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Review

Cell Volume Regulation in the Epidermis

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Abstract
In order to cope with external stressors such as changes in humidity and temperature or irritating substances, the epidermis as the outermost skin layer forms a continuously renewing and ideally intact protective barrier. Under certain circumstances, this barrier can be impaired and epidermal cells have to counteract cell swelling or shrinkage induced by osmotic stress via regulatory volume decrease (RVD) or increase (RVI). Here, we will review the current knowledge regarding the molecular machinery underlying RVD and RVI in the epidermis. Furthermore, we will discuss the current understanding how cell volume changes and its regulators are associated with epidermal renewal and barrier formation.

The epidermal barrier - our protective cover

The epidermis, as the outmost layer of the skin not only plays an important role in sensory perception and temperature regulation, but also protects the body from dehydration and external insults such as pathogens, UV radiation and fluctuations in humidity. To maintain this protective function, the epidermis is constantly regenerated from keratinocytes of the basal layer. These cells are subjected to a strict control between proliferation and asymmetric cell division in the basal layer and differentiation and migration across the epidermis. During this process, cells undergo transcriptional and cell shape changes to form a stratified epithelium consisting of the stratum spinosum, stratum granulosum and the stratum corneum (Fig. 1).

Each layer is characterized by the expression of specific structure proteins such as keratins (KRT 1 and 10), involucrin (IVL), and transglutaminase-1 (TGM-1) in the stratum spinosum, while the late differentiation markers loricrin (LOR) and filaggrin (FLG) are expressed in the stratum granulosum (Fig. 1B) [1]. This differentiation process is mainly regulated by a calcium (Ca^{2+}) gradient with lowest Ca^{2+} levels in the basal layers and the highest peak in the stratum granulosum [2]. The gradient is formed by Ca^{2+} ion release from the endoplasmic reticulum (ER) and Ca^{2+} influx from the extracellular space and not only regulates keratinocyte differentiation but is altogether crucial for the formation of the skin barrier as well as epidermal homeostasis [3, 4].

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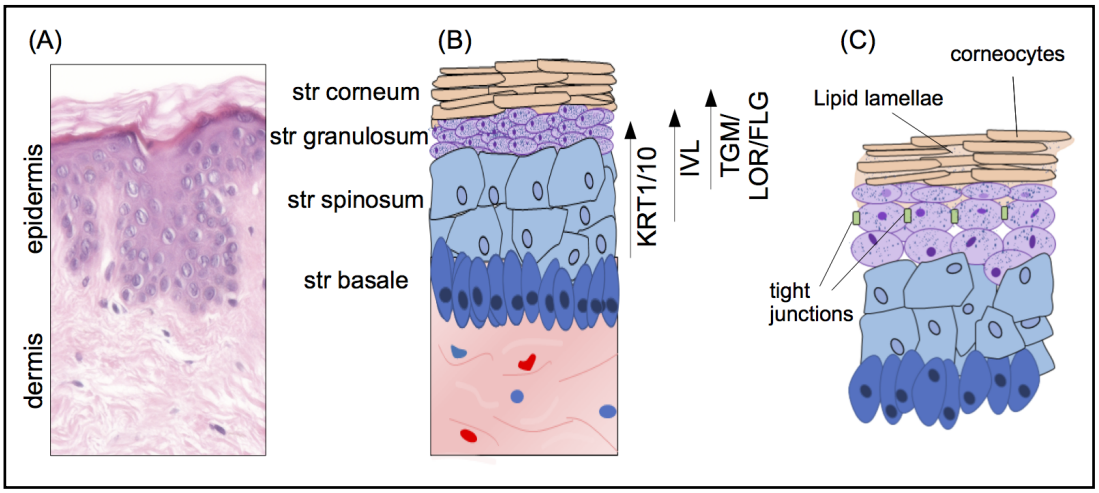

Fig. 1. Structure of the mammalian skin. Histological section (HE staining) (A) and schematic illustration (B, C) of human skin. Human skin consists of two main components: the dermis consisting of fibroblasts embedded in a fibrous network and the outermost epidermis that is stratified into four layers: stratum corneum, stratum granulosum, stratum spinosum and stratum basale (A, B). Each layer is characterized by the expression of specific differentiation marker proteins such as KRT1/10 (keratin 1/10), IVL (involucrin), TGM (transglutaminase), LOR (loricrin) and FLG (filaggrin) (B). The protective epidermal barrier is formed by corneocytes embedded in lipid lamellae and tight junctions (TJ) that seal cells of the stratum granulosum (C).

Terminally differentiated keratinocytes in the stratum corneum are called corneocytes that lack nuclei as well as other organelles. They are filled with bundled keratins, that together with structural filaggrin, involucrin, envoplactin, periplactin and several other proteins, which are cross-linked by TGM-1 form a rigid cornified envelope [5]. FLG is especially important as it is degraded into its component amino acids, which are highly hygroscopic and serve as natural moisturizing factors (NMFs). These NMFs together with a network of lamellar arranged lipids fill the extracellular area of the stratum corneum [6]. Thus, the stratum corneum is often described as a 'bricks and mortar' structure in which corneocytes being the bricks while intercellular lipid lamellae serve as the mortar [7]. In addition, tight junctions (TJs) in the stratum granulosum formed by occludin and claudin proteins contribute to the formation of a tight epidermal barrier [8, 9]. This barrier is further supported by other cell-cell junctions in lower cell layers such as desmosomes, that mainly provide mechanical strength and cadherin-based adherens junctions which regulate different aspects of epidermal physiology [10].

Disturbances in this tightly regulated differentiation and maturation process are associated with pathological dermal conditions such as psoriasis, atopic dermatitis or ichthyosis.

Thus, healthy skin forms a tight barrier to protect from external stress. However, under certain circumstances, such as injury, exposure to radiation or inflammation, the barrier can be impaired and keratinocytes are exposed to environmental influences. This not only leads to alterations in water content, but also induces osmotic stress leading to changes in cell volume. Keratinocytes can compensate this stress by regulatory processes called regulatory volume increase or decrease (RVI and RVD, respectively), which are fundamental mechanisms that guarantee cell volume homeostasis. However, cell volume regulation is not only a compensatory response, but also an integral part of many physiological processes contributing to proper tissue function [11].

In this review, we will discuss the current knowledge how cell volume is controlled in the skin and how cell volume regulation serves as a regulating mechanism during epidermal maturation, stratification and maintenance of the epidermal barrier. In addition, we will highlight the first few studies that link defects in cell volume regulation with pathological skin conditions.

Mechanisms of cell volume regulation in the epidermis – response to osmotic stress

Under the aforementioned circumstances, the epidermal barrier can become compromised and epidermal cells can be exposed to osmotic stress. Extracellular water can evaporate thus creating a hyperosmotic milieu. In contrast, a hypotonic milieu emerges if damaged skin is exposed to fresh water. Both conditions result in cell volume changes by water following the osmotic gradient across the membrane, leading to either cell shrinkage or cell swelling. Virtually all biological membranes are reasonably water permeable by the expression of aquaporins (AQPs) [12], which increase the osmotic water permeability coefficient by 5 to 50-fold depending on the cell type [13, 14]. Several AQPs were described to be selectively expressed in various cell types in the human skin, with AQP3 being the most abundant and playing a key role in skin hydration [15, 16]. Interestingly, AQP3 was found to be upregulated by osmotic stress [17]. Cell-intrinsic regulatory processes known as RVI or RVD actively counteract the stress induced changes in cell volume [18]. The mechanisms responsible for RVD and RVI may differ among different cell types and species, but in general both processes involve the activation of ion channels and transporters allowing the movement of ions and osmolytes across the cell membrane [11, 18]. Consequently, sodium (Na^+), potassium (K^+) and chloride (Cl^-) ions as well as organic osmolytes are either released from (in the case of RVD) or taken up by the cell (in the case of RVI). This creates an osmotic gradient, which in turn forces water influx or efflux leading to cell swelling or cell shrinkage, respectively, until the initial cell volume is restored (Fig. 2) [18].

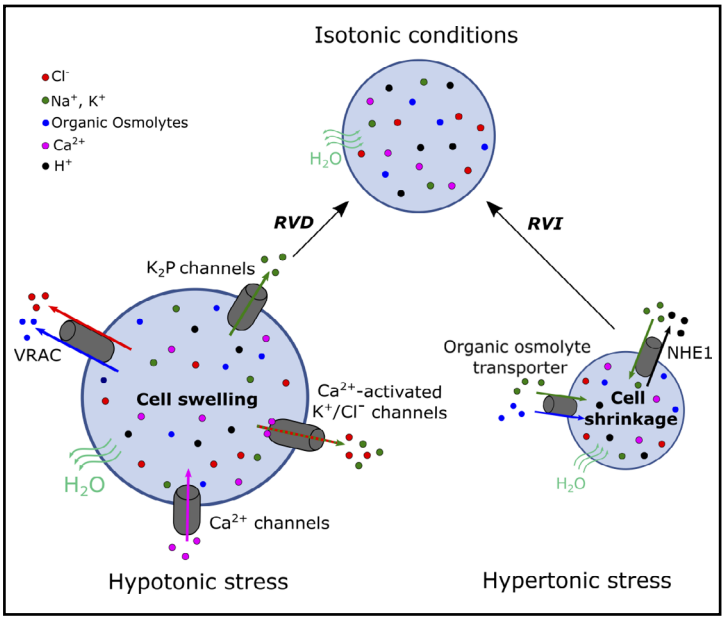

Fig. 2. Channels and transporters involved in cell volume regulation in keratinocytes (modified from [18]). Hypotonic stress leads to cell swelling by an influx of water, which is then counteracted by regulatory volume decrease (RVD) (left). In keratinocytes, RVD is mediated by release of K^+, Cl^- and organic osmolytes through volume-regulated anion channels (VRACs) such as LRRC8, two-pore-domain K^+ channels (K2P) and Ca^{2+}-activated K^+ or Cl^- channels (e.g. SK4 and BK). The efflux of osmolytes during RVD is supported by an influx of Ca^{2+} e.g. via TRPV4. Release of these osmolytes from the cytoplasm into the extracellular space alters the osmotic gradient and is therefore accompanied by water efflux. On the other hand, hypertonic stress leads to cell shrinkage due to water loss, which is counteracted by regulatory volume increase (RVI) (right). In keratinocytes, organic osmolyte transporters (like TAUT and BGT-1) can counteract this water movement by mediating the uptake of organic osmolytes. Additionally, activation of Na^+/H^+ exchanger 1 (NHE1) was described in keratinocytes and can mediate influx of Na^+. This increase in intracellular osmolyte concentrations mediates an influx of water, which restores the initial cell volume.

Regulatory volume increase (RVI) in the skin

A hypertonic environment occurs frequently in the skin. Disruption of the epidermal barrier leads to evaporation of water, thus causing a hyperosmotic milieu. Cells respond by water efflux leading to cell shrinkage, which in turn is compensated by RVI. So far, two classes of transporters have been identified in keratinocytes that contribute to this volume increase: transporters of organic osmolytes and NHE1 (Na^+/H^+ exchanger 1) ion transporters (Fig. 2). Other ion transporters, that are involved in RVI in other tissues, like NKCC ($Na^+/K^+/2Cl^-$ cotransporter), have either not been analysed or were not found in epidermal cells [19].

Transport of organic osmolytes via their transporters TAUT, BGT-1, HMIT and SMIT. Besides ions, organic osmolytes such as betaine, myo-inositol or taurine are transported over the cell membrane to counteract cell volume changes (Fig. 2). These molecules are electroneutral and can therefore be transported without disruption of membrane potential or ionic strength [20]. The taurine transporter TAUT (also known as SLC6A6) and the betaine transporter BGT-1 (SLC6A12) are highly expressed in granular keratinocytes [21], while SMIT (SLC5A3) and HMIT (SLC2A13), the transporters for myo-inositol, mainly localize to basal layers [22]. Intracellular accumulation of organic osmolytes involves uptake and intracellular synthesis by metabolic reactions [23]. Most organic omolytes are synthesized in the liver and/or the kidney and released into the blood, e.g. taurine is synthesized from cysteine in the liver, while betaine is synthesized from choline both in liver and kidney [24].

Hyperosmotic stress enhances the expression of these transporters and leads to an increase in intracellular levels of organic osmolytes [21]. Consequently, osmolytes are able to rescue detrimental effects on proliferation and cell death induced by hypertonic stress in keratinocytes [25].

Mechanistically organic osmolytes seem to contribute to epidermal homeostasis by different modes of action. First, transport of organic osmolytes significantly mediates RVI as aged or UV radiated skin displays reduced levels of TAUT and SMIT, which is accompanied by deterioration in their ability to regulate cell volume *in vivo* and *in vitro*. Consequently, aged keratinocytes comprise a smaller area than young cells within the epidermis and show reduced rates of RVI upon a hypertonic stimulus. Treatment with organic osmolytes could rescue these cell volume responses [26]. Secondly, the transport of organic osmolytes has a role beyond osmo-regulation as it affects the structure and function of tight junctions, which are an essential component of the epidermal barrier (Fig. 1) [22]. Thus, cell volume homeostasis of the skin is negatively influenced by ageing to which keratinocytes respond via increased uptake of organic osmolytes. Interestingly, early observations in psoriasis, a chronic inflammatory skin disease characterized by red, scaly lesions, showed a negative impact of oral taurine uptake while elimination of taurine led to remission of psoriatic skin lesions [27, 28]. However, a series of following studies in the 80s did not confirm these results so far [29].

The Na^+/H^+ exchanger NHE1. Another potential molecule involved both in epidermal cell volume regulation and in epidermal homeostasis is NHE1. The Na^+/H^+ exchangers of the NHE (SLC9A) family consist of nine different family members. The widely expressed isoform NHE1 is expressed in epidermal cells such as keratinocytes and melanocytes [30] and shows highest expression in the outer cell layers [31]. So far, no involvement of NHE1 in epidermal cell volume regulation was found. Interestingly, NHE1 is also permeable to and activated by Ca^{2+}, giving a potential link to epidermal differentiation, supported by findings that blocking NHE1 inhibits Ca^{2+} induced differentiation in keratinocytes [32]. In addition, NHE1 preferentially acidifies extracellular domains at the stratum granulosum/stratum corneum junction, which is important for lipid processing, thus contributing to epidermal barrier homeostasis [31, 33]. The important role for NHE1 for stratum corneum function is further underlined by findings that NHE1 was upregulated, in a FLG knockout ex vivo skin model, in order to maintain the skin pH and to compensate the FLG deficiency [34].

Regulatory volume decrease (RVD) in the skin

A hypo-osmotic extracellular milieu leads to water influx into cells resulting in cell swelling. Most cells compensate this by RVD, which involves efflux of K^+ and Cl^-, as well as organic osmolytes, followed by water flow from the cytoplasm into the extracellular space and cell shrinkage (Fig. 2). First experiments in the 1990s using electronic cell sizing showed, that confluent keratinocytes increased their cell volume following exposure to hypotonic medium but are not able to return to their normal cell size within half an hour. This led to the hypothesis, that keratinocytes are not able to undergo RVD [35]. However, current research using flow cytometry or volume sensitive imaging techniques indicates that keratinocytes are able to undergo RVD and restore their initial cell volume [36-38]. The actual reason for the discrepancy between the early and later studies remains unclear but may be due to the different experimental setups and measurement technologies.

Several ion channels and transporters involved in RVD (Fig. 2) are expressed in keratinocytes and will be discussed in this section. While in other organs K^+/Cl^- cotransporters have been found, only independent K^+ and Cl^- channels have been described in the skin. These include two-pore-domain K^+ (K_2P) and Ca^{2+} activated Cl^- and K^+ channels. In addition, Ca^{2+} currents via TRPV4 were described to be an important part of RVD in keratinocytes. Finally, VRACs (volume regulated anion channels) such as LRRC8 play a crucial role during hypotonic stress response.

Ca^{2+} activated Cl^- and K^+ channels. In many cells and also in keratinocytes, swelling is associated with an increase in intracellular Ca^{2+}. This in turn can induce Ca^{2+} activated Cl^- and K^+ channels [18]. Of the K^+ channels known to be involved in RVD in general (see [18]) the two-pore domain K_2P channels [39] and the two Ca^{2+} activated K^+ channels BK and SK4 have been shown to be expressed in keratinocytes [40, 41], but their role in RVD has not been investigated. SK4 is downregulated in differentiating keratinocytes, suggesting that SK4 mediated hyperpolarization in proliferating basal keratinocytes could be a critical determinant for their further biological fate [40]. In addition, overexpression of SK4 in murine epidermis induced hyperplasia and hyperkeratosis resembling an eczematous dermatitis indicating an involvement in proper regulation of epidermal regeneration [42]. Several publications also show Ca^{2+} activated Cl^- currents as well as faster RVD in response to increasing Ca^{2+} concentrations in keratinocytes [36, 40], but the molecular identity of the involved channels as well as their involvement in RVD is not clear.

TRPV4 and other Ca^{2+} channels. Epidermal RVD was proposed to involve an intracellular increase of Ca^{2+} [37, 38, 43, 44]. The first candidate channel suggested to be involved in RVD in keratinocytes was the cation channel TRPV4 (transient receptor potential vanilloid 4) that increases its conductance for Ca^{2+} after a hypotonic stimulus [37]. Interestingly, TRPV4 also works as a volume-sensor suggesting that osmostress also acts as mechanical stress by changing cell tension [45]. Moreover, it was speculated that TRPV4 or other ion channels may functionally interact with actin filaments to sense hypotonicity and mediate RVD, however, direct evidence for this hypothesis is still missing [43, 46]. TRPV4 contributes to epidermal barrier homeostasis by upregulation of the tight junction proteins occludin and claudin-4 and promotes intercellular barrier integrity [47, 48], while TRPV4 has a diverse impact on barrier integrity in other tissues. Thus, epidermal cells are able to respond to hypo-osmotic challenges not only by RVD, but also by reinforcing the epidermal barrier to prevent further osmotic assault. An alternative Ca^{2+} influx mechanism involves ATP, which is released due to mechanical stress triggered by a hypotonic milieu. Here ATP can function as an autocrine messenger to induce Ca^{2+} release from ER stores as well as store-operated Ca^{2+} entry [49].

Volume regulated anion channels (VRACs). Besides Ca^{2+}-activated Cl^- currents (see *Ca^{2+} activated Cl^- and K^+ channels*), also volume-sensitive Cl^- currents have been well described in keratinocytes [35, 36, 44]. Interestingly, both type of Cl^- currents are inversely regulated [36]. Although the swelling activated anion channels (VRACs) were first described in the late 1980s and shown to contribute to cell volume regulation, the molecular identity of these channels has long remained elusive [50, 51]. VRACs are activated within minutes after hypotonic stimulation, whereby the activation kinetics as well as the maximal current amplitude are

strongly dependent on cell type and cell cycle phase [52]. Also their inactivation kinetics vary significantly between different cell types from virtually absent in T lymphocytes [53] to highly pronounced in myoblasts [54]. Besides inorganic anions, VRACs also conduct various organic osmolytes and metabolites such as taurine, D-aspartate, D-glutamate, myo-inositol, and ATP as well as the cancer-drug cisplatin and its derivatives [55-57].

LRRC8 - the molecular identity of VRACs. Only in 2014 two groups identified simultaneously LRRC8A (Leucine-rich repeat-containing protein 8A), also known as SWELL1, as the first major component of VRACs [58, 59]. LRRC8A belongs to the LRRC8 protein family with four other family members (LRRC8B-E) that share a conserved domain structure consisting of transmembrane domains in the N-terminus followed by up to 17 leucine-rich repeats (LRR domain) (Fig. 3) [60, 61]. In order to build a functional VRAC LRRC8A and at least one other member of the LRRC8 family take up a hexameric structure (Fig. 3) [58, 59, 62, 63]. The pore is generated by the four transmembrane domains (TM1-4) of the six LRRC8 subunits, while the extracellular domain forms the entrance to the channel pore (Fig. 3) [64-67]. The intracellular LRR domains assemble as trimers of dimers and carry several threonine and serine residues, which can most likely be modified by phosphorylation [67]. Interestingly, the LRRC8 subunit composition differs among cell types and influences VRAC properties such as inactivation kinetics, voltage-dependence and preference of the transported organic osmolytes [56, 57, 62, 63, 68]. For example, channels composed of LRRC8A and LRRC8D were shown to mainly release uncharged osmolytes like taurine [56, 62]. Different subunit compositions also provide an explanation for the involvement of VRACs in a large number of physiological processes in different cells and tissues.

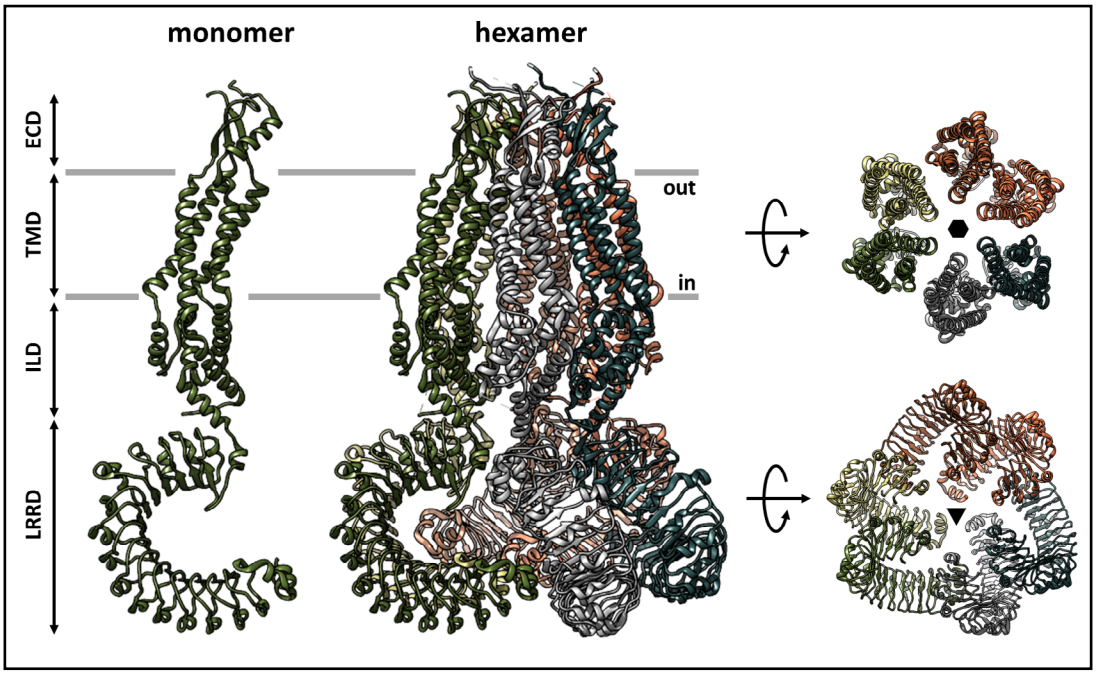

Fig. 3. Structure of the human LRRC8 channel (PDB 5ZSU) [66]. The monomer (left) can be divided into four structural domains: the extracellular domain (ECD), the transmembrane domain (TMD), the intracellular linker domain (ILD), and the leucine-rich repeat domain (LRRD). The functional LRRC8 channel shows a hexameric structure (middle) and is formed by LRRC8A subunits and at least one other LRRC8 isoform (LRRC8B-LRRC8E). The hexameric structure displays a 6-fold symmetry on the level of the TMD and a 3-fold symmetry on the level of the LRRD (right). Consequently, the cytosolic domain exhibits the structure of a trimer of dimers.

LRRC8 proteins are ubiquitously expressed and are shown to be relevant in various physiological processes in different mammalian cell types [69, 70]. However, we were the first to provide a basic description of the essential function of LRRC8 in the hypotonic stress response of the human epidermis. We have recently shown that LRRC8 ion channels are expressed in the native human epidermis [38]. In isolated primary keratinocytes, RNA sequencing showed that the essential LRRC8A subunit is more strongly expressed than the other subunits (Fig. 4A). The second most abundant subunit is LRRC8D (Fig. 4A) [38]. Interestingly, we found a particular focused expression of LRRC8A in the basal epidermal layer and declining LRRC8A protein levels towards more differentiated keratinocytes in suprabasal layers (Fig. 4B) [38]. In contrast, LRRC8D was uniformly distributed across the epidermis (Fig. 4C). To determine the contribution of LRRC8A to the hypotonic stress response, we generated LRRC8A knockout keratinocytes using CRISPR/Cas9 technology. We could show that LRRC8A is crucially important for mediating VRAC activity in these cells and that LRRC8A substantially contributes to RVD [38]. However, the exact function and regulation of LRRC8A is still poorly understood.

In summary, the epidermis possesses its own unique set of proteins, which are involved in cell volume regulation and potentially contribute to epidermal homeostasis partly even independent from the osmotic stress response, which is discussed further in the next section.

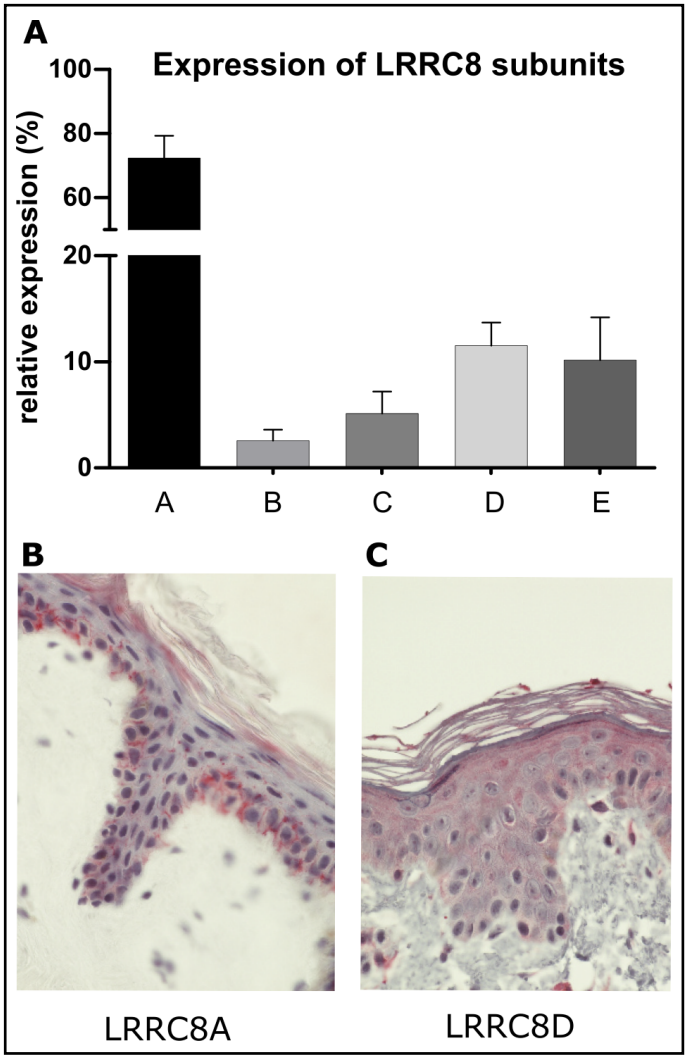

Fig. 4. Expression of LRRC8 in the human epidermis. (A) RNA was isolated from human primary keratinocytes (n=4) and subjected to RNA-Seq. Bars represent transcript levels of LRRC8A-E subunits depicted as relative abundance in relation to all subunits. Human keratinocytes express all LRRC8 subunits, with the highest abundance of LRRC8A and LRRC8D (see also [38]). (B+C) Punch biopsies were taken from healthy donors (approval 144/12 Clinic of the Goethe-University). The Declaration of Helsinki protocols were followed. Specimen were fixed in 4% PFA, paraffin embedded and 4 µm sections were processed routinely. Immunohistochemical stainings were performed with α-LRRC8A (NBP2-32158 from Novus Biologicals) (B) and LRRC8D (11537-1-AP from Proteintech) (C) antibodies. Histofine Simple Stain AP Multi (Nichirei Bioscience) was used for detection. Nuclei were stained with hematoxylin. Images were acquired by using a Nikon Eclipse slide scanning microscope. LRRC8A is preferentially expressed in the basal layer (B), while LRRC8D is more uniformly distributed throughout all epidermal layers (C).

Cell volume regulation as an integral part of epidermal physiology

Beyond the responses to osmotic challenges, changes in cell volume can occur in the absence of any osmotic imbalances and cell volume regulation is an integral part of many physiological processes [11, 71]. It is not only a passive adaptation or result of these physiological processes but can rather be considered as an active principle involved in driving these mechanisms. For example during cell migration, the local intracellular volume is increased at the leading edge and decreased at the trailing edge, which influences cytoskeletal organization, that is crucial for cell movement [72]. In addition, cell swelling is associated with necrosis, while apoptotic volume decrease (AVD) involving VRACs contributes to apoptosis [73]. Most importantly, cell volume regulation is an integral part of cellular proliferation and differentiation. A well-regulated balance between proliferation in the basal layer and ordered differentiation in suprabasal layers is necessary to form a functional epidermal barrier, thus aberrant volume regulation might contribute to epidermal pathologies.

Keratinocyte proliferation

To generate daughter cells of similar size as the parental cells, proliferation has to be coupled with increases in cell volume. It was already described in 1985 that the cell volume of freshly isolated keratinocytes correlated with their proliferative behavior: Cells with a bigger cell volume showed a higher proliferative potential than cells with a smaller cell volume [74]. This is in concert with findings describing cell stretch as a trigger for proliferation [75]. Interestingly, long-term exposure of keratinocytes to hypertonic stress inhibits proliferation [25] and is associated with a transient elevation of Ca^{2+} currents [76]. In contrast, keratinocytes respond to hypotonic stress with increased proliferation as well as an elevation of intracellular Ca^{2+} [44]. Interestingly, both mechanisms involved an increase in intracellular Ca^{2+} levels, which indicates that Ca^{2+} per se is not solely sufficient to control these opposing processes, but their regulation probably involves additional downstream effectors and pathways.

So far, the role of specific ion channels involved in cell volume regulation during proliferation has only been studied in other cell types, but not in epidermal cells. During proliferation most cells display an initial volume increase, followed by a transient activation of Cl^- channels leading to a decrease in cell volume [77]. VRACs have been proposed to be involved in these Cl^- currents during proliferation. However, LRRC8A knockdown in myoblast, colon cancer and glioblastoma cell lines showed no effect on proliferation [78], while others found an effect of LRRC8A on proliferation in hepatocellular carcinoma [79] or primary glioblastoma cells [80]. In addition, K^+ channels are involved in regulating cell migration and proliferation in epithelial cells through modulation of membrane potential, cell volume, intracellular Ca^{2+} and various signaling pathways [81].

Epidermal maturation and barrier formation

Osmotic stress is being discussed as a signal for epidermal differentiation and barrier formation. Applying hyperosmotic stress to keratinocytes induces the expression of differentiation markers such as KRT1/10, IVL, TGM-1 and FLG [82]. This is further underlined by the fact that in order to generate reconstructed 3D epidermal models keratinocytes are lifted to the air-liquid interface, thus promoting dehydration and hyperosmotic stress, which in turn induces stratification and differentiation. If such models are kept under dry conditions, the stratum corneum is thickened and barrier function is improved [83]. Induction of differentiation by hyperosmotic stress is associated with elevated intracellular Ca^{2+} levels [76]. Ca^{2+} ions and the associated concentration gradient are important factors in epidermal homeostasis by regulating keratinocyte behaviour and barrier formation [4]. This is maintained by Ca^{2+} release from intracellular stores such as the ER and influx of extracellular Ca^{2+} into keratinocytes. Thus, it would be conceivable that cells migrating through the epidermis are exposed to increasing osmotic stress. The resulting cell volume

regulation involves the opening of Ca^{2+} channels, which in turn induces the expression of differentiation markers.

In addition to transport of Ca^{2+} itself, it is possible, that ion channels involved in cell volume regulation could influence the membrane potential. Keratinocytes are non-excitable cells, which have a resting membrane potential of -30 to -40 mV, however a hypotonic stimulus causes a sustained, partially reversible hyperpolarization of keratinocytes [44]. Changes in K^+ or Cl^- levels mediate depolarization or hyperpolarization of the membrane potential, which is associated with proliferation and differentiation [84]. Interestingly, it was also described, that cells run through different stages of membrane potential while differentiating. For example, murine embryonic stem cells become more depolarized within the first day of a differentiation stimulus, repolarize and finally reach a hyperpolarized membrane potential compared to non-differentiated cells after two weeks [85]. Changes in the osmotic milieu could also directly influence the formation of the epidermal barrier. Hypoosmotic stress induces the expression of E-cadherin [86], which plays a key role in TJ positioning and barrier formation [87].

How specific channels, such as LRRC8, are mechanistically triggering epidermal maturation is currently under investigation. In myoblasts it was shown that LRRC8A is necessary for an initial hyperpolarization and a following Ca^{2+} increase, both necessary for initiation of differentiation in this cell type [88]. In addition, it can be imagined that similar to myoblast differentiation, membrane potential changes by LRRC8 activate voltage-dependent K^+ and Ca^{2+} channels leading to the influx of Ca^{2+}, a major trigger for keratinocyte differentiation. In adipocytes the LRR domain of LRRC8A is physically interacting with the adaptor molecule IRS of the insulin receptor. This permits the interaction with the PI3K/AKT signaling pathway, which plays an important role in keratinocyte maturation by controlling mTORC1 signaling, the major control hub for initiating the switch from proliferation to differentiation in the epidermis [89]. As mTORC1 signaling is upregulated in psoriasis and contributes to the epidermal defects in psoriatic skin [89, 90], one could speculate that aberrant LRRC8A function might also contribute to the pathogenesis. This is further underlined by different findings where impaired responses to osmotic stress contribute to pathological skin conditions. For example, in a rat model of disrupted skin barrier function hypoosmotic stress induced an inflammatory response in the epidermis [91]. In addition, it has been shown in cultured keratinocytes and ex vivo human skin models that hypoosmotic stress induces the expression of the alarmin IL-33 in a Ca^{2+} dependent manner [92]. Interestingly, the cutaneous irritant heptylamine blocks VRACs and induces Ca^{2+} store depletion [93], which could explain the dermatitis inducing effect of this compound and further underlines the important role of cell volume control for epidermal homestasis. Furthermore, cell lines derived from patients with EBS (epidermolysis bullosa simplex), a skin blistering disease, harbouring different keratin mutations, showed an impaired response to hypotonic stress that correlated with the clinical severity of the mutation carried [94]. Correspondingly, these cells showed changes in mechano-signaling suggesting that keratins may play a role in sensing the mechanical load caused by an osmotic assault [95].

In summary, mechanisms involved in cell volume control also contribute to epidermal homeostasis by regulating barrier function and controlling the balance between keratinocyte proliferation and differentiation.

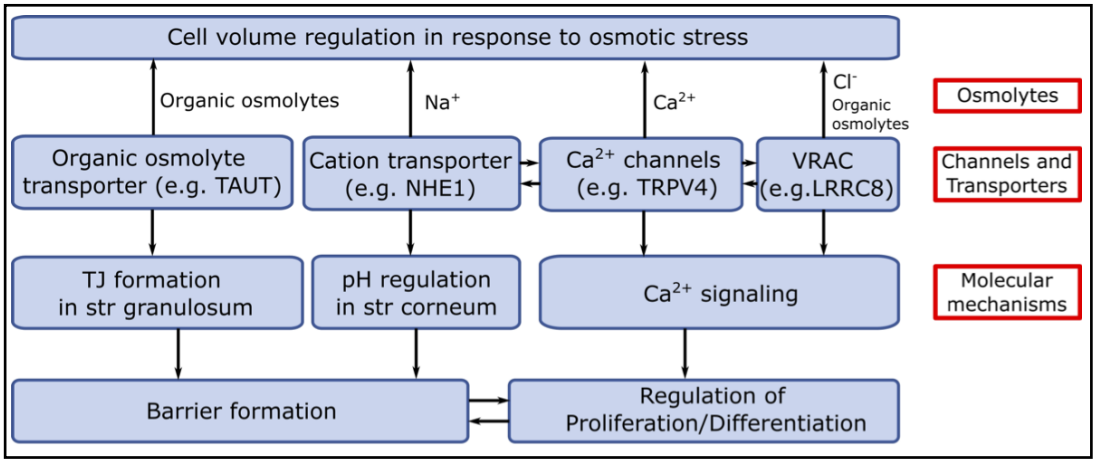

Fig. 5. Overview of the epidermal transporters and channels and their function. The depicted osmolyte transporters and ion channels are important for epidermal cell volume regulation in response to osmotic stress. At the same time, these molecules contribute to the maintenance of epidermal homeostasis by regulating keratinocyte maturation and differentiation as well as barrier formation.

Conclusion and perspectives

The epidermis is able to respond to osmotic stress by transporting ions and organic osmolytes through a specific set of channels and transporters (Fig. 5). This not only enables the adaption of cell volume, but also represents crucial mechanisms to maintain epidermal homeostasis by regulating TJ function, pH stability and Ca^{2+} levels (Fig. 5). To understand the interaction of different mechanisms and the precise function of specific channels and transporters within the skin, further research is necessary. However, one could speculate that every epidermal layer has evolved slightly different ways to adopt to varying osmotic conditions by regulating the expression of different ion channels and transporters to optimally cope with osmotic perturbations and regulate epidermal barrier function. In addition, further investigations are needed to understand how dysfunction in epidermal volume control contributes to different skin pathologies.

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Author Contributions
MJ and CB conceptualized, wrote and revised the manuscript. TF and OR wrote and revised the review.

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Statement of Ethics
The authors have no ethical conflicts to disclose.

Disclosure Statement

CB and TF are inventors of patent application WO 2019/158696.

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