Calcium, Orai1 and Epidermal Proliferation

DD Bikle\textsuperscript{1} and T Mauro\textsuperscript{2}

T Mauro: maurot@derm.ucsf.edu

\textsuperscript{1}Department of Medicine, Endocrine, Research Unit and Department of Dermatology, VA Medical Center, University of California San Francisco, 4150 Clement St (111N), San Francisco, CA 94121, USA

\textsuperscript{2}Department of Medicine, Dermatology, Research Unit and Department of Dermatology, VA Medical Center, University of California San Francisco, 4150 Clement St (111N), San Francisco, CA 94121, USA

Abstract

Ca\textsuperscript{2+} influx controls essential epidermal functions, including proliferation, differentiation, cell migration, itch, and barrier homeostasis. The Orai1 ion channel allows capacitive Ca\textsuperscript{2+} influx after Ca\textsuperscript{2+} release from the endoplasmic reticulum, and it has now been shown to modulate epidermal atrophy. These findings reveal new interactions among various Ca\textsuperscript{2+} signaling pathways and uncover novel functions for Ca\textsuperscript{2+} signaling via the Orai1 channel.

INTRODUCTION

Epidermal Ca\textsuperscript{2+} has long been recognized as an essential signal for many epidermal functions. Beginning with early descriptions of the keratinocyte differentiation response, changes in extracellular and intracellular Ca\textsuperscript{2+} have been shown to direct keratinocyte proliferation, differentiation and barrier homeostasis (reviewed in Mascia et al 2012) (Mascia, et al., 2012). The marked Ca\textsuperscript{2+} gradient present in the epidermis, almost four-fold higher in the stratum granulosum than in the basal layer, suggests that Ca\textsuperscript{2+} signaling seen in the culture dish is reflected in the in vivo responses of the epidermis. This report, “Reversal of Murine Epidermal Atrophy by Topical Modulation of Calcium Signaling”, by Darbellay et al (Darbellay, et al., 2013) reveals that Ca\textsuperscript{2+} flux through the plasma membrane Orai1 channel additionally controls epidermal proliferation and thickness, particularly when the epidermis atrophies in response to aging or chronic corticosteroid topical application. Related recent reports demonstrate further that the Orai1 channel also controls keratinocyte focal adhesion turnover (Vandenberghe, et al., 2013) and modulates early aspects of keratinocyte differentiation (Numaga-Tomita and Putney, 2013).

Ca\textsuperscript{2+} STORE RELEASE

Keratinocytes, like many other non-excitable cells, employ Ca\textsuperscript{2+} signaling through a variety of pathways. Many of these pathways share common components (Figure 1). A variety of stimuli (growth factors such as EGF, ATP PAR2 receptor agonists, or raised extracellular Ca\textsuperscript{2+}) bind to their receptors and generate IP\textsubscript{3}, leading to Ca\textsuperscript{2+} release from both the endoplasmic reticulum and the Golgi. As opposed to many other mammalian cells, both of
these cellular Ca\textsuperscript{2+} stores are important in keratinocytes, as mutations in either of the Ca\textsuperscript{2+} 
ATPases that restore these Ca\textsuperscript{2+} stores cause the blistering diseases Darier’s Disease or 
Hailey Hailey Disease (reviewed in Foggia and Hovnanian 2004)(Foggia and Hovnanian, 2004). However, much less is known about Golgi Ca\textsuperscript{2+} signaling in keratinocytes, and this 
review will concentrate on the interplay between ER Ca\textsuperscript{2+} release, store-operated Ca\textsuperscript{2+} entry 
(SOCE) through plasma membrane ion channels, and the multiple downstream effects that 
are mediated by these processes. Other important signaling mediators, in particular, 
diacylglycerol (DAG), a protein kinase C (PKC) activator, interact with Ca\textsuperscript{2+} signaling to 
modulate keratinocyte and epidermal proliferation, differentiation and cell-to-cell adhesion 
(Figure 1).

**BOTH Ca\textsuperscript{2+} RELEASE AND Ca\textsuperscript{2+} INFLUX ARE REQUIRED FOR NORMAL 
BIOLOGIC RESPONSES**

ER Ca\textsuperscript{2+} release leads to a transient spike in cytosolic Ca\textsuperscript{2+}, which has rapid effects on actin 
reorganization and the initiation of cell-to-cell junctions. Activation of growth factor 
receptors such as EGFR promotes these transient spikes of calcium. Raised cytosolic Ca\textsuperscript{2+} 
also increases nuclear Ca\textsuperscript{2+} concentrations, which control synthesis of differentiation 
specific proteins such as involucrin via AP-1 binding sites (Ng, et al., 2000). However, this 
rapid cytosolic increase must be augmented by a subsequent and longer-lasting influx of 
Ca\textsuperscript{2+} through plasma membrane ion channels to effectively promote differentiation, 
mediated at least in part by the formation of the Ecadherin/catenin membrane complex 
(Bikle, et al., 2012). The calcium sensing receptor is instrumental in promoting these 
processes (Tu, et al., 2012). ER Ca\textsuperscript{2+} release also promotes epidermal permeability barrier 
homeostasis, as simply releasing ER Ca\textsuperscript{2+} by topically applying low concentrations of the 
irreversible SERCA2 inhibitor thapsigargin mimics lamellar body and lipid secretion, and 
stimulates the formation of transitional cells seen after experimental barrier perturbation 
(Celli, et al., 2011). ER Ca\textsuperscript{2+} release also signals antimicrobial peptide (AMP) synthesis and 
secretion, via ceramide metabolism through the C1P/STAT1/3 and NF-kB pathways (Park, 
et al., 2011). While extracellular Ca\textsuperscript{2+} seems to be required, whether and how the Orai1 
channel modulates these processes is unknown. Ca\textsuperscript{2+} flux through the Orai1 channel, 
signaling via the NFAT pathway, has recently been shown to regulate TSLP release from 
k eratinocytes. TSLP then is secreted from the keratinocytes, and it subsequently activates 
TRPA1-positive sensory neurons to trigger itch (Wilson, et al., 2013). This signaling 
pathway has been shown to be central to the pathogenesis of atopic dermatitis.

**DIFFERENT Ca\textsuperscript{2+} SIGNALING PROCESSES YIELD DIFFERENT EPIDERMAL 
RESPONSES**

The Ca\textsuperscript{2+} signaling processes described above display many areas of overlap, and it has not 
been clear how diametrically opposite results (eg. proliferation and differentiation) could 
result from similar signaling pathways. However, from this and other reports, it is becoming 
increasingly clear that Ca\textsuperscript{2+} influx through the Orai1 channels appears to enhance epidermal 
proliferation and migration. These processes are regulated by activation of receptors such as 
EGFR. In contrast, Ca\textsuperscript{2+} influx through the TRP channels, in particular TRPC1 and TRPC4,
appear to direct keratinocyte differentiation (Tu, et al., 2005). Recent studies show that these different outcomes may be due to the Ca\(^{2+}\) pools that are accessed, the duration of Ca\(^{2+}\) influx, ratio of STIM to Orai1 proteins, relative activity of TRP vs Orai1 channels controlled by membrane depolarization, and possible direct interactions between TRP and Orai1 channels (reviewed in Saul et al 2013)(Saul, et al., 2013).

TRANSLATION TO THERAPY?

How these findings may be translated to therapy is not yet clear. This report demonstrates that ER Ca\(^{2+}\) release and subsequent Orai1 activation, via transient SERCA2 inhibition, leads to epidermal proliferation and reversal of corticosteroid-induced epidermal atrophy. However, caution is required before attempting to treat epidermal atrophy with SERCA2 inhibitors. First, while minor SERCA2 inhibition promotes many beneficial effects, such as barrier homeostasis and normalization of epidermal atrophy, major SERCA2 inhibition is the cause of Darier Disease, a blistering skin disease caused by mutations in SERCA2 (reviewed in Foggia and Hovnanian, 2004)(Foggia and Hovnanian, 2004). Second, heterozygous SERCA2 mice spontaneously develop cutaneous squamous cell carcinomas, with increased expression of the oncogene K-ras (Prasad, et al., 2005). Thus, activating Orai1 by inhibiting SERCA2 will require more selective SERCA2 inhibitors or more selective Orai1 agonists.

Acknowledgments

We gratefully acknowledge the superb editorial assistance of Ms Joan Wakefield and Ms Jerelyn Magnusson. This work was supported by NIH grants R01AR051930 and R01AG028492, which were administered by the Northern California Institute for Research and Education, and with resources of the Research Service, Department of Veterans Affairs. These sponsors had no role in writing this Commentary or in the decision to submit it for publication.

Abbreviations

| Abbreviation | Description                                           |
|--------------|-------------------------------------------------------|
| AMP          | Antimicrobial peptide                                  |
| CN           | Calcineurin                                             |
| DAG          | diacylglycerol                                          |
| ER           | endoplasmic reticulum                                   |
| FAK          | Focal Adhesion Kinase                                   |
| IP3          | inositol 1,4,5-trisphosphate                            |
| LB           | Lamellar Body                                           |
| NFAT         | nuclear factor of activated T cells                    |
| PIP2         | phosphatidylinositol 4,5-bisphosphate                   |
| PKC          | protein kinase C                                        |
| PLC          | SERCA, sarco (endo)plasmic reticulum Ca2+ ATPase       |
| SOCE         | store-operated calcium entry                           |
**References**

Bikle DD, Xie Z, Tu CL. Calcium regulation of keratinocyte differentiation. Expert Rev Endocrinol Metab. 2012; 7:461–472. [PubMed: 23144648]

Celli A, Mackenzie DS, Crumrine DS, Tu CL, Hupe M, Bikle DD, Elias PM, Mauro TM. Endoplasmic reticulum Ca2+ depletion activates XBP1 and controls terminal differentiation in keratinocytes and epidermis. Br J Dermatol. 2011; 164:16–25. [PubMed: 20846312]

Darbellay B, Barnes L, Boehncke WH, Saurat JH, Kaya G. Reversal of Murine Epidermal Atrophy by Topical Modulation of Calcium Signaling. J Invest Dermatol. 2013

Foggia L, Hovnanian A. Calcium pump disorders of the skin. Am J Med Genet C Semin Med Genet. 2004; 131C:20–31. [PubMed: 15468148]

Mascia F, Denning M, Kopan R, Yuspa SH. The black box illuminated: signals and signaling. J Invest Dermatol. 2012; 132:811–9. [PubMed: 22710487]

Ng DC, Shafae S, Lee D, Bikle DD. Requirement of an AP-1 site in the calcium response region of the involucrin promoter. J Biol Chem. 2000; 275:24080–8. [PubMed: 10816578]

Numaga-Tomita T, Putney JW. Role of STIM1- and Orai1-mediated Ca2+ entry in Ca2+-induced epidermal keratinocyte differentiation. J Cell Sci. 2013; 126:605–12. [PubMed: 23203806]

Park K, Elias PM, Oda Y, Mackenzie D, Mauro T, Holleran WM, Uchida Y. Regulation of Cathelicidin Antimicrobial Peptide Expression by an Endoplasmic Reticulum (ER) Stress Signaling, Vitamin D Receptor-independent Pathway. J Biol Chem. 2011; 286:34121–30. [PubMed: 21832078]

Prasad V, Boivin GP, Miller ML, Liu LH, Erwin CR, Warner BW, Shull GE. Haploinsufficiency of Atp2a2, encoding the sarco(endo)plasmic reticulum Ca2+-ATPase isofrom 2 Ca2+ pump, predisposes mice to squamous cell tumors via a novel mode of cancer susceptibility. Cancer Res. 2005; 65:8655–61. [PubMed: 16204033]

Saul S, Stanisz H, Backes CS, Schwarz EC, Hoth M. How ORAI and TRP channels interfere with each other: Interaction models and examples from the immune system and the skin. Eur J Pharmacol. 2013

Tu CL, Chang W, Bikle DD. Phospholipase cgamma1 is required for activation of store-operated channels in human keratinocytes. J Invest Dermatol. 2005; 124:187–97. [PubMed: 15654973]

Tu CL, Crumrine DA, Man MQ, Chang W, Elalieh H, You M, Elias PM, Bikle DD. Ablation of the calcium-sensing receptor in keratinocytes impairs epidermal differentiation and barrier function. J Invest Dermatol. 2012; 132:2350–9. [PubMed: 22622426]

Vandenberghe M, Raphael M, Lehen’kyi V, Gordienko D, Hastie R, Odds T, Rao A, Hogan PG, Skryma R, Prevorskaya N. ORAI1 calcium channel orchestrates skin homeostasis. Proc Natl Acad Sci U S A. 2013; 110:E4839–48. [PubMed: 24277812]

Wilson SR, The L, Batia LM, Beattie K, Katibah GE, McClain SP, Pellegrino M, Estandian DM, Bautista DM. The epithelial cell-derived atopic dermatitis cytokine TSLP activates neurons to induce itch. Cell. 2013; 155:285–95. [PubMed: 24094650]
Clinical Implications

1. Changes in extracellular and intracellular Ca2+ have been shown to direct keratinocyte proliferation, differentiation and barrier homeostasis.

2. Both Ca2+ release from intracellular stores and Ca2+ influx from extracellular sources are required for normal biologic responses.

3. Ca2+ influx through the Orai1 channels enhances keratinocyte and epidermal proliferation and migration. In contrast, Ca2+ influx through TRPC1 and TRPC4 channels appears to direct keratinocyte differentiation.
Figure 1.
Agonists (e.g. EGF, ATP, Ca\(^{2+}\), PAR2 receptor agonists) bind to their receptors and activate PLC. PLC activation, via PIP2, generates IP3, which binds to IP3 receptors and leads to ER and Golgi Ca\(^{2+}\) release. PLC also generates DAG, which, in turn activates PKC. The ER Ca\(^{2+}\) and Golgi Ca\(^{2+}\) stores are refilled by the translocation of STIM to the plasma membrane, activating the Orai1 and TRP ion channels to generate Store Operated Ca\(^{2+}\) Entry. Ca\(^{2+}\) ATPases SPCA1 and SERCA2 also replenish Golgi and ER Ca\(^{2+}\) stores, respectively.

ER Ca\(^{2+}\) release depletes ER Ca\(^{2+}\) stores, leading immediately to lamellar body/antimicrobial peptide secretion, and also modulating cell-to-cell adhesion and migration via cytosolic Ca\(^{2+}\) and PKC or FAK activation. ER Ca\(^{2+}\) release then activates several pathways. First, Ca\(^{2+}\) entry causes nuclear translocation of NFAT via calcineurin, inducing transcription of various proteins that control differentiation and proliferation, and also TSLP (Wilson, et al., 2013). Next, PKC activation leads to NF-KB activation, which in turn leads to various genes that control proliferation and differentiation (reviewed in Masica et al 2012) (Mascia, et al., 2012). Ca\(^{2+}\) also modulates cell to cell adhesion through direct action on junctions and also through Ca\(^{2+}\) influx through Orai1 channels acting on FAK signaling pathways (Vandenberghe, et al., 2013). Finally, ER Ca\(^{2+}\) release generates ceramide signaling pathways, via the STAT1/3 and NF-KB signaling pathways, which in turn generate antimicrobial peptide synthesis (Park, et al., 2011).