Calcium - a central regulator of keratinocyte differentiation in health and disease

Regular keratinocyte differentiation is crucial for the formation of an intact epidermal barrier and is triggered by extracellular calcium. Disturbances of epidermal barrier formation and aberrant keratinocyte differentiation are involved in the pathophysiology of several skin diseases, such as psoriasis, atopic dermatitis, basal and squamous skin cancer, and genetic skin diseases such as Darier’s disease and Olmstedt syndrome. In this review, we summarize current knowledge about the underlying molecular mechanisms of calcium-induced differentiation in keratinocytes. We provide an overview of calcium’s genomic and non-genomic mechanisms to induce differentiation and discuss the calcium gradient in the epidermis, giving rise to cornified skin and lipid envelope formation. We focus on the calcium-sensing receptor, transient receptor potential channels, and STIM/Orai as the major constituents of calcium sensing and calcium entry in the keratinocytes. Finally, skin diseases linked to impaired differentiation will be discussed, paying special attention to disturbed TRP channel expression and TRP channel mutations.

Key words: keratinocyte, differentiation, calcium, TRP channels

Keratinocyte differentiation in health

Skin consists of three different layers, called the subcutis, the dermis and the epidermis. The epidermis is the outermost layer and the place of keratinocyte differentiation [1]. In the epidermis, keratinocyte proliferation is restricted to the basal cell layers [2], whereas more superficial layers differentiate [3] and finally die. After mitosis in the basal layer, keratinocytes differentiate across the epidermis toward the stratum corneum. During differentiation, several keratinocyte layers develop in the epidermis, starting with the stratum basale and then the stratum spinosum, the keratinocyte layers develop in the epidermis, starting with the stratum basale and then the stratum spinosum, expressing distinctive marker genes at each differentiation stage [4, 5]. Keratins are mainly expressed by basal (keratin 5 and keratin 14) and spinous keratinocytes (keratin 1 and keratin 10; see also figure 1). Transglutaminase and involucrin are generated in spinous keratinocytes, whereas granular keratinocytes produce loricrin and filaggrin [6]. Besides these proteins, keratinocytes in the stratum granulosum synthesize sphingolipid precursors, which are stored in lamellar bodies. These lamellar bodies fuse with the plasma membrane during the differentiation process and release lipid precursors into the extracellular space in the stratum granulosum and corneum. After enzymatic processing, they are incorporated in lipid lamellar membranes, which embed together with the cornified envelope keratin-filled corneocytes, forming the permeability barrier [7].

The main trigger for keratinocyte differentiation is calcium. In 1980, Hennings et al. demonstrated that keratinocyte differentiation and proliferation depend on extracellular calcium concentration: After elevating extracellular calcium from 0.09 mM to 1.2 mM, the differentiation of murine keratinocytes began immediately, accompanied by a decline in DNA, RNA and protein synthesis. In contrast, more than 90% proliferate in the presence of 0.09 mM calcium [8]. These findings were later confirmed for human keratinocytes [9]. The mechanisms by which calcium induces differentiation are multifarious and include genomic and non-genomic pathways [4]. An example of a non-genomic mechanism is desmosome formation: In the presence of 1.2 mM extracellular calcium, murine keratinocytes form desmosomes as early as five minutes later. After two to five hours, desmosomes are symmetrical and functional [8, 10]. Desmosomes give mechanical strength to the epidermis but might also provide a signaling complex for differentiating keratinocytes [11].

Genomic mechanisms involve the activation of calcium-responsive promoters such as activator protein 1 (AP1) sites. AP1 sites are found in the involucrin [12] and in the keratin 1 gene [13, 14]. Both genes code for differentiation markers expressed in response to high extracellular calcium. It remains to be elucidated whether other differentiation-related genes contain calcium-sensitive promoters. Using a subtraction hybridization technique, Seo et al. found that 290 genes up-regulated in response to calcium. Most were differentiation related, whereas genes involved in metabolism, DNA repair, transcription, and translation decreased [15].

In the epidermis, extracellular calcium is provided by a calcium gradient with peaking calcium concentrations in the granular layer and a steep drop-off in the stratum corneum,
as demonstrated using a variety of techniques such as ion capture cytochemistry, microbeam proton induced x-ray emission or calcium-sensitive fluorescent dyes in murine and human skin [16-19]. In contrast, very low extracellular calcium levels are demonstrated in the basal layer, where low calcium keeps keratinocytes proliferating. Recent studies by Behne et al. [20] and Celli et al. [21] used a combination of two-photon microscopy, fluorescence lifetime imaging and phasor analysis in ex vivo unfixed skin biopsies of the epidermis to determine the intracellular calcium concentration. They confirmed previous findings showing that calcium concentrations in the upper layers of the viable epidermis are higher than in the stratum corneum. However, the calcium gradient was not as steep as previously described, and most epidermal calcium was found in intracellular organelles such as the endoplasmic reticulum or the Golgi apparatus. The authors suggested that keratinocytes might not only differentiate simply by responding to extracellular calcium but also by changing the composition of plasma membrane ion channels or by changing the intracellular or plasma membrane calcium sensing capacity.

The mechanisms of the formation and maintenance of the calcium gradient are still under investigation. In rodents, the calcium gradient develops before birth [22]. Gradient maintenance is achieved by tight junctions, preventing calcium loss [21]. The calcium gradient is vulnerable to skin damage, as injuring murine skin by tape-stripping markedly decreased the calcium gradient [23, 24]. A decreased calcium gradient results in reduced levels of the differentiation markers profilaggrin, loricrin and involucrin [25]. In addition, barrier insult by tape-stripping also resulted in a mobilization of calcium from intracellular calcium stores [20]. These findings are not surprising, as graded levels of extracellular calcium elicit a graded differentiation response in keratinocytes, the buffering of intracellular calcium prevents the terminal differentiation of keratinocytes, and the expression of early and late differentiation markers is controlled by intracellular calcium compartments [19]. However, a more comprehensive understanding of barrier development and maintenance is still lacking.

**Which receptors and ion channels take part in differentiation?**

**The calcium-sensing receptor (CaSR)**

Elevated extracellular calcium concentrations and keratinocyte differentiation are closely linked. Keratinocytes sense changes in the extracellular calcium concentration via the G-protein coupled calcium-sensing receptor (CaSR) [26]. The CaSR is predominantly expressed in the suprabasal keratinocyte layers. Several groups have provided evidence for the expression of the CaSR in primary human keratinocytes isolated from foreskin as well as in gingival keratinocytes from adult patients or primary neonatal keratinocytes [27-29]. The CaSR is involved in the mobilization of intracellular calcium as well as E-cadherin-mediated cell adhesion. Both pathways are important for calcium-mediated keratinocyte differentiation [5, 30]. Full-length CaSR was found to be essential for calcium-induced keratinocyte differentiation, as its ablation by the cDNA technique reduced the response to extracellular calcium and decreased the differentiation markers involucrin and transglutaminase [31]. These findings were confirmed by transgenic CaSR−/− mice displaying ultrastructural changes in the epidermis and reduced levels of loricrin and filaggrin [32]. However, these animals died early (mostly 5-7 days after birth), making the characterization of the skin phenotype in adulthood impossible. Therefore, Tu et al. [5] generated a transgenic mouse model with a keratinocyte-specific knockout of CaSR in the stratum basale. These animals were characterized by reduced epidermal differentiation, reflected in a reduced expression of the late