Chapter 5

Keratins in Skin Epidermal Development and Diseases

Ling-juan Zhang

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.79050

Abstract

Epidermal keratinocyte (KC), the major cell type in the skin epidermis, plays critical roles in forming a permeability barrier to separate internal organs from external stimuli. Keratins, constituting about 30–80% of the total protein in KCs, form the major intermediate filament cytoskeleton of KC. Keratins consist of 54 unique genes in humans and they are expressed in cell-, differentiation- and development-dependent manner. While keratin pairs K5-K14 and K1-K10 are normally associated with KCs at different cell differentiation stages, other keratin pairs such as K6-K16/K17 and K8–K18 and are usually not expressed in normal skin interfollicular epidermis, but are elevated during wounding, inflammatory skin diseases such as psoriasis or malignant conversion of KC. The expression and function of keratins are tightly regulated at both transcriptional and post-transcriptional levels. Inherited or spontaneous mutations in keratins or abnormal keratin regulations or modifications can cause KC and cutaneous tissue fragility, skin hypertrophic and inflammatory conditions or malignant transformation of KC, therefore accounting for a large number of disorders in human skin. Here we review the recent literature on how keratins are normally expressed during skin development and how mutations or misregulations of these keratins are involved in the pathogenesis of skin diseases.

Keywords: keratins, keratinocytes, skin barrier, differentiation, skin blistering diseases, wounding, psoriasis, skin tumor

1. Introduction: keratins—principal structural proteins of the skin epidermis

Locating at the outermost layer of the skin, the epidermis plays a critical role protecting our body against environmental pathogens and insults by forming a physical and immunological barrier. This protective role of skin epidermis is manifested by extensive cytoskeletal architecture, and keratins represent its principle structural protein, contributing to 30–80% of the total

protein and forming the major intermediate filament cytoskeleton of the epidermis. Since keratin family proteins were initially characterized based on their mobility on 2D SDS-PAGE back in 1980s, more than 50 mammalian keratins have been identified and characterized. Keratins can be sub-classified into two distinct classes: Type I keratins, including K9–K40, are relative acidic (pKi = 4.5–5.5) and small (40–56.5 kDa) whereas type II keratins, including K1–K8 and K71–86), are more basic (pKi = 5.5–7.5) and larger (53–67 kDa) [1–3]. The active keratin genes are clustered into two dense region of the chromosome: all type II keratins plus one type I keratin (K18) are located on chromosome 12q, and the remaining type I keratins are all on chromosome 17q. Despite the fact that type I and type II keratins are located at distinct region of the chromosome, they show beautifully specific patterns of gene expression within adjacent epidermal cell layers and a specific pair of keratins are usually co-expressed as a heterodimer between one acidic (type I) and one basic (type II) keratin [4]. These keratin heterodimers self-assemble into antiparallel, staggered tetramers, yielding intermediate filament through lateral and longitudinal interactions [4].

The primary function of the keratin intermediate filament cytoskeleton is to provide cells with structural resilience against mechanical trauma, and this is especially important for epidermal cells because as the outermost barrier tissue the epidermis has to have the ability to resist some of the most severe physical stress levels experienced by any human tissue. The basic structural organization of keratin intermediate filaments contains four helical or coiled-coil segments flanked by N- and C-terminal glycine rich sequence. And most disease-causing mutations occur in the well-conserved coiled-coil domains of keratins, leading to disruption of secondary structure formation between the heterodimer and the subsequent aggregation of the keratin filaments. In addition to structural and mechanical support, cell-specific keratin expression also modulates growth, adhesion, migration and invasion of epithelial cells. Thus, dysfunction or mutations of keratin proteins are associated with a remarkable variety of skin disorders, such as skin blistering, inflammatory disorders and skin tumors. So far more than 100 different disorders (termed as keratinopathies) have been linked to inherited keratin changes (www.interfil.org).

In this chapter we will first describe how keratins are normally expressed and regulated during epidermal development and in adult homeostasis, then we will describe how mutation or abnormal expression or modification of keratin proteins are associated with various skin diseases, and their function and regulatory mechanisms during disease pathogenesis. Keratin mutations and related diseases in skin appendages, including hair and nail, will not be reviewed here.

2. Keratin expression during epidermal development

2.1. Keratin expression kinetics during epidermal development

The barrier function of epidermis is mainly provided by keratinocytes (KC), the predominant cell type in the epidermis, and it is maintained by a tightly controlled balance between

proliferation and differentiation of KC [5, 6]. As shown in **Figure 1**, the murine epidermal development begins at embryonic day (E) 8.5 from a single layer of progenitor cells that express keratin pair K8–K18, and around E 9.5 these progenitor cells are specified to an epidermal cell fate and switch to express a different pair of keratins, K5–K14 [7–10]. Between E 10.5–E15.5, the committed KC begin a process of upward stratification and differentiation leading to the generation of suprabasal cells, in which cell cycle is arrested and early differentiation program is initiated and K5/K14 are substituted by K1 and K10. Studies have suggested that the ectopic expression of K10 inhibits cell cycle progression and proliferation and thus promotes terminal differentiation of keratinocytes [11, 12]. At E 16.5, the suprabasal KC commit to terminal differentiation and continue to migrate upward forming the granular layer, and these granular KCs express late differentiation markers, such as Filaggrin (FLG), Loricrin (LOR) and Involucrin (INV). By E 18.5, epidermis becomes fully mature, and terminally differentiated KCs become enucleated corneocytes forming the outer most cornified layer with complete barrier function.

Note that K15 is an additional type I keratin protein co-expressed with K5/K14 in the basal keratinocytes of stratified epithelia and in the bulge in hair follicles [13], and K2 is an additional type II keratin protein co-expressed with K1/K10 in the differentiated keratinocytes [14]. Although K15 or K2 are generally considered as minor keratins, but the ratio of K15 to K14 or K2 to K1 can vary dramatically during development or upon disease condition at different skin sites [15]. In adult skin, K15 expression is restricted to hair follicle stem cells [16, 17]. This cell-stage specific expression pattern of keratins is precisely maintained through postnatal development and adulthood under homeostatic condition in both human and mouse skin epidermis. Therefore K5 and/or K14 are highly specific markers for basal proliferative KC, K15 is generally used as a marker for epithelial stem cells in the hair follicle bulge, and K1 and/or K10 have been robustly used to mark KC in the early differentiation stage (**Figure 2**).

Figure 1. Overview of keratin expressions during different stage of mouse epidermal development. Murine epidermis develops from a single layer ectodermal keratinocyte (KC) progenitor cells which commit to an epidermal fat around embryonic day 8–9 (E 8–9). These KC progenitors express simple epithelia cell marker keratin pair of K18-K8. Around E 10.5 and the expression of K18/K8 starts to be substituted by K14/K5 in the committed KC, and stratification process starts leading to the formation of suprabasal cells. Around E15.5, early differentiation starts in the spinous/suprabasal layer (SL) and K10/K1 are expressed in the differentiated suprabasal cells while the expression of K14/K5 is restricted to basal layer (BL). At E16.5, suprabasal cells committed to terminal differentiation move upwards to form granular layer (GL) and K10/K1 expression are replaced by late differentiation markers. Finally cornification starts around E 16.5–17.5 and by E 18.5 epidermis becomes fully mature with intact cornified layer and complete barrier function.

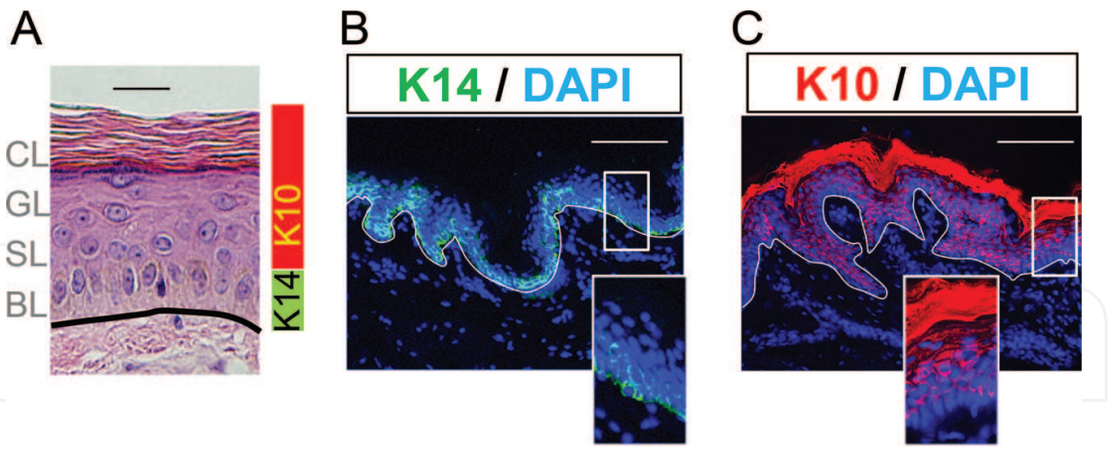

Figure 2. Expression of K14 and K10 in adult human skin epidermis. (A). H&E staining of adult human skin epidermis showing distinct epidermal layers including basal layer (BL), spinous (suprabasal) layer (SL), granular layer (GL) and cornified layer (CL). The dashed line marks the epidermal-dermal junction. Boxes on the right panel indicate the location of K14 and K10 expression in distinct epidermal layers. While K14 is restricted to cells in the basal layer, K10 is induced in early differentiated cells at the spinous layer and is maintained through all differentiated layers. Scale bar = 20 μm. (B-C). Adult human skin epidermis was immunostained by antibody specific for K14 (green in B) or K10 (red in C). Zoom-in pictures highlighted in white box are shown to illustrate distinct localization of K14 and K10 in skin epidermis. Scale bars = 100 μm.

2.2. Regulation of keratin expression during development

Keratin synthesis is regulated at the level of transcription by a characteristic constellation of transcription factors. Regulatory mechanisms for the expression of K5 and K14, the key keratins forming the cytoskeletal IF network in mitotically active basal cells, have been extensive studied. Studies have shown that the promoter activities of K5 and K14 genes are collaboratively regulated by several transcription factors, including AP1, AP2, NFκB, Skn-1a, Tst-1, RAR (nuclear receptor for retinoic acid), T3R (receptor for thyroid hormone), GR (glucocorticoid receptor) and coactivator CBP/p300 in response to many extracellular signals, such as growth factor, vitamins (retinoic acid/VitA), thyroid hormone, or glucocorticoids [18–22] .

The expression of K1 and K10 is upregulated during early differentiation process. Forced expression of transcription factor C/EBPβ in keratinocytes arrested growth and induced the expression of K1 and K10 but had a minimal effect on the expression of late differentiation markers [23]. In consistent, Cebpb-deficient mice had reduced levels of K1 and K10 but not of Lor or Inv, suggesting that C/EBPβ modulates K1 and K10 expression during early events of keratinocyte differentiation. Other transcription factors, such as C/EBPα and AP2 are also required for K10 expression during differentiation [24]. Therefore, C/EBPβ, C/EBPα and AP2 are considered the differentiation-associated transcription factors controlling early differentiation process of keratinocytes. We have shown previously that in cultured primary mouse basal keratinocytes, while elevating extracellular calcium robustly triggers stratification and the expression of late differentiation markers, K10 expression is only moderately increased by high calcium [6, 25]. In contrast, growth factor depletion/starvation strongly induced K10 expression [6, 25], suggesting that growth arrest but not high calcium is the key signal to turn on K10 expression. Indeed, studies have shown that C/EBPβ expression localizes in the upper

differentiated layers of human skin epidermis [26] and C/EBP family of transcription factors inhibit proliferation by blocking cell cycle progression [27, 28]. Therefore it is possible that growth factor depletion condition in culture induces and activates C/EBPs which in turn trigger cell cycle arrest and induction of K10 gene.

K8 and K18 are the keratin pair that is expressed earliest during embryonic development of the epidermis and their expressions are suppressed upon epidermal progenitor cells commitment to keratinocyte lineage [8, 29]. Several transcription factors, such as p63, Ovol2 and Ctip2, have been shown to suppress K8 expression during epidermal development [6, 9, 30]. It has been shown that developing murine p63−/− epidermis expressed high level of K8 and failed to develop a fully mature stratified epidermis [9], suggesting that p63 may regulate epidermal development through suppressing K8 transcription. We have also shown that in wild-type mouse keratinocytes K8 promoter was repressed by transcription factor Ctip2, and upon Ctip2 depletion K8 expression became strongly upregulated in both developing epidermis and in primary mouse keratinocytes at both transcript and protein levels, demonstrating that Ctip2 functions as a transcription suppressor of K8 gene [6].

3. Keratin disorders and skin diseases

3.1. Epidermolysis bullosa simplex diseases and K5/K14

Epidermolysis bullosa simplex (EBS) is an autosomal dominant skin disorder, manifests itself upon trauma in the form of epidermal basal cell death leading to skin blisters [31]. The primary cause of EBS is dominant mutations in either of the genes encoding keratins K5 or K14 [32]. This pair of type I (K14) and type II (K5) is specifically expressed in the basal cell layer of the skin epidermis, which is in direct contact with the basement membrane of the underlying dermis. Therefore, loss of function or mutation of K5 or K14 leads to defects in keratin filament formation in basal keratinocytes and thus triggers skin blistering due to fragility of the basal cell compartment in either human or mouse skin epithelium [33–35] (**Figure 3**). Additionally, it has been shown that overexpression of transcription factor Ovol2 in mouse basal keratinocytes caused reduced K5 and K14 expression, leading to a severe blistering phenotype that resembles the clinical features of EBS [30].

Although K5/K14 mutations are associated with the majority of EBS population, about a quarter of patients with EBS do not have genomic mutations nor abnormal transcript expressions of K5 or K14 [36], suggesting that alternative pathways may be involved in the pathogenesis of these patients. Indeed, recent studies have suggested that in addition to genetic mutation, abnormal K5/K14 functions in basal keratinocytes can also be regulated at post-transcriptional level through post-translational modifications, such as phosphorylation and disulfide bonding. Abnormal phosphorylation of keratins contributes to the pathogenesis and progression of EBS [37]. For example, threonine 150 (T150) in the head domain of K5 was found to be phosphorylated in human EBS keratinocytes. Expression of phosphomimetic T150D K5 mutant in keratinocytes arrested keratin heterodimer assembly leading to impaired

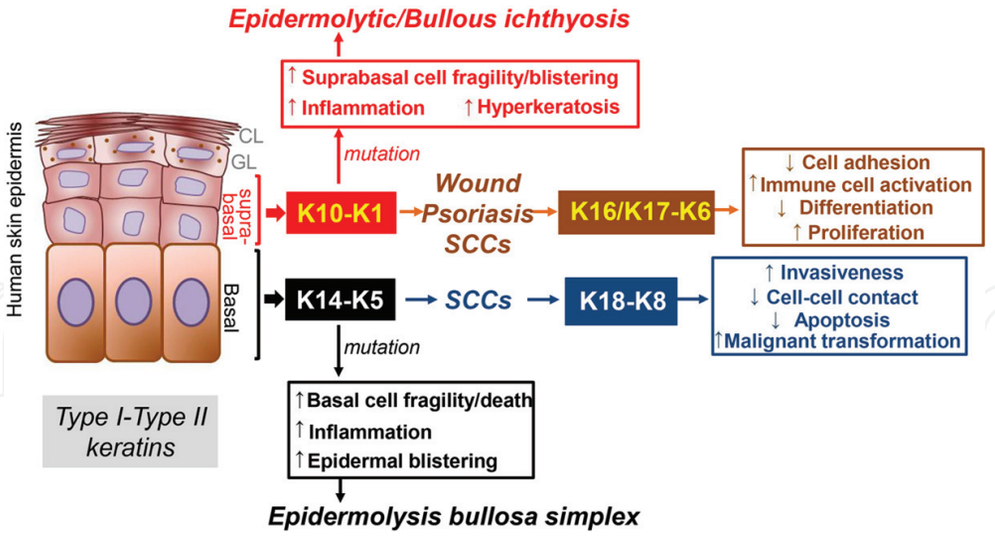

Figure 3. Keratins and skin diseases. Autosomal dominant mutations of basal cell keratins K14 or K5 are associated with skin blistering disease "Epidermolysis Bullosa simplex" and are caused by an increase in basal cell fragility/death, epidermal inflammation and epidermal blistering. Autosomal mutations of the suprabasal KC markers K10 and K1 are associated with hyperkeratosis skin disease "Epidermolytic/Bullous ichthyosis" and are caused by an increase in suprabasal cell fragility/blistering, inflammation, proliferation of basal cells and hyperkeratosis of epithelium. In skin wounds, inflammatory skin diseases such as psoriasis, or squamous cell carcinomas (SCCs), suprabasal cells express high levels of K6, K16 and K17 (keratins associated with activated KC), and these keratins decrease KC adhesion and differentiation and promote immune cell activation and KC proliferation. K8/K18 expression is found in poorly differentiated SCCs with increased invasiveness. K8 or K18 expression in KC leads to decreased cell–cell contact and an increase protection against apoptosis, as well as invasiveness and malignant transformation potential of KC.

keratin filament formations and reduced cell viability and elevated response to stressors [38], suggesting a possible role of K5 T150 phosphorylation in EBS pathogenesis. In addition to phosphorylation, it has been shown that proper disulfide bonding between K14-K5 heterodimers is required to maintain keratin intermediate filament organization and dynamics in primary mouse skin keratinocytes, and disruption of this K14-dependent disulfide linkages may lead to keratinopathies, such as EBS [39].

3.2. Epidermolytic hyperkeratosis diseases and K1/K10

When basal keratinocytes migrate to suprabasal layer of the epidermis, supra basal cells cease transcription of K5 and K14 but instead express K1 and K10. Mutations in either K1 or K10 cause several human skin diseases, such as Epidermolytic ichthyosis (EI), Bullous ichthyosis (BI), palmar-plantar keratoderma and Epidermolytic nevus (EN). BI/EI are caused by rare autosomal dominant mutations of either of K1 or K10 that manifest at birth with fragile blisters and erosions that develop into hyperkeratotic lesions. X-ray crystal structure analysis of K1-K10 heterodimer suggested that point mutation of these keratins may disrupt the disulfide linkage and secondary structure formation between the heterodimer leading to aggregation of the keratin filaments [40]. In contrast to EBI, only the K1 or K10 expressing suprabasal cells are affected in EI/BI patients, and the basal proliferative compartment are not affected. However, these unaffected basal proliferating cells are bathed beneath the rupturing suprabasal cells with inflammatory cytokines, leading to over-proliferation of the basal cells and epithelium – known

as hyperkeratosis (**Figure 3**). Therefore, BI skin contains a highly thicken epidermis made up of fragile cells and it is highly susceptible to bacterial and fungal colonization and is highly disfiguring and debilitating for the patient.

In contrast to EI or BI, Epidermolytic nevus (EN) is caused by somatic mutation of either K1 or K10, and the skin blistering phenotype is only affecting part of the body [41, 42]. Because germ line cells are not affected in EN patient, EN parents usually do not transmit the mutations and disease to next generation. However, it has been reported that under rare conditions EN can produce EI in the next generation through transmission from mosaic to germ line. And the risk of disease transmission to the next generation can be evaluated by next generation sequencing of mutation rate in sperm, leukocytes and lesional skin of the EN patients who wish to bear children [43].

3.3. Superficial epidermolytic ichthyosis (SEI) and K2

Superficial epidermolytic ichthyosis (SEI), previously known as ichthyosis bullosa of Siemens (IBS), is an autosomal dominant skin disorder linked to K2 mutations and it is characterized by superficial epidermal fragility and desquamation that lead to characteristic denuded areas. In SEI, aggregates of KF bundles and cytolysis are confined to the upper spinous and granular layers of the epidermis where K2 is expressed [44, 45]. In human, K2 is expressed later in differentiation in the upper spinous and granular layers of skin collected from different body sites [14], but SEI patients usually develop more severe symptoms in palms and soles compared to other body sites suggesting that K2 may play a major role to support tissue integrity in these areas. The tissue specific expression pattern of K2 has been better characterized in mouse skin: in regions of the soles (except foodpad which expresses K2-K9/K10), ears and tail of the mouse, K2 instead of K1 is the major type II keratin that pairs with K10. K2−/-K10−/− double knockout mice or K2−/−mice developed epidermal acanthosis and hyperkeratosis in the tail epidermis, ear epidermis and inter-footpad epidermis of the soles [46, 47], demonstrating that K2-K10 keratin pairs are essential for the epidermal integrity of plantar skin.

3.4. Epidermal palmoplantar keratoderma (EPPK) and K9

Epidermal palmoplantar keratoderma (PPK) is an autosomal dominant skin disorder that develops shortly after birth, and manifests as diffuse hyperkeratosis of the palms and soles and showing sharp demarcations with erythematous margin. Mutations of K9, which is expressed specifically in the suprabasal keratinocytes of the glabrous skin epidermis (palms and soles), are the major cause for EPPK [48, 49]. In human plantar and palmar epidermis, K9 functions as the additional type I keratin, besides K10, to partner with type II keratin K1 [50]. Therefore, mutations of K9 cause pathological epidermal thickening on palms and soles, manifesting as different forms of palmoplantar keratodermas [49]. In mice, K9 expression is restricted to skin epidermis of the footpad, and K9 deficient mice developed calluses marked by hyperpigmentation that are exclusively localized to the stress-bearing footpad. Additionally, hyperproliferation, impaired terminal differentiation, and abnormal expression of K5, K14 and K2 were found in the lesions of K9 deficient mice [51]. Together, these evidence demonstrate that K9 is required for the structural integrity and terminal differentiation of the palmoplantar epidermis.

4. Skin wounds and inflammatory skin diseases and K6/K16/K17

In mammals, the skin of the palm is uniquely adapted to withstand remarkable physical stress, and the palmoplantar epidermis contains a more complex pattern of keratins than thin skin and it is characterized by the constitutive expression of K6, 16, 17 and K9 [52, 53]. Mutations in K6, K16 and K17 genes cause pachyonychia congenita (thick nails, plantar keratoderma) [49].

Distinct from the palmoplantar epidermis, the interfollicular epidermis normally does not express K6, 16 or K17 under homeostatic conditions but these genes can be induced in interfollicular keratinocytes upon activation and reflects a hyper-proliferative state of keratinocytes under wounding or inflammatory conditions [54–56] (**Figure 3**). Upon injury, keratinocytes at the wound edge quickly downregulate K1/K10 and markedly induce K6 (type II)-K16 (type I) keratin heterodimer along with cytoplasmic K17 within 2–6 h of wounding [57], and therefore K6/K16/K17 have been widely used as markers for wound-activated keratinocytes in both human and mouse skin (**Figure 4**). The expressions of K6, 16 and 17 are also elevated in the hyperproliferative epithelium of inflammatory skin diseases such as psoriasis (**Figures 3** and **4**), which shares many inflammatory features with normal skin wounding, including elevated proinflammatory cytokines such as interleukin 1(IL1), tumor necrosis factor α (TNFα), type 1 interferons, interferon γ (IFNγ), IL17 and IL22 [58–60].

Hallmarks of activated keratinocytes include cell hypertrophy, altered cell adhesion and juxtanuclear reorganization of the keratin intermediate filament network, allowing activated keratinocytes to quickly migrate into the wound site, repopulate the skin and restore the epithelial lining and barrier function. In vitro study of mouse keratinocyte overexpressing

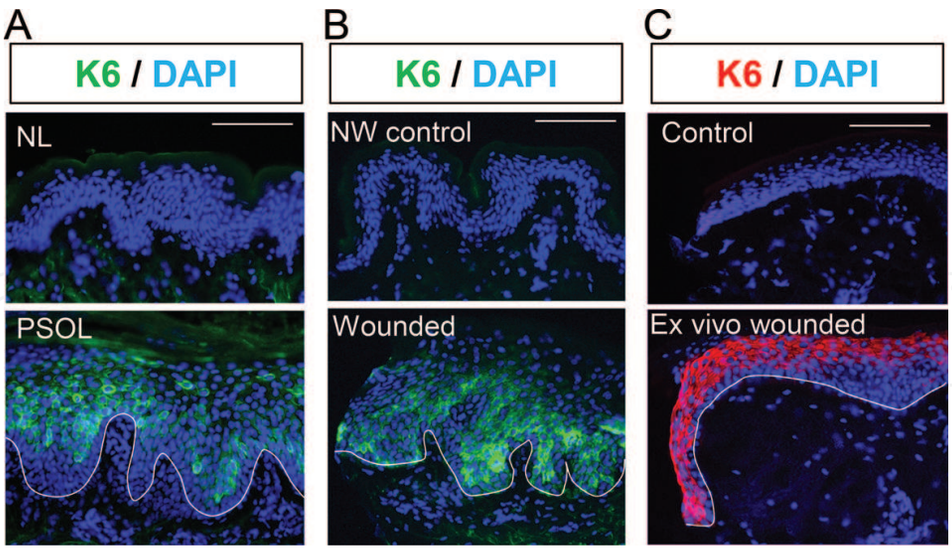

Figure 4. The expression of K6 is elevated in both wounded and psoriatic human skin epidermis. Human skin sections from (A) psoriasis lesional (PSOL), (B) in vivo wounded skin (by punch biopsy and collected at day3), or (C) ex vivo wounded skin were stained with K6 antibody in green or red as indicated. Non-wounded nuclei were counterstained with DAPI (blue). Scale bar, 100 μm. In A, non-lesional skin (NL) from the same patient was used as control for PSO. In B, skin biopsy collected at day 0 was used as non-wounded control. Note that while K6 expression was not detected in all control skin epidermis, strong K6 staining was detected in the suprabasal layers of both wounded and psoriatic skin epidermis in similar patterns. Also K6 was strongly elevated in the migrating tongue of ex vivo wounded human skin at the wound edge. Details of these samples can be found in our previous published work [58].

K16 revealed that while forced expression of K16 did not alter cell proliferation, it caused a reduction in cell adhesion and K10 expression (early differentiation) [61]. K10 expression inhibits cell proliferation, but ectopic expression of K16 promotes cell proliferation and diminishes the inhibitory function of K10 on cell proliferation when K6 and K10 are co-expressed [11] . K6−/− mouse keratinocytes migrated faster than control wild-type cells [56], and K6 negatively regulates the migratory potential of skin keratinocytes by inhibiting Src kinase [62], suggesting that the migratory feature of activated keratinocytes may be regulated by an K6/K16 independent pathway or by a non-cell autonomous manner. In contrast to K6−/− keratinocytes, K17−/− mouse keratinocytes show a delay in the closure of wounds [63], and protein translation, AKT activity and cell proliferation are suppressed in K17−/− keratinocytes [64]. These results suggest that in activated keratinocytes while the cell adhesion and differentiation maybe regulated by K6, cell hyper-proliferation and migration in response to wound maybe controlled by K17.

4.1. Regulation of K6/16/17 expression

Our group has shown that damage-associated molecular patterns "DAMPs", such as double-stranded RNA, are the first signals released from necrotic cells to rapidly initiate the inflammatory cytokine production from surrounding undamaged human keratinocyte upon injury [58, 65]. Inflammatory cytokines released either from activated keratinocytes or from recruited immune cells initiate expression of hyperproliferative keratins and keratinocyte hyperproliferation. It has been shown that cytokines interleukin 1(IL1) and tumor necrosis factor α (TNFα) can synergistically induce the transcription of K6 through transcription factor C/EBPβ and NFκB [66]. Transcription factor AP1 (c-Fos + c-Jun) can also activate K6 promoter synergistically with NFκB under inflammatory conditions [19]. Additionally, transcription factor NRF2 translocated from cytoplasm to nucleus upon stimulation with proinflammatory cytokines such as IL 17 or IL 22, and upregulated the expression of K6, K16 and K17 genes via the antioxidant responsive element (ARE)-binding region, promoting proliferation of keratinocytes in psoriasis [67]. K17 expression could also be induced by IFNγ in skin epidermis, and the K17 in turn function as an auto-antigen to stimulate proliferation of T cells and IFN expression, contributing to the amplification of the autoimmune response and immunopathogenesis of psoriasis [68, 69]. Accumulating recent evidences have suggested that microRNAs also play important roles in modulating keratinocyte activation and skin inflammation [70, 71]. Keratin expression can be regulated by microRNA in the contact of psoriasis. For example, miR-486-3p targeted K17 mRNA for degradation and it was identified as the top downregulated microRNAs in psoriasis, leading to K17 overproduction and hyperproliferation in psoriatic keratinocytes [72].

5. Keratins and cutaneous squamous cell carcinomas

5.1. SCCs and K5/K14/K15 and K6/K16/17

Cutaneous squamous cell carcinoma (SCC) is the second most common skin cancers and represents about 20% of all skin cancer, with up to 700,000 new cases annually diagnosed in the USA, and it is associated with a substantial risk of metastasis [73]. SCC is characterized by

extensive expression of K5/K14 through the epidermis and the expression of hyperproliferative keratin K6, K16 and K17 [50], which are not only upregulated in inflammatory skin, but often upregulated in many tumors originating in stratified and pseudostratified epithelia. K1/K10 may also be focally expressed in SCCs, and K8/K18 is often detected in poorly differentiated SCCs, and the role of K8/K18 will be discussed in more details next.

5.2. SCCs and K8/K18

As we have described in chapter 2, the simple epithelia-specific keratin pair, K8/K18, are expressed in keratinocyte progenitors, early on during embryonic skin development, and upon vertical epidermal stratification K8/K18 expression is then substituted by K5/K14, and becomes eventually lost in fully mature skin epidermis [9, 10, 74]. Overexpressing human K8 in mouse epidermis (TGHK8 mice) lead to severe epidermal phenotypes including epidermal hyperplasia associated with orthokeratotic hyperkeratosis, dysplastic hair follicles and altered expression terminal differentiation markers [75]. The severity of these skin phenotypes increased during aging, and the aged TGHK8 mice developed spontaneous premalignant skin tumors, and TGHK8 mice showed a drastic increase in the malignant progression of skin tumors in mouse model of chemical skin carcinogenesis. Previously, we have shown that Ctip2−/− mouse keratinocytes that overexpressed K8 exhibited loss of cell–cell contact and contain much thicker central stress fibers [6], indicating that aberrant K8 expression may decrease cell–cell adhesion and trigger an EMT (epithelial–mesenchymal transition) phenotype in epidermal keratinocytes. These results suggest that expression of K8 in adult epidermis impairs the normal epidermal differentiation program and may be responsible for the invasive behavior of transformed epidermal keratinocytes. Indeed, in adult skin epidermis, aberrant K8/K18 expression is broadly correlated with increased invasiveness and poor prognosis of squamous cell carcinomas [76]. In addition, K8/K18 protect epithelial cells against apoptosis mediated by proapoptotic signals, such as TNFα [77] released by macrophages and T lymphocytes and Fas [78], and may enable cancer cells to resist immune cell-mediated cell death and escape immune surveillance.

5.3. Regulation of keratin expression and function in SCCs

Phosphorylation is the key post-translational modification that regulates keratin functions, and phosphorylation of K8 is among the most well studied in keratin family [79]. More than a dozen phosphorylation sites have been identified on serine residues of K8 [79], and phosphorylation of K8 enhances the migratory, proliferative and invasive potential of epithelial cells, therefore promoting the malignant transformation of cancer cells [80]. Keratin expression can be altered through epigenetic modification during malignant transformation. A recent study investigating genome-wide DNA methylation changes in the progression from healthy human epidermis to cSCC reveals that DNA methylation profiles of cSCC epidermis display classical features of cancer methylomes compared to normal epidermis samples [81]. Further analyses of DNA methylation patterns of keratin gene clusters (including basal cell keratins K5 and K14 which are ectopically expressed throughout cSCC epidermis) identified major DNA methylation differences between healthy donors and cSCC patients, suggesting that abnormal keratin expression in SCCs may be regulated through epigenetic mechanism such as DNA methylation.

6. Conclusion

Tissue and cell differentiation specific expression of pair between type I and type II Keratins play essential roles in forming the intermediate filaments and providing cytoskeletal and structural support and mechanical resilience for epithelia tissues. In addition to these structural roles, keratins also control cell migration, cell adhesion, proliferation and differentiation processes in keratinocytes and modulate immune system in various settings. The transcriptions of keratins are tightly controlled by a series of transcription factors, and keratin functions are also regulated by post-translational modifications such as phosphorylation and intermolecular disulfide bond formation. Mutation or dysfunction of basal keratins K14/K5 or suprabasal keratins K1/K10 lead to severe skin blistering diseases, whereas K6, K16 and K17 are rapidly induced in activated keratinocytes upon skin wounding and are also expressed in inflammatory skin diseases, such as psoriasis. Understanding keratin functions and related regulatory mechanisms will help to design new therapeutic interventions for keratin-related skin diseases.

Acknowledgements

This work was supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases grant (R01AR069653).

Conflict of interest

The author has nothing to disclose.

Author details

Ling-juan Zhang[1,2]

Address all correspondence to: lingjuan.zhang@xmu.edu.cn

1 School of Pharmaceutical Sciences, Xiamen University, Xiamen, China

2 Department of Dermatology, School of Medicine, UC San Diego, USA

References

[1] Franke WW et al. Diversity of cytokeratins - differentiation specific expression of cytokeratin polypeptides in epithelial-cells and tissues. Journal of Molecular Biology. 1981;**153**(4):933-959

[2] Moll R et al. The catalog of human cytokeratins - patterns of expression in normal epithelia, tumors and cultured-cells. Cell. 1982;**31**(1):11-24

[3] Schweizer J et al. New consensus nomenclature for mammalian keratins. The Journal of Cell Biology. 2006;**174**(2):169-174

[4] Herrmann H et al. Intermediate filaments: Primary determinants of cell architecture and plasticity. Journal of Clinical Investigation. 2009;**119**(7):1772-1783

[5] Sambandam SAT et al. 14-3-3 sigma regulates keratinocyte proliferation and differentiation by modulating Yap1 cellular localization. Journal of Investigative Dermatology. 2015; **135**(6):1621-1628

[6] Zhang LJ et al. Ctip2 is a dynamic regulator of epidermal proliferation and differentiation by integrating EGFR and notch signaling. Journal of Cell Science. 2012;**125**(23):5733-5744

[7] Fuchs E. Scratching the surface of skin development. Nature. 2007;**445**(7130):834-842

[8] Nagarajan P, Romano RA, Sinha S. Transcriptional control of the differentiation program of interfollicular epidermal keratinocytes. Critical Reviews in Eukaryotic Gene Expression. 2008;**18**(1):57-79

[9] Romano RA et al. Delta Np63 knockout mice reveal its indispensable role as a master regulator of epithelial development and differentiation. Development. 2012;**139**(4):772-782

[10] Liu S, Zhang H, Duan E. Epidermal development in mammals: Key regulators, signals from beneath, and stem cells. International Journal of Molecular Sciences. 2013;**14**(6): 10869-10895

[11] Paramio JM et al. Modulation of cell proliferation by cytokeratins K10 and K16. Molecular and Cellular Biology. 1999;**19**(4):3086-3094

[12] Chen JL et al. An unexpected role for keratin 10 end domains in susceptibility to skin cancer. Journal of Cell Science. 2006;**119**(24):5067-5076

[13] Bose A et al. Keratin K15 as a biomarker of epidermal stem cells. International Journal of Molecular Sciences. 2013;**14**(10):19385-19398

[14] Collin C et al. Characterization of human cytokeratin 2, an epidermal cytoskeletal protein synthesized late during differentiation. Experimental Cell Research. 1992;**202**(1):132-141

[15] Lloyd C et al. The basal keratin network of stratified squamous epithelia: Defining K15 function in the absence of K14. The Journal of Cell Biology. 1995;**129**(5):1329-1344

[16] Lyle S et al. The C8/144B monoclonal antibody recognizes cytokeratin 15 and defines the location of human hair follicle stem cells. Journal of Cell Science. 1998;**111**(Pt 21):3179-3188

[17] Liu Y et al. Keratin 15 promoter targets putative epithelial stem cells in the hair follicle bulge. The Journal of Investigative Dermatology. 2003;**121**(5):963-968

[18] Rossi A et al. Effect of AP1 transcription factors on the regulation of transcription in normal human epidermal keratinocytes. The Journal of Investigative Dermatology. 1998; **110**(1):34-40

[19] Ma S et al. Transcriptional control of K5, K6, K14, and K17 keratin genes by AP-1 and NF-kappaB family members. Gene Expression. 1997;**6**(6):361-370

[20] Tomic-Canic M et al. Novel regulation of keratin gene expression by thyroid hormone and retinoid receptors. The Journal of Biological Chemistry. 1996;**271**(3):1416-1423

[21] Tomic-Canic M et al. Identification of the retinoic acid and thyroid hormone receptor-responsive element in the human K14 keratin gene. The Journal of Investigative Dermatology. 1992;**99**(6):842-847

[22] Perez P et al. Altered skin development and impaired proliferative and inflammatory responses in transgenic mice overexpressing the glucocorticoid receptor. The FASEB Journal. 2001;**15**(11):2030-2032

[23] Zhu S et al. C/EBPbeta modulates the early events of keratinocyte differentiation involving growth arrest and keratin 1 and keratin 10 expression. Molecular and Cellular Biology. 1999;**19**(10):7181-7190

[24] Maytin EV et al. Keratin 10 gene expression during differentiation of mouse epidermis requires transcription factors C/EBP and AP-2. Developmental Biology. 1999;**216**(1):164-181

[25] Li F, Adase CA, Zhang LJ. Isolation and culture of primary mouse keratinocytes from neonatal and adult mouse skin. Journal of Visualized Experiments. 2017;**125**:1-6, e56027

[26] Sikder H et al. A central role for transcription factor C/EBP-beta in regulating CD1d gene expression in human keratinocytes. Journal of Immunology. 2009;**183**(3):1657-1666

[27] Johnson PF. Molecular stop signs: Regulation of cell-cycle arrest by C/EBP transcription factors. Journal of Cell Science. 2005;**118**(Pt 12):2545-2555

[28] Timchenko NA et al. CCAAT/enhancer-binding protein alpha (C/EBP alpha) inhibits cell proliferation through the p21 (WAF-1/CIP-1/SDI-1) protein. Genes & Development. 1996; **10**(7):804-815

[29] Oshima RG et al. Intermediate filament protein synthesis in preimplantation murine embryos. Developmental Biology. 1983;**99**(2):447-455

[30] Lee B et al. Overexpression of transcription factor Ovol2 in epidermal progenitor cells results in skin blistering. The Journal of Investigative Dermatology. 2017;**137**(8):1805-1808

[31] Coulombe PA. Discovery of keratin function and role in genetic diseases: The year that 1991 was. Molecular Biology of the Cell. 2016;**27**(18):2807-2810

[32] Bonifas JM, Rothman AL, Epstein EH, Epidermolysis bullosa simplex: Evidence in two families for keratin gene abnormalities. Science. 1991;**254**(5035):1202-1205

[33] Coulombe PA, Lee CH. Defining keratin protein function in skin epithelia: Epidermolysis bullosa simplex and its aftermath. The Journal of Investigative Dermatology. 2012;**132**(3 Pt 2): 763-775

[34] Coulombe PA et al. Point mutations in human keratin 14 genes of epidermolysis bullosa simplex patients: Genetic and functional analyses. Cell. 1991;**66**(6):1301-1311

[35] Vassar R et al. Mutant keratin expression in transgenic mice causes marked abnormalities resembling a human genetic skin-disease. Cell. 1991;**64**(2):365-380

[36] Bolling MC et al. Mutations in KRT5 and KRT14 cause epidermolysis bullosa simplex in 75% of the patients. British Journal of Dermatology. 2011;**164**(3):637-644

[37] Sawant MS, Leube RE. Consequences of keratin phosphorylation for cytoskeletal organization and epithelial functions. International Review of Cell and Molecular Biology. 2017; **330**:171-225

[38] Sawant M et al. Threonine 150 phosphorylation of keratin 5 is linked to Epidermolysis Bullosa simplex and regulates filament assembly and cell viability. The Journal of Investigative Dermatology. 2018;**138**(3):627-636

[39] Feng X, Coulombe PA. A role for disulfide bonding in keratin intermediate filament organization and dynamics in skin keratinocytes. Journal of Cell Biology. 2015;**209**(1):59-72

[40] Bunick CG, Milstone LM. The X-ray crystal structure of the keratin 1-keratin 10 Helix 2B heterodimer reveals molecular surface properties and biochemical insights into human skin disease. The Journal of Investigative Dermatology. 2017;**137**(1):142-150

[41] Tsubota A et al. Keratin 1 gene mutation detected in epidermal nevus with epidermolytic hyperkeratosis. The Journal of Investigative Dermatology. 2007;**127**(6):1371-1374

[42] Paller AS et al. Genetic and clinical mosaicism in a type of epidermal nevus. The New England Journal of Medicine. 1994;**331**(21):1408-1415

[43] Kono M et al. A child with epidermolytic ichthyosis from a parent with epidermolytic nevus: Risk evaluation of transmission from mosaic to Germline. The Journal of Investigative Dermatology. 2017;**137**(9):2024-2026

[44] Rothnagel JA et al. Mutations in the rod domain of keratin 2e in patients with ichthyosis bullosa of Siemens. Nature Genetics. 1994;**7**(4):485-490

[45] Tsubota A et al. Congenital ichthyosiform erythroderma mimicking ichthyosis bullosa of Siemens. The British Journal of Dermatology. 2008;**158**(1):191-194

[46] Fischer H et al. Keratins K2 and K10 are essential for the epidermal integrity of plantar skin. Journal of Dermatological Science. 2016;**81**(1):10-16

[47] Fischer H et al. Loss of keratin K2 expression causes aberrant aggregation of K10, hyperkeratosis, and inflammation. The Journal of Investigative Dermatology. 2014;**134**(10):2579-2588

[48] Langbein L et al. Molecular characterization of the body site-specific human epidermal cytokeratin 9: cDNA cloning, amino acid sequence, and tissue specificity of gene expression. Differentiation. 1993;**55**(1):57-71

[49] Toivola DM et al. Keratins in health and disease. Current Opinion in Cell Biology. 2015;**32**: 73-81

[50] Moll R, Divo M, Langbein L. The human keratins: Biology and pathology. Histochemistry and Cell Biology. 2008;**129**(6):705-733

[51] Fu DJ et al. Keratin 9 is required for the structural integrity and terminal differentiation of the Palmoplantar epidermis. Journal of Investigative Dermatology. 2014;**134**(3):754-763

[52] Swensson O et al. Specialized keratin expression pattern in human ridged skin as an adaptation to high physical stress. British Journal of Dermatology. 1998;**139**(5):767-775

[53] Maruthappu T et al. Rhomboid family member 2 regulates cytoskeletal stress-associated keratin 16. Nature Communications. 2017;**8**:1-11, 14174

[54] Paladini RD et al. Onset of re-epithelialization after skin injury correlates with a reorganization of keratin filaments in wound edge keratinocytes: Defining a potential role for keratin 16. The Journal of Cell Biology. 1996;**132**(3):381-397

[55] Leigh IM et al. Keratins (K16 and K17) as markers of keratinocyte hyperproliferation in psoriasis in vivo and in vitro. The British Journal of Dermatology. 1995;**133**(4):501-511

[56] Wong P, Coulombe PA. Loss of keratin 6 (K6) proteins reveals a function for intermediate filaments during wound repair. The Journal of Cell Biology. 2003;**163**(2):327-337

[57] McGowan K, Coulombe PA. The wound repair-associated keratins 6, 16, and 17. Insights into the role of intermediate filaments in specifying keratinocyte cytoarchitecture. Sub-Cellular Biochemistry. 1998;**31**:173-204

[58] Zhang LJ et al. Antimicrobial peptide LL37 and MAVS signaling drive interferon-beta production by epidermal keratinocytes during skin injury. Immunity. 2016;**45**(1):119-130

[59] Nickoloff BJ et al. Lessons learned from psoriatic plaques concerning mechanisms of tissue repair, remodeling, and inflammation. Journal of Investigative Dermatology Symposium Proceedings. 2006;**11**(1):16-29

[60] Tampa M et al. The pathophysiological mechanisms and the quest for biomarkers in psoriasis, a stress-related skin disease. Disease Markers. 2018;**2018**:5823684

[61] Wawersik M, Coulombe PA. Forced expression of keratin 16 alters the adhesion, differentiation, and migration of mouse skin keratinocytes. Molecular Biology of the Cell. 2000; **11**(10):3315-3327

[62] Rotty JD, Coulombe PA. A wound-induced keratin inhibits Src activity during keratinocyte migration and tissue repair. The Journal of Cell Biology. 2012;**197**(3):381-389

[63] McGowan KM et al. Keratin 17 null mice exhibit age- and strain-dependent alopecia. Genes & Development. 2002;**16**(11):1412-1422

[64] Kim S, Wong P, Coulombe PA. A keratin cytoskeletal protein regulates protein synthesis and epithelial cell growth. Nature. 2006;**441**(7091):362-365

[65] Lai Y et al. Commensal bacteria regulate toll-like receptor 3-dependent inflammation after skin injury. Nature Medicine. 2009;**15**(12):1377-1382

[66] Komine M et al. Interleukin-1 induces transcription of keratin K6 in human epidermal keratinocytes. The Journal of Investigative Dermatology. 2001;**116**(2):330-338

[67] Yang L et al. Nrf2 promotes keratinocyte proliferation in psoriasis through up-regulation of keratin 6, keratin 16, and keratin 17. The Journal of Investigative Dermatology. 2017; **137**(10):2168-2176

[68] Gudmundsdottir AS et al. Is an epitope on keratin 17 a major target for autoreactive T lymphocytes in psoriasis? Clinical and Experimental Immunology. 1999;**117**(3):580-586

[69] Shen Z et al. Altered keratin 17 peptide ligands inhibit in vitro proliferation of keratinocytes and T cells isolated from patients with psoriasis. Journal of the American Academy of Dermatology. 2006;**54**(6):992-1002

[70] Hawkes JE et al. microRNAs in psoriasis. The Journal of Investigative Dermatology. 2016; **136**(2):365-371

[71] Hermann H et al. miR-146b probably assists miRNA-146a in the suppression of keratinocyte proliferation and inflammatory responses in psoriasis. The Journal of Investigative Dermatology. 2017;**137**(9):1945-1954

[72] Jiang M et al. TGFbeta/SMAD/microRNA-486-3p signaling Axis mediates keratin 17 expression and keratinocyte Hyperproliferation in psoriasis. The Journal of Investigative Dermatology. 2017;**137**(10):2177-2186

[73] Alam M, Ratner D. Cutaneous squamous-cell carcinoma. The New England Journal of Medicine. 2001;**344**(13):975-983

[74] Zhao Q et al. Spatiotemporal expression of p63 in mouse epidermal commitment. International Journal of Molecular Sciences. 2015;**16**(12):29542-29553

[75] Casanova ML et al. Epidermal abnormalities and increased malignancy of skin tumors in human epidermal keratin 8-expressing transgenic mice. The FASEB Journal. 2004;**18**(13): 1556-1558

[76] Karantza V. Keratins in health and cancer: More than mere epithelial cell markers. Oncogene. 2011;**30**(2):127-138

[77] Caulin C et al. Keratin-dependent, epithelial resistance to tumor necrosis factor-induced apoptosis. The Journal of Cell Biology. 2000;**149**(1):17-22

[78] Gilbert S et al. Simple epithelium keratins 8 and 18 provide resistance to Fas-mediated apoptosis. The protection occurs through a receptor-targeting modulation. The Journal of Cell Biology. 2001;**154**(4):763-773

[79] Kim HJ, Choi WJ, Lee CH. Phosphorylation and reorganization of keratin networks: Implications for carcinogenesis and epithelial mesenchymal transition. Biomolecules & Therapeutics (Seoul). 2015;**23**(4):301-312

[80] Tiwari R et al. Quantitative phosphoproteomic analysis reveals system-wide signaling pathways regulated by site-specific phosphorylation of Keratin-8 in skin squamous cell carcinoma derived cell line. Proteomics. 2017;**17**(7):1-17, 1600254

[81] Rodriguez-Paredes M et al. Methylation profiling identifies two subclasses of squamous cell carcinoma related to distinct cells of origin. Nature Communications. 2018;**9**(1):1-9, 577

IntechOpen