



INVITED REVIEW ARTICLE

Skin Barrier and Calcium

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Epidermal barrier formation and the maintenance of barrier homeostasis are essential to protect us from the external environments and organisms. Moreover, impaired keratinocytes differentiation and dysfunctional skin barrier can be the primary causes or aggravating factors for many inflammatory skin diseases including atopic dermatitis and psoriasis. Therefore, understanding the regulation mechanisms of keratinocytes differentiation and skin barrier homeostasis is important to understand many skin diseases and establish an effective treatment strategy. Calcium ions (Ca^{2+}) and their concentration gradient in the epidermis are essential in regulating many skin functions, including keratinocyte differentiation, skin barrier formation, and permeability barrier homeostasis. Recent studies have suggested that the intracellular Ca^{2+} stores such as the endoplasmic reticulum (ER) are the major components that form the epidermal calcium gradient and the ER calcium homeostasis is crucial for regulating keratinocytes differentiation, intercellular junction formation, antimicrobial barrier, and permeability barrier homeostasis. Thus, both Ca^{2+} release from intracellular stores, such as the ER and Ca^{2+} influx mechanisms are important in skin barrier. In addition, growing evidences identified the functional existence and the role of many types of calcium channels which mediate calcium flux in keratinocytes. In this review, the origin of epidermal calcium gradient and their role in the formation and regulation of skin

barrier are focused. We also focus on the role of ER calcium homeostasis in skin barrier. Furthermore, the distribution and role of epidermal calcium channels, including transient receptor potential channels, store-operated calcium entry channel Orai1, and voltage-gated calcium channels in skin barrier are discussed. (**Ann Dermatol 30(3) 265~275, 2018**)

-Keywords-

Barrier, Calcium, Endoplasmic reticulum, Keratinocytes, Orai1, Transient receptor potential channels

INTRODUCTION

Calcium ions (Ca^{2+}) serve as the universal signal to modulate various aspects of cellular functions in keratinocytes. The distribution and dynamic of Ca^{2+} in skin play an important role in epidermal homeostasis. In mammalian epidermis, a characteristic calcium gradient exists between lower and upper layers of epidermis, with low levels in basal and spinous layers and progressively increasing levels towards the stratum granulosum, and declining again in the stratum corneum (SC)¹⁻³. The gradient of calcium across the epidermis plays a crucial role in the processes of keratinocytes differentiation and formation of the epidermal permeability barrier and allows the dynamic changes of calcium ions to generate calcium signaling^{4,8}. Recent evidences suggest that both Ca^{2+} release from intracellular stores and Ca^{2+} influx from extracellular sources are important for the regulation of epidermal structures and functions⁹⁻¹². In this review, we focused on the origin and formation mechanism of epidermal calcium gradient and its roles in epidermal barrier homeostasis, keratinocytes differentiation, wound healing, and epidermal hyaluronan metabolism. We also discuss the homeostasis of Ca^{2+} in endoplasmic reticulum (ER) and ER stress response in keratinocytes and their implications in keratinocytes differentiation, permeability and anti-

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microbial barrier homeostasis, and cell-to-cell adhesion. The calcium-sensing receptor, transient receptor potential (TRP) channels, and Orai1 channel are also highlighted as the major constituents of calcium sensing and calcium influx in the keratinocytes.

EPIDERMAL CALCIUM GRADIENT

Calcium gradients, formed by the relative concentrations of calcium ions in the extracellular and intracellular spaces, form the basis of skin function. Epidermal calcium gradients are generated by several mechanisms. Based on the Ca^{2+} gradient dynamics following acute SC barrier disruption (rapid disappearance and reappearance after 6 hours in parallel with barrier repair) and the evidence that reappearance of Ca^{2+} gradient following barrier disruption was accelerated by artificial barrier restoration, whereas delayed by inhibition of barrier recovery, Elias et al.³ suggested low rate of sustained transepidermal water loss with restriction of ion movement by an intact epidermal SC barrier accounts for the formation of the epidermal Ca^{2+} gradient. They also considered that these passive processes are sufficient to generate the Ca^{2+} gradient. A recent study suggested that epidermal tight junctions (TJs) also contribute to the generation/maintenance of the epidermal Ca^{2+} gradient. In addition to the SC barrier, TJs in the stratum granulosum function as a secondary barrier in skin by restricting the movement of ion, macromolecule, and pathogenic microbes¹³. Kurasawa et al.¹⁴ demonstrated that epidermal TJs contribute to Ca^{2+} gradient formation and epidermal differentiation in reconstructed human epidermis. These findings suggest that both the SC permeability barrier and the TJs are crucial to generate and maintain the epidermal calcium gradient by preventing Ca^{2+} diffusion from the stratum granulosum to the SC. Recent advances in calcium imaging techniques have advanced understanding of the main calcium sources required for the formation of the epidermal calcium gradient. Early investigations which employed Ca^{2+} capture cytochemistry and proton-induced X-ray emission method have suggested that the extracellular calcium content is critical cellular compartment for the epidermal calcium gradient^{1,15}. However, these previous calcium measurement methods measure total Ca^{2+} , not free Ca^{2+} , and they require dehydration or fixation of tissues for measurement. To overcome these limitations, Celli et al.¹⁰ employed a fast fluorescence lifetime imaging system which measures ionic concentration from the decay of the ion-sensitive dye lifetime rather than its intensity which enables to visualize and quantify the spatial distribution of calcium in unfixed *ex vivo* epidermis and demonstrated

that the majority of the Ca^{2+} in the stratum granulosum is found in intracellular stores such as the Golgi and the ER rather than in extracellular spaces. These findings suggest that ER calcium stores contribute to the epidermal calcium gradient. Calcium gradient and calcium signaling is crucial for the regulation of many skin functions. Below, we review the role of epidermal calcium ion and its gradients in permeability barrier homeostasis, keratinocytes differentiation and proliferation, cell-to cell adhesion, wound healing, and hyaluronan metabolisms in the skin.

Role of calcium in skin barrier homeostasis

The skin barrier function resides in the epidermis, particularly in the SC, the outermost cornified layer. SC is composed of corneocytes, which is the end product of keratinocytes terminal differentiation and SC intercellular lipid, the lamellar bilayers composed of ceramides, cholesterol, and free fatty acid. Lamellar bodies (LBs), the specialized organelles in the skin play a central role in the formation of the SC lipid, protein and anti-microbial barrier via delivering pro-barrier lipids and enzymes crucial for lipid processing along with proteases and anti-microbial peptides to the SC¹⁶. Prior studies demonstrated that the rate of LBs secretion, lipid synthesis and permeability barrier homeostasis are regulated by the changes in extracellular calcium concentration of the upper epidermis, which is triggered by permeability barrier disruption⁵⁻⁷. Acute barrier disruption by topical solvent application or tape-stripping induces an immediate depletion of both extracellular calcium ions in the epidermis, especially in the upper granular layers, and results in the loss of normal epidermal calcium gradient^{6,17,18}. The calcium levels in the upper epidermis then progressively restored over 6~24 hours in parallel with barrier recovery^{6,7,17}. Inhibition of extracellular calcium loss in the upper epidermis by immersion of barrier disrupted skin in the high calcium-containing solutions or occlusion with a vapor-impermeable membrane impairs the barrier recovery^{7,19,20}. These findings indicate that acute loss of calcium concentration in the stratum granulosum following barrier disruption is an important regulatory signal, initiating the immediate release of pre-stored LBs contents to the SC interstices, accelerated synthesis of new LBs and epidermal lipid synthesis leading to barrier repair. In addition to the extracellular calcium contents, calcium influx into keratinocytes also regulates barrier recovery. Lee et al.^{4,5} demonstrated that L-type Ca^{2+} channel blocker, verapamil reverses the high extracellular Ca^{2+} -induced inhibition of barrier recovery. Both extracellular calcium and calcium influx via calcium channels regulates epidermal permeability barrier homeostasis. Later, Choi et al.²¹ demonstrated that high frequency sono-

phoresis or iontophoresis at energies that do not cause alterations in skin barrier function can trigger LBs secretion and cytokines expression to an extent comparable with barrier disruption via triggering the change in the epidermal calcium gradient. These observations suggest that alteration of calcium levels in the outer epidermis without influencing skin barrier function can be a strategy to enhance permeability barrier function.

Keratinocyte differentiation

Calcium ion is a major regulator in keratinocyte differentiation and proliferation^{8,22,23}. The skin is characterized by the vertical differentiation from basal layer to SC. The basal layer contains proliferating cells. As differentiation proceeds, keratinocytes progress upwards through the different epidermal layers along with the stage-specific changes in expression of numerous differentiation markers, and finally become the terminally differentiated corneocytes in the cornified layer of the SC. Calcium plays critical roles in the all processes of keratinocyte differentiation from the commitment to differentiation in the basal and spinous layer to the terminal differentiation in the stratum granulosum. Calcium regulates the transcription of all genes encoding keratinocyte differentiation-specific proteins. Activator protein-1 (AP-1) transcription factors are present in many keratinocyte-specific genes, including transglutaminase, loricrin, involucrin, profilaggrin, and other keratins to control the transcription of various differentiation markers²⁴. Ng et al.²⁵ revealed that gene encoding involucrin has AP-1 responsive element in the promoter region and the AP-1 site in the involucrin gene is essential for the calcium response, suggesting that nuclear Ca^{2+} regulates synthesis of differentiation specific proteins.

The calcium contents required for the stage-specific expression of differentiation-related proteins is different between each layers of the epidermis. For example, the extracellular calcium content required for expression of profilaggrin, the late differentiation marker, is higher than that required for the keratin 1 and keratin 10 expression⁸. These findings suggest that the epidermal calcium gradient is essential for proper epidermal differentiation and barrier formation. Calcium is also important in posttranslational processing of profilaggrin to filaggrin. Profilaggrin has N-terminal domain containing Ca^{2+} -binding motifs, which share similarity with the EF-hands of the S100 Ca^{2+} -binding protein family. Calcium, via binding to the head domain of profilaggrin, induces conformational changes, thereby exposing the crucial cleavage sites of profilaggrin to initiate the processing pathway²⁶. During the terminal differentiation, many envelope precursor proteins, includ-

ing involucrin, loricrin, elafin, small proline-rich proteins, filaggrin, and keratin are covalently cross-linked to form the cornified envelope by transglutaminase 1 and the transglutaminases mediate crosslinking of cornified envelope precursors in a calcium dependent-manner²⁷. Therefore calcium modulates cornified envelope formation during terminal differentiation.

In addition to extracellular calcium ions, calcium sensitive proteins are also known to induce keratinocyte differentiation. Protein kinase C (PKC), which is activated by the rise in diacylglycerol and intracellular calcium induces differentiation markers of granular keratinocytes, including loricrin, filaggrin, and transglutaminases²⁸⁻³⁰. Among the isozymes, PKC α and δ are activated by calcium ions in human and murine epidermis and regulate the extracellular calcium-induced transcription of these differentiated genes^{29,30}.

1) Extracellular calcium and the calcium-sensing receptor

The mechanism of increased intracellular Ca^{2+} ($[Ca^{2+}]_i$) in the stratum granulosum layer in response to the elevation of extracellular calcium is now explained by the calcium-sensing receptor (CaSR) expressed in granular layer³¹⁻³⁶. CaSR, a subfamily of G protein-coupled receptors, in the plasma membrane senses the rise in extracellular calcium levels and activates phospholipase C, most likely through G α_q , which in turn generates inositol triphosphate (IP₃), thereby releasing calcium from intracellular stores such as ER and Golgi and also stimulates Ca^{2+} influx via store-operated calcium channels³⁴⁻³⁶. Previous studies suggested that the CaSR is involved in mediating calcium signaling during keratinocyte differentiation^{31-35,37-39}. Tu et al.⁴⁰ evaluated the role of CaSR *in vivo* by generating keratinocyte-specific CaSR knockout mice and demonstrated that deletion of CaSR in keratinocytes causes the loss of epidermal calcium gradient, increased proliferation and a significant decrease in the amounts of the mid to late differentiation markers, and reductions in the number and the secretion of LBs at the stratum granulosum-SC interface, suggesting that CaSR is important for normal epidermal differentiation and barrier function *in vivo*.

2) Intracellular calcium and ER stress-role in differentiation and barrier homeostasis

Earlier studies have indicated that extracellular calcium is the major component forming the epidermal calcium gradient and critical for the signals of barrier repair in response to barrier perturbation^{1,4-7,15}. However, recent studies from Celli et al.¹⁰ has demonstrated that the bulk of Ca^{2+} measured in the epidermis comes from intracellular Ca^{2+} stores such as the ER and the ER calcium depletion is

an important signal for the terminal differentiation and epidermal barrier homeostasis. They found that external barrier perturbation by tape-stripping results in a marked loss of intracellular Ca^{2+} and activation of ER stress marker, XBP1 in murine skin to a comparable extent when treated with the sarco/endoplasmic reticulum Ca^{2+} -ATPase isoform 2 (SERCA2) inhibitor thapsigargin, which depletes ER calcium, indicating that most of the Ca^{2+} depletion from the stratum granulosum after barrier perturbation is derived from the intracellular ER calcium stores, triggering ER stress¹¹. The same study also demonstrated that the ER stress induction in skin by chemical trigger, thapsigargin at the concentration that does not activate the apoptotic pathway but instead triggered physiologic unfolded protein response (UPR) stimulates the LBs secretion and the expression of caspase 14 and loricrin, mimicking the physiologic processes of barrier recovery independent of barrier disruption¹¹. These results emphasize the important contributions of the intracellular calcium release, particularly from the ER in the regulation of keratinocyte differentiation and barrier homeostasis (Fig. 1). Release of ER calcium also has been demonstrated to be involved in the synthesis of antimicrobial peptide, such as cathelicidin and human beta-defensins via ER stress-induced ceramide metabolites (sphingosine-1-phosphate and ceramide-1-phos-

phate) pathways, indicating that various stresses to induce physiologic ER stress can enhance antimicrobial barrier function^{41,42}. However, when the ER stress is persistent or severe to exceed a given threshold, abnormal cell-to-cell adhesion, abnormal keratinization, or apoptosis can be triggered in keratinocytes⁴³⁻⁴⁶. Indeed, Savignac et al.⁴⁵ demonstrated that the keratinocytes from patients with Darier's disease caused by mutations in the ER Ca^{2+} ATPase SERCA2 showed constitutive ER stress and increased sensitivity to ER stressors, which in turn, lead to abnormal cell-to-cell adhesion via impaired redistribution of desmoplakin, desmoglein 3, desmocollin 3, and E-cadherin. They also found that a pharmacological ER stress chaperone, Miglustat improved cell-to-cell adhesion in Darier's disease keratinocytes. Furthermore, the authors recently demonstrated that the effect of ER calcium release in keratinocytes by SERCA2 inhibitor thapsigargin on epidermal TJ barrier is different, depending on the degrees of ER Ca^{2+} depletion. Physiologic ER stress enhances TJ barrier, in contrast severe ER stress to induce abnormal UPR disrupts the structure and function of epidermal TJ partly via disorganization of perijunctional actin cytoskeleton. Taken together, ER Ca^{2+} homeostasis and ER stress are also crucial for the regulation of barrier formation, cell-to cell adhesion, antimicrobial barrier, and permeability bar-

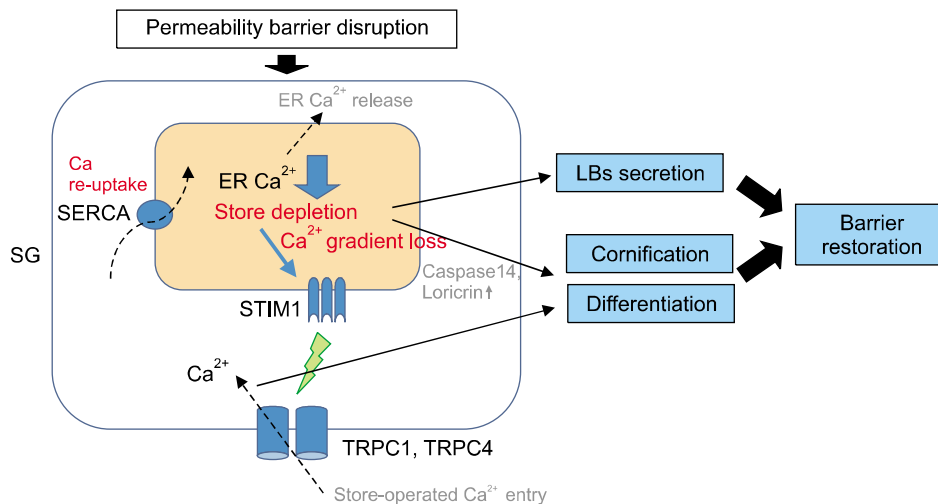


Fig. 1. Proposed role of endoplasmic reticulum (ER) calcium signaling in the permeability barrier homeostasis. Recent studies suggested that the intracellular calcium store such as the ER is the major compartment which forms the epidermal calcium gradient. Permeability barrier disruption stimulates Ca^{2+} release from ER in keratinocytes of stratum granulosum (SG), causing ER Ca^{2+} depletion and the loss of epidermal calcium gradient. ER Ca^{2+} depletion stimulates lamellar bodies (LBs) secretion and the expression of caspase 14 and loricrin, indicating that ER Ca^{2+} change is a critical signal for initiating the two key metabolic responses (lipid and protein barrier restoration) that lead to barrier recovery. The next physiological response to ER Ca^{2+} depletion is a rapid increase in store-operated Ca^{2+} entry, a mechanism involved in refilling of ER Ca^{2+} stores. Stromal interaction molecule 1 (STIM1) is an ER Ca^{2+} sensor that triggers the store-operated Ca^{2+} entry. In the SG, this store-operated Ca^{2+} entry is mediated by TRPC1 and TRPC4. This calcium influx through TRPC1 and TRPC4 further stimulates keratinocyte differentiation. SERCA: sarco/endoplasmic reticulum Ca^{2+} -ATPase isoform.

rier homeostasis.

Wound healing and cell migration

Moreover, intracellular calcium dynamics play a role in keratinocyte migration and wound healing⁴⁷. It is well known that asymmetries in the distribution of the intracellular Ca^{2+} concentration regulate cellular polarity, guidance, and migration via providing spatial and temporal information to control cellular extension and migration^{48,49}. Previous study has observed the asymmetric distributions of lamellipodial Ca^{2+} sparks in frequency during keratinocyte migration⁴⁷. They also demonstrated that Ca^{2+} -permeable channels within these cells are mechanically activated and among the mechanosensitive TRP channels, TRPV1 was revealed to be involved in the Ca^{2+} influx in response to the membrane tension during keratinocyte migration. These findings suggest that modulations of calcium and mechanosensitive TRP channels can be the potential strategies for wound healing, especially during the proliferation phase.

Epidermal hyaluronan metabolism

Recently, it is recognized that epidermal hyaluronan regulates epidermal differentiation and lipid synthesis/secretion, which in turn influence permeability barrier homeostasis through the interaction with its receptor, CD44⁵⁰. Previously, our group demonstrated that permeability barrier perturbation induces the expression of hyaluronan and CD44 in murine epidermis and the epidermal calcium gradient change hyaluronan is the important signal for the barrier disruption-induced hyaluronan synthesis in keratinocytes⁵¹. In addition, the authors demonstrated that high frequency sonophoresis at the intensity that do not cause alterations in barrier function also induces epidermal hyaluronan and CD44 expression via triggering the change in the epidermal calcium gradient.

CALCIUM CHANNELS IN SKIN

The effects of extracellular and intracellular Ca^{2+} on the keratinocyte differentiation and barrier homeostasis suggest that Ca^{2+} channels which mediate the Ca^{2+} influx into the cells exist in keratinocytes and regulate many functions in skin barrier homeostasis. Indeed, keratinocytes functionally express several types of calcium channels including TRP channels, components of the store-operated calcium entry (SOCE) pathway such as Ca^{2+} influx channel (Orai1) and endoplasmic Ca^{2+} depletion sensor (stromal interaction molecule 1 [STIM1]), and voltage-gated calcium channels (VGCCs) such as L-type calcium channel. The distribution of these channels are different in each lay-

ers of epidermis, therefore the response to calcium is distinct in different layers (Fig. 2).

TRP CHANNELS

TRP channels are widely expressed in the nervous systems and play an important role in processing sensory information such as itch and pain in response to a variety of environmental factors, such as temperature, physical or chemical stimuli⁵²⁻⁵⁴. Growing evidences have indicated that most TRP channels are also expressed in keratinocytes and play an important role in the regulation of skin barrier homeostasis, keratinocytes differentiation/proliferation, and inflammation⁵⁵⁻⁵⁷. TRP channels in keratinocytes also act as 'cellular sensors' that respond to changes in the environment, including temperature, mechanical, chemicals, osmolarity and pH and process those informations⁵²⁻⁵⁴. Among the six subfamilies of TRP channels, we focus on the role of TRPV (vanilloid), TRPC (canonical), TRPA (ankyrin), and TRPM (melastatin) in skin barrier function.

TRP channels and keratinocyte differentiation/proliferation

Keratinocytes express five TRPV subfamilies including four nonselective cation channels (TRPV1, TRPV2, TRPV3, and TRPV4) and one highly Ca^{2+} selective channel (TRPV6)^{58,59}. Among the TRPV channels, TRPV1, TRPV3, and TRPV6 were demonstrated to regulate keratinocyte differentiation/proliferation. TRPV1 is activated by heat ($>43^{\circ}\text{C}$), capsaicin and low pH and showed stronger expression in the stratum basale compared to upper layers of the skin⁶⁰⁻⁶². TRPV1 was demonstrated to be required for the endocannabinoid-mediated suppression of keratinocyte proliferation and induction of apoptosis⁶³. Among TRP channels, TRPV3 is primarily expressed in the skin, especially in epidermal and follicular keratinocytes and activated by innocuous warm temperatures ($>33^{\circ}\text{C}$), chemicals, and inflammatory mediators such as arachidonic acids⁶⁴. The role of TRPV3 in keratinocyte differentiation, proliferation, and skin barrier function has been unraveled from TRPV3 knockout mice model and human disease caused by the mutations in TRPV3, the so-called "TRPV3 channelopathy"⁶⁵⁻⁷¹. TRPV3-deficient mice show dry skin phenotype with defective barrier formation and altered late terminal differentiation along with abnormal hair morphogenesis⁶⁸. Cheng et al.⁶⁸ demonstrated that TRPV3 forms a signaling complex with transforming growth factor- α /epidermal growth factor receptor, two growth factors that regulate keratinocyte proliferation in basal layer and differentiation in suprabasal layers, to modulate the activity of transglutaminases to induce terminal differ-

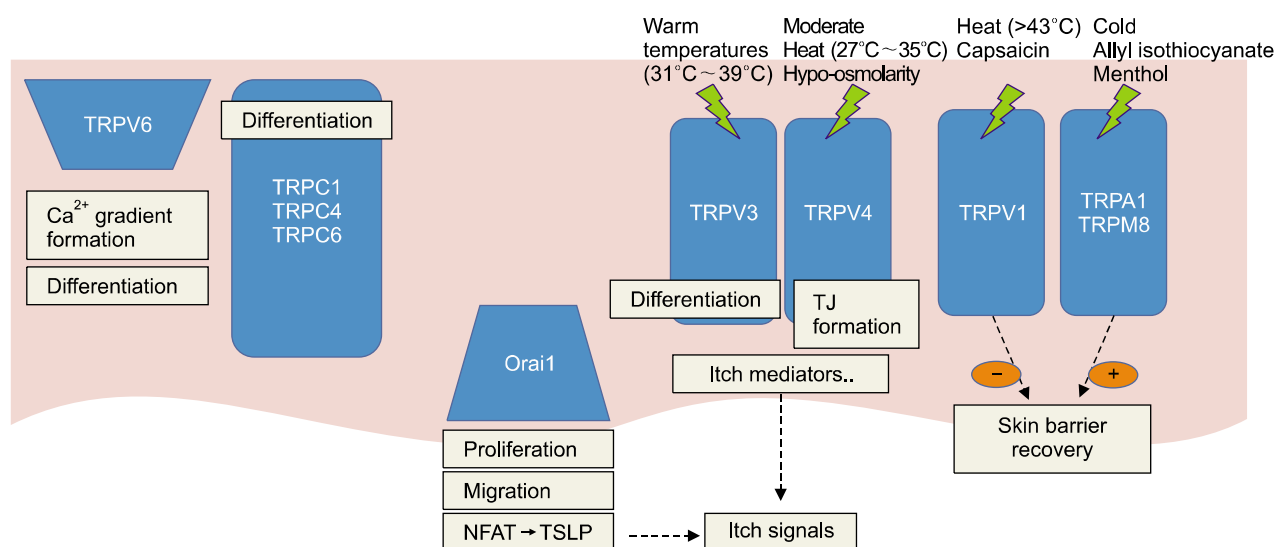


Fig. 2. The role of various calcium channels in skin barrier homeostasis and itch. Epidermal keratinocytes functionally express many calcium channels including transient receptor potential (TRP) channels and the components of the store-operated calcium entry (SOCE) pathway such as Ca^{2+} influx channel Orai1. TRPV6 expression is upregulated in the differentiated keratinocytes, where TRPV6 is implicated in the formation of epidermal calcium gradient and differentiation. TRPC channels in skin play a role in store-operated channels (SOC)-related Ca^{2+} entry pathways. TRPC1, TRPC4, and TRPC6 play a role in differentiation. In addition to TRPC channels, important player of SOC in skin is Orai1, which is mainly expressed in basal cell layer. Orai1 plays important roles in cellular proliferation, migration, and itch signals. TRPV3 and TRPV4 are activated by warm temperatures. TRPV3 plays a role in skin barrier formation and differentiation in keratinocyte through a transforming growth factor- α /epidermal growth factor receptor-complex, however, overactive TRPV3 stimulates abnormal keratinocytes proliferation. TRPV4 plays a role in the formation of epidermal tight junction (TJ). TRPV3 and TRPV4, which are strongly expressed in keratinocytes, have been implicated in itch, possibly by releasing various mediators to stimulate the sensory nerves. The heat-sensitive channel TRPV1 delays barrier recovery, in contrast the cold-sensitive channels TRPA1 and TRPM8 accelerate barrier recovery. TSLP: thymic stromal lymphopoietin.

entiation and cornified envelope formation. In addition, transgenic mice with the gain-of-function mutation of the TRPV3 gene (*TRPV3^{Gly573Ser}*) and patients with Olmsted syndrome (OMIM 607066) caused by the identical or other 'gain-of-function' mutation of TRPV3 showed similar clinical features characterized by a pruritic and hyperkeratotic skin inflammation with massive acanthosis and hyperkeratosis in histopathological examination⁶⁹⁻⁷¹. These findings indicate that hyperactive TRPV3 in keratinocytes disrupts the balance of keratinocyte proliferation and differentiation and emphasize its relevance in inflammation and pruritus. TRPV6, a highly Ca^{2+} -selective channel, has been shown to be expressed in keratinocytes and play a crucial role in Ca^{2+} /1,25-dihydroxyvitamin D₃-induced differentiation of keratinocytes⁷². Knockdown of TRPV6 in human keratinocytes has been shown to impair the Ca^{2+} -induced differentiated phenotype with inhibited expression of differentiation markers as involucrin, transglutaminase-1, and cytokeratin-10. 1,25-Dihydroxyvitamin D₃ increases the expression of TRPV6 in human keratinocytes, which in turn mediates, at least in part, the pro-differentiating effects of 1,25-dihydroxyvitamin D₃ by increasing Ca^{2+} entry, thereby promoting differentiation⁷³.

Store-operated channels (SOC)-related Ca^{2+} entry is a Ca^{2+} entry pathway that is activated in response to depletion of Ca^{2+} stores within the ER, and contributes to the control of various cellular functions. Among the TRP channels, TRPC subfamily has been suggested to participate in SOC-related Ca^{2+} entry^{74,75}. TRPC1, TRPC4, and TRPC6 have been also implicated in the CaSR triggered elevation of $[\text{Ca}^{2+}]_i$ and keratinocytes differentiation⁷⁶⁻⁷⁹. Knockdown of TRPC1 and TRPC4 in human keratinocytes has been shown to prevent the induction of Ca^{2+} -induced differentiation⁷⁶. It was also demonstrated that activation of TRPC6 with hyperforin induces full differentiation and inhibits proliferation similar to high $[\text{Ca}^{2+}]_{\text{ex}}$ ⁷⁹. Previous studies demonstrated a defective SOC-related Ca^{2+} entry and reduced expression of TRPC1, TRPC4, and TRPC6 in psoriatic keratinocytes. TRPC6 activation was observed to partly restore the disturbed differentiation and proliferation in psoriatic keratinocytes⁸⁰. Furthermore, an up-regulation of TRPC1 was observed in keratinocytes of SERCa2^{+/-} mice and Darier's disease patients and this upregulated TRPC1 was thought to augment cell proliferation and restrict apoptosis. These findings indicate an important role of TRPC channels-induced calcium influx in keratinocyte

differentiation and proliferation⁸¹. However, it has also been demonstrated that ER Ca^{2+} release itself can promote keratinocyte differentiation, suggesting that keratinocyte differentiation is regulated by increased $[\text{Ca}^{2+}]_i$ via both Ca^{2+} release from intracellular stores, such as the ER and Ca^{2+} influx mechanisms.

TRP channels and skin barrier homeostasis

Denda et al.⁸² has demonstrated that several TRP channels such as TRPV1, TRPV4, and TRPA1 are involved in the regulation of epidermal permeability barrier homeostasis. They found that thermal (at 42°C) or pharmacological activation of TRPV1 (capsaicin) delayed barrier recovery, whereas thermal pharmacological activation of TRPV4 (4 α -Phorbol 12,13-didecanone) accelerated barrier recovery, suggesting that TRPV1 and TRPV4 play important roles in skin permeability barrier homeostasis. A later study reported that a TRPV1 inhibitor compound, PAC-14028 improved epidermal barrier function in Dermatophagoides farina-and hapten-induced atopic dermatitis murine models⁸³. TRPV4 that is activated by moderate heat (> 30°C), hypo-osmolarity, and inflammatory metabolites has been demonstrated to regulate skin barrier formation. TRPV4-deficient mice showed the impaired epidermal barrier and it was demonstrated that TRPV4 is functionally co-expressed and interacts with β -catenin and E-cadherin, the crucial components linking adherens junctions and the actin cytoskeleton, thereby enhancing the formation of the epidermal TJ barrier⁸⁴⁻⁸⁶. Further, pharmacological activation of TRPV4 has shown to strengthen the epidermal tight-junction barrier⁸⁷. Other TRP channels, TRPA1 (below 17°C) and TRPM8 (below 22°C), which are expressed in keratinocytes and activated by low temperature have been demonstrated to play a role in epidermal barrier homeostasis. Denda et al.⁸⁸ have demonstrated that brief exposure to cold (10°C to 15°C) or pharmacological activation of TRPA1 (allyl isothiocyanate or cinnamaldehyde) accelerated barrier recovery. They later found that exposure to low temperature (< 22°C) induced elevation of intracellular calcium in cultured human keratinocytes and topical application of TRPM8 agonists (menthol and WS 12) accelerated barrier recovery *in vivo*⁸⁹. These findings indicate that modulation of TRP channels can be a therapeutic approach for skin diseases with barrier impairment such as atopic dermatitis and psoriasis.

TRP channels in keratinocytes and itch

Although many TRP channels in sensory nerve contribute to itch, TRPV3 and TRPV4 which are mainly expressed in keratinocytes, at much higher levels than those seen in neurons, have been suggested to be involved in non-hista-

minergic itch. The characteristic severe itch found in mice and humans with TRPV3 gain-of-function mutations strongly suggest the involvement of TRPV3 in keratinocytes in the production and transduction of itch signal possibly through the release of itch mediators to activate neurons in dorsal root ganglia. A number of candidate mediators such as prostaglandin E₂ (PGE₂), ATP, nerve growth factor, and thymic stromal lymphopoietin (TSLP) have been demonstrated to be released by TRPV3 activation on keratinocytes⁹⁰⁻⁹³. TRPV4 is an osmoreceptor in the skin and has been shown to be functionally required to generate dry skin-associated itch in mice. It was also found that TRPV4 mediate serotonin-evoked itch and 5-hydroxytryptamine signal is required for TRPV4-dependent chronic itch conditions⁹⁴. These findings suggest a role of TRPV3 and TRPV4 in the link between keratinocyte calcium signal, epidermal barrier, and itch.

SOCE: ORAI1 IN KERATINOCYTE DIFFERENTIATION/PROLIFERATION AND BARRIER HOMEOSTASIS

Gating of the Ca^{2+} release-activated Ca^{2+} (CRAC) channel is a classical instance of store-operated Ca^{2+} entry and recently Vandenberghe et al.⁹⁵ identified that Orai1 is the main component of the store-operated current in human keratinocytes. Orai1 is activated by STIM1, the Ca^{2+} sensor of the ER. Upon ER calcium store depletion, STIM1 senses the ER Ca^{2+} reduction, followed by a local redistribution at sites of ER-plasma membrane apposition and subsequently recruits Orai1 to ER-plasma membrane contacts, where Ca^{2+} enters the cell through the opened Orai1 channels. Vandenberghe et al.⁹⁵ also found that Orai1 is predominantly expressed in the basal layer of human epidermis and plays a critical role in the control of keratinocyte proliferation and polarized motility by enhancing focal adhesion turnover. Orai1 has shown to constitutively inhibit terminal keratinocyte differentiation. The keratinocytes from Orai1 knockout mice have also been shown as exhibit remarkably decreased migration, proliferation, and impaired differentiation, yielding impaired epidermis formation. Later, Darbellay et al.⁹⁶ demonstrated that activation of Orai1 channel by SERCA inhibitor BHQ, which causes passive Ca^{2+} releases from the ER stimulates human keratinocyte proliferation and reverses corticosteroid-induced skin atrophy, suggesting that topical modulation of Orai1-mediated calcium influx can be a strategy to stimulate epidermal proliferation. The authors recently demonstrated that Orai1 is induced by ultraviolet B (UVB) in keratinocytes and plays a critical role in UVB-induced change such as epithelial proliferation, differentiation, bar-

rier homeostasis and induction of TSLP and cyclooxygenase 2 in murine skin (not published yet).

Both Orai1 and TRPC channels are involved in the activation of store-operated Ca^{2+} entry in keratinocytes, however TRPC channels trigger keratinocytes differentiation, whereas Orai1/STIM1-mediated Ca^{2+} entry induces proliferation with suppressed terminal differentiation. The different effect of these two calcium channels-mediated SOCE on keratinocytes can be explained by the different distribution in epidermis. Orai1 is expressed mainly in the basal layer and TRPC channels are expressed more differentiated layers, thus the keratinocyte response to SOCE through these two channels might be different.

Furthermore, Wilson et al.⁹⁷ demonstrated that Orai1 channel-mediated Ca^{2+} influx stimulate TSLP release from keratinocytes via the NFAT signaling and Orai1 channel is required for protease-activated receptor 2-evoked SOCE and TSLP secretion by keratinocytes, suggesting a role Orai1 in inflammation and itch in atopic dermatitis.

VGCC: L-TYPE CALCIUM CHANNEL IN SKIN BARRIER HOMEOSTASIS

VGCC is categorized into the subtypes, L, P/Q, N, and R type. The L-type calcium channel, a high-voltage activated family of voltage-dependent calcium channel, is originally assumed to be expressed in excitable cells, but earlier studies have shown that the changes in skin surface electric potential regulate barrier homeostasis and the L-type channel blockers such as verapamil and nifedipine reversed the high extracellular calcium-induced delayed barrier recovery, suggesting the existence of L-type calcium channel in the epidermis^{4,98}. Later, Denda et al.⁹⁹ demonstrated the existence of functional L-type calcium channel in epidermal keratinocytes and their role in skin barrier homeostasis.

CONCLUDING REMARKS

In conclusion, epidermal calcium gradient, ER calcium homeostasis, and calcium influx through TRP channels, Orai1, or VGCCs play a crucial role in keratinocyte differentiation, barrier formation, wound healing, and skin barrier homeostasis. In addition, keratinocytes by expressing numerous calcium channels can act as a biosensor that mediates, processes, or transmits the sensory signal in response to various physical or chemical stimuli. From the therapeutic perspective, it is of great importance to reveal the regulatory mechanisms and functions of calcium and related channels in skin barrier homeostasis.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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