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Role of the calcium-sensing receptor in calcium regulation of epidermal differentiation and function

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Abstract

The epidermis is a stratified squamous epithelium composed of proliferating basal and differentiated suprabasal keratinocytes. It serves as the body's major physical and chemical barrier against infection and harsh environmental insults, as well as preventing excess water loss from the body into the atmosphere. Calcium is a key regulator of the proliferation and differentiation in keratinocytes. Elevated extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) raises the levels of intracellular free calcium ($[Ca^{2+}]_i$), promotes cell-cell adhesion, and activates differentiation-related genes. Keratinocytes deficient in the calcium-sensing receptor fail to respond to $[Ca^{2+}]_o$ stimulation and to differentiate, indicating a role for the calcium-sensing receptor in transducing the $[Ca^{2+}]_o$ signal during differentiation. The concepts derived from *in vitro* gene knockdown experiments have been evaluated and confirmed in three mouse models *in vivo*.

Keywords

calcium-sensing receptor; keratinocyte differentiation; permeability barrier; intracellular free Ca^{2+} ($Ca^{2+}{}_i$); E-cadherin

Introduction

The epidermis consists of multiple layers of keratinocytes, which differentiate and produce a permeability barrier that provides protection against desiccation and xenobiotic penetration. Extracellular calcium ($Ca^{2+}{}_o$) is essential for initiating keratinocyte differentiation and maintaining epidermal functions (1, 2). Elevating the extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) triggers an increase in the level of intracellular free Ca^{2+} ($[Ca^{2+}]_i$) (3) and induces cell-cell adhesion (2), two key signaling events promoting keratinocyte differentiation. The increased $Ca^{2+}{}_i$ level is due to Ca^{2+} release from internal stores and Ca^{2+} influx through channels in the plasma membrane (4). Raising $[Ca^{2+}]_o$ also induces E-cadherin-mediated cell-cell adhesion by activating RhoA GTPase and Src/Fyn tyrosine kinase signaling pathways (5, 6). The E-cadherin-mediated cell adhesion recruits and activates phosphatidylinositol 3-kinase (PI3K) and phospholipase C (PLC) γ1 (7, 8), important

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regulators for cell survival and differentiation. Recent studies have begun to unveil the mechanisms transducing $[Ca^{2+}]_o$ signals to cellular responses in keratinocytes.

The $[Ca^{2+}]_o$-sensing receptor (CaSR) in keratinocytes (9) not only localizes on the cell membrane to detect changes in $[Ca^{2+}]_o$, but also regulates intracellular Ca^{2+} handling by interacting with other modulators of intracellular Ca^{2+} stores and membrane channels (10). Inhibiting CaSR expression *in vitro* markedly suppresses $[Ca^{2+}]_i$ responses to $[Ca^{2+}]_o$ and impairs cell differentiation by reducing intracellular Ca^{2+} pools (10), and blocking E-cadherin-mediated signaling (11). Abrogating CaSR *in vivo* perturbs the epidermal $[Ca^{2+}]_o$ gradient and compromises differentiation and barrier functions (12).

A. Epidermal differentiation and permeability barrier

The mammalian epidermis is a highly organized, stratified squamous epithelium consisting of basal, spinous, granular, and cornified keratinocyte layers. After mitosis in the basal layer, keratinocytes differentiate progressively across the epidermis toward the stratum corneum (SC). Each layer is defined by characteristic morphology and biochemical features indicative of its state of differentiation (13).

a. Cornified envelope, epidermal sphingolipids, and permeability barrier

Keratinocytes at each differentiation stage express distinctive marker genes. Keratin 5 (K5) and 14 (K14) are predominantly expressed by basal keratinocytes. Markers of early differentiation, Keratin 1 (K1) and 10 (K10) are made in spinous cells (14). The cornified envelope (CE) precursors involucrin and transglutaminase 1 (TG1), the enzyme responsible for the ε-(γ-glutamyl)-lysine cross-linking of these protein substrates into the insoluble CE, are also present in the spinous layer (15, 16). Late differentiation markers including the CE protein loricrin (17), and the precursor of the keratin cross-linking protein filaggrin (18), are expressed in granular keratinocytes. In corneocytes, involucrin, loricrin and other structural proteins are extensively cross-linked by TG1 to form the CE (19). The CE serves not only as a mechanical barrier but also as a scaffold necessary for the organization of secreted lipids into lamellar membrane structures and as a repository of antimicrobial peptides providing the first defense against infectious organisms.

The function of the epidermal barrier in mammalian SC resides primarily in the extracellular lipid domains between the outermost, enucleated corneocytes. Ceramides (Cer), cholesterol, and free fatty acids (FFA) are organized into the extracellular lamellar membrane structures (20). Precursor lipids, including glucosylceramide (GlcCer), sphingomyelin, glycerophospholipids, and cholesterol sulfate, are packaged into LB within the upper granular keratinocyte layer by ABC transporter proteins localized in the membranes of the LB (21). Fusion of the LB with the apical plasma membrane in the uppermost SG keratinocytes allows extrusion of lipid precursors into the extracellular space (22). Subsequent enzymatic processing of precursor lipids generates the major lipids (i.e., Cer, cholesterol, and FFAs) essential for effective permeability barrier formation (20).

Mammalian SC also functions as an antimicrobial barrier. Three lipids (FFAs, GlcCer, and sphingosine, a product of Cer hydrolysis) exhibit strong antibacterial and antiviral activity (23, 24). Human epidermis expresses two major families of small, highly hydrophobic AMPs, the β-defensins, hBD 1–4, and cathelicidin, hCAP-18. The C-terminal cleavage product of hCAP18, LL-37, and hBD2 are co-packaged along with lipids within LBs (25, 26) before their secretion into the extracellular lipid matrix in SC membrane domains. Although the constitutive expression of both of these AMPs is low, an upregulated innate immune system consisting of epidermal AMPs, Toll-like receptors (TLRs), and chemokines forms the next level of the cutaneous antimicrobial defense (27).

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b. Epidermal calcium gradient

Ion capture cytochemistry and proton-induced X-ray emission analyses have demonstrated that calcium forms a steep gradient in the human and mouse epidermis, increasing from the stratum basale (SB) to the outer SG, where it reaches its maximum, then decreasing in the SC (28, 29). Calcium level in the SC is very low partly because those relatively desiccated cells are not able to dissolve the ions. Recent fluorescence-lifetime imaging microscopy studies suggested that the bulk of Ca^{2+} measured in the epidermis comes from intracellular Ca^{2+} stores (i.e., ER and Golgi), with $[Ca^{2+}]_o$ making little contribution to the epidermal calcium gradient (30). This method enables calcium concentrations to be calculated, and they range from less than 3μM in the SC to over 20μM in the SG and dermis (30). Formation of the Ca^{2+} gradient coincides with key developmental milestones of barrier formation and development of SC (31). Skin diseases characterized by an abnormal barrier, such as essential fatty acid deficiency and psoriasis, are accompanied by a loss of the calcium gradient (32). Perturbation in permeability barrier by tape stripping or ionophoresis leads to a decline in calcium levels in the outer epidermis and disruption of the epidermal calcium gradient, causing increased LB secretion and cell proliferation with disarray of the differentiated cell layers (33). Re-establishment of the normal calcium gradient in 6–24 hours after barrier disruption corresponds with barrier recovery (1). Though the factors that establish and maintain the calcium gradient are as yet unidentified; these observations support a pivotal role for calcium in epidermal differentiation *in vivo*.

B. Calcium-induced keratinocyte differentiation

Primary cultures of human and mouse keratinocytes are an excellent *in vitro* model for studying epidermal differentiation, as they recapitulate the steps of cell differentiation in the epidermis (34). When cultured at a low $[Ca^{2+}]_o$ (<0.07 mM), epidermal keratinocytes proliferate rapidly and express the phenotype of basal cells. Raising $[Ca^{2+}]_o$ above 0.1 mM (calcium switch) promotes cell differentiation as indicated by the formation of adherens junctions (AJ) and desmosomes, along with stratification and cornification of cell layers (35). Changes in gene expression in cultured keratinocytes occur hours after the calcium switch with a defined temporal order, first the K1 and K10, followed by involucrin and TG1, and finally profilaggrin and loricrin (36), reflecting the sequence of cell differentiation in the epidermis.

The calcium switch also activates phospholipase C family members (PLC) and increases phosphoinositide metabolism (37). PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) to diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3), which activate protein kinase C (PKC) and mobilize intracellular Ca^{2+} (38), respectively. In conjunction with increases in $[Ca^{2+}]_i$, elevated $[Ca^{2+}]_o$ rapidly induces cadherin-mediated cell-cell adhesion between neighboring cells (39). E-cadherin-mediated intercellular adhesion recruits and activates downstream signaling molecules, such as phosphatidylinositide 3-kinase (PI3K), Akt, and PLCγ1, which are critical for maintaining proper keratinocyte differentiation and cell survival (40, 41).

a. $[Ca^{2+}]_i$ signaling in keratinocytes

Elevation of $[Ca^{2+}]_o$ triggers a transient and then a sustained increase in $[Ca^{2+}]_i$ in keratinocytes (3). Raising $[Ca^{2+}]_o$ rapidly increases levels of IP_3 via activation of the PLC pathway (4). IP_3 binds to IP_3R in the intracellular Ca^{2+} stores, triggering a Ca^{2+} release that accounts for the acute, transient phase of $[Ca^{2+}]_i$ increase (42). ER is generally considered as the major agonist-sensitive intracellular Ca^{2+} store in most cell types (43). In keratinocytes, Golgi is also a key intracellular Ca^{2+} pool that modulates Ca^{2+} mobilization (44). Golgi uses the secretory pathway Ca^{2+}-ATPase (SPCA) to take up Ca^{2+} (45) and IP_3R to release Ca^{2+}

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(46, 47). The role of ER and Golgi in modulating $[Ca^{2+}]_i$ signaling and epidermal differentiation is illustrated by two skin disorders, Darier's disease (DD) and Hailey-Hailey disease (HHD) (48). These diseases are caused by inactivating mutations in ATP2A2 and ATP2C1, genes that encode the sarco/endoplasmic reticulum Ca^{2+}-ATPase 2 (SERCA2) and the SPCA1, respectively. Keratinocytes from the patients' skin manifest dysregulated cell adhesion and differentiation due to the loss of the ability of ER and Golgi to store Ca^{2+} (48).

The sustained phase of Ca^{2+} mobilization induced by elevated $[Ca^{2+}]_o$ is likely due to increased Ca^{2+} influx through Ca^{2+} release-activated Ca^{2+} entry mediated by the store-operated channels (SOCs). These are activated by the depletion of intracellular Ca^{2+} stores and activation of IP_3R (49). In keratinocytes, SOC activity is mediated by the canonical transient receptor potential channels 1 and 4 (TRPC1 and 4) (50, 51). The SOC-mediated Ca^{2+} entry may further promote Ca^{2+} influx through the voltage-independent nonselective cation channels regulated by a Ca^{2+}/calmodulin mechanism (52) and/or Ca^{2+}-sensitive chloride channels via hyperpolarizing the plasma membrane (53). Blocking the rise in $[Ca^{2+}]_i$ with $Ca^{2+}{}_i$ chelators such as BAPTA or blocking Ca^{2+} influx with the channel blocker La^{3+} prevents $[Ca^{2+}]_o$-induced keratinocyte differentiation (54, 55). Furthermore, ATP which triggers only a transient increase in $[Ca^{2+}]_i$ fails to induce differentiation (56), demonstrating that a sustained increase in $[Ca^{2+}]_i$ is essential for keratinocyte differentiation. Activation of PLCγ1 via a Src-family kinase-dependent PI3K-mediated mechanism is required for the prolonged increase in $[Ca^{2+}]_i$ (41), since direct interaction of the TRPC1 channel with PLCγ1 is essential for initiating SOC-mediated Ca^{2+} influx (50). Inhibition of PLCγ1 function or expression blocks the $[Ca^{2+}]_o$-induced sustained increases in $[Ca^{2+}]_i$ and cell differentiation (57). In contrast, the initial acute surge in IP_3 and $[Ca^{2+}]_i$ after the calcium switch is likely mediated by PLCβ, as it is blocked by the general PLC inhibitor U73122 but is not deterred by PLCγ1 knockdown (Xie and Bikle, unpublished observation).

b. E-cadherin-mediated cell-cell adhesion

Another $[Ca^{2+}]_o$-induced cellular response in keratinocytes is the development of intercellular attachments through desmosomes and AJs, processes that are mediated by adhesion molecules of the cadherin family (58). Particularly, E-cadherin-mediated adhesion plays a key role in maintaining the tissue integrity and differentiation of epidermal keratinocytes (59, 60). Whereas distribution of P-cadherin is restricted to the SB, E-cadherin is the predominant AJ cadherin in suprabasal layers (61). In epithelial cells, raising $[Ca^{2+}]_o$ stimulates the binding of E-cadherin to its counterpart on the surface of neighboring cells, and its interactions with β- (or γ-), α-, p120-catenins, and the actin cytoskeleton to form AJs (58). These interactions stabilize AJs and support cell stratification.

Activated E-cadherin initiates signaling responses that are crucial for cell survival and differentiation (58). Through interactions with β- (and/or γ-) and p120-catenin, PI3K is recruited to the E-cadherin/catenin complex at the cell membrane (8, 40), where PI3K is activated resulting in conversion of PIP_2 to phosphatidylinositol 3,4,5-triphosphate (PIP_3). PIP_3 in turn activates PLCγ1 (41), which stimulates Ca^{2+} release from stores and influx through SOC channels. In addition, phosphatidylinositol-4-phosphate 5-kinase 1α (PIP5K1α) is recruited by the E-cadherin-catenin complex to the plasma membrane where it converts PIP to PIP_2 providing the substrate for both PI3K and PLCγ1 (62). Though keratinocyte intercellular adhesion is independent of the level of $[Ca^{2+}]_i$ (11), the E-cadherin-dependent signaling cascade is critical for $[Ca^{2+}]_o$-induced generation of the second messengers IP_3 and $[Ca^{2+}]_i$. PI3K also activates the downstream effector Akt to promote cell differentiation and survival (7, 40). Inactivating E-cadherin function prevents $[Ca^{2+}]_o$-induced activation of PI3K and keratinocyte differentiation (7, 8).

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Rho-like GTPase (63) and Src-family tyrosine kinases (5) are two major families of regulators of E-cadherin-mediated cell-cell adhesion in keratinocytes. Src-family tyrosine kinases phosphorylate β-, γ- and p120-catenin after $[Ca^{2+}]_o$ stimulation to promote their interaction with E-cadherin (5). In mouse keratinocytes, $[Ca^{2+}]_o$ selectively activates Fyn kinase and induces its association with the E-cadherin/catenin complex at the cell membrane (6). Tyrosine phosphorylation of catenins, cell-cell adhesion, and cell differentiation are compromised in Fyn-deficient keratinocytes (5). Rho-GTPases are upstream mediators of Fyn in regulating cell-cell adhesion in keratinocytes (6). Whereas Rac1 facilitates the actin-cytoskeleton reorganization, RhoA is required for E-cadherin clustering at cell-cell junctions (63). Raising $[Ca^{2+}]_o$ activates RhoA and induces its translocation from cytosol to the cell-cell contacts. Inactivating RhoA blocks the formation of AJs and keratinocyte differentiation (6, 64). Blocking RhoA activity prevents the prolonged increase in $[Ca^{2+}]_i$ level following the calcium switch, although the initial rise in $[Ca^{2+}]_I$ remains intact (64) consistent with the concept that different mechanisms are involved in the acute vs. prolonged rise in $[Ca^{2+}]_i$ following the calcium switch, the latter necessary for differentiation and requiring the E-cadherin signaling pathway.

c. Protein kinase C-mediated regulation of keratinocyte differentiation

The rise in DAG and $[Ca^{2+}]_i$ following the calcium switch leads to PKC activation (65).4 Human and mouse epidermis express PKCα, δ, ε, η and ζ, but only PKCα and δ respond to calcium (66). The activation of involucrin, loricrin, and profilaggrin genes after the calcium switch is accompanied by translocation of PKCα from the cytosol to the plasma membrane (66). Blocking PKCα or PKCδ expression inhibits $[Ca^{2+}]_o$-induction of these differentiation genes (65, 67).

The effect of PKCs on keratinocyte differentiation is in part mediated by the activator protein-1 (AP-1) transcription factors, which include Fos and Jun families. Genes encoding involucrin and loricrin have AP-1 responsive elements in their promoter regions (68, 69). PKC activation results in a rapid increase in expression of c-Fos and c-Jun and the occupying of AP-1 binding sites (70). Other members of the Fos and Jun families, with either stimulatory or inhibitory functions, are also present in keratinocytes and are involved in transcriptional regulation of differentiation related genes. JunB, JunD, c-Fos and Fra1 are found to bind both of the proximal and distal AP-1 sites in the involucrin gene promoter region (70). Gel-shift analyses demonstrate a strong binding of the proximal AP1 site by JunB and Fra1 in proliferating keratinocytes. As keratinocytes differentiate, c-Fos replaces JunB and Fra1 to stimulate involucrin expression (71). These findings demonstrate that PKCs are able to differentially regulate the same target genes via controlling the expression of various AP-1 factors.

C. Roles of the calcium-sensing receptor (CaSR) in keratinocyte differentiation

The $[Ca^{2+}]_i$ response to $[Ca^{2+}]_o$ in keratinocytes resembles that in parathyroid cells, which sense $[Ca^{2+}]_o$ via the CaSR (72). The ability of a selective activator of the CaSR, NPS R-467, to increase the $[Ca^{2+}]_i$ response to $[Ca^{2+}]_o$ and to activate the differentiation marker genes (i.e. involucrin and TG1) in keratinocytes supports the notion that CaSR mediates $[Ca^{2+}]_i$ signaling during keratinocyte differentiation (73). The keratinocyte CaSR is identical to that expressed in the parathyroid gland and kidney (9). Inactivation of CaSR in keratinocytes blunts the $[Ca^{2+}]_i$ response to $[Ca^{2+}]_o$, blocks the calcium-dependent inhibition of cell proliferation and prevents calcium-stimulated differentiation (74).

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a. Expression of CaSR in keratinocytes

Keratinocytes produce two variants of the CaSR: the full-length CaSR and a smaller alternatively spliced variant lacking exon 5, CaSRalt. The amino acid residues 460 to 536 in the extracellular domain are lost in the CaSRalt. The full-length CaSR proteins are expressed as two major forms, 140-kDa and 160-kDa, whereas the CaSRalt is expressed as a single protein of 130-kDa (9). Deglycosylation analyses revealed that the full length CaSR has two different N-glycosylated oligosaccharide chains whereas the CaSRalt has only high mannose type oligosaccharide chains (9). The change of glycosylation pattern in the CaSRalt is probably attributed to the deletion of the 2 N-glycosylation sites at Asn468 and Asn488 within exon 5. When exogenously expressed in keratinocytes, only the full-length CaSR is able to mediate calcium-stimulated inositol phosphate (IP) production and transcriptional activation of involucrin and TG1 genes (9). The strictly intracellular localization of CaSRalt (75) suggests the change in glycosylation limits the surface expression of the CaSR spliced variant, leading to its failure to mediate the cellular response to $[Ca^{2+}]_o$. In addition, the CaSRalt moderately reduces the IP response of the full length CaSR to $[Ca^{2+}]_o$ when they are coexpressed in HEK293 cells (9), suggesting that the ratio of the two forms of CaSR may be a factor regulating calcium responsiveness in cells. The full-length CaSR and CaSRalt are expressed differentially during differentiation. Whereas the level of CaSRalt remains relatively unchanged, the level of full-length CaSR gradually decreases in the terminally differentiated keratinocytes (75). This expression pattern of CaSR is consistent with the steady decline in $[Ca^{2+}]_i$ and IP responses to $[Ca^{2+}]_o$ in differentiated cells (9) but is somewhat different from the *in vivo* situation discussed subsequently.

In the parathyroid cells and in HEK293 cells expressing full-length CaSR cDNA, CaSR was extensively expressed on the plasma membrane. However, substantial perinuclear cytoplasmic localization of CaSR is commonly observed in other cell types, i.e. rat chondrogenic RCJ.C5.18 cells (76), mouse osteoblastic MC3T3-E1 cells (77), osteoblasts, articular and growth plate chondrocytes (78), and pancreatic acinar cells (79). In keratinocytes, CaSR localization is predominantly intracellular and to a lesser extent in the plasma membrane (29, 74, 75). It is likely that the CaSR on the cell surface senses changes in $[Ca^{2+}]_o$, but the function of the CaSR in the intracellular compartments needs elucidation.

b. CaSR regulates intracellular Ca^{2+} handling

There are several mechanisms by which CaSR mediates $[Ca^{2+}]_o$-induced increases in $[Ca^{2+}]_i$, thereby promoting differentiation. CaSR in the plasma membrane directly activates PLC, most likely through Gαq (80), in response to elevated $[Ca^{2+}]_o$. PLC in turn generates IP_3, which then releases Ca^{2+} from intracellular stores and stimulates Ca^{2+} influx. Inactivation of CaSR in keratinocytes by transfecting a full-length antisense CaSR cDNA construct greatly reduces Ca^{2+} release in response to elevated $[Ca^{2+}]_o$ (10, 74). This is a consequence of a profound depletion of Ca^{2+} pools in cells lacking the CaSR, as indicated by the reduced amount of Ca^{2+} released by $[Ca^{2+}]_o$, ionomycin, or thapsigargin (10). Consistent with its effect to deplete intracellular Ca^{2+} stores, inhibition of CaSR expression also augments the SOC-mediated Ca^{2+} influx (10). This result is probably a mechanism compensating for the extended loss of Ca^{2+} from internal stores. Moreover, CaSR null keratinocytes demonstrate increased calcium accumulation by ER and Golgi most likely due to their reduced calcium stores and/or loss of regulation by CaSR. These are acute responses. The data showing increased SOC-mediated calcium influx and accumulation by ER and Golgi in the absence of CaSR but overall loss of intracellular calcium stores suggests that CaSR enables calcium entry through channels that we have not yet identified, but which are important for the maintenance of the calcium stores.

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Similar impairments in $[Ca^{2+}]_o$-stimulated $[Ca^{2+}]_i$ responses are exhibited in keratinocytes from HHD patients that have reduced Ca^{2+} levels in their Golgi because of mutations in SPCA1 (44). The shared characteristics of CaSR-deficient and SPCA1-deficient keratinocytes in Ca^{2+} handling suggest that the CaSR and SPCA1 are likely parts of an apparatus that transduces $[Ca^{2+}]_o$ signals into cellular responses in keratinocytes. Fluorescence immunostaining and co-immunoprecipitation studies reveal that CaSR co-localizes and forms a protein complex with several $[Ca^{2+}]_i$ modulators, i.e. PLCγ1, IP_3R and SPCA1, in the *trans*-Golgi (10). Since PLCγ1 and IP_3R are crucial for instigating both Ca^{2+} release from stores and Ca^{2+} entry through membrane channels (50, 81), while SPCA1 is responsible for refilling the intracellular Ca^{2+} stores (44), the CaSR may serve as a coordinator of these signaling events due to its intrinsic ability to sense $[Ca^{2+}]_o$ and possibly the Ca^{2+} level in the lumen of intracellular stores.

c. CaSR-dependent regulation of E-cadherin signaling and cell-cell adhesion

Besides the initial Ca^{2+} release via PLC activation, the CaSR sustains increases in $[Ca^{2+}]_i$ levels via E-cadherin-mediated signaling (11). CaSR regulates E-cadherin-mediated cell-cell adhesion through the Rho/Fyn-dependent pathway as previously described. Inhibiting CaSR expression blocks the $[Ca^{2+}]_o$-induced activation of Rho and Fyn, the tyrosine phosphorylation of β-, γ-, and p120-catenin, the formation of E-cadherin/catenin complex, and activation of PI3K (11).

In keratinocytes, the transduction of $[Ca^{2+}]_o$ signals to the downstream Rho pathway requires the physical interaction of the C-terminal region of CaSR with an actin-binding protein filamin A (64). Filamin A is known to interact with intracellular signaling proteins including the Rho-like GTPase, guanine nucleotide exchange factor (GEF), Rho kinase, and MAPK (82). Filamin A also directly interacts with a variety of transmembrane proteins including β integrins, Ca^{2+} and K^+ channels, and CaSR (82). In keratinocytes, raising $[Ca^{2+}]_o$ stimulates interactions among CaSR, filamin A, RhoA, and E-cadherin at the cell membrane and facilitates CaSR signaling through the Rho A pathway (64). Disrupting CaSR-filamin interaction by expressing a dominant-negative filamin A or a CaSR C-terminus peptide inhibits the membrane localization of CaSR, $[Ca^{2+}]_o$-activation of RhoA, and consequently the E-cadherin-mediated cell-cell junctions (64). $[Ca^{2+}]_o$-activation of RhoA in keratinocytes also involves Trio, a Rho-GEF that binds directly with filamin (Tu and Bikle, unpublished observation). These findings demonstrate that through forming a signaling complex with filamin, GEF, and Rho the CaSR elicits the $[Ca^{2+}]_o$-activation of the E-cadherin-mediated pathway required for keratinocyte differentiation.

d. Effect of CaSR abrogation in keratinocyte proliferation, differentiation and cell death

Raising $[Ca^{2+}]_o$ inhibits cell proliferation and promotes differentiation in keratinocytes, but inactivation of CaSR blocks these calcium-dependent processes. The inability of intracellular Ca^{2+} stores to properly handle Ca^{2+} mobilization in the CaSR-deficient keratinocytes profoundly inhibits the ability of $[Ca^{2+}]_o$ to raise $[Ca^{2+}]_i$ (64). The ineffectual RhoA/Fyn signaling after CaSR knockdown fails to stabilize the E-cadherin/catenin adhesion complex and to activate PI3K and PLCγ1 (11). Consequently, the basal $[Ca^{2+}]_i$ is decreased, the transcriptional activation of involucrin and TG1 promoters by $[Ca^{2+}]_o$ is blocked (10, 74), and the $[Ca^{2+}]_o$-stimulated protein expressions of late differentiation genes involucrin, TG1, loricrin, profilaggrin, and caspase 14 are greatly reduced in these cells (11). Moreover, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining shows that knockdown of CaSR significantly increases the number of apoptotic cells in cultured keratinocytes (11).

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D. Calcium-sensing receptor regulates epidermal functions

Immunocytochemistry and *in situ* hybridization confirmed the expression of CaSR message and proteins in mammalian skin in the interfollicular suprabasal keratinocyte layers with little CaSR in the basal layer (29, 83). The level of CaSR expression increases as cell differentiation continues across the epidermis, with the strongest expression in the cells of the upper SG (29), whereas the corneocytes display weak staining. Like the distribution of CaSR in cultured keratinocytes, most of the CaSR proteins in epidermal layers localize intracellularly (29). Overall, the expression of CaSR closely parallels the Ca^{2+} gradient in the epidermis. The importance of CaSR in the epidermal differentiation is made clear in three animal models: transgenic mice with epidermal overexpression of CaSR, full-length CaSR deficient (*Casr–/–*) mice, and keratinocyte-specific CaSR knockout (*^{Epid}CaSR–/–*) mice.

a. Transgenic mice overexpressing CaSR in the epidermis

To investigate the role of CaSR, Truksen and Troy generated transgenic mice with constitutive overexpression of CaSR in the basal keratinocytes in the epidermis (84). The CaSR transgenic mice manifest advanced differentiation and epidermal permeability barrier formation during embryologic development and accelerated hair growth at birth. Several morphological and biochemical changes are found in the epidermis from these mice. Suprabasal keratinocyte layers are expanded as cells become hypertrophic, whereas the basal layer remains unaltered. The granular keratinocytes exhibit an increase in keratohyalin granules, consistent with an upregulated filaggrin expression. Furthermore, the levels of early (keratin K1) and late (small proline-rich protein1A, involucrin, loricrin and filaggrin) differentiation markers, as well as the hyperproliferation marker keratin 6, are significantly augmented in the transgenic epidermis. The upregulation of EGF and non-canonical Wnt signaling pathways in the CaSR transgenic epidermis suggest that an enhanced cross talk between CaSR and other signaling pathways may contribute to the changes in epidermal differentiation.

b. The full-length CaSR deficient (*Casr –/–*) mice

A global CaSR "knockout" (*Casr–/–*) mouse model was developed by Ho *et al* to assess CaSR functions *in vivo* (85). In *Casr–/–* mice the CaSR gene is disrupted by insertion of a neomycin resistance gene into exon 5, leading to a complete loss of the full-length CaSR expression. The homozygous knockout mice die shortly (mostly 5-7 days) after birth with a phenotype of neonatal severe hyperparathyroidism, and heterozygous knockout mice manifest the phenotypes of familial benign hypocalciuric hypercalcemia (85), confirming a role for the CaSR in the kidney and parathyroid glands. However, immunocytochemistry, Western analyses, and *in situ* hybridization detect the continuous expression of CaSRalt in these animals (83, 86), which may compensate for the loss of the full-length CaSR in tissues outside of parathyroid and kidney (86).

Nevertheless, the *Casr–/–* mice manifest various epidermal abnormalities. *Casr–/–* epidermis shows a modest reduction in the number of nucleated cell layers with abnormal polarity and flattening of basal and suprabasal keratinocytes (83). Proliferating cell nuclear antigen (PCNA) staining indicates a modest increase in the proliferation of the basal layer in *Casr–/–* epidermis (29). Electron microscopy studies demonstrate that the epidermis of *Casr –/–* mice has a looser and less organized SC, an expanded SG/SC interface, abnormal accumulation of large keratohyalin granules and premature LB secretion in the keratinocytes of the lower granular layers (29). Furthermore, the expression levels of differentiation markers loricrin and filaggrin are greatly decreased in the epidermis of *Casr–/–* mice. The epidermis of *Casr–/–* mice exhibits a steeper Ca^{2+} gradient (29), perhaps due to the

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hypercalcemia and hyperparathyroidism in these mice, even though the keratinocytes from *Casr–/–* mice fail to respond to $[Ca^{2+}]_o$ with a significant rise in $[Ca^{2+}]_i$ (83). These results indicate that the full-length CaSR is required for normal epidermal differentiation and that this function cannot be replaced with the alternatively spliced CaSR.

c. The keratinocyte-specific CaSR knockout (^{Epid}*CaSR–/–*) mice

The early death of the *Casr–/–* mice precludes detailed analysis of the skin phenotype in adults. Also, it is unclear whether the epidermal defects in *Casr–/–* mice are strictly due to the lack of $[Ca^{2+}]_o$-sensing in keratinocytes or are caused by severe metabolic disturbances. To address these issues, we have generated a keratinocyte-specific CaSR knockout (^{Epid}*CaSR–/–*) mouse (12) by breeding a floxed CaSR (CaSRfl/fl) mouse line (87) with mice expressing Cre recombinase under the control of the human K14 promoter (88). In ^{Epid}*CaSR–/–* mice, exon 7 of the CaSR gene encoding the entire transmembrane domain and intracellular portion of the receptor is deleted in the keratinocytes in the epidermal basal layer. These mice have no overt growth phenotype or metabolic derangement (12).

Ion capture cytochemistry reveals that the epidermal Ca^{2+} gradient is lost in the ^{Epid}*CaSR–/–* mice (12). Although ^{Epid}*CaSR–/–* epidermis displayed relatively normal organization of the nucleated cellular layers, cell proliferation is increased and the expression levels of mid to late differentiation markers are significantly decreased (12). Deletion of CaSR also causes marked reductions in the number of lamellar bodies in the keratinocytes in the upper SG and their secretion at the SG/SC junction in ^{Epid}*CaSR–/–* epidermis (12). The levels of the lamellar body lipid transporter and sphingolipid processing enzymes are decreased in the ^{Epid}*CaSR–/–* epidermis, whereas the sphingolipid biosyntheses are essentially normal. As a result, ^{Epid}*CaSR–/–* epidermis manifested thinner corneocyte lipid envelopes (12). The formation of the epidermal permeability barrier is normally completed by E17.5 during embryonic development (89); however, it is delayed in the ^{Epid}*CaSR–/–* mice (12). The integrity of the epidermal permeability barrier in the adult ^{Epid}*CaSR–/–* mice is compromised due to the defects in CE and lipid lamellar bilayers. The permeability barrier defects in ^{Epid}*CaSR–/–* epidermis are especially aggravated in mice subjected to dietary Ca^{2+} restriction. Under such a condition, ^{Epid}*CaSR–/–* mice display a clear retardation in the recovery of the permeability barrier after barrier perturbation and a down-regulated innate immune response to wounding (12). These phenotypes in ^{Epid}*CaSR–/–* skin are consistent with a continuous suppression of epidermal differentiation in these animals (12).

Aligned with the *in vivo* results, epidermal keratinocytes cultured from ^{Epid}*CaSR–/–* mice display blunted $[Ca^{2+}]_I$ response to elevated $[Ca^{2+}]_o$ and reduced intracellular Ca^{2+} pools (12), indicative of abnormal intracellular Ca^{2+} handling. ^{Epid}*CaSR–/–* keratinocytes also manifest defective cell-cell junction formation indicated by the diminished membrane-association of Rho, Fyn with E-cadherin. Additionally, $[Ca^{2+}]_o$-induced expression of late differentiation markers in the ^{Epid}*CaSR–/–* keratinocytes is decreased, verifying that the defect in epidermal differentiation is a direct consequence of CaSR knockout in keratinocytes.

d. Interaction of calcium and 1,25-dihydroxyvitamin D_3 (1,25D_3) in epidermal differentiation

1,25D_3 potentiates differentiation through many of the same pathways as calcium, such as by increasing $[Ca^{2+}]_i$ levels and activating PKC and PLC signaling (90, 91). Calcium and 1,25D_3 synergistically activate transcription of the involucrin promoter that contains an AP-1 binding site necessary for calcium and 1,25D_3 induction and an adjacent vitamin D_3 receptor (VDR)-responsive element (VDRE) (68, 92, 93). 1,25D_3 augments the sensitivity to the prodifferentiating actions of calcium in keratinocytes by increasing the expression of CaSR (94), likely via the VDRE in the CaSR promoter (95). Inhibiting VDR in

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keratinocytes suppresses the expression of CaSR and its translocation to the plasma membrane after calcium stimulation *in vitro* (96). Conversely, dietary Ca^{2+} supplementation has been shown to up-regulate VDR expression in the epidermis of the vitamin D-deficient rat (97). VDR null and 25-hydroxyvitamin D3 1-α-hydroxylase (CYP27B1)-deficient mice share similar epidermal phenotypes as $^{Epid}CaSR{-/-}$ mice: abnormal epidermal Ca^{2+} gradient, decreased LB numbers and secretion, delayed recovery of permeability barrier function, and defective differentiation (12, 98, 99), indicative of important roles for $1{,}25D_3$/VDR as well as calcium/CaSR in keratinocyte differentiation and permeability barrier homeostasis.

Summary

$[Ca^{2+}]_o$ signaling regulates proliferation, differentiation and apoptosis in most, if not all, cell types including keratinocytes. $[Ca^{2+}]_o$ stimulates the keratinocytes to differentiate in large part through the CaSR, which regulates cell survival and keratinocyte differentiation by two major pathways (Figure 1). First, CaSR controls intracellular Ca^{2+} stores and Ca^{2+} handling by interacting with various $[Ca^{2+}]_i$ modulators. Second, via Rho- and Fyn/Src-mediated signaling the CaSR controls the $[Ca^{2+}]_o$-induced formation of E-cadherin/catenin complexes, which underlie cell-cell adhesion and serve as a scaffold for recruiting and activating other signaling molecules critical for differentiation. Inhibiting CaSR expression *in vitro* reduces the $[Ca^{2+}]_o$-induced increase in $[Ca^{2+}]_i$, blocks E-cadherin-mediated cell-cell adhesion and PI3K activation, decreases differentiation, and increases apoptosis. Abrogating CaSR *in vivo* perturbs the epidermal Ca^{2+} gradient, inhibiting differentiation-associated cellular functions including the innate immune response and permeability barrier function. Complementary to the phenotypes of CaSR knockout mice, mice with CaSR over-expression in the epidermis display accelerated epidermal differentiation and permeability barrier formation. These findings demonstrate that the CaSR is required for normal epidermal differentiation and barrier function *in vivo.*

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References

1. Menon GK, Elias PM, Lee SH, Feingold KR. Localization of calcium in murine epidermis following disruption and repair of the permeability barrier. Cell and Tissue Research. 1992; 270:503–512. [PubMed: 1486603]
2. Hennings H, Holbrook KA. Calcium regulation of cell-cell contact and differentiation of epidermal cells in culture. An ultrastructural study. Experimental Cell Research. 1983; 143:127–142. [PubMed: 6186504]
3. Bikle DD, Ratnam A, Mauro T, et al. Changes in calcium responsiveness and handling during keratinocyte differentiation. Potential role of the calcium receptor. The Journal of Clinical Investigation. 1996; 97:1085–1093. [PubMed: 8613532]
4. Lee E, Yuspa SH. Changes in inositol phosphate metabolism are associated with terminal differentiation and neoplasia in mouse keratinocytes. Carcinogenesis. 1991; 12:1651–1658. [PubMed: 1893524]
5. Calautti E, Cabodi S, Stein PL, et al. Tyrosine phosphorylation and src family kinases control keratinocyte cell-cell adhesion. Journal of Cell Biology. 1998; 141:1449–1465. [PubMed: 9628900]
6. Calautti E, Grossi M, Mammucari C, et al. Fyn tyrosine kinase is a downstream mediator of Rho/PRK2 function in keratinocyte cell-cell adhesion. Journal of Cell Biology. 2002; 156:137–148. [PubMed: 11777936]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

7. Calautti E, Li J, Saoncella S, et al. Phosphoinositide 3-kinase signaling to Akt promotes keratinocyte differentiation versus death. The Journal of Biological Chemistry. 2005; 280:32856–32865. [PubMed: 16036919]
8. Xie Z, Bikle DD. The recruitment of phosphatidylinositol 3-kinase to the E-cadherin-catenin complex at the plasma membrane is required for calcium-induced phospholipase C-gamma1 activation and human keratinocyte differentiation. The Journal of Biological Chemistry. 2007; 282:8695–8703. [PubMed: 17242406]
9. Oda Y, Tu CL, Pillai S, Bikle DD. The calcium sensing receptor and its alternatively spliced form in keratinocyte differentiation. The Journal of Biological Chemistry. 1998; 273:23344–23352. [PubMed: 9722568]
10. Tu CL, Chang W, Bikle DD. The role of the calcium sensing receptor in regulating intracellular calcium handling in human epidermal keratinocytes. The Journal of Investigative Dermatology. 2007; 127:1074–1083. [PubMed: 17124506]
11. Tu CL, Chang W, Xie Z, Bikle DD. Inactivation of the calcium sensing receptor inhibits E-cadherin-mediated cell-cell adhesion and calcium-induced differentiation in human epidermal keratinocytes. The Journal of Biological Chemistry. 2008; 283:3519–3528. [PubMed: 18065418]
12. Tu CL, Crumrine DA, Man MQ, et al. Ablation of the calcium-sensing receptor in keratinocytes impairs epidermal differentiation and barrier function. The Journal of Investigative Dermatology. 2012; 132:2350–2359. [PubMed: 22622426]
13. Fuchs E. Epidermal differentiation: the bare essentials. Journal of Cell Biology. 1990; 111:2807–2814. [PubMed: 2269655]
14. Schweizer J, Winter H. Keratin biosynthesis in normal mouse epithelia and in squamous cell carcinomas. mRNA-dependent alterations of the primary structure of distinct keratin subunits in tumors. The Journal of Biological Chemistry. 1983; 258:13268–13272. [PubMed: 6195160]
15. Rice RH, Green H. Presence in human epidermal cells of a soluble protein precursor of the cross-linked envelope: activation of the cross-linking by calcium ions. Cell. 1979; 18:681–694. [PubMed: 42494]
16. Thacher SM, Rice RH. Keratinocyte-specific transglutaminase of cultured human epidermal cells: relation to cross-linked envelope formation and terminal differentiation. Cell. 1985; 40:685–695. [PubMed: 2578891]
17. Hohl D, Lichti U, Breitkreutz D, et al. Transcription of the human loricrin gene in vitro is induced by calcium and cell density and suppressed by retinoic acid. The Journal of Investigative Dermatology. 1991; 96:414–418. [PubMed: 2007780]
18. Lonsdale-Eccles JD, Resing KA, Meek RL, Dale BA. High-molecular-weight precursor of epidermal filaggrin and hypothesis for its tandem repeating structure. Biochemistry. 1984; 23:1239–1245. [PubMed: 6712945]
19. Hohl D. Cornified cell envelope. Dermatologica. 1990; 180:201–211. [PubMed: 2192924]
20. Holleran WM, Takagi Y, Uchida Y. Epidermal sphingolipids: metabolism, function, and roles in skin disorders. FEBS letters. 2006; 580:5456–5466. [PubMed: 16962101]
21. Sakai K, Akiyama M, Sugiyama-Nakagiri Y, et al. Localization of ABCA12 from Golgi apparatus to lamellar granules in human upper epidermal keratinocytes. Experimental Dermatology. 2007; 16:920–926. [PubMed: 17927575]
22. Vielhaber G, Pfeiffer S, Brade L, et al. Localization of ceramide and glucosylceramide in human epidermis by immunogold electron microscopy. The Journal of Investigative Dermatology. 2001; 117:1126–1136. [PubMed: 11710923]
23. Miller SJ, Aly R, Shinefeld HR, Elias PM. In vitro and in vivo antistaphylococcal activity of human stratum corneum lipids. Archives of Dermatology. 1988; 124:209–215. [PubMed: 3341800]
24. Bibel DJ, Aly R, Shinefeld HR. Antimicrobial activity of sphingosines. The Journal of Investigative Dermatology. 1992; 98:269–273. [PubMed: 1545135]
25. Oren A, Ganz T, Liu L, Meerloo T. In human epidermis, beta-defensin 2 is packaged in lamellar bodies. Experimental Molecular Pathology. 2003; 74:180–182. [PubMed: 12710950]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

26. Braff MH, Di Nardo A, Gallo RL. Keratinocytes store the antimicrobial peptide cathelicidin in lamellar bodies. The Journal of Investigative Dermatology. 2005; 124:394–400. [PubMed: 15675959]
27. Lehrer RI. In defense of skin. The Journal of Investigative Dermatology. 2005; 125:viii–ix. discussion x-xi. [PubMed: 15982292]
28. Menon GK, Grayson S, Elias PM. Ionic calcium reservoirs in mammalian epidermis: ultrastructural localization by ion-capture cytochemistry. The Journal of Investigative Dermatology. 1985; 84:508–512. [PubMed: 3998499]
29. Komuves L, Oda Y, Tu CL, et al. Epidermal expression of the full-length extracellular calcium-sensing receptor is required for normal keratinocyte differentiation. Journal of Cell Physiology. 2002; 192:45–54.
30. Celli A, Sanchez S, Behne M, et al. The epidermal Ca^{2+} gradient: Measurement using the phasor representation of fluorescent lifetime imaging. Biophysical Journal. 2010; 98:911–921. [PubMed: 20197045]
31. Elias PM, Nau P, Hanley K, et al. Formation of the epidermal calcium gradient coincides with key milestones of barrier ontogenesis in the rodent. The Journal of Investigative Dermatology. 1998; 110:399–404. [PubMed: 9540982]
32. Menon GK, Elias PM. Ultrastructural localization of calcium in psoriatic and normal human epidermis. Archives of Dermatology. 1991; 127:57–63. [PubMed: 1986708]
33. Menon GK, Elias PM, Feingold KR. Integrity of the permeability barrier is crucial for maintenance of the epidermal calcium gradient. British Journal of Dermatology. 1994; 130:139–147. [PubMed: 8123567]
34. Eckert RL, Crish JF, Robinson NA. The epidermal keratinocyte as a model for the study of gene regulation and cell differentiation. Physiology Review. 1997; 77:397–424.
35. Braga VM, Hodivala KJ, Watt FM. Calcium-induced changes in distribution and solubility of cadherins, integrins and their associated cytoplasmic proteins in human keratinocytes. Cell Adhesion and Communication. 1995; 3:201–215. [PubMed: 8846022]
36. Yuspa SH, Kilkenny AE, Steinert PM, Roop DR. Expression of murine epidermal differentiation markers is tightly regulated by restricted extracellular calcium concentrations in vitro. Journal of Cell Biology. 1989; 109:1207–1217. [PubMed: 2475508]
37. Jaken S, Yuspa SH. Early signals for keratinocyte differentiation: role of Ca2+-mediated inositol lipid metabolism in normal and neoplastic epidermal cells. Carcinogenesis. 1988; 9:1033–1038. [PubMed: 2453303]
38. Berridge MJ. Cell signalling. A tale of two messengers. Nature. 1993; 365:388–389. [PubMed: 8413581]
39. Pece S, Gutkind JS. Signaling from E-cadherins to the MAPK pathway by the recruitment and activation of epidermal growth factor receptors upon cell-cell contact formation. The Journal of Biological Chemistry. 2000; 275:41227–41233. [PubMed: 10969083]
40. Pece S, Chiariello M, Murga C, Gutkind JS. Activation of the protein kinase Akt/PKB by the formation of E-cadherin-mediated cell-cell junctions. Evidence for the association of phosphatidylinositol 3-kinase with the E-cadherin adhesion complex. The Journal of Biological Chemistry. 1999; 274:19347–19351. [PubMed: 10383446]
41. Xie Z, Singleton PA, Bourguignon LY, Bikle DD. Calcium-induced human keratinocyte differentiation requires src- and fyn-mediated phosphatidylinositol 3-kinase-dependent activation of phospholipase C-gamma1. Molecular Biology of the Cell. 2005; 16:3236–3246. [PubMed: 15872086]
42. Berridge MJ. Inositol trisphosphate and calcium signalling. Nature. 1993; 361:315–325. [PubMed: 8381210]
43. Berridge MJ. The endoplasmic reticulum: a multifunctional signaling organelle. Cell Calcium. 2002; 32:235–249. [PubMed: 12543086]
44. Behne MJ, Tu CL, Aronchik I, et al. Human keratinocyte ATP2C1 localizes to the Golgi and controls Golgi Ca2+ stores. The Journal of Investigative Dermatology. 2003; 121:688–694. [PubMed: 14632183]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

45. Callewaert G, Parys JB, De Smedt H, et al. Similar Ca^{2+}-signaling properties in keratinocytes and in COS-1 cells overexpressing the secretory-pathway Ca^{2+}-ATPase SPCA1. Cell calcium. 2003; 34:157–162. [PubMed: 12810057]
46. Pinton P, Pozzan T, Rizzuto R. The Golgi apparatus is an inositol 1,4,5-trisphosphate-sensitive Ca2+ store, with functional properties distinct from those of the endoplasmic reticulum. EMBO Journal. 1998; 17:5298–5308. [PubMed: 9736609]
47. Surroca A, Wolff D. Inositol 1,4,5-trisphosphate but not ryanodine-receptor agonists induces calcium release from rat liver Golgi apparatus membrane vesicles. Journal of Membrane Biology. 2000; 177:243–249. [PubMed: 11014862]
48. Foggia L, Hovnanian A. Calcium pump disorders of the skin. Amerian Journal of Medical Genetics Part C: Seminars in Medical Genetics. 2004; 131C:20–31.
49. Putney JW Jr. Capacitative calcium entry: sensing the calcium stores. Journal of Cell Biology. 2005; 169:381–382. [PubMed: 15866892]
50. Tu CL, Chang W, Bikle DD. Phospholipase cgamma1 is required for activation of store-operated channels in human keratinocytes. The Journal of Investigative Dermatology. 2005; 124:187–197. [PubMed: 15654973]
51. Fatherazi S, Presland RB, Belton, et al. Evidence that TRPC4 supports the calcium selective I(CRAC)-like current in human gingival keratinocytes. Pflugers Archiv. 2007; 453:879–889. [PubMed: 17031666]
52. Fatherazi S, Belton CM, Izutsu KT. Sequential activation of store-operated currents in human gingival keratinocytes. The Journal of Investigative Dermatology. 2003; 121:120–131. [PubMed: 12839572]
53. Denda M, Fuziwara S, Inoue K. Influx of calcium and chloride ions into epidermal keratinocytes regulates exocytosis of epidermal lamellar bodies and skin permeability barrier homeostasis. The Journal of Investigative Dermatology. 2003; 121:362–367. [PubMed: 12880429]
54. Li L, Tucker RW, Hennings H, Yuspa SH. Chelation of intracellular Ca2+ inhibits murine keratinocyte differentiation in vitro. Journal of Cell Physiology. 1995; 163:105–114.
55. Pillai S, Bikle DD. Lanthanum influx into cultured human keratinocytes: effect on calcium flux and terminal differentiation. Journal of Cell Physiology. 1992; 151:623–629.
56. Pillai S, Bikle DD. Adenosine triphosphate stimulates phosphoinositide metabolism, mobilizes intracellular calcium, and inhibits terminal differentiation of human epidermal keratinocytes. The Journal of Clinical Investigation. 1992; 90:42–51. [PubMed: 1321844]
57. Xie Z, Bikle DD. Phospholipase C-gamma1 is required for calcium-induced keratinocyte differentiation. The Journal of Biological Chemistry. 1999; 274:20421–20424. [PubMed: 10400667]
58. Wheelock MJ, Johnson KR. Cadherins as modulators of cellular phenotype. Annual Review of Cell and Developmental Biology. 2003; 19:207–235.
59. Young P, Boussadia O, Halfter H, et al. E-cadherin controls adherens junctions in the epidermis and the renewal of hair follicles. EMBO Journal. 2003; 22:5723–5733. [PubMed: 14592971]
60. Tinkle CL, Lechler T, Pasolli HA, Fuchs E. Conditional targeting of E-cadherin in skin: insights into hyperproliferative and degenerative responses. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101:552–557. [PubMed: 14704278]
61. Furukawa F, Fujii K, Horiguchi Y, et al. Roles of E- and P-cadherin in the human skin. Microscopy Research and Technique. 1997; 38:343–352. [PubMed: 9297684]
62. Xie Z, Chang SM, Pennypacker SD, et al. Phosphatidylinositol-4-phosphate 5-kinase 1alpha mediates extracellular calcium-induced keratinocyte differentiation. Molecular Biology of the Cell. 2009; 20:1695–1704. [PubMed: 19158393]
63. Braga VM, Machesky LM, Hall A, Hotchin NA. The small GTPases Rho and Rac are required for the establishment of cadherin-dependent cell-cell contacts. Journal of Cell Biology. 1997; 137:1421–1431. [PubMed: 9182672]
64. Tu CL, Chang W, Bikle DD. The calcium-sensing receptor-dependent regulation of cell-cell adhesion and keratinocyte differentiation requires Rho and filamin A. The Journal of Investigative Dermatology. 2011; 131:1119–1128. [PubMed: 21209619]

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65. Lee YS, Dlugosz AA, McKay R, et al. Definition by specific antisense oligonucleotides of a role for protein kinase C alpha in expression of differentiation markers in normal and neoplastic mouse epidermal keratinocytes. Molecular Carcinogenesis. 1997; 18:44–53. [PubMed: 9022812]
66. Denning MF, Dlugosz AA, Williams EK, et al. Specific protein kinase C isozymes mediate the induction of keratinocyte differentiation markers by calcium. Cell Growth and Differentiation. 1995; 6:149–157. [PubMed: 7756173]
67. Deucher A, Efimova T, Eckert RL. Calcium-dependent involucrin expression is inversely regulated by protein kinase C (PKC)alpha and PKCdelta. The Journal of Biological Chemistry. 2002; 277:17032–17040. [PubMed: 11864971]
68. Ng DC, Shafaee S, Lee D, Bikle DD. Requirement of an AP-1 site in the calcium response region of the involucrin promoter. The Journal of Biological Chemistry. 2000; 275:24080–24088. [PubMed: 10816578]
69. Jang SI, Steinert PM. Loricrin expression in cultured human keratinocytes is controlled by a complex interplay between transcription factors of the Sp1, CREB, AP1, and AP2 families. The Journal of Biological Chemistry. 2002; 277:42268–42279. [PubMed: 12200429]
70. Welter JF, Crish JF, Agarwal C, Eckert RL. Fos-related antigen (Fra-1), junB, and junD activate human involucrin promoter transcription by binding to proximal and distal AP1 sites to mediate phorbol ester effects on promoter activity. The Journal of Biological Chemistry. 1995; 270:12614–12622. [PubMed: 7759510]
71. Sinitsyna NN, Reznikova TV, Qin Q, et al. Arsenite suppression of involucrin transcription through AP1 promoter sites in cultured human keratinocytes. Toxicology and Applied Pharmacology. 2009; 243:275–282. [PubMed: 20006635]
72. Brown EM, MacLeod RJ. Extracellular calcium sensing and extracellular calcium signaling. Physiology Review. 2001; 81:239–297.
73. Tu CL, Oda Y, Bikle DD. Effects of a calcium receptor activator on the cellular response to calcium in human keratinocytes. The Journal of Investigative Dermatology. 1999; 113:340–345. [PubMed: 10469331]
74. Tu CL, Chang W, Bikle DD. The extracellular calcium-sensing receptor is required for calcium-induced differentiation in human keratinocytes. The Journal of Biological Chemistry. 2001; 276:41079–41085. [PubMed: 11500521]
75. Tu CL, Oda Y, Komuves L, Bikle DD. The role of the calcium-sensing receptor in epidermal differentiation. Cell Calcium. 2004; 35:265–273. [PubMed: 15200150]
76. Chang W, Tu C, Bajra R, et al. Calcium sensing in cultured chondrogenic RCJ3.1C5.18 cells. Endocrinology. 1999; 140:1911–1919. [PubMed: 10098531]
77. Kanatani M, Sugimoto T, Kanzawa M, et al. High extracellular calcium inhibits osteoclast-like cell formation by directly acting on the calcium-sensing receptor existing in osteoclast precursor cells. Biochemical and Biophysical Research Communinations. 1999; 261:144–148.
78. Chang W, Tu C, Chen TH, et al. Expression and signal transduction of calcium-sensing receptors in cartilage and bone. Endocrinology. 1999; 140:5883–5893. [PubMed: 10579354]
79. Bruce JI, Yang X, Ferguson CJ, et al. Molecular and functional identification of a Ca2+ (polyvalent cation)-sensing receptor in rat pancreas. The Journal of Biological Chemistry. 1999; 274:20561–20568. [PubMed: 10400686]
80. Blank JL, Ross AH, Exton JH. Purification and characterization of two G-proteins that activate the beta 1 isozyme of phosphoinositide-specific phospholipase C. Identification as members of the Gq class. The Journal of Biological Chemistry. 1991; 266:18206–18216. [PubMed: 1655741]
81. Boulay G, Brown DM, Qin N, et al. Modulation of Ca(2+) entry by polypeptides of the inositol 1,4, 5-trisphosphate receptor (IP3R) that bind transient receptor potential (TRP): evidence for roles of TRP and IP3R in store depletion-activated Ca(2+) entry. Proceedings of the National Academy of Sciences of the United States of America. 1999; 96:14955–14960. [PubMed: 10611319]
82. Feng Y, Walsh CA. The many faces of filamin: a versatile molecular scaffold for cell motility and signalling. Nature Cell Biology. 2004; 6:1034–1038.
83. Oda Y, Tu CL, Chang W, et al. The calcium sensing receptor and its alternatively spliced form in murine epidermal differentiation. The Journal of Biological Chemistry. 2000; 275:1183–1190. [PubMed: 10625662]

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84. Turksen K, Troy TC. Overexpression of the calcium sensing receptor accelerates epidermal differentiation and permeability barrier formation in vivo. Mechanisms of Development. 2003; 120:733–744. [PubMed: 12834872]
85. Ho C, Conner DA, Pollak MR, et al. A mouse model of human familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. Nature Genetics. 1995; 11:389–394. [PubMed: 7493018]
86. Rodriguez L, Tu C, Cheng Z, et al. Expression and functional assessment of an alternatively spliced extracellular Ca2+-sensing receptor in growth plate chondrocytes. Endocrinology. 2005; 146:5294–5303. [PubMed: 16166224]
87. Chang W, Tu C, Chen TH, et al. The extracellular calcium-sensing receptor (CaSR) is a critical modulator of skeletal development. Science Signaling. 2008; 1:ra1. [PubMed: 18765830]
88. Vasioukhin V, Degenstein L, Wise B, Fuchs E. The magical touch: genome targeting in epidermal stem cells induced by tamoxifen application to mouse skin. Proceedings of the National Academy of Sciences of the United States of America. 1999; 96:8551–8556. [PubMed: 10411913]
89. Hardman MJ, Sisi P, Banbury DN, Byrne C. Patterned acquisition of skin barrier function during development. Development. 1998; 125:1541–1552. [PubMed: 9502735]
90. Bikle DD, Gee E, Pillai S. Regulation of keratinocyte growth, differentiation, and vitamin D metabolism by analogs of 1,25-dihydroxyvitamin D. The Journal of Investigative Dermatology. 1993; 101:713–718. [PubMed: 8228333]
91. Pillai S, Bikle DD, Su MJ, et al. 1,25-Dihydroxyvitamin D3 upregulates the phosphatidylinositol signaling pathway in human keratinocytes by increasing phospholipase C levels. The Journal of Clinical Investigation. 1995; 96:602–609. [PubMed: 7615834]
92. Su MJ, Bikle DD, Mancianti ML, Pillai S. 1,25-Dihydroxyvitamin D3 potentiates the keratinocyte response to calcium. The Journal of Biological Chemistry. 1994; 269:14723–14729. [PubMed: 7910167]
93. Bikle DD, Ng D, Oda Y, et al. The vitamin D response element of the involucrin gene mediates its regulation by 1,25-dihydroxyvitamin D3. The Journal of Investigative Dermatology. 2002; 119:1109–1113. [PubMed: 12445200]
94. Ratnam AV, Bikle DD, Cho JK. 1,25 dihydroxyvitamin D3 enhances the calcium response of keratinocytes. Journal of Cell Physiology. 1999; 178:188–196.
95. Canaff L, Hendy GN. Human calcium-sensing receptor gene. Vitamin D response elements in promoters P1 and P2 confer transcriptional responsiveness to 1,25- dihydroxyvitamin D. The Journal of Biological Chemistry. 2002; 277:30337–30350. [PubMed: 12036954]
96. Oda Y, Chalkley RJ, Burlingame AL, Bikle DD. The transcriptional coactivator DRIP/mediator complex is involved in vitamin D receptor function and regulates keratinocyte proliferation and differentiation. The Journal of Investigative Dermatology. 2010; 130:2377–2388. [PubMed: 20520624]
97. Zineb R, Zhor B, Odile W, Marthe RR. Distinct, tissue-specific regulation of vitamin D receptor in the intestine, kidney, and skin by dietary calcium and vitamin D. Endocrinology. 1998; 139:1844–1852. [PubMed: 9528970]
98. Bikle DD, Chang S, Crumrine D, et al. 25 Hydroxyvitamin D 1 alpha-hydroxylase is required for optimal epidermal differentiation and permeability barrier homeostasis. The Journal of Investigative Dermatology. 2004; 122:984–992. [PubMed: 15102089]
99. Oda Y, Uchida Y, Moradian S, et al. Vitamin D receptor and coactivators SRC2 and 3 regulate epidermis-specific sphingolipid production and permeability barrier formation. The Journal of Investigative Dermatology. 2009; 129:1367–1378. [PubMed: 19052561]

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Research Agenda

- The interaction between vitamin D signaling and calcium/CaSR signaling requires further elucidation at the molecular level.
- The role of CaSRalt in the epidermis and other tissues needs further exploration
- The function of the intracellular CaSR and its relationship with other calcium signaling molecules requires further investigation.
- Novel drugs that target the epidermal CaSR may result in better treatment of diseases including Darier's Disease and Hailey-Hailey disease, atopic dermatitis or psoriasis.

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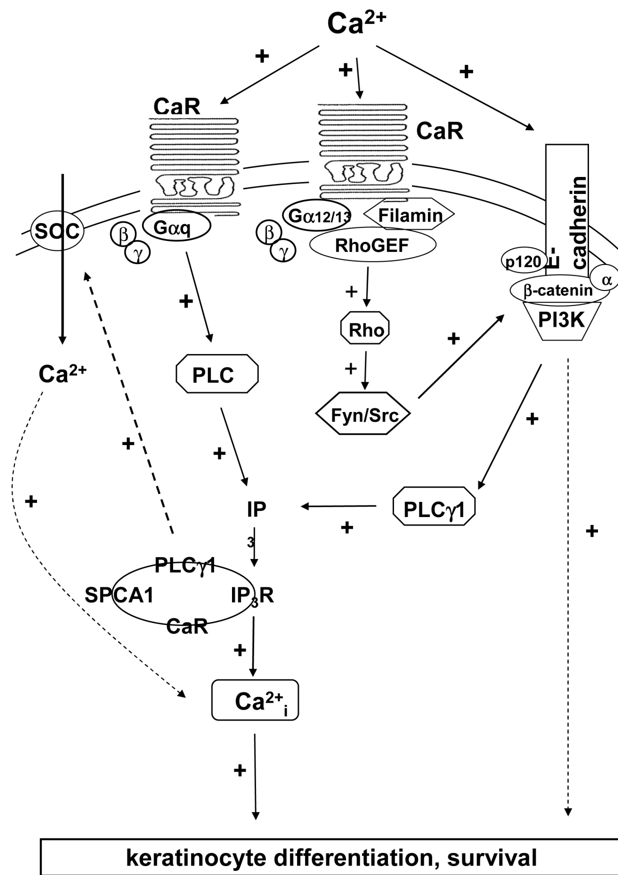

Figure 1.

$[Ca^{2+}]_o$-stimulated signaling responses in keratinocytes. Membrane CaSR senses changes in $[Ca^{2+}]_o$ and activates two signaling pathways. One is mediated by the Ga_q-activated PLC, which generates IP_3 to trigger Ca^{2+} release from internal stores including ER and Golgi via IP_3R. Depletion of Ca^{2+} stores stimulates Ca^{2+} influx through SOCs by an IP_3R- and PLCγ1-mediated mechanism to further raise $[Ca^{2+}]_i$. The second involves activation of Rho through interaction with Ga, e.g. Ga_q or $Ga_{12/13}$, filamin A, and RhoGEF to stimulate Fyn/Src kinases. Fyn/Src kinases phosphorylate catenins, promoting the formation of E-cadherin/catenin complex at the cell membrane and activation of PI3K. PI3K in turn activates PLCγ1 to further increase $[Ca^{2+}]_i$. The activation of PI3K downstream effectors and rise in $[Ca^{2+}]_i$ stimulate the expression of genes essential for differentiation and cell survival. Finally, the intracellular CaSR forms complexes with IP_3R, PLCγ1, and SPCA1 in the Golgi and regulates Ca^{2+} uptake and release from intracellular Ca^{2+} stores, as well as Ca^{2+} entry via SOC.