EPIDERMAL CORNEOCYTES: DEAD GUARDS OF THE HIDDEN TREASURE

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Abbreviations:
7-DHC, 7-dehydrocholesterol; VDR, vitamin D receptor; PLC, phospholipase C, PIP2, phosphatidylinositol; IP3, inositol triphosphate; DAG, diacylglycerol; TGM, transglutaminase(s); ALP, antileukoproteinase; LEKTI, lymphoepithelial Kazal-type 5 serine protease inhibitor; SCCE, corneum chymotryptic enzyme; SCTE, stratum corneum tryptic enzyme; MT-SP1, Matriptase.

Abstract

Gradual transformation of the epidermal stem cells to corneocytes involves a chain of chronologically well-arranged events that mostly stimulated locally by their neighbors. Cell diversity that observed during the differentiation through the different epidermal cell layers included the consisted changes of cell shape, intercellular contacts and proliferation. However, the most dramatically these changes appeared at the molecular level through gene expression, catalysis and intraprotein interactions. The proposed review explains these changes by switching systemic transcription factors that unlike their counterparts those role is limited to a contribution to gene expression also prepare cells to the next step of differentiation via modification of the chromatin pattern. Since primary epidermal keratinocytes are one of the most easy available type of the stem cells, a better understanding of the epidermal differentiation will benefit the research in the other areas by a discovery of basic coordinating mechanisms that stand behind such distinct molecular events as cell signaling and gene expression, and formulate basic principles for a smart therapeutic correction of the metabolism.

Introduction

As the most outer tissue of the body, the epidermis protects it from physical and chemical insults and infections. In the other words, the epidermis keeps inside what do we need and outside - whatever can harm us. It also maintains homeostasis through thermoregulation and prevents dehydration. Besides, the epidermis contributes to the immune surveillance and also employed as a sensory organ. Successful functioning of the epidermis is based on its ability to the continuous self-renewal that obviously requires a balance between cellular proliferation and cell death. Disturbance of the balance leads to life-threatening conditions: excessive cell proliferation causes a development of tumors and prevalence of the cell death causes the tissue atrophy. The barrier function of the epidermis is based on a balance between established cell junctions and protease inhibitors from one side and proteases- from another one. The necessity to connect cells in the internal layers requires different kind of cell-cell contacts. These areas are protected from degradation by protease inhibitors unless the arrest of gene expression that normally occurs in the upper layers will raise proteolytic activity and disrupts the cell junctions.
Figure 1 A. Interfollicular epidermis and hair follicle: location of stem cells in the epidermis. Stem cells are located within the bulge region of the hair follicle and at the interfollicular space of the basement membrane. In the picture: E-epidermis; H- hair; SG- sebaceous gland; B-bulge; M-matrix; ORS- outer root sheath; DP- dermal papilla; CL- cornified layer, stratum corneum; GL- granular layer, stratum granulosum; SL- spinous layer, stratum spinosum; BL- basal layer, stratum basale. B. Hemidesmosomes: connection of cells to the basal membrane. In the picture: KF- keratin filaments; α6 and β4- α6 and β4 integrins; L5- laminin 5; BM- basal membrane.

Proliferating cells of the basal membrane

Proliferating cells of a self-renewing tissue should maintain its thickness and prevent life-threatening changes in the genome. In the epidermis, this attained due to a coordinated work of two different kinds of proliferating cells: epidermal stem cells that retain genome integrity and transient amplifying cells that are responsible for the most of cell divisions. Stem cells are located within the bulge region of hair follicles and at interfollicular space of the basement membrane (Figure 1A). They are pluripotent cells that divide infrequently [1]. Proliferation of these slow-cycling cells occurs asymmetrically and gives a life to one new stem cell and one differentiating (transit amplifying) cell (Figure 2A). Unlike the stem cells, transit amplifying cells divide symmetrically and one parent cell produces two differentiating cells (Figure 2B). Until epidermal cells express integrins α6, β1 and β4 [2] they continue to proliferate. Mice conditionally null for β1 integrin exhibit an impaired proliferation in the epidermis and expose defects under the assembly of the basement membrane [3-5].

In stratum basale, cells are of cuboidal shape and maintain a spatio-temporal orientation. They are attached to the basal membrane through hemi-desmosomes. Hemi-desmosomes (Figure 1B) are made of laminin 5 that is anchored in the basal membrane, integrin α6β4 and intercellular keratin filaments [6] The α6β4 integrin-null mice show epidermal blistering, which is consistent with the role for α6β4 integrin in the anchorage [7]. Besides, cells located on the basal membrane receive signals from the extracellular matrix to regulate organization of the cytoskeleton, proliferation and differentiation [8].
Figure 2 Division and differentiation of epidermal stem and transit amplifying cells. A. Dividing epidermal stem cell divides asymmetrically to one stem cell and one transit amplifying cell. B. Transit amplifying cell divides symmetrically to two differentiating cells; C. Differentiating cells lose a connection with the basal membrane, interrupt cell division and move to the spinous layer.

Analysis of the signaling pathways employed in differentiation will reveal what and how drives the stem cells to corneocytes. Unfortunately, the mechanisms that control population of stem cells in the epidermis are not yet uncovered [1]. However, two signaling pathways (Wnt and Myc) seem to be implicated in this process. The key element of the canonical Wnt signaling pathway, β-catenin, plays a dual role in the epidermis. First, β-catenin was identified as a part of adherent junctions between the cells of the basal membrane [9] where it links actin filaments of the cytoplasm and cadherins of the plasma membrane. The cytoplasmic pool of β-catenin is phosphorylated by the kinase, GSK-3, and degrades in the cell proteasomes (Figure 3A). Activation of Wnt pathway inhibits the phosphorylation and leads to a translocation of β-catenin to the nucleus where it interacts with transcription factor Lef/Tcf (Fig 3B). This complex binds to the DNA and activates the expression of numerous genes including sonic hedgehog (SHH). After secretion from the cells, SHH binds to the receptor Patched at the surface of another cell. Patched is an integral membrane protein that acts as an inhibitor of Smoothened activation. The pathway downstream of the Smoothened receptor remains unclear. However, it involves the Gli family of transcriptional activators, including Gli-1, Gli-2, and Gli-3 and induces the proliferative response (Figure 3C) [10]. Interestingly, interaction of β-catenin with the repressive form of Lef/Tcf overlaps with epigenetic control of gene expression since it displaces HDAC complex (Figure 3D) [11; 12]. Constitutive activation of β-catenin in the epidermis of transgenic mice leads to a highly enriched stem cell population. On the other hand, dominant-negative β-catenin favors cell differentiation and stimulates cells to leave the stem cell compartments [10].
Figure 3  Role of the canonical Wnt signaling pathway in gene expression and proliferation.

A. Cell with deactivated Wnt signaling pathway: excess of free (unbound) β-catenin degrades in proteasomes. B. Cell after activation of Wnt-signaling pathway: β-catenin binds to Tcf/Lef factor, causing dissociation of the transcriptional repressors and induces the gene expression.

In the picture: β-cat, β-catenin; Dsh- mediator of Wnt signaling; Axin, Gsk3, and APC- subunits of the β-catenin degradation complex; Brg1- SWI/SNF chromatin-remodeling protein, repressor; p300- coactivator; HDAC, histone deacetylase, histone modifying enzyme, repressor; Gro, member of groucho family of protein repressors. C. Sonic hedgehog pathway: cell prior activation by SHH D. Activation of sonic hedgehog pathway by SHH.
One of the β-catenin downstream targets is oncogene, c-Myc [13]. c-Myc is required for the transition of the cell from G1 to S phase of the cell cycle and it promotes proliferation of the epidermal transit amplifying cells. It was shown that activation of c-Myc pathway in the epidermis stimulates cell differentiation and departure from the basal membrane [14; 15]. The majority of the genes suppressed by c-Myc in the epidermis encodes cell adhesion and cytoskeleton proteins. Besides, c-Myc downregulates the expression of the α6β4 integrin, decreases the formation of hemi-desmosomes and delays the assembly of the actomyosin cytoskeleton. Level of c-Myc also has a reverse correlation with decreased levels of the other target proteins. Size and shape of the keratinocytes become variable and nuclear atypia appears as well [14; 15]. The skin loses melanocytes and Langerhans cells. Response to the injury [16], chemical clearance and immune response are also slower than in control [13;14]. The epidermis becomes more fragile and more frequently develops a cancer [13]. On the other hand, action of c-Myc, as it was shown recently, depends on the presence of another protein, Miz1. The transgenic mice with a lack of functional Myc-Miz1 heterodimer restored normal keratinocyte adhesion, polarization of the basal membrane despite of overexpression of c-Myc [17].

Conversion of stem cells to transit amplifying cells is likely to consider as the first step of keratinocyte differentiation. They still reside on the basal membrane, the innermost layer of the epidermis, and migrate on it laterally. However, their protein expression profile has already changed due to an induction of markers of early differentiation: keratins 5 and 14 (K5 and K14) and p63 [2].

**Keratinocytes in transition to the squamous cells**

After they lose a connection to the basal membrane, the differentiating cells enter the suprabasal layer and become squamous cells (Figure 2C). Despite they experience similar external and intrinsic signals as their multipotent embryonic counterparts, the differentiating cells undergo changes in morphology and gene expression as well as gradual changes in their junctions and water impermeability. In stratum spinosum, cells increase their size and cytoplasm: nucleus ratio. They also gain an extensive cytoskeleton which is made of keratin filaments and linked to tightly adhesive desmosomes [18; 19]. Unlike the basal membrane that composed of the only layer of cells, the spinous layer is about four to six cells thick.

Cell proliferation in basal and suprabasal layers of the epidermis can be activated through vitamin D pathway by the sun light that converts 7-dehydrocholesterol (7-DHC) to vitamin D-3 in a photochemical reaction [20, 21]. The following hydroxylation of D-3 produces calcitriol, 1,25(OH)₂D₃, the active form of vitamin D, that induces gene expression through the binding to vitamin D receptor (VDR). After the binding, the receptor forms either homo- or heterodimer with different binding partners: VDR₂; VDR-RXR or VDR-RAR in favor of VDR-RXR. The dimers translocate to the nucleus where they interact to the DNA binding either transcriptional repressor DRIP or coactivator p160/SRC at the DNA. The dimers translocate to the nucleus where they interact to the DNA binding either transcriptional repressor DRIP or coactivator p160/SRC. In undifferentiated keratinocytes, the dimers presumably bind to DRIP while in differentiating cells binding to p160/SRC takes over due to the level of DRIP falls down [rev. in 21]. Interestingly, higher doses of Vitamin D have a negative effect on the proliferation [compare 22 and 23]. Administration of 1,25(OH)₂D₃ blocks the cells at the transition from G₀/G₁ to S phase of the cell cycle [24] due to an induction of the cell cycle inhibitor, p21 [25]. Influence of VDR signaling on keratinocyte differentiation was recently confirmed by characterization of VDR-null phenotype in the mice. VDR-null mice exhibit a defect in epidermal differentiation, it believed, due to reduced levels of involucrin, profilaggrin, and loricrin, and lack of keratohyalin granules in the epidermis [26].
Desmosomes in keratinocytes: assembly and schematic representation. A. Merging keratinocytes form filopodia that embed into the neighbor cell. B. Formation of two-row adhesion zipper between the merging cells. C. Stabilization of the desmosomes. D. Parts of the mature desmosome and their interaction.

Figure 4

Activation of NFkB pathway drives the cells to the quiescent state [27]. Normally, it occurs in the suprabasal layer while in the cells of the basal membrane, NFkB remains inactive. Inhibition of NFkB leads to hyperproliferation of keratinocytes and thickening of the epidermis while its overexpression thins the tissue and causes hypoproliferation [28].

One of the interesting phenomena observed in the spinous layer was the formation of desmosomes (Figure 4). Desmosomes are the molecular complexes of linking and cell adhesion proteins that connect cells to each other and secure the connection through the binding to the intermediate cytoskeletal filaments. In the epidermis, the desmosomes are composed of transmembrane desmosomal cadherins (desmocollin and desmoglein) and two linker proteins called desmoplakin and plakophilin. Desmoplakin attaches cytoplasmic parts of the cadherins to the intermediate filament network. There are two options that can be used by the cell to form a link between desmosomal cadherins and intrafilaments. Both involve C-terminal domain of the linker protein, desmoplakin [29; 30]. Desmoplakin either binds the filaments directly to desmocollin [31] or connects them to desmogleins through the binding to plakoglobin and plakophilin.

Formation of adherens junctions and desmosomes requires extracellular calcium. Raising the calcium concentration in the cell culture medium from 0.05 to 1.2mM [32] stimulates keratinocytes to form strong cell-cell adhesions in vitro. The adherent junctions appear in the cell prior the desmosomes [33] and are necessary for arrangement the last [34-37]. The calcium switch leads to a rapid reorganization of actin and intermediate filaments and relocation of junctional and desmosomal proteins towards cell-cell borders [33; 38]. Assembly of a desmosome starts from a generation of
filopodia (Figure 4A). The filopodia penetrate and embed into the adjacent cell. Adherent junctional proteins concentrate at the tip of the filopodia and generate a two-row adhesion zipper (Figure 4B) [36]. Then, the desmosome binds the opposing cells together and stabilizes the junction [36; 37] until the directed actin polymerization will transform the two-row adhesion zipper into a single row (Figure 4C).

Moving upward through the spinous layer, the cells initiate the program of terminal differentiation. In stratum spinosum, keratinocytes express the following differentiation markers: K1 and K10, involucrin (Ivl), envoplakin, periplakin and 14-3-3σ, a nuclear export protein implicated in the Ras/MAPK pathway [39]. Expression of Ivl, envoplakin and periplakin appears to be the beginning of cornification.

**Keratinocytes in the granular layer, Ca^{2+} influx and its role in differentiation**

In the next epidermal level, stratum granulosum, cells have an elongated shape and contain multiple keratohyaline granules in the cytoplasm. These granules mostly consist of loricrin and profilaggrin, the precursor of filaggrin, a bundling protein of the keratin filaments. The cells of stratum granulosum also contain lamellar bodies mostly composed of lipids and lipid-processing enzymes whose contents will be secreted later into the junction between stratum granulosum and stratum corneum and become a mortar between the corneocyte "bricks". Usually, the granular layer composed of two- four layers of cells. Cells contain keratohyalin granules and number of the granules as well as their size increases toward the skin surface. The granules tend to accumulate in the outer apical side of the cytoplasm in each cell.

The majority of regulatory proteins employed in cornification depend on Ca^{2+}. Outside the cell, calcium concentration rises toward the stratum corneum and keratinocyte differentiation is tightly linked to the calcium extracellular contents [rev. in 21]. Upwelling extracellular calcium causes a calcium influx into the cell [40-42]. This influx is mediated by the calcium receptor (CaR, Figure 5) [43, 44], which is normally expressed in the suprabasal keratinocytes [45] and upregulated by the active form of vitamin D [46]. With progress of differentiation, keratinocytes lose their sensitivity to calcium [47], and this loss coincides with a switch in CaR processing: from the long to the alternatively spliced short one that lacks of one of the exons [43]. While several ion channels of the cell plasma membrane were identified as potential gates for the calcium invasion [48-52], their role has still to be characterized. Absence of the full-length CaR in the epidermis resulted in hyperproliferation and ultrastructural changes of the granular keratinocytes such as abnormal keratohyalin granule formation and premature secretion of lamellar body. Late-differentiation proteins (Flg, Lor and Ivl) were downregulated while number of proliferating cells was dramatically increased even in the presence of well-formed Ca^{2+} gradient across the epidermis [45].

In the cells, calcium activates phospholipase C (PLC), particularly the isoforms PLCβ and PLCγ1 [53, 54]. PLC is the key enzyme of phosphoinositol phosphates metabolism (Figure 5) that hydrolyzes the membrane lipids, phosphatidylinositols (PIP₂), into two different secondary messengers: inositol triphosphate (IP₃) and diacylglycerol (DAG). The first compound, water-soluble IP₃ diffuses through the cytoplasm to the endoplasmic reticulum where it binds to and opens calcium channels, releasing calcium from the intercellular storages into the cytoplasm. Interaction with both: DAG and calcium activates PKC increasing the kinase activity. This leads to phosphorylation of many other proteins, including the transcription factor AP1, altering their activity. The AP1-dependent transcriptional activation upregulates the expression of the cornified envelope precursors and transglutaminases (TGM). This accelerates the formation of the cornified envelope.
Figure 5. Ca\textsuperscript{2+}-mediated signaling in keratinocytes. Calcitriol, 1,25(OH)\textsubscript{2}D\textsubscript{3}, activates CaR and accelerates conversion of phosphatidylinositol bisphosphate to IP\textsubscript{3} and DG. Their rise leads to activation of protein kinase (PKC) by translocating PKCs to their specific membrane receptors (RACK). Opening of calcium channels in the plasma membrane and the following downstream activation of AP-1 transcription factors induces expression of cornified envelope precursors and transglutaminase (TG) and leads to formation of cornified envelope (CE).

Assembling of the cornified envelope

At the step of terminal differentiation, keratinocytes assemble the cornified cell envelope, an insoluble, about 10-nm thick protein layer (Figure 6E). In the epidermis, the cornified envelop also includes 5-nm thick lipid layer made of the ceramides that are covalently bound to the proteins [55; 56]. There are at least two types of chemical bonds that connect the components of the cornified envelope: N\textsuperscript{ε}-γ-glutamyl)lysine isopeptide bonds produced during the crosslinking by transglutaminases and disulfide (S-S) bonds [55; 57]. Once the cornified envelop seals the body surface, the epidermal cells start carrying their barrier function [58].

Cornification starts in the upper part of the spinous layer with an induction of the early differentiation genes. At the stage, called “initiation”, keratinocytes express envoplakin and periplakin (Figure 6A and B) [59]. This followed by the expression of involucrin that binds to the cell membranes in a Ca\textsuperscript{2+}-dependent manner [60] and members of S100 family: S100a7, S100a8, S100a9 and S100a10 [51]. While S100a8 and S100a9 can be considered as stress-induced proteins, S100a10 is interesting by its ability to interact with annexin II and form Ca\textsuperscript{2+}- channels in the plasma membrane promoting the cornification (Figure 6C).
Figure 6  Appearance and growth of the cornified envelope. A.- keratinocyte entering cornification; B- initiation of cornification: cells express envoplakin and periplakin; 
C- early cornification: involucrin binds to the plasma membrane, cell forms lamellar bodies and expresses S100 proteins; D- crosslinking: transglutaminases oligomerize precursors of the cornified envelope and incorporate them into the plasma membrane; E- late cornification: enucleation and degradation of cell organelles, proteolysis and desquamation.

Another essential element of the cornified envelope is TGM (Figure 6D). There are four transglutaminase isoforms expressed in the epidermis: TGM 1, TGM2, TGM3 and TGM5. TGM3 cross-links proteins to short oligomers and TGM1 connects them to the cornified envelope [61]. Specificity of another epidermal TGM isoform, TGM5, seems to be similar to TGM1 [62] while it can't replace it in crosslinking of lipids and doesn't incorporate itself into the plasma membrane [rev. in 63]. Contribution of TGM2 to cornification is not yet proven. Besides its participation in protein crosslinking, TGM1 also crosslinks ω-hydroxy-ceramides to involucrin [64]. During the terminal differentiation, rise of the calcium concentration results in proteolytic cleavage and about 1000 fold activation of TGM1 [65; 66]. Recently obtained data suggest that cathepsin D, an aspartate protease, can be involved in proteolytic activation of TGM1 [67]. In cathepsin D-deficient mice, TGM1 activity was sufficiently reduced, likely due to an abnormal processing of this enzyme.

Members of epidermal differentiation complex that have sequence similarities such as glutamine- and lysine-rich tracts become good substrates for TGM (Figure 6D). After Ca$^{2+}$-dependent activation, TGM1 incorporates itself into the membranes and links to the cornified envelope other proteins like envoplakin, periplakin and involucrin [68]. Shortly, members of small proline rich proteins, Sprr, become covalently bound to involucrin and envoplakin. Sprrs are very important for cornification due to their ability to form back links between larger proteins [rev in 69 and 70]. Then, the complex of cross-linked proteins spreads across the inner surface of the plasma membrane,
penetrates the desmosomes and binds to the intermediate filaments. The lamellar bodies fuse with the plasma membrane and ω-hydroxy ceramides appear in the cytoplasm. Since they become available to TGM1, it links them to the protein layer. At the later stages of keratinocyte differentiation, when the cornified envelope becomes stable and insoluble, it also recruits late-differentiation proteins: loricrin, the major component of the cornified envelope that composes about 70% of the total crosslinked protein and repetin (Figure 6D) [68]. Surprisingly, the epidermis of loricrin knockout mice looked almost normal [71] as it believed due a compensatory effect that appears in upregulation of the certain Sprr proteins and repetin. Interestingly, the knockout mice that lacked an expression of involucrin [72] and envoplakin [73] also did not have serious impairments in the epidermis. On the other hand, targeting structural proteins of the cornified envelope, desmosomal and cytoskeletal proteins often led to severe pathology and disturbance of barrier function. This was demonstrated numerous times by a targeted mutation or an ablation of keratins (K10, K2e and K1), desmoplakin, desmoglein and desmocollin-1 [rev. in 63].

Role of proteolysis in differentiation

Regulation of the protease activity in cells and tissues is a crucial element of their proper functioning that relays on a fragile balance of multiple factors and can be easy destroyed by a sudden shift at wrong place and time. In the most cases, proteolytic enzymes are carefully isolated from their potential targets. Besides, the level of protease inhibitors expressed by the cells is usually sufficient to prevent an occasional leak of endogenous or deactivate exogenous (microbial) proteases. Protease inhibitors may also reside in the intracellular space to keep the secreted enzymes neutralized. In stratum corneum, they are represented by antileukoproteinase (ALP) known also as secretory leukocyte protease inhibitor (Slpi); elafin, formerly known as SKALP, skin-derived-antileukoprotease; lymphoepithelial Kazal-type 5 serine protease inhibitor (LEKTI) and two specific cysteine protease inhibitors: cystatin and cystatin M/E. ALP and elafin inhibit the stratum corneum chymotryptic enzyme, SCCE, preventing the detachment of corneocytes from human plantar epidermis [74]. LEKTI (protein product of the gene SPINK-5) targets SCCE and SCTE, stratum corneum trypsin like enzyme. Cystatin expression was detected in the spinous layer [75]. During the cornification, cystatin interacts with epidermal TGM and becomes crosslinked to the cornified envelope as one of the minor components [69] Expression of cystatin M/E is restricted to the stratum granulosum [76]. Mutations in this gene impair desquamation and may lead to life-threatening conditions [74, 77, 78].

In this context, cornification can be considered as a serious challenge for the cell antiproteolytic defense. Gradual disruption of cell organelles due to the rapid growth of the cornified envelope releases proteases from their storages. Interfusion of different compartments, particularly lysosomes and cytoplasm, causes a release of potentially disruptive enzymes and involves in the degradation the proteins those function was to suppress it. Indeed, the granular layer of the epidermis is stained positively for a variety of proteases that normally do not appear in the cytoplasm including the lysosomal proteolytic enzymes [79]. On the other hand, the calcium influx decreases the ratio of protease inhibitors to other cytoplasmic proteins. Finally, degradation of the nucleus contributes to the shift. It shouts down the gene expression and it leaves the cell without de novo synthesized protein in conditions when it faces a great variety of fully-activated proteolytic enzymes.

Interestingly, two major proteases of stratum corneum SCCE/KLK7/hK7 and SCTE/KLK5/hK5 together can destroy three major components of the corneodesmosomes: DSC1, DSG1 and CDSN [80-82]. These enzymes belong to kallikrein family of serine proteases. Their expression starts in suprabasal keratinocytes where their inactive precursors undergo a processing by an unidentified trypsin-like protease [83; 84]. In stratum corneum, these enzymes appear in the intercellular spaces [85; 86] suggesting their involvement in the desquamation.

Previously performed analysis of protease inhibitors expressed in the skin, especially the
studies carried on the transgenic mice led to the surprising conclusion that other proteolytic enzymes also take a part in cornification and desquamation. This group of enzymes includes cathepsins C and D [87], L and L2 [88], legumain and serine proteinase MT-SP1. Some authors question a sufficiency of cathepsin E for desquamation while even they do not deny its "minor" contribution to the digestion of desmosomes [87].

Cathepsin C (CTSC) is a lysosomal cysteine protease that participates in degradation of the intracellular proteins. Cathepsin C seems to be essential for processing of keratins and contributes to the digestion of desmosomes [89]. Besides, it is important for establishing and maintenance of the structural organization in the tissues that surround the teeth [90]. Mutations in cathepsin C are associated with Haim-Munk and Papillon-Lefevre syndromes [91] that appear in severe early onset periodontitis, premature tooth loss, and thickening and scaling of the skin on palms and soles. Besides, patients with Haim-Munk syndrome (HMS) also have some other clinical features such as nail deformities, acroosteolysis. Surprisingly, CTSC-deficient mice do not resemble the human disorders and do not have severe abnormalities in teeth and skin [92].

Cathepsin D (CSTD) is a lysosomal enzyme and an aspartatic protease. In the epidermis, CSTD performs the final stage of desquamation [87]. It is also important for activation of TGM1 [67]. Ablation of cathepsin D in the mice sufficiently reduced TGM1 activity and also decreased the levels of involucrin and loricrin. Besides, it affected the morphology of stratum corneum, which was composed of an increased number of corneocyte layers. Cathepsin D- null phenotype had a similarity to the phenotype observed for the human skin disease, lamellar ichthyosis. Surprisingly, these findings also suggested a link between protease activity and regulation of the gene expression while the molecular mechanism underlying this interesting phenomenon still remains uncovered [93].

Cathepsin L (CTSL) is a lysosomal cysteine protease that participates in processing of trichohyalin [94] and profilaggrin [95]. Cathepsin L-deficient mice develop epidermal hyperplasia, acanthosis, hyperkeratosis and periodic hair loss [96]. Furless (fs) and nackt (nkt) mice with a mutation in Cathepsin L and lock of Cathepsin L activity develop similar phenotype [95; 96].

Cathepsin L2 formerly known as stratum corneum thiol protease (SCTP) [88; 97] and cathepsin V, has 75% sequence homology with cathepsin L. In mice, cathepsin L2 is not found to the time, while in humans, Cathepsin L2 has even higher activity than its closest homolog, cathepsin L. Surprisingly, the human cathepsin L2 expressed from K14 promoter can compensate the absence of the murine cathepsin L in cathepsin L deficient mice [98].

Legumain is a cysteine protease that hydrolyzes the proteins at Asp and Asn sites. In lysosomes, legumain processes precursors of other cathepsins and legumain deficiency results in accumulation of unprocessed cathepsins B, H and L. In stratum corneum, legumain interacts with cystatin M/E and cystatin M/E deficiency is incompatible with the life. The cystatin M/E knockout mice that survived up to 5-12 day had aberrant cornification in hyperplastic and hyperkeratotic epidermis. Moreover, unrestricted legumain causes premature TGM3 processing and abnormalities in processing of loricrin. On the other hand ablation of MT-SP1 seems doesn't affect other epidermal proteins or proteolytic processing of epidermal TGM [rev. in 100].

Type II transmembrane serine protease Matriptase (MT-SP1) is involved in numerous biological programs such as hair follicle development and epidermal differentiation [99]. Targeted deletion of this enzyme is lethal for the mice due to dehydration and loss of epidermal barrier function. Besides, it disturbs the formation of lipid matrix, impairs morphogenesis of the cornified envelope and affects desquamation. Some of these consequences can be explained by a defective processing of profilaggrin and loss of mature filaggrin monomer and filaggrin S-100 protein. On the other hand ablation of MT-SP1 seems doesn't affect other epidermal proteins or proteolytic processing of epidermal TGM [rev. in 100].

Formation of the mature cornified envelope and intercellular lipid layer accomplishes the transition of cells into flat, keratin-filled corneocytes. Instead of degraded subcellular organelles
corneocytes become tightly packed by the keratin fibrils that are oriented roughly parallel to the long dimension of the cell. Between keratin fibrils there is a matrix consisting of the remains of keratohyalin. As soon as corneocytes reach the skin surface their connection to the neighbor cells becomes weaker and finally they take out off it.

**Conclusion**

Despite the recent progress in our understanding of epidermal differentiation, the mechanism that coordinates gene expression remains unknown. In the other words, list of the differentiation participants that includes multiple categories such as receptors, kinases, transcription factors and the others still can tell us a little of the mysterious motive that enforces all these diverse parts to work together. That could be caused by several reasons. First, we can’t be sure that we know all of them, while studies of epidermal differentiation continue for a long period of time. Indeed, role of many genes remains unclear and it is very unlikely that we are going to know soon what most of them are doing in the cell. Secondary, role of some factors can be different from what we think of them now. Indeed, several proteins that we previously considered as “only regulatory” (ex. transcription factor Clock) demonstrated the enzyme (histone acetyl transferase) activity. *En contra*, other proteins that were initially described as enzymes also appeared as regulatory factors (ex. epidermal protease Cathepsin D that is also a receptor ligand). Third, some parts of the mechanism still can be missing or we previously didn’t pay them enough attention. The mechanisms of epigenetic control such as histone modification can be a good example of it. Fourth, we honestly do not know how the previous step of differentiation predetermines the next one. Finally, we should know sufficiently more of the external stimuli that come into the epidermis from outside and push the trigger of the differentiation program. On the other hand, the existence of a common coordinating center for gene expression mentioned above can be proved *via* establishing a new category of the systemic transcription factors. These factors suppose to connect epidermis with the surrounding tissues and rapidly react to the incoming stimuli. Besides, their principle role in the cell would be in preparing the chromatin to the next differentiation step *via* histone modification. The future search for these class of regulatory elements already begun [101] and it expects to be a challenging task.

**References:**

1. Alonso L, Fuchs E. Stem cells in the skin: waste not, Wnt not. Genes Dev. 2003; 17: 1189-200.

2. Morasso MI, Tomic-Canic M. Epidermal stem cells: the cradle of epidermal determination, differentiation and wound healing. Biol Cell. 2005; 97: 173-83.

3. Brakebusch C, Grose R, Quondamatteo F, Ramirez A, Jorcano JL, Pirro A, Svensson M, Herken R, Sasaki T, Timpl R, Werner S, Fassler R. Skin and hair follicle integrity is crucially dependent on beta 1 integrin expression on keratinocytes. EMBO J. 2000; 19: 3990-4003.

4. Raghavan S, Bauer C, Mundschau G, Li Q, Fuchs E. Conditional ablation of beta1 integrin in skin. Severe defects in epidermal proliferation, basement membrane formation, and hair follicle invagination. J Cell Biol. 2000; 150; 1149-60.

5. Lopez-Rovira T, Silva-Vargas V, Watt FM. Different consequences of beta1 integrin deletion in neonatal and adult mouse epidermis reveal a context-dependent role of integrins in regulating proliferation, differentiation, and intercellular communication. J Invest Dermatol. 2005; 125: 1215-27.
6. Borradori L, Sonnenberg A. Structure and function of hemidesmosomes: more than simple adhesion complexes. J Invest Dermatol. 1999; 112: 411-8.

7. DiPersio CM, van der Neut R, Georges-Labouesse E, Kreidberg JA, Sonnenberg A, Hynes RO. alpha3beta1 and alpha6beta4 integrin receptors for laminin-5 are not essential for epidermal morphogenesis and homeostasis during skin development. J Cell Sci. 2000; 113: 3051-62.

8. Mainiero F, Pepe A, Yeon M, Ren Y, Giancotti FG. The intracellular functions of alpha6beta4 integrin are regulated by EGF. J Cell Biol. 1996; 134: 241-53.

9. Fuchs E, Raghavan S. Getting under the skin of epidermal morphogenesis. Nat Rev Genet. 2002; 3: 199-209.

10. Watt FM. The stem cell compartment in human interfollicular epidermis. J Dermatol Sci. 2002; 28: 173-80.

11. Daniels DL, Weis WI. ICAT inhibits beta-catenin binding to Tcf/Lef-family transcription factors and the general coactivator p300 using independent structural modules. Mol Cell. 2002; 10: 573-84.

12. Daniels DL, Weis WI. Beta-catenin directly displaces Groucho/TLE repressors from Tcf/Lef in Wnt-mediated transcription activation. Nat Struct Mol Biol. 2005; 12: 364-71.

13. de Nigris F, Balestrieri ML, Napoli C. Targeting c-Myc, Ras and IGF cascade to treat cancer and vascular disorders. Cell Cycle. 2006; 5(15): 1621-8.

14. Waikel RL, Wang XJ, Roop DR. Targeted expression of c-Myc in the epidermis alters normal proliferation, differentiation and UV-B induced apoptosis. Oncogene. 1999; 18: 4870-8.

15. Frye M, Gardner C, Li ER, Arnold I, Watt FM. Evidence that Myc activation depletes the epidermal stem cell compartment by modulating adhesive interactions with the local microenvironment. Development. 2003; 130: 2793-808.

16. Waikel RL, Kawachi Y, Waikel PA, Wang XJ, Roop DR. Deregulated expression of c-Myc depletes epidermal stem cells. Nat Genet. 2001; 28: 165-8.

17. Gebhardt A, Frye M, Herold S, Benitah SA, Braun K, Samans B, Watt FM, Elsasser HP, Eilers M. Myc regulates keratinocyte adhesion and differentiation via complex formation with Miz1. J Cell Biol. 2006; 172: 139-49.

18. Fuchs E, Cleveland DW. A structural scaffolding of intermediate filaments in health and disease. Science. 1998; 279: 514-9.

19. Kirfel G, Herzog V. Migration of epidermal keratinocytes: mechanisms, regulation, and biological significance. Protoplasma. 2004; 223: 67-78.

20. Lamprecht SA, Lipkin M. Chemoprevention of colon cancer by calcium, vitamin D and folate: molecular mechanisms. Nat Rev Cancer. 2003; 3: 601-14.

21. Bikle DD. Vitamin D and skin cancer. J Nutr. 2004; 134: 3472S-3478S.
22. Wood AW, Chang RL, Huang MT, Uskokovic M, Conney AH. 1 alpha, 25-Dihydroxyvitamin D3 inhibits phorbol ester-dependent chemical carcinogenesis in mouse skin. Biochem Biophys Res Commun. 1983; 116: 605-11.

23. Kensler TW, Dolan PM, Gange SJ, Lee JK, Wang Q, Posner GH. Conceptually new deltanoids (vitamin D analogs) inhibit multistage skin tumorigenesis. Carcinogenesis. 2000; 21: 1341-5.

24. Sebag M, Henderson J, Rhim J, Kremer R. Relative resistance to 1,25-dihydroxyvitamin D3 in a keratinocyte model of tumor progression. J Biol Chem. 1992; 267: 12162-7.

25. Liu M, Lee MH, Cohen M, Bommakanti M, Freedman LP. Transcriptional activation of the Cdk inhibitor p21 by vitamin D3 leads to the induced differentiation of the myelomonocytic cell line U937. Genes Dev. 1996; 10: 142-53.

26. Xie Z, Komuves L, Yu QC, Elalieh H, Ng DC, Leary C, Chang S, Crumrine D, Yoshizawa T, Kato S, Bikle DD. Lack of the vitamin D receptor is associated with reduced epidermal differentiation and hair follicle growth. J Invest Dermatol. 2002; 118: 11-6.

27. Schmidt-Ullrich R, Tobin DJ, Lenhard D, Schneider P, Paus R, Scheidereit C. NF-kappaB transmits Eda A1/EdaR signalling to activate Shh and cyclin D1 expression, and controls post-initiation hair placode down growth. Development. 2006; 133: 1045-57.

28. Seitz CS, Lin Q, Deng H, Khavari PA. Alterations in NF-kappaB function in transgenic epithelial tissue demonstrate a growth inhibitory role for NF-kappaB. Proc Natl Acad Sci U S A. 1998; 95: 2307-12.

29. Stappenbeck TS, Lamb JA, Corcoran CM, Green KJ. Phosphorylation of the desmoplakin COOH terminus negatively regulates its interaction with keratin intermediate filament networks. J Biol Chem. 1994; 269: 29351-4.

30. Kouklis PD, Hutton E, Fuchs E. Making a connection: direct binding between keratin intermediate filaments and desmosomal proteins. J Cell Biol. 1994; 127: 1049-60.

31. Smith EA, Fuchs E. Defining the interactions between intermediate filaments and desmosomes. J Cell Biol. 1998; 141: 1229-41.

32. Hennings H, Michael D, Cheng C, Steinert P, Holbrook K, Yuspa SH. Calcium regulation of growth and differentiation of mouse epidermal cells in culture. Cell. 1980; 19: 245-54.

33. Green KJ, Geiger B, Jones JC, Talian JC, Goldman RD. The relationship between intermediate filaments and microfilaments before and during the formation of desmosomes and adherens-type junctions in mouse epidermal keratinocytes. J Cell Biol. 1987; 104: 1389-402.

34. Wheelock MJ, Jensen PJ. Regulation of keratinocyte intercellular junction organization and epidermal morphogenesis by E-cadherin. J Cell Biol. 1992; 117: 415-25.

35. Lewis JE, Jensen PJ, Wheelock MJ. Cadherin function is required for human keratinocytes to assemble desmosomes and stratify in response to calcium. J Invest Dermatol. 1994; 102: 870-7.
36. Vasioukhin V, Bauer C, Yin M, Fuchs E. Directed actin polymerization is the driving force for epithelial cell-cell adhesion. Cell. 2000; 100: 209-19.

37. Vasioukhin V, Bowers E, Bauer C, Degenstein L, Fuchs E. Desmplakin is essential in epidermal sheet formation. Nat Cell Biol. 2001; 3: 1076-85.

38. O'Keefe EJ, Briggaman RA, Herman B. Calcium-induced assembly of adherens junctions in keratinocytes. J Cell Biol. 1987; 105: 807-17.

39. Pellegrini G, Dellambra E, Golisano O, Martinelli E, Fantozzi I, Bondanza S, Ponzin D, McKeon F, De Luca M. p63 identifies keratinocyte stem cells. Proc Natl Acad Sci U S A. 2001; 98: 3156-61.

40. Su MJ, Bikle DD, Mancianti ML, Pillai S. 1,25-Dihydroxyvitamin D3 potentiates the keratinocyte response to calcium. J Biol Chem. 1994; 269: 14723-9.

41. Ng DC, Su MJ, Kim R, Bikle DD. Regulation of involucrin gene expression by calcium in normal human keratinocytes. Front Biosci. 1996; 1: a16-24.

42. Pillai S, Bikle DD. Role of intracellular-free calcium in the cornified envelope formation of keratinocytes: differences in the mode of action of extracellular calcium and 1,25 dihydroxyvitamin D3. J Cell Physiol. 1991; 146: 94-100.

43. Oda Y, Tu CL, Pillai S, Bikle DD. The calcium sensing receptor and its alternatively spliced form in keratinocyte differentiation. J Biol Chem. 1998; 273: 23344-52.

44. Oda Y, Tu CL, Chang W, Crumrine D, Komuves L, Mauro T, Elias PM, Bikle DD. The calcium sensing receptor and its alternatively spliced form in murine epidermal differentiation. J Biol Chem. 2000; 275: 1183-90.

45. Tu CL, Chang W, Bikle DD. The extracellular calcium-sensing receptor is required for calcium-induced differentiation in human keratinocytes. J Biol Chem. 2001; 276: 41079-85.

46. Ratnam AV, Bikle DD, Cho JK. 1,25 dihydroxyvitamin D3 enhances the calcium response of keratinocytes. J Cell Physiol. 1999; 178: 188-96.

47. Pillai S, Bikle DD, Mancianti ML, Cline P, Hincenbergs M. Calcium regulation of growth and differentiation of normal human keratinocytes: modulation of differentiation competence by stages of growth and extracellular calcium. J Cell Physiol. 1990; 143: 294-302.

48. Mauro TM, Isseroff RR, Lasarow R, Pappone PA. Ion channels are linked to differentiation in keratinocytes. J Membr Biol. 1993; 132: 201-9.

49. Grando SA, Horton RM, Mauro TM, Kist DA, Lee TX, Dahl MV. Activation of keratinocyte nicotinic cholinergic receptors stimulates calcium influx and enhances cell differentiation. J Invest Dermatol. 1996; 107: 412-8.

50. Oda Y, Timpe LC, McKenzie RC, Sauder DN, Largman C, Mauro T. Alternatively spliced forms of the cGMP-gated channel in human keratinocytes. FEBS Lett. 1997; 414: 140-5.
51. Eckert RL, Crish JF, Efimova T, Dashti SR, Deucher A, Bone F, Adhikary G, Huang G, Gopalakrishnan R, Balasubramanian S. Regulation of involucrin gene expression. J Invest Dermatol. 2004; 123: 13-22.

52. Tu CL, Chang W, Bikle DD. Phospholipase c gamma1 is required for activation of store-operated channels in human keratinocytes. J Invest Dermatol. 2005; 124: 187-97.

53. Jaken S, Yuspa SH. Early signals for keratinocyte differentiation: role of Ca\(^{2+}\)-mediated inositol lipid metabolism in normal and neoplastic epidermal cells. Carcinogenesis. 1988; 9: 1033-8.

54. Xie Z, Bikle DD. Phospholipase C gamma1 is required for calcium-induced keratinocyte differentiation. J Biol Chem. 1999; 274: 20421-4.

55. Elias PM, Menon GK. Structural and lipid biochemical correlates of the epidermal permeability barrier. Adv Lipid Res. 1991; 24: 1-26.

56. Marekov LN, Steinert PM. Ceramides are bound to structural proteins of the human foreskin epidermal cornified cell envelope. J Biol Chem. 1998; 273: 17763-70.

57. Polakowska RR, Goldsmith LA. The cell envelope and transglutaminases. In: Goldsmith LA. ed. Physiology, Biochemistry, and Molecular Biology of the Skin, Second Edition. New York: Oxford University Press, Inc., 1991, pp 168 - 201.

58. Kalinin AE, Kajava AV, Steinert PM. Epithelial barrier function: assembly and structural features of the cornified cell envelope. BioEssays. 2002; 24: 789-800.

59. Ruhrberg C, Hajibagheri MA, Parry DA, Watt FM. Periplakin, a novel component of cornified envelopes and desmosomes that belongs to the plakin family and forms complexes with envoplakin. J Cell Biol. 1997; 139: 1835-49.

60. Nemes Z, Steinert PM. Bricks and mortar of the epidermal barrier. Exp Mol Med. 1999; 31: 5-19.

61. Steinert PM, Candi E, Kartasova T, Marekov L. Small proline-rich proteins are cross-bridging proteins in the cornified cell envelopes of stratified squamous epithelia. J Struct Biol. 1998; 122, 76-85.

62. Candi E, Oddi S, Paradisi A, Terrinoni A, Ranalli M, Teofoli P, Citro G, Scarpato S, Puddu P, Melino G. Expression of transglutaminase 5 in normal and pathologic human epidermis. J Invest Dermatol. 2002; 119: 670-7.

63. Zeeuwen PL. Epidermal differentiation: the role of proteases and their inhibitors. Eur J Cell Biol. 2004; 83: 761-73.

64. Nemes Z, Marekov LN, Fesus L, Steinert PM. A novel function for transglutaminase 1: attachment of long-chain omega-hydroxyceramides to involucrin by ester bond formation. Proc Natl Acad Sci U S A. 1999; 96: 8402-7.

65. Kim SY, Chung SI, Steinert PM. Highly active soluble processed forms of the transglutaminase 1 enzyme in epidermal keratinocytes. J Biol Chem. 1995; 270: 18026-35.
66. Steinert PM, Chung SI, Kim SY. Inactive zymogen and highly active proteolytically processed membrane-bound forms of the transglutaminase 1 enzyme in human epidermal keratinocytes. Biochem Biophys Res Commun. 1996; 221: 101-6.

67. Egberts F, Heinrich M, Jensen JM, Winoto-Morbach S, Pfeiffer S, Wickel M, Schunck M, Steude J, Saftig P, Proksch E, Schutze S. Cathepsin D is involved in the regulation of transglutaminase 1 and epidermal differentiation. J Cell Sci. 2004; 117, 2295-307.

68. Steinert PM, Marekov LN. Initiation of assembly of the cell envelope barrier structure of stratified squamous epithelia. Mol Biol Cell. 1999; 10: 4247-61.

69. Steinert PM, Marekov LN. Direct evidence that involucrin is a major early isopeptide cross-linked component of the keratinocyte cornified cell envelope. J Biol Chem. 1997; 272: 2021-30.

70. Djalilian AR, McGaughey D, Patel S, Seo EY, Yang C, Cheng J, Tomic M, Sinha S, Ishida-Yamamoto A, Segre JA. Connexin 26 regulates epidermal barrier and wound remodeling and promotes psoriasiform response. J Clin Invest. 2006; 116: 1243-53.

71. Koch PJ, de Viragh PA, Scharer E, Bundman D, Longley MA, Bickenbach J, Kawachi Y, Suga Y, Zhou Z, Huber M, Hohl D, Kartasova T, Jarnik M, Steven AC, Roop DR. Lessons from loricrin-deficient mice: compensatory mechanisms maintaining skin barrier function in the absence of a major cornified envelope protein. J Cell Biol. 2000; 151: 389-400.

72. Djian P, Easley K, Green H. Targeted ablation of the murine involucrin gene. J Cell Biol. 2000; 151: 381-8.

73. Maatta A, DiColandrea T, Groot K, Watt FM. Gene targeting of envoplakin, a cytoskeletal linker protein and precursor of the epidermal cornified envelope. Mol Cell Biol. 2001; 21: 7047-53.

74. Franzke CW, Baici A, Bartels J, Christophers E, Wiedow O. Antileukoprotease inhibits stratum corneum chymotryptic enzyme. Evidence for a regulative function in desquamation. J Biol Chem. 1996; 271: 21886-90.

75. Jarvinen M, Rinne A, Hopsu-Havu VK. Human cystatins in normal and diseased tissues-a review. Acta Histochem. 1987; 82: 5-18.

76. Zeeuwen PL, Van Vlijmen-Willems IM, Jansen BJ, Sotiropoulou G, Curfs JH, Meis JF, Janssen JJ, Van Ruissen F, Schalkwijk J. Cystatin M/E expression is restricted to differentiated epidermal keratinocytes and sweat glands: a new skin-specific proteinase inhibitor that is a target for cross-linking by transglutaminase. J Invest Dermatol. 2001; 116: 693-701.

77. Zeeuwen PL, van Vlijmen-Willems IM, Hendriks W, Merkx GF, Schalkwijk J. A null mutation in the cystatin M/E gene of ichq mice causes juvenile lethality and defects in epidermal cornification. Hum Mol Genet. 2002; 11: 2867-75.

78. Chavanas S, Bodemer C, Rochat A, Hamel-Teillac D, Ali M, Irvine AD, Bonafe JL, Wilkinson J, Taieb A, Barrandon Y, Harper JI, de Prost Y, Hovnanian A. Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. Nat Genet. 2000; 25: 141-2.
79. Marks R. The stratum corneum barrier: the final frontier. J Nutr. 2004; 134: 2017S-2021S.

80. Jamora C, Fuchs E. Intercellular adhesion, signalling and the cytoskeleton. Nat Cell Biol. 2002; 4: E101-8.

81. Egelrud T. Desquamation in the stratum corneum. Acta Derm Venereol Suppl (Stockh). 2000; 208: 44-5.

82. Caubet C, Jonca N, Brattsand M, Guerrin M, Bernard D, Schmidt R, Egelrud T, Simon M, Serre G. Degradation of corneodesmosome proteins by two serine proteases of the kallikrein family, SCTE/KLK5/hK5 and SCCE/KLK7/hK7. J Invest Dermatol. 2004; 122: 1235-44.

83. Backman A, Strandén P, Brattsand M, Hansson L, Egelrud T. Molecular cloning and tissue expression of the murine analog to human stratum corneum chymotryptic enzyme. J Invest Dermatol. 1999; 113: 152-5.

84. Brattsand M, Egelrud T. Purification, molecular cloning, and expression of a human stratum corneum trypsin-like serine protease with possible function in desquamation. J Biol Chem. 1999; 274: 30033-40.

85. Sondell B, Thornell LE, Egelrud T. Evidence that stratum corneum chymotryptic enzyme is transported to the stratum corneum extracellular space via lamellar bodies. J Invest Dermatol. 1995; 104: 819-23.

86. Ekholm IE, Brattsand M, Egelrud T. Stratum corneum tryptic enzyme in normal epidermis: a missing link in the desquamation process? J Invest Dermatol. 2000; 114: 56-63.

87. Igarashi S, Takizawa T, Takizawa T, Yasuda Y, Uchiwa H, Hayashi S, Brysk H, Robinson JM, Yamamoto K, Brysk MM, Horikoshi T. Cathepsin D, but not cathepsin E, degrades desmosomes during epidermal desquamation. Br J Dermatol. 2004; 151: 355-61.

88. Bernard D, Mehul B, Thomas-Collignon A, Simonetti L, Remy V, Bernard MA, Schmidt R. Analysis of proteins with caseinolytic activity in a human stratum corneum extract revealed a yet unidentified cysteine protease and identified the so-called "stratum corneum thiol protease" as cathepsin 12. J Invest Dermatol. 2003; 120: 592-600.

89. Itin PH, Fistarol SK. Palmoplantar keratodermas. Clin Dermatol. 2005; 23: 15-22.

90. Nuckolls GH, Slavkin HC. Paths of glorious proteases. Nat Genet. 1999; 23: 378-80.

91. Hart TC, Hart PS, Michalec MD, Zhang Y, Firatli E, Van Dyke TE, Stabholz A, Zlotogorski A, Shapira L, Soskolne WA. Haim-Munk syndrome and Papillon-Lefevre syndrome are allelic mutations in cathepsin C. J Med Genet. 2000; 37: 88-94.

92. de Haar SF, Tigchelaar-Gutter W, Everts V, Beertsen W. Structure of the periodontium in cathepsin C-deficient mice. Eur J Oral Sci. 2006; 114: 171-3.

93. Fusek M, Vetvicka V. Dual role of cathepsin D: ligand and protease. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2005; 149: 43-50.
94. Tobin DJ, Foitzik K, Reinheckel T, Mecklenburg L, Botchkarev VA, Peters C, Paus R. The lysosomal protease cathepsin L is an important regulator of keratinocyte and melanocyte differentiation during hair follicle morphogenesis and cycling. Am J Pathol. 2002; 160: 1807-21.

95. Benavides F, Starost MF, Flores M, Gimenez-Conti IB, Guenet JL, Conti CJ. Impaired hair follicle morphogenesis and cycling with abnormal epidermal differentiation in nackt mice, a cathepsin L-deficient mutation. Am J Pathol. 2002; 161: 693-703.

96. Roth W, Deussing J, Botchkarev VA, Pauly-Evers M, Saftig P, Hafner A, Schmidt P, Schmahl W, Scherer J, Anton-Lamprecht I, Von Figura K, Paus R, Peters C. Cathepsin L deficiency as molecular defect of furless: hyperproliferation of keratinocytes and pertubation of hair follicle cycling. FASEB J. 2000; 14: 2075-86.

97. Watkinson A. Stratum corneum thiol protease (SCTP): a novel cysteine protease of late epidermal differentiation. Arch Dermatol Res. 1999; 291: 260-8.

98. Hagemann S, Gunther T, Dennemarker J, Lohmuller T, Bromme D, Schule R, Peters C, Reinheckel T. The human cysteine protease cathepsin V can compensate for murine cathepsin L in mouse epidermis and hair follicles. Eur J Cell Biol. 2004; 83: 775-80.

99. List K, Haudenschild CC, Szabo R, Chen W, Wahl SM, Swaim W, Engelholm LH, Behrendt N, Bugge TH. Matriptase/MT-SP1 is required for postnatal survival, epidermal barrier function, hair follicle development, and thymic homeostasis. Oncogene. 2002; 21: 3765-79.

100. List K, Bugge TH, Szabo R. Matriptase: Potent Proteolysis on the Cell Surface. Mol Med. 2006 Jun 20;

101. Geng S, Mezentsev A, Kalachikov S, Raith K, Roop DR, Panteleyev AA. Targeted ablation of Arnt in mouse epidermis results in profound defects in desquamation and epidermal barrier function. J Cell Sci. 2006; 4901-12.

102. Cowling VH, Cole MD. Mechanism of transcriptional activation by the Myc oncoproteins. Semin Cancer Biol. 2006, 16(4); 242-52.

103. Zanet J, Pibre S, Jacquet C, Ramirez A, de Alboran IM, Gandarillas A. Endogenous Myc controls mammalian epidermal cell size, hyperproliferation, endoreplication and stem cell amplification. J Cell Sci. 2005; 118(Pt 8); 1693-704.

104. Orian A, Eisenman RN. TGF-beta flips the Myc switch. Sci STKE. 2001; 2001(88); PE1.

105. Watt, F.M. Role of integrins in regulating epidermal adhesion, growth and differentiation. EMBO J. 2002; 21; 3919–3926.