up to 10% of the European population). In a recent series of 11 Korean XLI patients, 7 had a prior history of atopic disease, while only one displayed flexural involvement, a reliable clinical marker of AD. Thus, *filaggrin* represents a genetic modifier of the XLI phenotype.

Routine histopathology in XLI typically shows moderate hyperkeratosis with mild acanthosis and partial accentuation of the granular cell layer. While these features are nonspecific, they can help to exclude IV or filaggrin-deficient AD, which should instead display decreased keratohyalin granules. Measurement of substrate accumulation in skin (cholesterol sulfate) or blood (cholesterol sulfate or other sulfated steroid hormones) is diagnostic, as is the assay of SSase activity in epidermis [13,14], cultured fibroblasts, or leukocytes [15,16]. Serum lipoprotein electrophoresis is also diagnostic, demonstrating more

Abbreviations: CSO_4, cholesterol sulfate; FISH, fluorescence *in situ* hybridization; IV, ichthyosis vulgaris; Klk, kallikreins; PSD, placental sulfatase syndrome; SC, stratum corneum; SCCE, stratum corneum chymotryptic enzyme; SCTE, stratum corneum tryptic enzyme; SSase, STS, steroid sulfatase; SULT2B1b, cholesterol sulfotransferase; XLI, x-linked ichthyosis

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rapid mobility of the LDL (beta) and pre-LDL (pre-beta) fractions due to an increase in sulfated sterol content [15,17]; however, this assay is no longer widely available. Because most XLI cases arise from deletion of the STS gene [18–26], fluorescence *in situ* hybridization (FISH) analysis is commonly employed for diagnosis of XLI and its carrier state [27], but FISH testing provides false negatives in XLI patients who have point mutations (≈10% of affected XLI subjects).

3. Syndromic features of XLI

XLI is considered a systemic, albeit usually mild, syndromic disorder [28]. *Placental sulfatase deficiency syndrome (PSD)*, which occurs in pregnancies of XLI fetuses, can manifest as failure of labor either to initiate or to progress, defective cervical softening, and a poor response to exogenous pitocin. PSD syndrome can be detected prior to the development of these complications by low maternal urinary and blood estriol levels due to the placenta's (a largely fetal structure) failure to desulfate estrone sulfate [19,29,30]. Since maternal estriol levels are part of the so-called 'triple screen' employed to detect pregnancies at risk for trisomies 18/21, Smith–Lemli–Opitz syndrome, and neural tube defects, many XLI fetuses are now detected in this manner [31–34].

In contrast to earlier estimates, the incidence of *cryptorchidism* (testicular maldescent) does not appear to exceed 5–10% [35,36]. Moreover, earlier reports of *testicular cancer* in a few XLI patients with normally descended testes [37] have not been confirmed as a true association. These associations instead could be due to a contiguous gene syndrome, with loss of pseudouridine-phosphate phosphatase [38]. Small, entirely asymptomatic, comma-shaped *corneal opacities* develop in the posterior capsule of Descemet's membrane in ≈ 25% of adult XLI patients, but these often are not present in affected children. But when present, these opacities are diagnostic, and some female carriers display similar opacities [9,36]. A small number of XLI patients have developed *nephrotic syndrome*, attributable to the deleterious effects of excessive cholesterol sulfate on glomerular cell function [38]. Finally, in recent reports, as many as 40% of XLI patients demonstrated cognitive behavioral abnormalities, such as *attention-deficit disorder* [39,40], but the actual incidence may be closer to 25%. These abnormalities have been attributed to altered sterol metabolism in the central nervous system [40–42]. Inactivation of SSase increases aggressive behavior in rodents [43], providing some further support for this new association.

Because the STS locus lies on the distal tip of the short arm of the X chromosome, deletion of this locus is often accompanied by additional deletions of flanking regions, resulting in a series of in contiguous gene syndromes. In these instances, XLI males present with an ichthyosiform phenotype in the setting of multisystem disease [44–47]. One commonly reported association is with Kallman's syndrome, displaying anosmia, hypogonadism. *Mental retardation*, linked to deletions in the neighboring VCX gene has also been observed [48–50], as have contiguous gene syndromes with chondrodysplasia punctata, autism cerebellar ataxia, and albinism. In one large series, 8% of STS-deficient fetuses exhibited deletions of additional contiguous genes [51].

4. Molecular Biology and Regulation of SSase

SSase (EC 3.1.6.2, arylsulfatase-C) is a member of a superfamily of 12 different mammalian sulfatases that hydrolyze alkyl steroid sulfates (e.g., dehydroepiandrosterone sulfate [DHEAs]) and aryl steroid sulfates (e.g., estrone sulfate) to their unconjugated forms (Fig. 1). The STS gene that encodes SSase, which is located distally on the short arm of the X chromosome, consists of 10 exons that span 146 kb, while the cDNA encodes a protein with 583 amino acids, as well as four potential glycosylation sites [52]. The promoter region of the SSase gene is unusual in that it resembles neither a housekeeping gene nor other tightly-regulated genes, and also lacks binding sites for Sp1 and other common transcription factors [53]. It is located proximal to the portion of the gene that encodes enzyme expression, but the promoter sequence varies in a tissue-specific fashion [54]. While cytokines such as TNFα and IL-6 upregulate enzyme activity, IL-1β instead reportedly downregulates SSase activity [55], although IL-1β (as well as interferon γ) reportedly down-regulate SSase expression by inhibiting NFkB,while activating the glucocorticoidreceptor [55]. Finally, both retinoids and $1,25(OH)_2$ vitamin D3 induce both SSase activity and expression [56].

5. Enzyme characteristics and epidermal localization

SSase is a 65 kDa microsomal enzyme that localizes to the endoplasmic reticulum, Golgi, and endosomal membranes, including coated pits (but not in lysosomes) of placenta and several other tissues [52,57,58]. A key feature of SSase is that exogenous substrates, such as estrone sulfate [59] and cholesterol sulfate [60], induce enzyme activity. In addition, specific, high-affinity sterol sulfate transporters can be activated by their substrates [61].

In normal epidermis, SSase protein and enzyme activity are low in the basal and lower spinous layers, but both increase in the outer nucleated cell layers, where they peak in the granular layer [14]. Enzyme activity persists into the stratum corneum, where it continues to desulfate cholesterol sulfate, contributing to the pool of cholesterol available to form the extracellular lamellar bilayers (Figs. 2 & 3). In ultrastructural cytochemical studies, SSase activity localizes not only within the cytosol, but also within lamellar bodies, followed by its exocytosis from lamellar bodies into the interstices of the lower stratum corneum [62] (Fig. 3). Thus, SSase, like other lipid hydrolases that are involved in the processing of polar lipids to more hydrophobic species in the stratum corneum, utilizes the lamellar body secretory system to reach the extracellular domain, where it can participate in the regulation of permeability barrier homeostasis and desquamation [63]. In contrast to SSase, cholesterol sulfate exploits its extreme amphilicity to diffuse into the extracellular domains from the cytosol of granular cells ([64]; see also below). Finally, while we are focusing here on cholesterol sulfate, epidermal SSase also could increase the bioavailability of androgens not only in epidermis, but also in hair follicles [65,66], where it has been implicated in the pathogenesis of androgenetic alopecia [67].

6. Cholesterol sulfotransferase

Cytosolic sulfotransferases (SULTs) represent a superfamily of enzymes that catalyze the sulfoconjugation of hormones, neurotransmitters, drugs, xenobiotics, and sterols [68,69]. The SULT superfamily of enzymes is composed of five families, of which the SULT2 family is primarily responsible for the sulfation of endogenous steroids and sterols. The SULT2 family is further divided into SULT2A1 and SULT2B1. SULT2A1 catalyzes the conversion of DHEA to DHEA sulfate and is commonly referred to as DHEA sulfotransferase. The SULT2B1 subfamily consists of two isoforms, SULT2B1a and SULT2B1b, derived from the same gene via differential splicing. The SULT2B1a isoform preferentially sulfonates pregnenolone, but not cholesterol, while SULT2B1b preferentially catalyzes the conversion of cholesterol to cholesterol sulfate. Thus, the SULT2B1b isoform accounts for the majority of cholesterol sulfotransferase activity. The human SULT2B1b gene is localized to chromosome 19, where it encodes a protein that contains 365 amino acids [69].

In the epidermis and in keratinocytes SULT2B1b is expressed, while neither SULT2B1a nor SULT2A1 is observed by either PCR or Western blotting [70]. The expression of SULT2B1b and cholesterol sulfotransferase activity increases in keratinocytes subjected to calcium-induced differentiation [70]. Using immunocytochemistry, SULT2B1b is observed in both the basal and suprabasal layers of the epidermis [70,71]. Retinoic acid, which inhibits differentiation, also inhibits cholesterol sulfotransferase activity, while PPARα, PPARβ/d, PPARg, and LXR activators, which stimulate differentiation, instead increase SULT2Blb expression and cholesterol sulfotransferase activity [72–74]. Similarly, TPA and TNF, which also stimulate keratinocyte