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The Epithelial Sodium Channel ENaC and its Regulators in the Epidermal Permeability Barrier Function

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Abstract: The highly amiloride-sensitive epithelial sodium channel ENaC is well known to be involved in controlling whole body sodium homeostasis and lung liquid clearance. ENaC expression has also been detected in the skin of amphibians and mammals. Mice lacking ENaC expression lose rapidly weight associated with an epidermal barrier defect that develops following birth. This dehydration is accompanied with a highly abnormal lipid matrix composition and an impaired skin surface acidification. This strongly suggests a role of ENaC in the maturation of barrier function rather than in the prenatal generation of the barrier, and may be as such an important modulator for skin hydration. In parallel, gene targeting experiments of regulators of ENaC activity, membrane serine proteases, also termed channel activating proteases, like CAP1/Prss8 and matriptase/MT-SP1 by themselves have been shown to be crucial for the epidermal barrier function. In our review, we mainly focus on the role of ENaC and its regulators in the skin and discuss their importance in the epidermal permeability barrier function.

Keywords: Sodium channel, transmembrane ion flux, regulators or ENaC, serine protease, barrier dysfunction.

INTRODUCTION

The Barrier Function of the Skin

The skin barrier protects against extensive water loss in one direction (outward barrier) and against the invasion of harmful substances from the environment (inward barrier). The complex cellular organization in the epidermis, and the way keratinocytes interconnect, contribute to the epidermal permeability barrier. It has been proposed that during phylogenetic evolution, the mammalian skin has developed at least two independent systems for forming an efficient barrier, namely the *cornified envelope* (a protein-lipid layer) in the stratum granulosum and corneum and the *tight junctions* in the second last nucleated layer of the stratum granulosum [1].

More than 20 intercellular proteins, connecting adjacent corneocytes, maintain the integrity of the stratum corneum and are directly related to desquamation processes. Thus, concurrently, a series of structural proteins, including involucrin, loricrin and the class of small proline-rich proteins (SPRs) and S100 proteins are synthesized and sequentially cross-linked by transglutaminases to reinforce the cornified envelope just beneath the plasma membrane [2]. There is a functional redundancy, which is mainly due to the overlapping functions of the transglutaminase substrates, like e.g., loricrin and involucrin [3, 4]. Evidence that this cornified envelope assembly is necessary for barrier development in skin was obtained from the study of transglutaminase 1-deficient mice that lack cornified envelopes resulting in neonatal lethality [5]. Alterations of corneocyte morphology have also been described in mouse models with epidermal permeability barrier defects as, e.g., irregular shaped corneocytes in Klf4 null mice [6], and volume-enlarged corneocytes in serine protease matriptase/ST14 -/- [7] and CAP1/Prss8 -/- [8] animal models. Barrier lipids are formed in mice only few days before birth [9] and their deficiency in pSAP- and beta-glucocerebrosidase results in disruption of the water permeability barrier and in early death [10, 11]. Equally, mice with a targeted disruption of the fatty acid transport protein 4 (Fatp 4) showed a disturbed fatty acid composition of epidermal ceramides, despite a normal distribution of tight junction proteins [12]. Thus, mice with impaired skin barrier function either showed a defect in only one system or in the two components of the barrier in parallel. For example, Klf4 knock-out [6] and transgenic mice expressing desmoglein-3 ectopically [13] showed postnatal lethality due to dehydration, whereas layered organization of the keratinocytes was not affected. Mice deficient for the membrane-bound serine protease CAP1/Prss8 showed a defect in the two systems, a disturbed stratum corneum lipid composition as well as functionally defective tight junctions [8], whereas mice deficient for the tight junction protein claudin-1 exhibited only a defect in the tight junctions [14]. This underlies the importance that both systems are needed and might work concurrently. Equally important is the natural moisturizing factor composed of amino acids and their derivatives, such as pyrrolidone carboxylic acid, together with lactate, sugars and urea and act as efficient humectants to maintain free water in the stratum corneum [15]. The natural moisturizing factor is derived from filaggrin, synthesized in the granular layer as profilaggrin, a large histidine-rich and heavily phosphorylated protein that functions to aggregate keratins. At the transition between the granular layer and the stratum

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corneum, filaggrin is subjected to further modifications through protein phosphatase PP2A and at least three different proteases, including profilaggrin proteinase 1, calpain and furin, resulting in filaggrin peptides of 27 kDa in mice. Proteolyzed into free amino acids, these are thought to provide high osmolarity necessary for the retention of water and maintenance of tissue flexibility. Not surprisingly, mouse models with defect in filaggrin gene (flaky tail, ft) [16] exhibit severe impaired epidermal barrier function. Additionally, animal models of caspase-14 [17], involucrin, envoplakin, periplakin [18], lipoxygenases [19] and of serine proteases discussed later in this review, display defective filaggrin processing and impaired epidermal permeability.

The major lipid classes in the stratum corneum are ceramides, free fatty acids and cholesterol. Ceramides are amide-linked fatty acids containing a long-chain amino alcohol called sphingoid base and account for 30 to 40% of stratum corneum lipids. Ceramides are generated by serine-palmitoyl transferase as rate-limiting enzyme and by hydrolysis of both glucosylceramide through beta-glucocerebrosidase and sphingomyelin through acid sphingomyelinase. The stratum corneum contains at least nine different free ceramides, covalently bound to cornified envelope proteins, e.g., involucrin [20, 21]. The epidermis contains free fatty acids as well as fatty acids bound in triglycerides, phospholipids, glycosylceramides and ceramides. Cholesterol is the third major lipid class in the stratum corneum. Although basal cells are capable of reabsorbing cholesterol from circulation, most cholesterol in the epidermis is synthesized in situ from acetate. Cholesterol levels are regulated by the membrane transporter ATP-binding cassette subgroup 1 member 12 transporter (ABCA12), responsible for cholesterol efflux, liver X receptor activators and peroxisome proliferator-activated receptors [12].

Tight junctions are cell-cell junctions connecting neighboring cells, controlling the paracellular pathway of molecules (barrier function) and separating the apical from the basolateral part of a cell membrane (fence function). In human epidermis, various tight junction proteins have been identified, including occludin, claudins 1, 4, and 7, JAM-1 (junctional adhesion molecule-1), ZO-1 (zonula occludens protein 1) and MUPP-1 (multi-PDZ protein-1) [22]. During epidermal re-generation, the synthesis of tight junction proteins precedes the formation of the stratum corneum [23]. Therefore, beside barrier and fence functions, tight junctions have been proposed to present a rescue system when epidermal barrier is perturbed, challenged or absent.

At birth, the epidermal barrier is normally formed consisting of the cornified envelope [24] that develops during the late stages of embryonic life [25]. Yet, it is known that a postnatal maturation of the skin exists as the skin surface pH is near neutral in humans [26-28] and rodents [29, 30] to reach more acidified levels after 10 days (human) [31] and in 7 days (rat) [32] and 2 days (mice) [33]. Thus, in human infants, the delay in acidification could explain the increased infantile risk for irritant/allergic contact dermatitis, infection, and cutaneous absorption of toxic chemicals [27]. Defects in acidification may lead to increased water loss [29] and delayed epidermal permeability barrier recovery [34] explained by the impairment of sphingomyelinase and beta-glucocerebrosidase [21, 35]. Acidification is defective in the sodium/hydrogen antiporter-1 knock-out mice [32, 34, 36] and in the phospholipase A_2-deficient mice [37]. Mice over-expressing the Ca^{2+} receptor showed an accelerated barrier function with a strong hyperkeratosis with increase of inositol-3-phosphate (IP3) [38]. Thus beyond the genes that are clearly known to be crucial for the prenatal barrier formation, there are genes/processes that are rather important for the postnatal maturation of the skin barrier function (e.g. ENaC and its regulator the membrane-bound serine protease CAP1/Prss8).

Barrier Dysfunctions in Skin Diseases

Several skin diseases are known to show defects in epidermal permeability barrier function. The most classical ones include atopic dermatitis and psoriasis, although the majority of the studies on the pathogenesis of atopic dermatitis and psoriasis concentrate on the primary role of the immune system abnormalities [39, 40]. However, it is still not clear whether permeability barrier defect is a cause or consequence of inflammation. Atopic dermatitis is characterized by chronic, pruritic, inflammatory dermatosis and the impaired barrier function is most likely caused by increased epidermal proliferation and disturbed differentiation, including changes in lipid composition [41, 42]. Loss-of-function genetic variants in the gene encoding filaggrin have been shown to be strong predisposing factors for atopic dermatitis [43]. In psoriasis, the level of transepidermal water loss is directly related to the clinical severity of the lesion [44]. The mode of inheritance of psoriasis is complex; classic genome-wide linkage analysis has identified at least nine chromosomal loci with statistically significant linkage to psoriasis, comprehending genes involved in immune functions and epidermal differentiation [45].

A disturbed skin barrier is relevant for the pathogenesis of ichthyoses, which involve several generalized genetic skin disorders. All types of ichthyosis exhibit dry, thickened, scaly skin. The severity of symptoms can greatly vary, from mild types as ichthyosis vulgaris, up to life-threatening conditions such as harlequin-type ichthyosis. Aberrant filaggrin expression has been found in ichthyosis vulgaris [46]. Several ichthyoses are associated with inherited disorders of lipid metabolism. Mutations in transglutaminase-1 and deficiency in the enzyme steroid sulfatase have been found defective in lamellar ichthyosis and recessive X-linked lamellar ichthyosis, respectively [47, 48]. Lamellar ichthyosis type 2 (LI2) and the harlequin fetus type of congenital ichthyosis are linked to mutations in ATP-binding cassette subgroup 1 member 12 transporter (ABCA12), a membrane transporter responsible for cholesterol efflux [49, 50].

Epithelial barrier function has emerged as a critical factor in the development and progression of allergic diseases. Importantly, patients with atopic dermatitis manifest compromised skin barrier associated with increased allergen sensitization that can augment the atopic inflammatory response [51]. This suggests a more global mechanism by which allergen sensitization could contribute to skin inflammation. Even though the epithelium was initially considered to function just as a physical barrier, it is now seen as a central player in sensitization processes. Allergens often interfere directly or indirectly with the innate immune

functions of airway epithelial cells and dendritic cells of the skin [52].

ENaC and its Role in the Epidermal Barrier Function

The non-voltage gated, highly amiloride-sensitive epithelial sodium channel (ENaC) has been extensively studied as a key regulator for sodium homeostasis [53, 54]. ENaC belongs to a gene family called ENaC/degenerin (DEG) encoding the ENaC α-, β-, γ- (rodents and human) and δ-subunits (humans) [55]. The δ-ENaC isoform can substitute the α-ENaC subunit, but is often found in organs distinct from the classical sodium absorbing epithelia [56]. Each subunit has two hydrophobic domains, corresponding to two transmembrane domains, TM1 and TM2, a large extracellular loop making more than one-half of the channel protein representing a structural feature unique to the ENaC/DEG family members with short cytoplasmic N- and C-termini [57, 58]. Electrophysiological characteristics of ENaC include a high sensitivity to the potassium-sparing diuretic amiloride (K_i 0.1 μM), a low conductance of about 5 pS [58], long opened and closed times (0.5-5 s), and a high selectivity for sodium over potassium (> 100:1) [55]. ENaC activity at the organ level seems regulated by the presence or absence of the channel at the membrane, and steroid hormones, like aldosterone or glucocorticoids, and membrane-bound serine protease (see for review [53]).

ENaC subunits are also expressed in the tongue where have been shown to be implicated in salt tasting [59, 60], in the inner ear [61] or the retina function [62]. The presence of an amiloride-sensitive current in the skin of amphibians is known since decades now [63-65]. The movements of ions and their distribution can lead to the formation of an electrical potential. The presence of a negative potential difference has been shown in skin [66]. This potential was impaired if treated with various channel blockers like ouabain (Na/K ATPase blocker) or L-type calcium channel blocker like verapamil, and nifedipin [67]. Thus, ENaC activity may play a role in the regulation of diverse cellular processes in the skin like e.g. barrier function, galvanotaxis and wound healing [68].

Different mouse lines have been established in which the ENaC activity has been altered, and genes encoding for *Scnn1a* (α-ENaC) [69], *Scnn1b* (β-ENaC) [70] and *Scnn1g* (γ-ENaC) [71] have been inactivated. The phenotypes observed in these mice demonstrate that each subunit is essential for survival and for regulation of sodium transport [72-75]. Inactivation of the β- and γ-ENaC subunit led to reduced ENaC activity, whereas gene targeting of the α-ENaC subunit resulted in completely abolished ENaC activity [53]. The presence of all three subunits of ENaC has been demonstrated in the epidermis of mouse, human and rat but also in hair follicles and in sweat glands (humans) [76, 77]. Moreover the expression of ENaC seems to increase with differentiation of the keratinocytes [78]. Patch clamp recordings on human keratinocytes reveal a sodium channel conductance that is blocked by benzamil with similar affinity and voltage dependence of the amiloride block as previously described for ENaC [78]. Further evidence that ENaC-mediated Na^+ transport may be implicated in keratinocyte and epidermal differentiation comes from the previous analysis of newborn α-ENaC knock-out mice which exhibit epidermal thickening and premature lipid secretion in the upper epidermis, suggesting that ENaC-mediated sodium ion fluxes control selective aspects of keratinocyte differentiation [79]. Gene inactivation of the alpha subunit of the highly amiloride-sensitive epithelial sodium channel (α-ENaC, *Scnn1a*) leads to distinct perinatal effects on epidermal development and homeostasis, which culminates in a barrier defect within the first 24h, characterized by a loss of body weight (by 6% in 6 hours) and an increased transepidermal water loss, which is accompanied by a higher skin surface pH in one day-old pups [33]. While early and late differentiation markers, as well as tight junction protein distribution and function seem not affected, deficiency of α-ENaC severely disturbs the stratum corneum lipid composition with decreased ceramide and cholesterol levels, and increased pro-barrier lipids glucosylceramide, sphingomyelin and cholesterol sulfate, while covalently-bound ceramide and ω-hydroxylated fatty acid are drastically reduced. Ultra-structural analysis revealed morphological changes in the formation of intercellular lamellar lipids and the lamellar body secretion. Extracellular formation of the lamellar lipids proved to be abnormal in the knock-outs. In conclusion, ENaC-deficiency results in progressive dehydration and consequently weight loss due to severe impairment of lipid formation and secretion (Table **1**). Our data further demonstrate that ENaC expression is required for the postnatal maintenance of the epidermal barrier function, but not for its generation.

Transmembrane ionic fluxes are controlling keratinocyte differentiation and the synthesis of cornified envelope and other differentiation-specific proteins, conversion of profilaggrin to filaggrin and secretion of stratum corneum lipid precursors [80]. Further evidence that ions may be important regulators in these processes is suggested by the presence of a calcium gradient within the epidermis, with higher quantities of Ca^{2+} in the upper than in the lower epidermis [81]. Moreover, Na^+ influx also modulates Ca^{2+}-induced keratinocyte differentiation. Thus, application of amiloride, the known inhibitor of ENaC, blocks Ca^{2+}-induced differentiation in keratinocytes. Only recently, mice deficient for ion/water channels and transporters are analyzed for their skin phenotype. Keratinocytes from mice deficient for the Ca^{2+}-sensing receptor did no longer respond to extracellular Ca^{2+}, and the mice exhibit disordered differentiation [82]. Mice deficient for the sodium channel ENaC show severe dehydration and mice lacking the NHE1 exchanger exhibit an impaired stratum corneum acidification [29, 34]. The water transporting protein aquaporin-3 functions as a glycerol transporter in mammalian skin and mice deficient in AQP3 exhibit dry skin with reduced stratum corneum hydration, decreased elasticity and impaired biosynthesis [83, 84]. In human, mutations in the different subunits of the channel are the cause of human hereditary diseases [85]. Hereditary mutations of ENaC subunits have been described leading to disturbed sodium homeostasis. First, in the Liddle's syndrome, the C-terminal tail of β or γ is mutated in the proline-rich domain, preventing ubiquitination by Nedd4-2 then causing hypertension and hypokaliemia due to the higher availability of ENaC at the membrane [53, 54]. Second, the pseudohypoaldosteronism type I (PHA-1), characterized by a decreased ENaC activity resulting in salt wasting and

Table 1. Comparative Phenotypes of ENaC, CAP1/Prss8 and CAP3/Tmprss14 Mouse Models

	Complete Alpha ENaC Knock-Out	Skin-Specific CAP1/Prss8 Knock-Out	Complete CAP3/Tmprss14 Knock-Out	Transgenic CAP3/Tmprss14 (K5 Promoter)
Epidermal Features	hyperplasia	hyperkeratosis	hyperkeratosis	hyperplasia, tumor formations
Barrier Function	impaired	impaired	impaired	n.d.
Differentiation Markers	K6 over-expression	filaggrin processing defect	filaggrin processing defect	K6 over-expression
Tight Junctions	functional	Severely impaired	n.d.	n.d.
Lipid Composition	severely affected	affected	affected	n.d.
References	[79, 33]	[8]	[7, 109]	[117]

n.d. = not determined.

hypotension, is due to mutations leading to expression of inefficient forms of the three subunits [75]. In PHA-1 patients carrying ENaC-mutations, macroscopic skin lesions like dermatitis have been described, although the skin phenotype was not further analyzed. Thus, it is still unknown whether dysfunction of ENaC channels in skin contributes to and/or is indeed causative for defined skin diseases [68].

ENaC Regulators in the Epidermal Permeability Barrier Function

To detect proteins involved in ENaC regulation, Vallet and colleagues screened a *Xenopus* A6 cell complementary DNA library allowing the isolation of a serine protease whose co-expression with ENaC induced a 3-fold increase in the ENaC sodium current [86]. Consequently, this serine protease was termed *Xenopus* channel-activating protease-1 (xCAP1), encoded by *Prss8* gene and ortologous to human prostasin, that presents a glycosyl-phosphatidyl-inositol (GPI)-anchored protein [86]. Two years thereafter, the mouse counterpart was cloned in a cortical collecting duct cell line derived from mouse kidney and identified as mouse CAP1 (mCAP1) [87]. CAP1/Prss8 appeared to be co-expressed in epithelial tissues with ENaC such as kidney, lung, colon, skin, ovary and salivary glands [86, 87]. CAP1/Prss8 is produced as zymogen and *in vitro* experiments indicated its inability to auto-activate, suggesting that its proteolytic activity is regulated by an upstream protease. Accordingly, no evidence of CAP1/Prss8 intramolecular cleavage was seen *in vitro* experiments in *Xenopus* oocytes [88, 89]. Hypertension and elevated levels of urinary CAP1/Prss8 have been reported in rats transiently over-expressing CAP1/Prss8 [90]. Increased urinary CAP1/Prss8 excretion also occurs in patients with hypertension from primary hyperaldosteronism and is stimulated by saline infusion or mineralcorticoids [91, 92]. CAP1/Prss8 is highly expressed in cystic fibrosis airways and it is a strong basal activator of ENaC in cystic fibrosis airway epithelial cells [93, 94]. These observations predict that CAP1/Prss8 has a critical role in regulating epithelial sodium transport in normal and pathological conditions. On the other hand, CAP1/Prss8 is down-regulated in hormone refractory prostate cancers, gastric and breast cancer [95-98] and it has been found over-expressed in epithelial ovarian cancer [99] suggesting an additional role of CAP1/Prss8 in tumor invasion. Carattino *et al*. claim that proteolytic processing of ENaC gamma subunit by CAP1/Prss8 has a dominant role in ENaC activation [100]. In contrast, Andreasen and colleagues have shown that catalytically inactive CAP1/Prss8 is still able to fully activate ENaC [89]. To ascertain the role of mCAP1 in the different tissues, an allelic series of mutations at the mouse *Prss8* gene locus were generated to delete its vital region (exon 3, 4 and 5) in a temporally and/or tissue-specific manner [101]. These animals allowed studying the consequences of CAP1/Prss8 deficiency in epidermal function by crossing them with keratin-14 Cre-recombinase transgenic mice [102]. Mice lacking CAP1/Prss8 in skin died 60 hours after birth [8]. A member of the epidermal proteins, the lipid constituent of the cornified envelope and the tight junction functionality were found defective in skin-specific CAP1/Prss8 knock-out mice, indicating that each component of the epidermal permeability barrier suffered from the lack of CAP1/Prss8 in the skin. The epidermis lacking CAP1/Prss8 presented an aberrant pattern of profilaggrin-derived proteolytic products, with nearly complete loss of filaggrin monomers. Corneocytes morphogenesis was perturbed and the level of pro-barrier and covalently bound lipids was altered in CAP1/Prss8-deficient epidermis. No expression of occludin was found in the CAP1/Prss8-deficient skin and the tight junction functionality, at least against molecules of about 600 Dalton, was severely affected. These defects can be causative of a more reddish and wrinkled skin evident few hours after birth, a hyperkeratotic stratum corneum and incompletely matured and reduced in number hair follicles and a severe impairment of both inward and outward barrier functions of the epidermis, which most likely lead to early postnatal death in mice lacking CAP1/Prss8 in the skin [8].

The ENaC-mediated sodium current, measured in the mouse cortical collecting duct cell line, from which mCAP1 was identified, appeared to be only 50% sensitive to the serine protease inhibitor aprotinin [87]. This suggested that ENaC activation depends on more than one serine protease with different sensitivity to aprotinin, and lead to the discovery of two additional membrane-bound serine proteases found to increase 6 to 10-fold ENaC currents [87]. Accordingly, these serine proteases were called mCAP2 and mCAP3. mCAP2 is a type II-oriented membrane-bound serine protease whom protease domain shares homology with xCAP1 (45%), mCAP1 (43%), and 80% with the human orthologue hTMPRSS4 [103]. CAP2/Tmprss4 requires catalytic activity to activate ENaC [89, 104] and it has been reported that CAP2 cleaves all three ENaC subunits, both with and without associated stimulation [104]. CAP2/Tmprss4 appeared to be highly expressed in lung

cancer tissues compared with normal tissues and was found to be broadly expressed in a variety of human cancer cell lines, resulting in an important mediator of invasion, metastasis, migration and adhesion [105]. However, the physiological functions of CAP2/Tmprss4 remain to be ascertained in the whole organism by, e.g., conditional gene targeting models.

mCAP3 is identical to the mouse epithin/Tmprss14 [106]. As CAP2/Tmprss4, CAP3/Tmprss14 is a type II serine protease which shares 47% homology with the mouse trypsinogen, 49% with xCAP1, 40% with mCAP2 and 83% with the human orthologue hMT-SP1 [107] also known as matriptase [108]. CAP3/Tmprss14 has an essential physiological role in profilaggrin processing, corneocyte maturation, and lipid matrix formation associated with terminal differentiation of the oral epithelium and the epidermis, and is also critical for hair follicle growth. In mice, targeted ablation of the serine protease CAP3/Tmprss14 leads to postnatal lethality within 48 hours, striking malformation of the stratum corneum, seriously compromised epidermal barrier function and loss of proteolytically processed filaggrin [7]. Interestingly, complete CAP3/Tmprss14 and skin specific CAP1/Prss8 knock-out mice exhibit similar phenotypes ([7, 8, 109] and Table **1**) and it has been proposed that CAP1/Prss8 presents the downstream substrate target of CAP3/Tmprss14 [110]. However, mice constitutively lacking CAP1/Prss8 are embryonic lethal (R.-P. Charles and E. Hummler manuscript in preparation), whereas mice deficient for matriptase/MT-SP1 go through the embryonic development and die after birth indicating that these serine protease might exhibit tissue/organ-specific roles independent from each other [7]. Despite the known structural (e.g. epidermal thickness) and functional (e.g. sweet gland) differences between mouse and human skin, the human skin disease the autosomal recessive ichthyosis with hypotrichosis, found to be caused by mutation in CAP3/Tmprss14 gene [111], shows close similarity with the CAP3/Tmprss14 deficiency in mouse skin [112]. CAP3/Tmprss14 is an efficient activator of pro-urokinase plasminogen activator (pro-uPA), hepatocyte growth factor/scatter factor (HGF/SF), and PAR2 *in vitro*, and CAP3/Tmprss14 could have pleiotropic functions in the activation of proteolytic cascades, growth factors, and G protein coupled receptors [113]. CAP3/Tmprss14 is universally co-expressed with its cognate inhibitor, hepatocyte growth factor activator inhibitor-1 (HAI-1) encoded by the serine protease inhibitor Kunitz type 1 (Spint1), in both normal and malignant tissues [114-116]. Modest CAP3/Tmprss14 orthotopic over-expression in the skin of transgenic mice caused spontaneous squamous cell carcinoma and dramatically potentiated carcinogen-induced tumor formation (Table **1**). Increasing epidermal HAI-1/Spint1 expression completely negated CAP3/Tmprss14 oncogenic effects [117]. HAI-1/Spint1 is a membrane-bound serine proteases inhibitor expressed in various epithelial tissues, such as the gastrointestinal tract, breast, prostate, lung and skin [118]. Several *in vitro* studies showed that HAI-1/Spint1 potently inhibits trypsin-like serine proteases such as hepatocyte growth factor activator, CAP3/Tmprss14, hepsin/Tmprss1, and CAP1/Prss8 [119-123]. Homozygous deletion of HAI-1/Spint1 in mice resulted in embryonic lethality attributable to impaired placental development [124]. High chimeric HAI-1/Spint1 knock-out newborns showed growth retardation and died by 16 days. These mice developed scaly skin because of hyperkeratinization, reminiscent of ichthyosis, and abnormal hair shafts that showed loss of regular cuticular septation. The interfollicular epidermis showed acanthosis and immunoblot analysis revealed altered proteolytic processing of profilaggrin in HAI-1/Spint1 deleted skin with impaired generation of filaggrin monomers [125] indicating an important role of this serine protease inhibitor for skin patho-physiology.

Defective ENaC processing and function has been observed in tissue kallikrein-deficient mice [126]. Tissue kallikreins are extracellular serine proteases secreted by keratinocytes into upper stratum granulosum and stratum corneum interstices of the epidermis and are also localized in appendages such as hair follicles and sweat glands [127-129]. The proteolytic activity of kallikreins is regulated in several ways including zymogen activation, endogenous inhibitors, such as serpins, and *via* internal (auto) cleavage leading to inactivation. Until recently, kallikrein proteolytic activity in the skin was exclusively ascribed to kallikrein-5 and kallikrein-7 (also known as stratum corneum tryptic enzyme, SCTE, and stratum corneum chymotryptic enzyme, SCCE, respectively) even though other kallikreins are expressed in the skin and its associated appendages [130] that show involvement in skin barrier functions [131]. It has been found increased epidermal expression of SCCE in psoriasis and in atopic dermatitis patients. Transgenic mice expressing SCCE in suprabasal epidermal keratinocytes develop pathologic skin changes with increased epidermal thickness, hyperkeratosis, dermal inflammation, and severe pruritus [132]. The activities of SCTE and SCCE are increased in SPINK5 (serine protease inhibitor Kazal-type 5, encoding the putative multi-domain serine protease inhibitor LEKTI) deficient mice, and these mice mimic the epidermal dysfunctions in human disease Netherton syndrome leading to a disruption in skin barrier function [133].

It has been recently found that the purified serine protease plasmin from nephrotic urine activates the epithelial sodium channel ENaC [134] and that plasmin activates ENaC in association with inducing cleavage of the gamma-subunit [135]. Plasminogen is a zymogen that is converted to the active enzyme plasmin by tissue plasminogen activator, urokinase plasminogen activator and factor XII [136]. However plasmin has been proposed as one of the main extracellular and cell surface proteases involved in wound healing [137] and its activity has been found significantly increased after disruption of the epidermal barrier [138] indicating additional ENaC regulators implicated in skin barrier function.

CONCLUSIONS AND PERSPECTIVES

In conclusion, our recent results unveil the physiological consequences in skin of the highly amiloride-sensitive epithelial sodium channel deficiency that leads to distinct phenotypes in the prenatal versus the postnatal period. We propose that ENaC plays an important role in the maintenance of the postnatal epidermal barrier function. The fine tuning between activation and inhibition of serine proteases, which present regulators of ENaC, appear to play a key role in epidermal homeostasis [139]. These serine

proteases are known to be deregulated during tissue damage and thereby contribute to injury, repair, and cell survival responses, although the *in vivo* molecular targets of these membrane-bound serine proteases are largely unknown. The cellular actions of these serine proteases may be mediated through activation of G-protein-coupled receptors like the protease-activated receptors (PARs), and regulated by serine protease inhibitors and associated proteins. It will be important to identify the remaining members of these cascades, as well as the signals that trigger their activation. This delicate balance can be disturbed by genetic defects or exogenous influences and has been shown as the underlying and promoting cause for a large number of different diseases. Abnormalities in skin-specific knock-outs of these serine proteases and its comparison with ENaC-deficient mutant mice will give new insights into molecular mechanisms of the epidermal permeability barrier function, its implication in genetic disorders, and the identification of putative target proteins. Recently, we could demonstrate *in vivo* a crucial role of CAP1/Prss8 in the regulation of ENaC in the lung being implicated in ENaC-mediated alveolar sodium and water transport and fluid balance [140]. Thus, a better understanding of these interactions, ascertained from animal and human studies will help to develop novel means of prevention and treatment.

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