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Review

Keratin function and regulation in tissue homeostasis and pathogenesis

Wera Roth[1], Mechthild Hatzfeld[2], Maik Friedrich[1], Sören Thiering[1] and Thomas M. Magin[1,*]

[1] Division of Cell and Developmental Biology, Translational Centre for Regenerative Medicine (TRM) and Biology, University of Leipzig, D-04103 Leipzig, Germany

[2] Institut für Molekulare Medizin, Sektion Pathobiochemie, Medizinische Fakultät, Martin-Luther-Universität Halle-Wittenberg, Hollystraße 1, 06097 Halle, Germany

* Corresponding author
e-mail: thomas.magin@trm.uni-leipzig.de

Abstract

Epithelial tissues act as hubs in metabolism and communication and protect the organism against dehydration, infections, pharmacological and physical stress. Keratin intermediate filament proteins are well established as major cytoskeletal players in maintaining epithelial integrity. More recently, an involvement of keratins in growth control and organelle functions has emerged. Disruption of the keratin cytoskeleton by mutations or its reorganization following posttranslational modifications can render epithelia susceptible to tissue damage and various stresses, while loss of keratin expression is a hallmark of epithelial-mesenchymal transition (EMT). To understand the molecular mechanisms by which keratins perform their functions remains a formidable challenge. Based on selected examples, we will discuss how cell-specific expression of keratin isotypes affects cytoarchitecture and cell behavior. Further, we ask how posttranslational modifications alter keratin organization and interactions during signaling. Next, we discuss pathomechanisms of epidermal keratin disorders in the light of novel data. Finally, we raise open questions and point out future directives.

Keywords: cell growth; keratins; posttranslational modification; protein biosynthesis; stress response.

Introduction

Including the skin and the liver, epithelial tissues are not only the largest organs of the mammalian body but also are tissues with an enormous regenerative potential. Epithelia protect the body against mechanical insult, physical and chemical stress, dehydration, and infection. Moreover, they act as hubs in metabolism and communication. We now begin to recognize that the keratin intermediate filament (IF) cytoskeleton modulates many of these functions in a cell type-specific manner (1–5). Keratin IF are well suited to do so as they are assembled from >50 different types I and II proteins expressed as specific heterodimer pair combinations. These proteins are encoded by types I and II gene families with 26 type I and 28 type II genes in humans and the mouse, respectively, which are predominantly expressed in epithelial cells and tissues (6). The domain organization of keratins – a central coil-coil α-helical rod domain of 310 amino acids in length that is flanked by non-α-helical head and tail domains – is similar to other IF proteins. The head is crucial for interdimer association and filament assembly, whereas the tail regulates filament diameter and bundling (7). Keratins assemble first into obligate, parallel heterodimers, next into antiparallel tetramers and through lateral and longitudinal interactions of tetramers into long, non-polar IF (8).

In many textbooks, keratins are still described as major contributors to viscoelastic properties of cells and of the resilience of epithelia against mechanical force. During the last decade, additional functions including cell growth, apoptosis, organelle transport, and cytoarchitecture emerged (1, 2, 5). Many of these observations stem from the analysis of genetically altered mice and mutations in so far 19 human keratin genes, either causing or predisposing their carriers to >50 diseases (9; http://www.interfil.org/index.php). Here, we focus on selected studies that strongly support a role for keratins in protein biosynthesis, signaling, and inflammation and help to shed light on keratin function in disease.

The tissue level: implications of keratin isotype expression and regulation

Multiprotein families offer a playing ground for the evolution of protein function and redundancy in a context-dependent manner. This is very true for keratins that form expression pairs indicating epithelial differentiation and vary in expression levels ranging from ~0.1% to 30% of total cellular proteins with low abundance in hepatocytes and highest levels in suprabasal keratinocytes of the epidermis. Transcription control is largely responsible for cell-specific variation in keratin isotypes and amounts, together with posttranslational modifications. To which extent do primary sequence, subunit composition, and abundance of the available >50 isotypes affect distinct keratin networks and cell function (1, 2, 10)? Here, we discuss mechanisms underlying keratin isotype

expression and discuss how single keratins affect cytoskeletal organization and cell function.

K8 and K18 (types II and I, respectively) are present in all embryonic and simple epithelia and represent the only keratins in hepatocytes, acinar, and islet cells of the pancreas and proximal tubular epithelial cells of the kidney (3). In all other simple epithelia, varying combinations of K19, K20, K23, and K80 are present, indicating distinct stages of differentiation and serving as excellent markers for the state of epithelial differentiation (3, 6, 11). K23 expression is elevated in a subset of colorectal tumors with microsatellites, along with the transcription factors FOXQ1 and CEBPA correlating with elevated cell proliferation (12). In a cell culture model, increase in the tumor suppressor SMAD4, acting downstream of TGF-β, coincides with elevated K23 expression. The concomitant identification of 14-3-3β as a K23-binding protein and its cytoplasmic re-localization upon K23 expression suggests a mechanism for K23 as tumor suppressor (13).

Like no other tissue, the epidermis illustrates how exquisite keratin isotype expression relates to their structural and regulatory functions and causes cell type-specific disorders following expression of mostly dominant mutations. Two resident cell populations are characterized by keratin expression. The predominating keratinocytes express between 3 and ≥10 different 'epidermal' keratins, whereas neurosensory Merkel cells build their IF cytoskeleton from 'simple epithelial type' keratins K8, K18, K19, and K20 (14). There are two types of slow-cycling stem cells in the vibrissae bulge, one expressing K5, K15, K17, and K19 organized into a loosely arranged keratin network. The second expresses K5/K17, correlating with densely bundled filaments (15). Notably, both lack K14, which together with K5 – and K15/K17 in subpopulations – is typical of interfollicular basal keratinocytes. This invites the hypothesis that keratin isotypes affect cytoarchitecture and epithelial-specific functions of the epidermal stem cell compartment. The K5/K14 network of interfollicular keratinocytes provides a mechanical connection between hemidesmosomes along the basement membrane, desmosomes at the lateral plasma membrane and the cell nucleus. As soon as these cells commit to terminal differentiation, basal keratins become sequentially replaced by the K1/K10 expression pair, complemented by K9 in palmoplantar epidermis (1, 16). The prominent bundling of some keratins is an intrinsic property of subunit interactions, although filaggrin might additionally contribute to filament bundling (7, 17). Bundling of IF most likely lowers subunit exchange and contributes to increased mechanical stability (7).

Owing to the distinct characteristics of keratin isotypes, changes in their expression pattern help cells to adapt to various stress conditions. The disruption of epidermal homeostasis by wounding, viral infection, UV exposure, in psoriasis, atopic dermatitis, and epidermolytic disorders is followed by profound changes in the transcription program including downregulation of the 'normal' set of keratins and transient expression of K6, K16, and K17 (18). These keratin isotypes have unique properties, supporting enhanced migration, filament dynamics, and increased protein biosynthesis in the case of K17, thus facilitating repair processes (19, 20).

How do conditions of acute and chronic stress affect keratin expression? Experimentally, the compound sulforaphane (SF) induces K16 and K17 transcription *in vivo* (21). Further dissecting the molecular mechanism revealed a modular regulation of both genes. In addition to a SF-Nrf2-dependent mechanism, K17 can be induced also by lower glutathione levels via activation of the MAP kinases p38 and JNK. Nuclear factor (erythroid-derived 2)-like 2 is a transcription factor that induces genes involved in the antioxidant response (22). In turn, these activate the transcription factor AP-1 that has binding sites on several keratin promoters (23) (Figure 1). What is the relationship between distinct keratins and lower glutathione levels? While regulation of the balance between reduced and oxidized glutathione (GSH/GSSG) in cells is complex, lower levels coincide with increased stress, characterized by reduced general protein biosynthesis and translation of select genes (24, 25). K17 promotes protein biosynthesis in activated keratinocytes through a 14-3-3-dependent mechanism (20). Analysis of SF-induced gene products in addition to K17 offers an attractive way to identify its isotype function during keratinocyte stress and a possible role in stress-related protein biosynthesis.

The recently described K80 (type II) is unique in several aspects. Whereas its primary sequence relates it to type II hair keratins, its expression in virtually all types of epithelia suggests a common epithelial function. Further, K80 exists in two splice variants, one of which is expressed in most epithelia, the smaller one restricted to subsets of hair follicle and tongue keratinocytes. Unlike any other keratin known to date, it shows differentiation-dependent subcellular distribution. In the duodenal epithelium, K80 appears confined to the apical domain. In cultured epithelial cells, it either was restricted to the desmosomal plaque or adapted the typical cytoplasmic

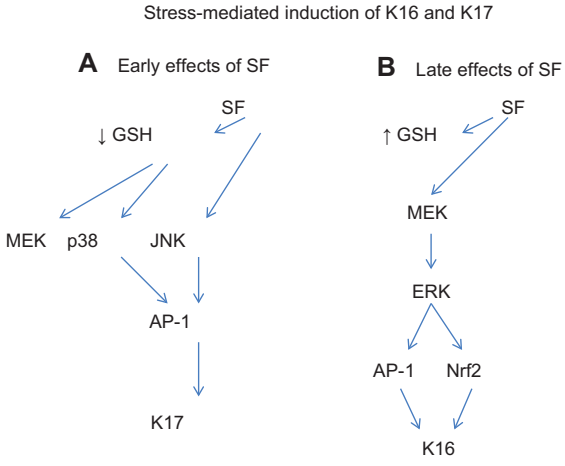

Figure 1 SF-mediated transcription control of K16 and K17. (A) Early effects, 3 h upon addition of SF, intracellular glutathione levels drop, coinciding with elevated activity of MAP kinases and of the transcription factor AP-1, which can activate K17. (B) Late effects, SF-mediated stimulation of transcription factor Nrf2 stimulating K16 transcription. Concomitant normalization of glutathione levels additionally contribute to K16 expression involving the MAP kinase-AP-1 axis.

keratin array upon terminal differentiation, suggesting that K80 may modulate keratin-desmosome interactions (11). This leads to the question for mechanisms involved in cell type-specific intracellular distribution of keratin isotypes (4, 26, 27). Although studied for many years, there is no information about the distribution of keratin isotypes in cells that express three or more keratins. In view of their sequence diversity and different affinities of heterodimeric complexes, the local composition of the keratin cytoskeleton has implications for assembly, turnover, protein interactions, and signaling (see Figure 2 for some major arrangements). The expression of keratins in non-epithelial mammalian tissues was first regarded as a rare event when presence of very low amounts of K8, K18, and K19 were identified in smooth muscle cells of umbilical cord in addition to the predominating IF protein vimentin (28). Also, transient coexpression and colocalization of desmin, K8, and K18 in developing myocardial cells of some vertebrate species was found, but both studies did not provide functional data (29). An important contribution of K19 and K8 for muscle integrity came into light upon genetic deletion of desmin in mice. These uncovered a crucial role for K8 and K19 in anchoring the myofiber to costameres at the sarcolemma at the Z and M line, complementing the function of the muscle-specific IF protein desmin. At the molecular level, keratins and desmin form distinct IF. The interaction between keratin IF and costameres is mediated through the actin-binding domain of dystrophin to K19, but not K8 and K18 (30). The tibialis anterior (TA) muscles of mice lacking K19 lost costameres, accumulated mitochondria under the sarcolemma and generated lower specific tension than controls (31). Subsequent analysis of mice double deficient for desmin and K19 revealed a synergy of K19 and desmin regarding costamere organization and contractile torque and independence of K19 toward the distribution of mitochondria (32). The notion that young K19$^{-/-}$ mice suffer from a mild myopathy may have implications for human disease and underlines a crucial function of a keratin isotype in a non-classic context. These studies provide convincing evidence that 'ectopic' expression of keratins has no detrimental consequences, provided balanced types I and II expression at physiological levels occurs.

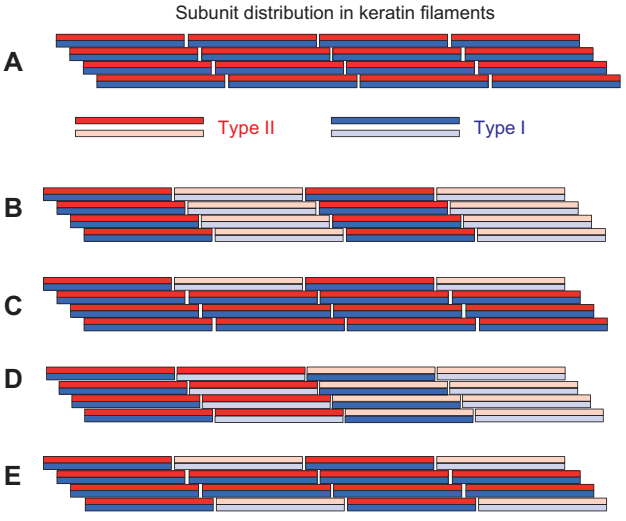

Figure 2 Potential distribution of isotypes in the keratin cytoskeleton in cells and *in vivo*.
(A) Equal distribution of heterodimers (red – type II, blue colors – type I) throughout filaments. For simplicity, antiparallel orientation of dimers at the tetramer level is omitted, and IF is depicted with only four instead of eight dimers in cross section. (B–E) Different modes of subunit distribution along a single keratin IF. As any types I and II keratin can heterodimerize, cells expressing four or more different isotypes can assemble homo- or heterogeneous IF. Non-equal distribution of subunits can result from assembly or reorganization of IF and can provide a mechanism for localized alterations of IF. Note that subunit affinity is determined by primary sequence and can be modified by posttranslational modifications and/or associated proteins.

Cellular level: posttranslational modifications and keratin isotypes in signaling and growth control

Whether the large number of proteins required in regulating actin dynamics at distinct levels is matched by keratin-associated proteins remains an open issue. Conversely, keratin assembly, reorganization, and function are regulated by subunit composition and posttranslational modifications. Phosphorylation at Ser, Thr, and Tyr residues, O-glycosylation, sumoylation, and ubiquitination represent the best known keratin modifications (4, 33). Through these modifications, keratins are targets and effectors of a large number of proteins involved in cytoskeletal organization, signaling, and stress.

K8/18 hyperphosphorylation correlates with mitosis, many settings of stress, e.g., disease progression in patients with chronic liver disease, expressing K8 sequence variants. Expression of non-phosphorylatable K8-S73A or -G61C in transgenic mice showed increased susceptibility to liver injury (34). The hypothesis that K8-S73 and K18-S52 act as a phosphate sponge protecting hepatocytes from proapoptotic effects of stress-activated protein kinases was further supported by reduced phosphorylation of the two p38/MK2 MAP kinase target proteins MSK1 and HSPB1 in the presence of K8 and K18. p38 and MK2 MAP kinases cooperate such that the former phosphorylates K8 at Ser-73 and the latter K18 at Ser-52 (34, 35). K8Ser73 phosphorylation can occur additionally upon phosphatase inhibition by c-jun-N-terminal kinase, upon stimulation of the proapoptotic receptor Fas/CD95/Apo-1 and by protein kinase Cd under mechanical stress (36–38). What are the effects of keratin hyperphosphorylation? In general, interkeratin interactions become weaker, and phosphorylated species enter a non-filamentous soluble fraction, from where they can either re-enter into IF or become degraded (39–41). Further, phosphorylation creates binding sites for members of the 14-3-3 family of adapter proteins, e.g., Thr-9/Ser-44 on K17 and Ser-33 on K18, respectively (42). It will be interesting to determine how much of the total cellular pool of 14-3-3, which can shuttle between the cytosol and the nucleus, is sequestered by keratins, too. Upon induction of K17, a yet unidentified kinase acts on the above

motif, leading to cytoplasmic recruitment of 14-3-3 on K17, followed by cytoskeletal sequestration of the TSC1/TSC2 complex and subsequent stimulation of protein biosynthesis via increased mTOR activity (20). Complete loss of keratins in mouse embryos lead to the downregulation of protein biosynthesis through a mechanism that involves mislocalization of apical glucose transporters GLUT1 and GLUT3, accompanied by activation of AMP kinase, indicative of metabolic stress. Phosphorylation of the mTOR regulator Raptor by AMPK subsequently downregulated protein biosynthesis in several mouse embryo tissues (43, 44). Additional experiments are required to resolve the roles and mechanism(s) by which distinct keratins regulate translation (Figure 3).

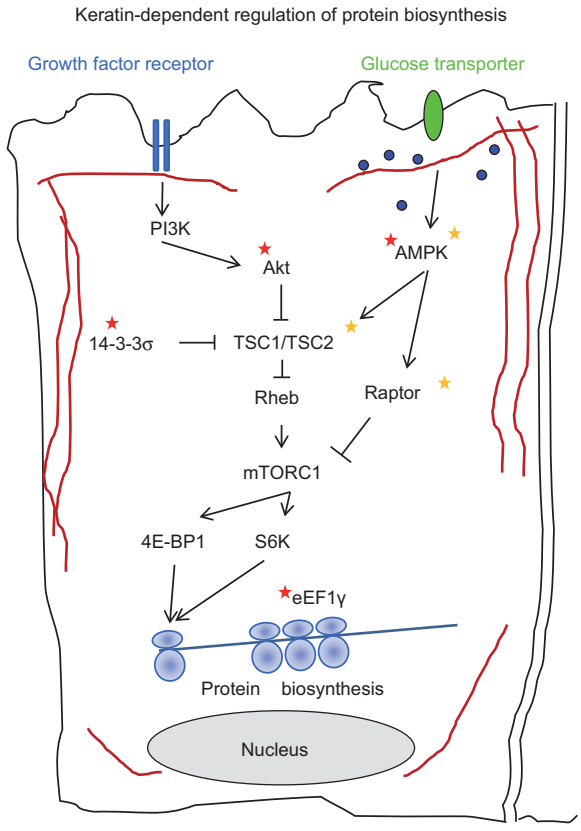

Figure 3 Keratins regulate protein biosynthesis. Keratins (red curved lines) affect the localization and activity of many cellular proteins.
Protein biosynthesis depends on the availability of nutrients (here, only glucose is depicted) and growth factors, mediated by growth factor receptors (blue), PI3 kinase, and transporters (green). Induction of K17 enables cytoplasmic sequestration of 14-3-3σ, which inactivates the TSC1/TSC2 complex. This allows activation of the kinase mTORC1 and stimulates translation initiation through S6K and 4E-BP-1. Keratins can also influence translation elongation through eEF1γ. Additionally, keratins link protein biosynthesis to intracellular glucose availability, mediating correct localization of GLUT transporters. Absence of keratins coincides with mislocalized GLUT transporters and impaired glucose metabolism, sensed by AMP kinase (AMPK). Activated AMPK inactivates mTORC1 by phosphorylating TSC2 and Raptor, lowering protein biosynthesis to reduced glucose levels. Red asterisks indicate in/direct keratin interaction and yellow asterisks denote phosphorylation.

A recent study on the function of O-glycosylation of K18 elegantly illustrates how keratins act as targets and effectors to mediate tissue protection. O-GlcNAc residues are added and removed from serine/threonine by O-GlcNAc transferase and N-acetyl-D-glucosaminidase (O-GlcNAcase), respectively. O-GlcNAcylation occurs on a large number of cytoplasmic and nuclear proteins, regulating protein turnover, transcription, and stress responses (45). O-GlcNAcylation and phosphorylation frequently occur at adjacent or identical serine and threonine residues, and each modification can interfere with the other. K18 and K8 phosphorylation and glycosylation likely occur at distinct sites and can be concurrent (46). To investigate human K18 O-glycosylation, which occurs at Ser-30, -31, and -49, the sites were mutated into -Ala, rendering them resistant to glycosylation without affecting other phosphorylation sites, and the resultant mutant was expressed in transgenic mice. Without pharmacological or metabolic stress, transgenic mice displayed no obvious phenotype. However, mutant mice in which K18 could not be glycosylated due to mutations were more susceptible to streptozotocin (STZ; an O-GlcNAcase inhibitor) and PUGNAc/Fas-induced liver and pancreatic injury compared to control animals (46). They died most likely from multiorgan failure, accompanied by apoptosis in the liver and the pancreas. *In vivo* and in cultured hepatocytes, decreased activity of Akt kinase, a positive regulator of growth and a negative regulator of apoptosis, was noted, suggesting a link between K18 glycosylation and cell survival. In support with decreased Akt activity, the executioner caspase-3 was elevated. In K18 Gly-deficient cells, phosphorylation of Akt and its substrate GSK-3 was diminished, while Akt glycosylation was enhanced. Based on the previous observation that interaction between K10 (a type I keratin) and Akt reduces kinase activity, Akt was found to bind K8 but not to K18 (47). In the context of K8 and K18, K8-Akt interaction was independent of K18 glycosylation and Akt1 Thr-308 phosphorylation (46). This suggests that signals which trigger K18 – and possibly – Akt glycosylation release Akt from the K8-K18 complex to contribute to full Akt stimulation (Figure 4). An open issue remains the extent to which total Akt is sequestered and regulated by interacting with K8. The newly discovered link between K18 O-glycosylation, Akt, and regulation of apoptosis might reflect a more general mechanism by which keratins, in general, protect tissues against damage and invites to re-envisage pathomechanisms in keratinopathies. Further, K18 O-glycosylation enhances the solubility of keratins, leading to the general question whether mechanical and signaling functions of keratins at least partially depend on the cytoskeletal and soluble fractions, respectively (48). Bioinformatic analysis of the 54 human keratins predicts O-glycosylation for 13 type I and 15 type II keratins (Scanhttp://cbsb.lombardi.georgetown.edu/OGAP.html). For K8, K13, and K18, several head domain sites have been experimentally verified (see Figure 5). Prediction locates the majority of sites to the head and tail domains, with most sites (7) occurring in the tailless K19. For both K80 isoforms, no O-glycosylation sites in the head domain are predicted. The helical rod

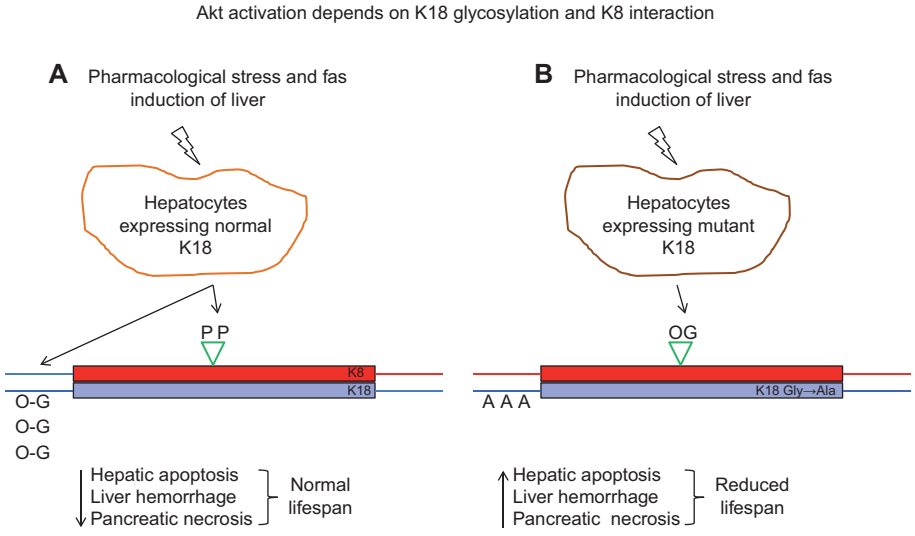

Figure 4 Activation of Akt kinase and protection against pharmacological stress depend on O-glycosylation of major K18 sites. (A) In response to an O-GlcNAcase inhibitor or to Fas, O-glycoslation of K18 and activation of K8-bound Akt are involved in protecting the mouse liver against liver damage. (B) Expression of a K18 transgene deficient in major O-glycosylation sites renders mouse liver and pancreas more susceptible to injury and apoptosis induced by an O-GlcNAcase inhibitor or to liver injury by combined O-GlcNAcase inhibition and Fas administration. Enhanced apoptosis coincides with lower activity and phosphorylation of K8-bound Akt. The data suggest that K18 hyperglycosylation promotes Akt hypoglycosylation and hyperphosphorylation and that K8 binding contributes to Akt activity in this setting.

domains may contain between 1 and 4 sites. Of the predicted sites in the head and tail regions, a few are predicted phosphorylation sites. Generation of site-specific antibodies should help to address the intracellular distribution of glycosylated keratin species and the potential relationship of keratin O-glycosylation to cell cycle, metabolic state, or exposure to stress.

Reversible covalent modification of Lys residues by small ubiquitin-like modifiers (SUMO-1, -2, and -3) regulates multiple molecular processes including localization, activity, and stability of proteins, mainly through altering interactions with other macromolecules (49). In analogy to ubiquitin, SUMO residues are added either as multi- or oligomers, with contrasting outcome for protein solubility and function. How sumoylation serves to regulate the IF cytoskeleton was first illustrated for the nuclear envelope protein lamin A, where SUMO-2 modification at Lys-201 in the consensus motif MKEE abrogates incorporation into the nuclear envelope and increases cell death by an unknown mechanism (50). Sumoylation of K8 at Lys-285/287, K18 at Lys-207/372, and K19 at Lys-208 is detectable under conditions of apoptosis, oxidative stress, and phosphatase inhibition in cells and tissues (33). K8 variants associated with chronic liver disorders become hypersumoylated and aggregate, possibly diminishing the cytoprotective function of K8/K18 discussed before, whereas modest sumoylation of unmutated K8 increases its solubility. Like glycosylation, sumoylation is regulated by other modifications, in particular phosphorylation (45). One can envisage a mechanism by which stress-induced modest sumoylation of K8 and K18 enhances their solubility, facilitating phosphorylation of K18-Ser33. This permits binding to 14-3-3 proteins, sequestering them in the cytosol with implications for translation and cell cycle control (20, 42). Support for a role of sumoylation in K8 function comes from genetic deletion of the SUMO E2 enzyme Ubc9 in mice, causing a wide range of defects including stem cell depletion in the crypts of the small intestine and fragility of enterocytes in which K8 sumoylation failed (51). In the nematode *Caenorhabditis elegans*, sumoylation has a general role regulating assembly and cytoarchitecture of IF. Here, the cytoplasmic intermediate filament (cIF) protein IFB-1, together with additional IF proteins, associates with hemidesmosome-like adhesion complexes in the worm epidermis and is essential for embryonic elongation and maintenance of muscle attachment to the cuticle (52). Repression of the only SUMO gene in *C. elegans* by RNAi or mutation of the Lys residue necessary for sumoylation of IFB-1, was followed by a decrease in the soluble IFB-1 fraction, accumulation of aggregated IF, and disorganization of hemidesmosome-like attachment sites (53). This implicates an involvement of IFB-1 dynamics in integrity of the latter. Whether sumoylation and phosphorylation of IFB-1 in *C. elegans* are interdependent has not been addressed but becomes highly relevant in the light of a recent report identifying hemidesmosomes in the worm as mechanosensors. In tissue morphogenesis, transduction of mechanical force into chemical signals is crucial for cell migration, interaction, and organ shape. Zhang and colleagues described a mechanotransduction pathway active between the body wall muscles of *C. elegans* and the epidermis. It involves a Rac GTPase and three signaling proteins located at the hemidesmosome, namely, p21-activated kinase (PAK-1), the adaptor GIT-1, and its partner PIX-1. Activation of this pathway also involves phosphorylation of IFA-3A at Ser-470 which,

together with additional events, promotes maturation of hemidesmosomes mechanical stress-resistant junctions and morphogenesis of epidermal and muscle tissues (54). Linking these events together puts IF in the worm and keratins in mammals, where they interact with *bona fide* hemidesmosmes, on a map, as transducers of mechanical signals, implicating that sumoylation and phosphorylation modulate mechanotransduction.

Pathomechanisms of keratin disorders: keratins as immune regulators

The identification of mutations in epidermal keratin genes causing disorders of skin and appendages marks an important breakthrough in the field, demonstrating the importance of the protein family in epithelial integrity and providing model system to investigate pathomechanisms. There is compelling evidence that most mutations weaken the keratin cytoskeleton's resilience against mechanical force, sever the connection to desmosomes and hemidesmosomes, and lead to formation of cytoplasmic aggregates (55). This said, we are far away from being able to predict effects of mutations on keratin assembly and interactions and on disease severity, owing to the lack of atomic structure and a comprehensive list of keratin-interacting proteins. The notion that even the most severe K14Arg$_{125}$→Cys mutation allows formation of normal keratin IF *in vitro* (56) and that the condition of some patients suffering from EBS improves with age suggest more complex pathomechanisms (EBS book). The action of genetic modifiers in EBS and other keratinopathies is indicated by phenotype variability and seasonal variation (e.g., the mutation K14Met$_{119}$→Thr; Arin et al., 2010). Below, we discuss accumulating evidence for additional pathomechanisms in keratin disorders, which also raise the issue whether mutations represent loss or gain of toxic functions.

The complexity of mechanisms underlying EBS is underscored by novel K14 null mutations. K14 haploinsufficieny is assumed to cause Naegeli-Franceschetti-Jadassohn syndrome (NFJS), a rare autosomal dominant form of ectodermal dysplasia that affects the skin, sweat glands, nails, and teeth. Major hallmarks are lack of dermatoglyphs and hyperpigmentation. This is distinct from severe, dominant EBS, caused by K14 missense mutations (9, 57). Now, Titeux and colleagues reported a severe case of EBS also resulting from K14 haploinsufficiency, challenging the haploinsufficiency

Keratin Type I	Head	Rod	Tail
K9 (4)		S155	
K10	S14	S247	S569, S570
K12		S332	S453, T472, S485
K13 isoform I (2)	S8	S203, S308	S421, S422, S430, T434, T436
K13 isoform II (2)	S8	S203, S308	
K14 (4)	T9, S51, S74	S213	T442
K15	S54	S154	
K16	T9, S73	T215	S448, S464
K17 (4)	T9, S32	S182	T404, T405
K18 (3)	S30(1), S31(1), S49(1)	S242	
K19	S3, S9, S46, S48, S54, S55, S56	S133	
K20	S13, S22	T108, T282, S293, S322	
K23	S6, S44	S316	
K24	S15, S121	S348, S401	S513, S514, S517

Keratin Type II	Head	Rod	Tail
K1	S75, S79	S324	S504, S506, T507, S508, S637
K2	S23, S30, S71	S249	S496, S597
K3	S24, S65, S69	S344, S354, T444	S528, S593, S619, S620
K4	S31, S60	S295, S305	S478, S482, S517
K5	S31, S50, S75	S232, S312, S322, S387	S492, S496, S528, S535, S547, T580, T581
K6A	S3, S5	S227, S307, S317	S491, S540, T552, T554, S555
K6B	S3, S5	S307, S317	S491, S540, T552, T554, S555
K6C	S3, S5	S227, S307, S317	S528, S540, T552, T554, S555
K7 (4)	S27	S135, S309	S444
K8 (3)	T6, S13(4), S15(4)		S432, S456
K76	S25	T427	S518, S530, S542, S543, S579, S590, S600, S608
K77	S20, S67	S308, S458	S488, S560, S568
K78	S13	T210	S494
K79	S34, S70, S72, S74	S288	S502, S508, S516, T525, T530, S531
K80 Isoform I		T184, S274, S291	T437
K80 Isoform II		T184, S274, S291	

Figure 5

Figure 5 (continued)

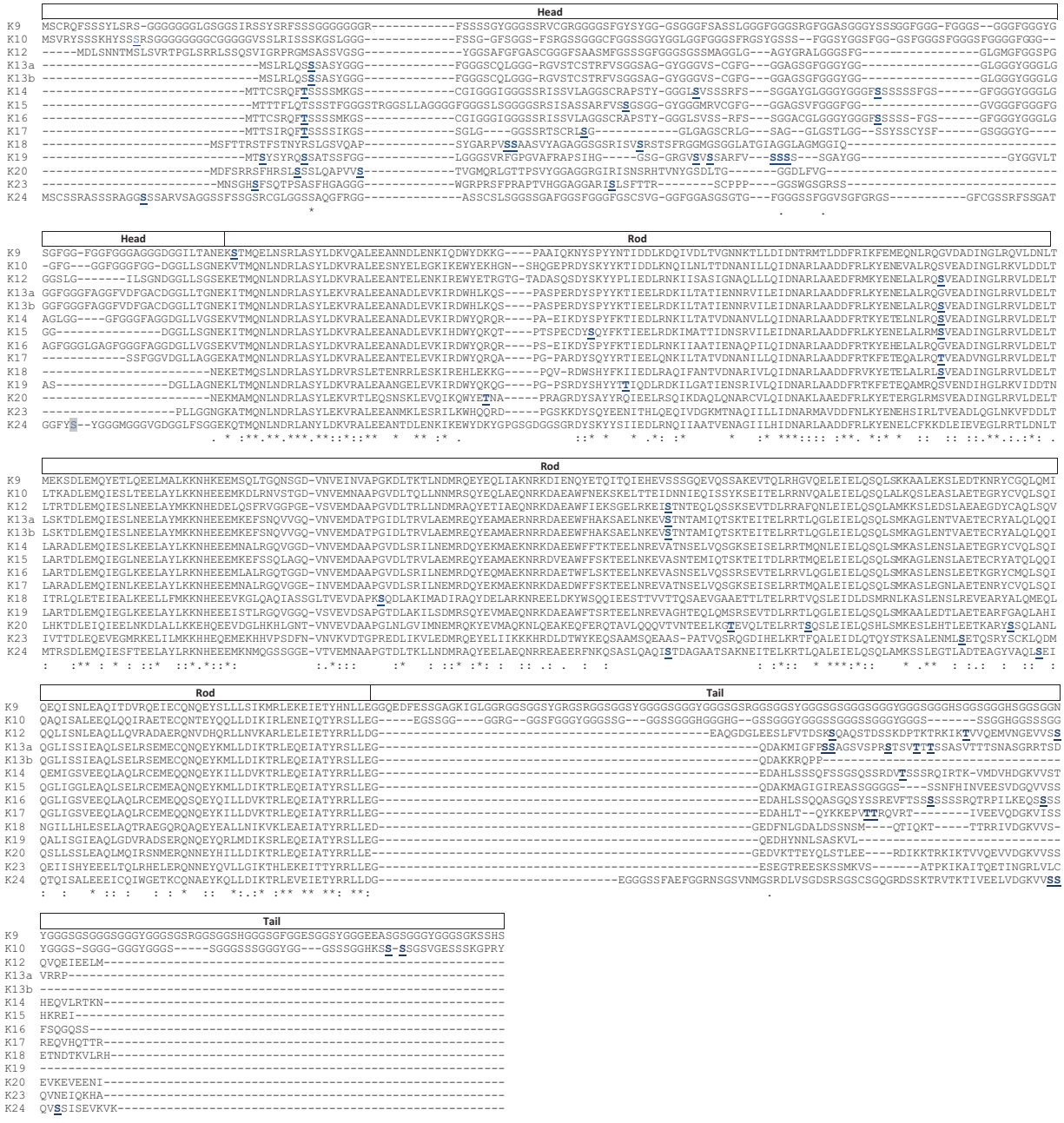

Figure 5

hypothesis (58). The authors propose that the synthesis of short serine-rich peptides encoded in the K14 head domain segment that could impair its assembly as shown for vimentin (59), or the alteration of a putative non-coding RNA arising from the 5′ terminus of KRT14 could cause NFJS. In view of the K17 data discussed below, a third mechanism may be the expression of K17 in an ectopic context, followed by alterations described.

Several studies suggest that a direct involvement of keratins in the inflammatory response contributes to the phenotype of keratin disorders or knockout mice. Transcriptome and cytokine profiling of K5$^{-/-}$ mice, serving as model for severe EBS, revealed upregulation of the proinflammatory cytokines IL-6 and IL-1β in neonatal mouse skin. Treatment with doxycycline extended the survival of neonatal K5$^{-/-}$ mice from <1 to up to 8 h, accompanied by downregulation of matrix metalloproteinase-13 and IL-1β, indicating for the first time a link between a keratin isotype and inflammation (60). A subsequent cytokine analysis of skin extracts identified an increase in the nuclear factor kappa B (NF-κB)-regulated cytokines CCL2, CCL19, and CCL20 in K5 knockout mice and EBS patients with K5 mutations. In line with their role in the recruitment, maturation, and migration of Langerhans cells (LCs) in the epidermis, their number was increased in mouse and human

Figure 5 (continued)

Figure 5

Figure 5 Predicted O-glycosylation sites in types I and II keratins.
Predicted sites in type I (blue) and type II (red) keratins excluding hair keratins. Tables in upper sections list Ser and Thr residue positions predicted as O-glycosylation sites. Experimentally confirmed sites in K8, K18 are marked with 1 and 2 in brackets, respectively. Site(s) in keratins 7, 9, 13, 14, and 17 were not identified so far. In addition, amino acid sequence comparisons of types I and II keratins for which O-glycosylation sites are predicted or confirmed are provided. Predicted positions are marked in blue (type I) and red (type II). Head, rod, and tail domains are marked above the sequence blocks. Asterisk: identical amino acids; dot: related amino acids; colon: highly similar amino acids (78–81).

skin with K5 defects. The unaltered cytokine profile and LC number in K14 knockout mice and patients suggested an isotype-specific involvement of keratins in these changes (61). These and related studies showing downregulation of junction proteins in keratinocytes with severe K14 mutations raise important issues about EBS pathology and the role of keratins in epidermal homeostasis (62, 63).

Given that inflammation can drive oncogenesis and angiogenesis, understanding how keratin isotypes affect inflammatory pathways in epithelia should contribute to understanding their role in malignancy. Here, we will not discuss keratin expression during malignant transformation and metastasis [for review, see (5)] but two studies merit closer scrutiny. Malignant rhabdoid tumors (MRT) are extremely rare; aggressive childhood tumors characterized by the presence of 'rhabdoid' cells. These are regarded as an indicator of a highly malignant potential in various types of specific malignant neoplasms of either mesenchymal or epithelial origin. MRTs are characterized by the presence of cytoplasmic accumulations of vimentin, K8, and K18. In a collection of tumor samples, missense mutations in the human *KRT*8 gene, Arg89→Cys, Arg251→Cys, Glu267→Lys, Ser290→Ile, Met and Arg301→His were identified (64). The Arg89→Cys localizes to the head domain, whereas Arg251→Cys alters the L1–2 linker sequence. The latter is equivalent to a K5 mutation in a mild form of EBS (IF database). All mutations may affect keratin organization and promote the formation of cytoplasmic keratin inclusions, composed of misfolded protein species. Although it is not clear how this contributes to malignancy, the misfolded keratin might induce protein stress and activate the chaperone machinery (65), a setting that is also characteristic of tumor cells (66).

Mutations in the sonic hedgehog regulator Gli lead to either basaloid follicular hamartoma, a rare benign tumor of hair follicles or to malignant basal cell carcinoma (67). Overexpression of Gli2 in transgenic mice causes basaloid follicular hamartoma in adult animals (68). Previous data suggested a link between K17 and TNFα signaling, possibly mediated by the interaction of the TNF receptor I adapter protein TRADD. In that setting, K17-deficient keratinocytes were more susceptible to TNFα, indicating a particular role of this keratin in inflammatory signaling (69). Now, DePianto and Coulombe examined mechanisms underlying keratin-dependent inflammation in the skin. To do so, they mated K17 knockout to Gli2 transgenic mice, based on the observation that K17 is a Gli target gene with elevated expression in this setting (70, 71). Without directly affecting hedgehog signaling, absence of K17 delayed onset of hamartoma formation in mice by 30–40% in a sex-dependent fashion. This coincided not only with a 3-fold decreased cell proliferation in the absence of K17, but also a reduction of innate immune and T cells and diminished angiogenesis. Loss of K17 in the Gli2 transgenic background coincided with a marked reduction in Th1- and Th17-related and induction of Th2-related cytokines. Th1 cytokines (e.g., IL-1β and CCL-4) mediate a proinflammatory and Th2 (e.g., CCL-24, CCL-4) an anti-inflammatory response, and Th17 (e.g., IL-6, MMP-9) is critical in mounting an antimicrobial response in barrier epithelia (72). Of note, changes in the cytokine profile occurred in K17 null, Gli2 transgenic mice before the onset of tumor formation. Thus, the K17 status exerts an immunomodulatory influence in inflamed epidermis. At least part of the underlying mechanism is cell autonomous, as keratinocytes from Gli2 mice displayed a K17-dependent cytokine profile similar to the one determined *in situ* (70). Remarkably, elevated K17 and Th1 activity coincide in psoriatic lesions; further, elevated K17 expression marks a poor prognosis in mammary and pancreatic cancer (73, 74). One of the future challenges remains to dissect the function of K17 in tissue homeostasis from its proinflammatory role in disease. In this regard, the molecular composition of heterodimeric keratin complexes containing K17, posttranslational modifications, or associated proteins are likely determinants.

In the light of these data, the finding that certain K5 mutations including K5Gly138→Glu act as a genetic modifier in basal cell carcinoma (67) is intriguing. Finally, our own lab has recently identified another link between keratins and immune control. We have found that K1, the major keratin involved in cornified envelope formation, controls release of the proinflammatory cytokine IL-18 and of the TLR-4 ligands S100A8/A9 in a cell-autonomous fashion in the mouse. This implies that K1 mutations not only give rise to epidermolytic ichthyosis but also predispose to local and/or systemic inflammation through a mechanism other than cell fragility (75). Of note, IL-18 and S100A8/A9 levels are low in the presence of K1, whereas expression of K17 stimulates synthesis of proinflammatory cytokines. Therefore, the mechanisms by which certain keratin isotypes regulate cytokine synthesis and release may be very different but lead to a similar outcome, namely, mounting a keratinocyte-dependent skin-specific immune response. Loss-of-function mutations in the filaggrin gene also support a primary role of keratinocyte-dependent functions in several chronic disorders involving the skin. One common denominator resulting from alterations in K1, K17, and filaggrin is a defective epidermal barrier resulting from impaired lipid envelope and tight junction functions (76). These defects can arise at several levels, including biosynthesis, intracellular transport, and secretion, processes in which the involvement of keratins at a mechanistic level awaits urgent clarification.

Conclusions/expert opinion

Here, we have discussed some recent findings that begin to shed light on mechanisms by which keratin isotypes act as targets and effectors of signals. There is mounting evidence that providing mechanical stability to epithelia is one of several crucial keratin functions. This has implications for therapy concepts of keratin-related disorders, adding small molecule-based approaches to gene therapy-based approaches (65, 77). We have emphasized that many keratin functions depend on isotypes, their intrafilament/intracellular distribution, and posttranslational modifications. At present, we lack knowledge of integration, distribution, and turnover of individual keratins into the cytoskeleton and the underlying mechanism (Figure 3). High-resolution microscopy, in conjunction with fluorescent tags and biochemical approaches, should help to clarify this issue. Further, despite many years of research, there is still limited knowledge on the biophysics of the keratin cytoskeleton. To which extent does composition and keratin network organization, i.e., bundling and intracellular organization, affect cell mechanics and behavior? This requires identification of mechanisms underlying cross talk with actin regulators, in particular, members of the Rho GTPase family and development of cell-based assays to measure forces depending on keratins. Finally, what are the mechanisms by which keratins, either in filamentous form or as soluble oligomers, contribute to signaling, protein trafficking, and inflammation? Resolving at least some of these issues will have major implications for epithelial disorders.

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Wera Roth studied Biology at the University of Cologne and received her PhD in Biochemistry at the University of Göttingen in 1996. After several years of postdoctoral research in Prof. C. Peters Laboratory at the University of Freiburg, Prof. R. Grosschedl's laboratory at the Gene Center, Munich and in Prof. M. Meisterernst laboratory at the Helmholtz Zentrum in Munich, she joined the group of Prof. T.M. Magin in Bonn in 2006. Since 2010 she is a senior post doc in the laboratory of Prof. T.M. Magin, division of Cell and Developmental Biology at the Translational Center of Regenerative Medicine and the Institute of Biology, University of Leipzig.

Mechthild Hatzfeld studied biochemistry at the University of Tübingen and received her PhD in Cell Biology in 1987, following thesis work on keratins in the group of Prof. W.W. Franke at the German Cancer Research Center, Heidelberg. As postdoctoral fellow, she joined the group of Prof. Klaus Weber at the Max Planck Institute of Biophysical Chemistry in Göttingen in 1987. In 1996, she was awarded a Heisenberg fellowship. From 1997–2002, she was an independent group leader at the Martin-Luther-Universität Halle-Wittenberg. In 2002, she was nominated professor of pathobiochemistry at the University of Halle. In 2011, she became director of the Institut für Molekulare Medizin, Sektion Pathobiochemie at the University Halle-Wittenberg. Her major research interest is on the regulation of cell growth and adhesion by armadillo proteins.

Maik Friedrich studied biology at the Universities of Jena and Leipzig. He received his PhD in biology in 2007. In 2007, he joined the MD/PhD program and received the Dr.rer. med. degree in 2009. From 2009–2010, he was postdoc with Prof. Engeland, University of Leipzig. In 2010, je joined the group of Prof. Magin at the division of Cell and Developmental Biology at the Translational Center of Regenerative Medicine and the Institute of Biology, University of Leipzig.

Sören Thiering studied biotechnology at the Hochschule Anhalt and received his BSc degree in November 2011.

Thomas Magin studied Biology at the University of Heidelberg and received his PhD in Cell Biology in 1987, following thesis work on keratins in the group of Prof. W.W. Franke at the German Cancer Research Center, Heidelberg. He was a DFG and Wellcome Research Fellow at the University of Edinburgh from 1990 to 1995. From 1995 to 2000, he was independent group leader at the Institute of Genetics, University of Bonn. From 2001 to 2010, he was Professor of Biochemistry at the Medical Faculty, University of Bonn. Since April 2010, he is Professor of Cell and Developmental Biology at the Translational Center for Regenerative Medicine and the Institute of Biology, University of Leipzig. His major research interest is on understanding the function of intermediate filament proteins during differentiation, regeneration, and pathogenesis of epithelia.