Keratin 17 in disease pathogenesis: from cancer to dermatoses

Luting Yang, Shaolong Zhang and Gang Wang*

Department of Dermatology, Xijing Hospital, Fourth Military Medical University, Xi'an, PR China

*Correspondence to: G Wang, Department of Dermatology, Xijing Hospital, Fourth Military Medical University, 127 West Changle Road, Xi'an 710032, PR China. E-mail: xjwgang@fmmu.edu.cn

Abstract

Keratin 17 (K17) is a type I intermediate filament mainly expressed in the basal cells of epithelia. As a multifaceted cytoskeletal protein, K17 regulates a myriad of biological processes, including cell proliferation and growth, skin inflammation and hair follicle cycling. Aberrant overexpression of K17 is found in various diseases ranging from psoriasis to malignancies such as breast, cervical, oral squamous and gastric carcinomas. Moreover, genetic mutation in KRT17 is related to tissue-specific diseases, represented by steatocystoma multiplex and pachyonychia congenita. In this review, we summarize our findings concerning the regulatory mechanisms of K17 overexpression in psoriasis and compare them to the literature relating to other diseases. We discuss data that proinflammatory cytokines, including interleukin-17 (IL-17), IL-22, interferon-gamma (IFN-γ), transforming growth factor-beta (TGF-β) and transcription factors glioma-associated oncogene homolog 1/2 (Gli1/2), Nrf2 and p53 can regulate K17 by transcriptional and translational control. Moreover, post-translational modification, including phosphorylation and ubiquitination, is involved in the regulation of K17 stability and biological functions. We therefore review the current understanding of the K17 regulatory mechanism and its pathogenic role in diseases from dermatoses to cancer. Prospects for anti-K17 therapy in diagnosis, prognosis and disease treatment are also discussed.

Copyright © 2018 Pathological Society of Great Britain and Ireland. Published by John Wiley & Sons, Ltd.

Keywords: Keratin 17; dermatoses; squamous cell carcinoma; breast; cervix; psoriasis; pachyonychia congenita; steatocystoma multiplex; p27

Received 5 July 2018; Revised 17 September 2018; Accepted 2 October 2018

No conflicts of interest were declared.

Introduction

Keratins belong to the superfamily of intermediate filament proteins. Based on the gene substructure and nucleotide sequence homology, keratins are divided into two groups: 28 type I acidic and 26 type II basic proteins. Type I and type II keratins form heterodimers for assembly of the 10 nm filaments that provide structural support for maintaining cellular integrity [1]. Keratin 17 (K17) belongs to the type I intermediate family. Like all members of the keratin family, K17 is a tripartite structure composed of 432 amino acids: non-helical head (1–83), α-helical rod (84–392) and non-helical tail domains (393–432). Within the rod domain, the heptad repeat-containing segments coils 1A (84–120), 1B (139–230) and 2 (251–392) are interrupted by linker sequences [2,3]. Genetic mutation in KRT17 that encodes K17 is related to tissue-specific diseases, represented by steatocystoma multiplex (SM) and pachyonychia congenita (PC) [4,5].

K17 is mainly present in the epithelial appendages, such as hair follicles, sebaceous glands and other glands [6]. However, K17 is unusual in that it shows no expression in epidermis of normal skin, but is inducible under stressful conditions such as skin injury [7], viral infections [8] and psoriasis [9,10]. In psoriasis, K17 is overexpressed in epidermis owing to the stimulation of proinflammatory cytokines such as interleukin-17 (IL-17), IL-22 and interferon-gamma (IFN-γ) [9–11]. Furthermore, K17 is upregulated in breast, cervical, oral squamous and gastric carcinomas [12]; K17 is a prognostic biomarker for these cancers.

K17 is a multifunctional protein that regulates a myriad of cellular processes, including cell proliferation and growth [13,14], skin inflammation [15] and the differentiation of skin appendages [16]. As an intermediate filament, K17 is thought to reside in the cytoplasm to exert its functions. However, recent findings indicated that K17 could shuttle in and out of the nucleus owing to the existence of a nuclear localization signal and a nuclear export signal [17]. This new idea of nuclear localized intermediate filaments raises the possibility for keratins to regulate additional cellular processes.

This review summarizes the transcriptional, translational and post-translational modification (PTM) of K17 expression and function in different diseases, from
cancer to psoriasis and other dermatoses. We also discuss the prospects for targeting K17 in disease treatment.

Regulation of K17

Cytokine regulation of K17

Several cytokines and growth factors such as IFN-γ, IL-17, IL-22, transforming growth factor-beta 1 (TGF-β1) and basic fibroblast growth factor (bFGF) are known to upregulate K17 expression via different molecular pathways. IFN-γ is a pluripotent cytokine mainly secreted by Th1 polarized CD4+ T helper cells [18]. Being recognized as a proinflammatory cytokine in psoriasis pathogenesis, IFN-γ is also implicated in cancer and autoimmune diseases [19]. In 1994, Jiang et al [20] discovered that IFN-γ could upregulate KRT17 transcription through signal transducer and activator of transcription 1 (STAT1) in human keratinocytes. STAT1 was specifically activated by IFN-γ, phosphorylated and translocated to the nucleus, where it bound to IFN-γ-activated sequence (GAS) within the promoter region of KRT17. IFN-γ-induced K17 was subsequently confirmed in the immortalized human keratinocyte cell line, HaCaT, which pinpointed the GAS position in the KRT17 promoter [21]. STAT3 is another pathway linking IFN-γ and K17, where interfering with STAT3 reduced the IFN-γ-induced K17 upregulation in HaCaT cells [22]. The elevation of K17 induced by IFN-γ was also shown to be mediated partially through IL-1, a cytokine known to induce the pathogenesis of psoriasis [11].

Our group identified that Th1-related cytokines IL-17 and IL-22 could also upregulate K17 expression in psoriatic keratinocytes through different signaling pathways. The molecular mechanism by which IL-17A upregulated K17 was characterized to be STAT1 and STAT3 [9]. However, IL-22 was found to promote the proliferation of keratinocytes and upregulate K17 through STAT3 and extracellular regulated protein kinases (Erk) 1/2 [10].

TGF-β1, a key cytokine in regulating inflammation and the tumor microenvironment [23,24], upregulates K17 in both human keratinocytes and cervical cancer cells via different molecular mechanisms. In psoriasis keratinocytes, our group delineated a TGF-β1/Smad3/miR-486-3p pathway that controls K17 overexpression and keratinocyte proliferation [25]. However, in cervical cancer cells, TGF-β1 K17 upregulation was mediated through the Erk1/2 pathway [26]. Another growth factor, bFGF, was demonstrated to upregulate K17 in endothelial cells and contributed to tumor angiogenesis [27].

In summary, IFN-γ, IL-17, IL-22, TGF-β1 and bFGF have been shown to upregulate K17 via different molecular pathways in keratinocytes and endothelial cells (Figure 1). Regarding the complexity of cancer and inflammatory microenvironments, other cytokines might also have a role in regulating K17 expression. Transcription factors involved in the regulation of K17 expression

Apart from cytokines, several transcription factors show strong binding to the promoter region of the KRT17 gene, including glioma-associated oncogene homolog 1/2 (Gli1/2), Nrf2 and p53 (Figure 1). The diversity of responsiveness to these transcription factors contributes to the pleiotropic functions of K17. Gli1 and Gli2 are members of the Gli family of transcription factors. As terminal activators of Sonic hedgehog signaling, Gli1 and Gli2 are implicated in the oncogenic transformation of several cancers [28,29]. The first study conducted in mice showed a Gli2-responsive element in a 48-bp fragment within the Krt17 promoter. Deletion of this fragment resulted in loss of K17 expression in most epithelial appendages. This 48 bp fragment encompassed binding sites for other transcription factors, including AP1 and SP2, which were essential for hair follicle development and cycling [30]. In Ewing sarcoma, K17 was confirmed to be a direct downstream target of Gli1, with evidence of Gli1 binding to the promoter-proximal region of KRT17. K17, in coordination with Gli1, mediated oncogenic transformation and cell adhesion in Ewing sarcoma [31]. Gli1 or Gli2 showed a strong correlation with K17 levels in oral squamous cell carcinoma (OSCC) and Gli inhibition suppressed K17 expression in OSCC cells [32].

In 2017, our group identified Nrf2 as another positive regulator of K17 expression [33]. As a transcription factor, Nrf2 is known to regulate cellular defenses against oxidative stress by binding to the antioxidant responsive element (ARE) located in the promoter region of target genes [34]. By chromatin immunoprecipitation, we identified that Nrf2 could bind to the ARE region located in the promoter of KRT17, regulating its transcription and translation. The increase in Nrf2 in psoriatic keratinocytes thus contributed to K17 upregulation and keratinocyte proliferation in psoriasis patients.

Apart from these positive regulators of K17, p53 negatively regulates K17. The tumor suppressor protein p53 is a transcription factor that regulates cell stress response, metabolism and cell cycle [35]. In radiation dermatitis, p53 repressed K17 transcription and K17 expression showed an inverse correlation with p53 activation in a mouse model. Mapping the promoter region of Krt17 revealed two putative p53 binding sites [36]. This p53−K17 negative regulatory mechanism raises the possibility of employing p53 for the treatment of K17-related dermatoses.

Translational and post-translational modification of K17

Apart from cytokines and transcription factors, proteomic analysis showed several proteins that interact with K17. Annexin A2 is a K17-interacting protein that is implicated in epidermal growth factor receptor (EGFR)-induced upregulation of K17 in cancer. Annexin A2 orchestrates multiple biological processes,
including regulation of keratin filament stability and cancer progression [37,38]. Interaction with annexin A2 promoted K17 stability, which prevented EGFR-induced K17 remodeling. K17 reciprocally impacted annexin A2 phosphorylation and subcellular distribution [39].

Among the few studies that have investigated PTM of K17, Pan et al. [40] examined phosphorylation. Phosphorylated forms of K17 were seldomly detectable in the steady-state of cultured mouse skin keratinocytes. However, two phosphorylation sites on K17, threonine 9 (Thr9) and serine 44 (Ser44), were seen in response to oxidative stress, ultraviolet irradiation, growth factors such as EGF and the tumor-promoting phorbol ester, TPA [40]. Phosphorylated K17 interacted with the scaffold protein 14-3-3 sigma and promoted cell growth. However, hypoactivation of these two phosphorylation sites resulted in a sequestration of 14-3-3 sigma in the nucleus, leading to suppression of cell growth and protein synthesis [7]. Another study from the same group identified that p90 ribosomal S6 kinase 1 (RSK1) was responsible for Ser44 phosphorylation of K17, controlling skin keratinocyte growth. These findings point to a low level of phosphorylated Ser44 of K17 under normal conditions, which is induced in a growth- and stress-dependent manner (Figure 2A). Other potential phosphorylation sites on K17 are predicted by PhosphositePlus and UniProt, suggesting further complexity of K17 phosphorylation. Extensive studies are required to clarify how phosphorylation occurs and how it regulates K17 and its multifaceted functions in different cell compartments.

Ubiquitination is another key PTM that modulates protein stability and protein–protein interactions [41]. Recently, our group delineated a mechanism in which K17 could be ubiquitylated in human keratinocytes. We firstly predicted the existence of K17 ubiquitination sites by UbiSite (http://csb.cse.yzu.edu.tw/ubisite/). Immunoprecipitation confirmed that K17 was ubiquitylated in HaCaT cells. Mass spectrometry identified E3-ligase Trim21 in K17 ubiquitination, which promoted the stability of K17 in psoriatic keratinocytes via K63-linked ubiquitination. The Trim21-induced ubiquitination of K17 interacted with STAT3, promoting its activation and cell proliferation in psoriasis keratinocytes (Figure 2B) [42]. This opens a new avenue for targeting ubiquitination in the potential treatment of psoriasis. Further studies are needed to explore whether K17 ubiquitination is a common PTM to regulate stability and function in other K17-related diseases.

K17 dysregulation in cancer pathogenesis

K17 in diagnosis and prognosis in OSCC, breast, cervical and other carcinomas

In neoplastic conditions, K17 is expressed in cervical carcinoma, breast carcinoma, gastric carcinoma, adenocarcinoma and squamous cell carcinomas (SCC) [43–46]. In the cervix, K17 immunohistochemistry showed stronger expression in SCC and high-grade squamous intraepithelial lesions than in
Keratin 17 in disease pathogenesis

Figure 2. PTM of K17. (A) Phosphorylation. In response to extracellular stimuli (EGF and TPA) and to cellular stresses (ultraviolet radiation [UVR] and oxidative stress), RSK1 is activated, which phosphorylates K17 on Thr9 and Ser44. Phosphorylated K17 promotes the relocalization of 14-3-3 sigma from the nucleus to the cytoplasm, where K17 interacts with 14-3-3 sigma and activates AKT/mTOR pathways, potentially regulating skin keratinocyte cell growth. (B) Ubiquitination. Upon stimulation with IFN-γ in psoriatic milieu, Trim21 is elevated and ubiquitylates K17 in keratinocytes. Ubiquitylated K17 interacts with and facilitates STAT3 activation, promoting keratinocyte proliferation.

K17 regulates cancer cell proliferation and tumor growth

The function of K17 in tumorigenesis needs to be clarified. In a Gli2 transgenic mouse model of basaloid skin tumors, Krt17 genetic ablation delayed tumor initiation and growth. In a mouse model of acute dermatitis induced by TPA, K17 loss reduced hyperplasia and normal squamous mucosa and low-grade squamous intraepithelial lesions, suggesting K17 as a diagnostic marker to distinguish malignant cervical lesions from non-malignant lesions or normal mucosa [44]. Similarly, K17 levels and its correlation with cancer metastasis suggest that K17 has potential as a diagnostic and prognostic biomarker in multiple other subtypes of carcinoma [47–49].
inflammation in a similar manner [13]. In carcinoma in situ and SCC tissues, K17 modulated cell proliferation through interaction with 14-3-3 sigma. In carcinoma in situ and SCC tissues and cell lines, K17 was co-expressed with 14-3-3 sigma in the cytoplasm. However, in SCC cells where K17 was knocked down, 14-3-3 sigma translocated from the cytoplasm to the nucleus, accompanied by decreased cell proliferation and cell size [14]. The K17-induced translocation of 14-3-3 sigma might induce the proliferation of carcinoma cells through activation of Akt/mTOR (mammalian target of rapamycin), a signaling pathway that has a central role in regulating cell growth [7].

In cervical cancer, K17 translocated to the nucleus where it bound the cell cycle inhibitor p27KIP1 and regulated cell cycle progression and tumor growth by promoting nuclear export of p27KIP1 [50]. The cytoplasmic sequestration of p27KIP1 further led to its degradation by a ubiquitination-proteasome-dependent system. In vivo, xenograft tumors derived from cancer cells expressing control shRNA were more than twice the size of those from shKrt17 cells after 30 days of implantation. Moreover, tumor cell proliferation was decreased in xenografts with K17 knockdown, with decreased expression of Ki67 and proliferating cell nuclear antigen [50]. Thus, K17 functions as an oncoprotein by promoting the nuclear export and degradation of cell cycle suppressor p27KIP1. In human cancers, p27 level or localization is frequently deregulated by SRC, MAPK and receptor tyrosine kinase pathways [51].

K17 induced inflammation in tumorgenesis

K17 promoted skin tumorigenesis in an inflammatory manner involving expression of CXCR3 ligands CXCL9, CXCL10 and CXCL11 [15,52]. In a human epidermoid carcinoma cell line, K17 interacted with heterogeneous nuclear ribonucleoprotein K (hnRNPK) and was responsible for cytoplasmic translocation of hnRNPK, where it regulated CXCL9, CXCL10 and CXCL11 and promoted tumor proliferation and metastasis. In the same year, it was found that K17 could physically bind to hnRNPK in both human and mouse tumor-prone keratinocytes and induced transcription of the transcriptional regulator Aire (autoimmune regulator) [53]. Meanwhile, K17 interacted with Aire in the nucleus of skin tumor keratinocytes and both bound to the same promoter regions of CXCL9, CXCL10 and CXCL11 and promoted tumor growth. This said, the nuclear and cytoplasmic form of K17 in tumor cells may be responsible for K17-induced inflammation and tumor growth.

K17 in dermatoses

K17 in psoriasis

In 2006, we proposed a K17/T cell/cytokine autoimmune loop in the pathology of psoriasis [54]. K17 shares similar epitopes with streptococci M6 protein, which is a superantigen in psoriasis. The T-cell activating epitopes of K17 might be recognized by dendritic cells and trigger their activation and maturation. The mature dendritic cells further secrete mediators that induce the differentiation of naive T cells into Th1 and Th17 cells, which produce high levels of IL-17, IFN-γ and IL-22 in psoriasis. These inflammatory factors in turn promote the expression of K17 in psoriatic epidermis [55]. This autoimmune positive feedback loop provides a model for which K17 is involved in the recurrence and development of psoriasis.

K17 in alopecia

Alopecia, or hair loss, is a common disease that affects 50% of men and 40% of women by age 50 and 70 years, respectively, with a prevalence of 0.1–0.2% in the general population. Inherited homozygous dominant mutations were detected in the coiled 1A domain of K17 in two families with early onset alopecia. In one, a homozygous dominant missense mutation (c.275A>G) was detected, which caused a substitution of asparagine to serine residue located at codon 92 of K17 (Asn92Ser). In the other, a homozygous dominant mutation (c.280C>T) that caused a substitution of arginine to cysteine at codon 94 (Arg94Cys) of K17 was identified [56].

Given the evidence of K17 mutation in alopecia, the function of K17 in hair follicle cycling is the issue of interest. The hair cycle-dependent regulation of K17 expression was first revealed in mice in 1997. Although hair follicles were the only skin structures to express K17, the expression profile and its localization were precisely regulated during murine hair cycling. In telogen, K17 was mainly present in a spatially confined portion of the outer root sheath isthmus. With the progression of the anagen phase, K17 was increased in the outermost cell layers of the outer root sheath, suggesting that K17 expression is hair cycle-dependent [57].

The crucial role of K17 in hair follicle maturation was later revealed in a mouse model. K17 null mice developed severe alopecia during the first week after birth, correlating with hair fragility, structural alteration in hair follicle compartments and apoptosis in matrix cells. Despite the obvious changes in hair follicles, even the most severe alopecia mice showed phenotypic recovery and normalized during the first postnatal anagen phase of the hair cycle, correlating with a marked increase in compensatory keratins, such as K16 [58]. Later it was found that K17 interacted with tumor necrosis factor-alpha (TNF-α) in hair follicle cycling. TNF-α signaling was increased in K17 null mice, whereas ablation of TNF-α in K17 null mice rescued the hair loss in K17 null mice [59].

K17 mutation in PC and SM

Missense mutation in K17 gives rise to two distinct disorders of the skin, each related to ectodermal dysplasia; PC and SM.
PC is divided into two types: PC-1 and PC-2. PC-2 is distinguished from PC-1 by the presence of pilosebaceous cysts. It is now recognized that PC-1 is caused by genetic mutation in the genes coding for K6a or K16 and PC-2 by mutation in K6b or K17 [60]. Several KRT17 mutations have been reported, with a c.275A>G missense mutation causing a substitution of asparagine to serine (Asn92Ser) the most frequent [4,61] and, to date, reported PC-2 mutations occur in the helix initiation motif of the 1A domain of K17. Although the molecular mechanism underlying the correlation between K17 mutation and PC-2 pathogenesis is still unclear, it is presumed that the distribution of K17 in pilosebaceous and glandular structures might account for cyst formation in PC-2 [62].

To date, eight KRT17 mutations have been identified in SM patients [63,64]. All of these mutations are missense mutations, with most located in the 1A domain. Among these, a heterozygous mutation (c.280C>T) causing a substitution of arginine to cysteine at codon 94 (Arg94Cys) was identified in a Chinese SM pedigree of 10 affected members [63]. A Dutch Caucasian woman with SM was also detected to have Arg94Cys in K17 [65]. This mutation abolishes a recognition site for the restriction enzyme Acil and this substitution might be detrimental to the integrity of keratin in K17-expressing tissues.

Concluding remarks

To summarize, we highlight the major functions of K17 in the pathogenesis of cancer and dermatoses. Ranging from regulation of immune responses in tumorigenesis and immune-related dermatoses, several basic cellular functions, such as cell cycle progression, are under the control of K17. This area of research will continue to improve our understanding of the role of K17 in disease pathogenesis. We have reviewed that K17 is subjected to transcriptional, translational and post-translational regulation in different diseases. By proteomics analysis, K17 is predicted to be modulated by PTMs such as ubiquitination, acetylation and sumoylation. Nevertheless, the current findings in various diseases are limited in terms of identifying new PTMs of K17 and their synergistic effects. Exploring key modulators of K17 in different diseases is also vital to direct anti-K17 therapies in clinical management.

The challenge in future studies is the effectiveness of anti-K17 therapies in disease treatment. Anti-K17 therapies have been shown to alleviate psoriasis symptoms in a SCID-hu xenogeneic transplantation mouse model [66]. K17 knockdown also reduced gastric adenocarcinoma cell proliferation and migration and reduced tumorigenicity in xenografts in vivo [12]. Although RNAi strategies targeting K17 hold tremendous potential for the treatment of cancers and psoriasis, the proper vehicles for delivery and the side-effects need to be explored. Equally important is to understand the value of K17 as a biomarker of cancer diagnosis and prognosis. Large sample analysis, molecular mechanisms regarding the role of K17 in cancer pathogenesis and animal model studies will be a prerequisite for applying K17 therapeutics in preclinical and combined therapy of cancer treatment.

Acknowledgements

The authors are grateful to Dr Liang Jin, Dr Wei Zhang and Dr Bing Li for their contributions to the research. This work was supported by the National Natural Science Foundation of China (no. 81502716, 81602749, 81672692 and 81430073).

Author contributions statement

All authors contributed to this review and reviewed the final manuscript.

References


sense mutation in keratin 17 leads to alopecia in addition to severe
mice exhibit age- and strain-dependent alopecia. Genes Dev 2002;
16: 1412–1422.
59. Tong X, Coulombe PA. Keratin 17 modulates hair follicle cycling in
K6a, K16 and K17 causing pachyonychia congenita. J Dermatol Sci
N92S mutation of keratin 17 gene: clinical features, mutation analysis
in a large family with pachyonychia congenita type 2. Arch Dermatol
63. Liu Q, Wu W, Lu J, et al. Steatocystoma multiplex is associated
with the R94C mutation in the KRT17 gene. Mol Med Rep 2015; 12:
5072–5076.
oligodontia and partial persistent primary dentition associated
with a novel keratin 17 mutation. Br J Dermatol 2009; 161:
1396–1398.
65. Covello SP, Smith FI, Sillevis S, et al. Keratin 17 mutations cause
either steatocystoma multiplex or pachyonychia congenita type 2. Br
with antisense and RNAi strategies: exploring novel therapy for

50 Years ago in The Journal of Pathology...

Hydroxyanisole depigmentation: In-vivo studies

P. A. Riley

Hydroxyanisole depigmentation: In-vitro studies

P. A. Riley

Skeletal muscle necrosis associated with carcinoma

Barbara Smith

To view these articles, and more, please visit:
www.thejournalofpathology.com

Click 'BROWSE' and select 'All issues', to read articles going right back to
Volume 1, Issue 1 published in 1892.