Phenotypes, genotypes and their contribution to understanding keratin function

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A large number of mutations in keratin genes underlie inherited tissue fragility disorders of epithelia. The genotype–phenotype correlations emerging from these studies provide a rich source of information about the function of keratins that would have taken decades to achieve by a purely transgenic approach. Human disease studies are being supplemented by engineered mouse mutant studies, which give access to the effects of genetic alterations unlikely to occur naturally. Evidence is emerging that the great diversity of keratins might be required to enable cells to adapt their structure in response to different signalling pathways.

The term cytoskeleton was coined to describe the scaffold-like appearance of the detergent-insoluble protein filaments that extend throughout the cell cytoplasm and nucleus, leading to the assumption that these filaments play a role in cellular architecture. Studies of these microfilament, microtubule and intermediate filament protein structures have indicated that the cell cytoskeleton is capable of constant remodelling to enable cell shape changes involved in differentiation and cell motility. Microtubules and microfilaments undergo constant, rapid remodelling and are intimately involved in signalling pathways for cell motility, proliferation, differentiation and cell death. The intermediate filaments, however, appear to be less dynamic, less well-conserved in evolution, and possibly not essential for individual cell survival. Compared with the huge and rapid fluxes between polymer and monomer that occurs in microfilament and microtubule systems, intermediate filaments are relatively stable, with most of the protein held in a filamentous state.

A structural role for keratins confirmed by mutations causing skin disorders

Keratins make up the two largest of the five or six classes of intermediate filament proteins, and, within the keratins alone, <49 genes have been identified in humans [1]. They are expressed in all epithelial cells from the single-layered epithelia of most internal organs (simple epithelia) to the complex multi-layered epithelium (stratified epithelium) of the epidermis. Recent discoveries that skin fragility disorders are often caused by disruptive mutations in keratins have led to the perception that intermediate filaments are the closest to a true 'skeletal' component of the cytoskeleton.

However, it remains unclear why so many different intermediate filament genes exist – 65 at the last count [1]. Has there been redundant gene duplication, or is there actually a need for subtly different filamentous scaffolds in different tissues? Newly discovered keratin disorders in humans, and keratin defects experimentally generated in mice have revealed just how much functional diversity and overlap exists in the keratin protein families. It is therefore important to consider the structural requirements of different tissues, and the requirement for a mechanical infrastructure to respond to cellular signals.

Keratin proteins share a common structure with other members of the intermediate filament family, as shown in Box 1. Keratins assemble into filaments from type I or type II heterodimers and are co-expressed in their type I/type II pairs in a tissue- and differentiation-specific manner (see Table 1); however, structural and sequence data do not provide the answer to why this should be so. The pathology caused by mutation in a keratin gene is primarily determined by the tissue-specific expression pattern of that keratin (Table 1). The epidermis has a particularly complicated keratin expression pattern (Fig. 1) and this, together with the accessibility of the skin for analysis, has resulted in a large number of genetic skin disorders recognized as being caused by different mutations in epidermal keratins (Table 1).

Mutations in EBS highlight functional clusters

The first genetic disorder to be identified as caused by keratin mutations was epidermolysis bullosa simplex (EBS). This skin blistering disorder is caused by mutations in keratin K5 (type II) or K14 (type I). K5 and K14 are the predominant keratin pair of the basal cell layer of the epidermis, and mutations in these keratins lead to fragility of basal keratinocytes. In terms of keratin function, EBS is a particularly informative disorder because different mutations in either of the two genes produce visibly diverse pathological phenotypes, and the disorder can be subdivided clinically according to severity. At the severe end of the spectrum, patients with the Dowling–Meara variant of EBS suffer from clusters of

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http://tigs.trends.com 0168-9525/03/$ - see front matter doi:10.1016/S0168-9525(03)00071-4

Box 1. Intermediate filament structure

Like all intermediate filament proteins, keratins have a central rod domain consisting of four highly conserved α-helical domains (1A, 1B, 2A and 2B) separated by non-helical linker regions (L1, L12 and L2). Non-helical head and tail domains, which vary greatly in size and sequence, flank the rod domain, and it is these domains that differ most between members of the keratin family. The end domains are made up of E1 (head) and E2 (tail) extreme end domains, the variable V1 (head) and V2 (tail) domains, and homology domains H1/H2 domains. The type I keratins are acidic and the type II are neutral to basic. Type I keratins, alone of all intermediate filament groups, do not have these H1/H2 domains, which are believed to be essential for filament assembly. Therefore, in the keratin heteropolymer, the H1/H2 function must be provided solely by the type II keratin.

The rod domain sequence defines the class of intermediate filaments that a protein belongs to (Fig. I). The α-helical domains consist of heptad repeats in which every first and fourth residue is apolar and positions 5 and 7 are often charged amino acids. The regularity of the repeats is interrupted (known as the stutter) in the 2B domain. The rod is involved in the polymerization of the keratin to form intermediate filaments. The apolar residues are on the outside of the α-helix and are thought to interact during dimerization. Unlike other intermediate filament proteins, keratins have to form a heterodimer between one type I and one type II keratin before higher-order structures can polymerize. At either end of the rod domain are highly conserved stretches of amino acids known as the helix initiation motif and helix termination motif (coloured red in the figure). These domains are crucial for the filament assembly process. The consensus sequence for the helix termination motif is most commonly EIATYRKLLEGE, with some variation at positions 7 and 12. The helix initiation motif differs between keratin types; in type I keratins it is LNDRLASY, but in type II it is LNNKFASY. Higher-order structures form by anti-parallel alignment of the keratin heterodimers with a staggered overlap of either 1B or 2B domains.

Although all type I keratins can form filaments with any type II keratin *in vitro*, *in vivo* specific pairs of keratins are expressed in a tissue type- and differentiation-specific manner (see Table 1).

Unfortunately, the nomenclature for keratins has become rather cumbersome owing to the family being much larger than first anticipated. The type II keratins are numbered K1–K8 for the cytokeratins, and Ha1–Ha6 for the hair keratins. Multiple keratins with a high degree of homology to K6 have been identified and have been called K6a–K6f, K6irs (inner root sheath of the hair follicle) and K6hf (hair follicle companion layer). The type I keratins are numbered K9–K23 (omitting K11, which was later shown not to exist) for cytokeratins, Ha1–Ha9 for hair keratins and IRSa1–IRSa3 for inner root sheath keratins.

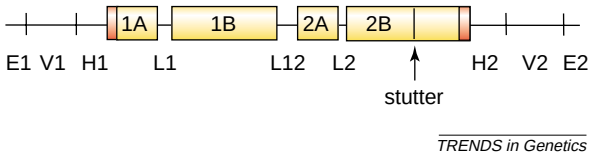

Fig. I. Domain structure of keratin proteins. The central rod domain consists of highly conserved α-helical domains 1A, 1B, 2A and 2B separated by non-helical linker regions L1, L12 and L2. The head and tail domains consist of end domains (E1/E2), variable domains (V1/V2) and homology domains (H1/H2). The interruption to the heptad repeats that occurs in 2B is known as stutter.

blisters that form readily upon mild physical trauma to the skin; the blistering is also often accompanied by palmoplantar (palms and soles) keratoderma (cornified thickening of the epidermis). The Köbner variant of EBS manifests itself as severe generalized (not clustered) blistering, and Weber–Cockayne EBS – the mildest form – is mostly restricted to blistering on the hands and feet, these being the most severely traumatized areas of the epidermis in the course of normal wear and tear.

As more mutations were identified, an emerging pattern suggested that the position of the mutation in the protein could predict the phenotypic severity (Fig. 2). The sequence changes were also noticeably clustered in specific regions of the keratin molecules (Fig. 2). Dowling–Meara EBS was associated with mutations in the helix boundary motifs (Box 1, Fig. I), particularly at a conserved arginine residue in the helix initiation motif of type I keratins (R125 in K14): this codon includes a highly mutable CpG dinucleotide in the DNA sequence, which partly accounts for the prevalence of mutations. By contrast, Köbner and Weber-Cockayne phenotypes were associated with mutations in the rod domain outside the helix boundary peptides, or in non-helical domains such as the linker regions or the H1 domain in the head (Fig. 2). The H1 domain has been implicated in filament assembly by many biochemical and transfection experiments, but the function of the linker regions is unclear. It has been thought that these regions might enable additional flexibility in the filament. Thus, a mutation that increases the probability of α-helical folding within a normally non-helical linker domain [2] might be detrimental to filament structure. Recent evidence suggests that interactions between helical and non-helical regions might be important for correct filament assembly, for example, a putative interaction between the arginine hotspot in the helix initiation motif and a glutamate residue in linker L2 [3]. Studies of mutations underlying keratin disorders have also confirmed the importance of another feature – a conserved discontinuity in the α-helical coiling of the 2B region, known as the 'stutter'. In one case, a mutation in K5 near the stutter was not detrimental alone, but combined with a mild (Weber-Cockayne) dominant negative mutation in helix 1A of K5, led to a more severe phenotype [4]. Weber–Cockayne and Köbner phenotypes have occasionally been found to be associated with different mutations in the same residue or a close neighbour [5,6]. The different clinical severity caused by these similar mutations is logically explained by the substitution of different amino acids that can distort the helix to differing degrees [7].

Functional insights from bullous congenital ichthyosiform erythroderma

The second keratin disease to be recognized was bullous congenital ichthyosiform erythroderma (BCIE), also referred to as epidermolytic hyperkeratosis (EHK). This disease is caused by mutations in keratins K1 and K10, expressed in keratinocytes as they move up into the suprabasal layers of the epidermis (Fig. 1). K1 and K10 are secondary, differentiation-specific keratins and are laid down on a template of primary keratins – K5 or K14 – synthesized when the cell was in the basal layer. Thus, cells expressing K1 or K10 also have some K5 or K14 in their filaments. The mutations lead to blistering in neonates owing to cytolysis of suprabasal epidermis. The blistering improves with age but is replaced by thickening and scaling of the epidermis (ichthyosis). This disorder is usually associated with mutations in the helix initiation

Table 1. Keratin tissue distribution and associated human disorders[a]

Keratin pair Type II	Type I	Tissue expression	Disorders resulting from mutations
K1	K10	Suprabasal cells of cornified stratified squamous epithelia	Bullous congenital ichthyosiform erythroderma (BCIE) or epidermolytic hyperkeratosis (EHK) Epidermolytic palmoplantar keratoderma (EPPK) Non-epidermolytic palmoplantar keratoderma (NEPPK) Ichthyosis hystrix of Curth-Macklin (IHCM)
K2e[b]		Upper suprabasal epidermis	Ichthyosis bullosa of Siemens (IBS)
K2p		Upper suprabasal cells in oral palate	?
K3	K12	Corneal epithelium	Meesman corneal dystrophy (MECD)
K4	K13	Suprabasal cells of orogenital stratified squamous epithelium	White sponge naevus (WSN)
K5	K14	Basal layer keratinocytes; basal cells in glands and stratified epithelia	Epidermolysis bullosa simplex (EBS): Dowling–Meara EBS; Weber–Cockayne EBS; Köbner EBS
	K15	Basal-layer keratinocytes	?
K6a	K16	Suprabasal cells in orogenital stratified squamous epithelium; palmoplantar epidermis; hair follicle outer root sheath; induced in interfollicular epidermis by trauma	Pachyonychia congenita type 1 (PC-1) Steatocystoma multiplex EPPK
K6b	K17	Suprabasal cells in orogenital stratified squamous epithelium Focal in palmoplantar epidermis; deep hair follicle; extensive in foetal stratified squamous epithelia; induced by trauma in interfollicular epidermis	PC-2 Steatocystoma multiplex
K6hf		Companion layer of the hair follicle	?
K7		Gland ducts; myoepithelia; many simple epithelia; hair follicle and nail bed	?
K8	K18	Simple epithelia, including early embryo; placenta	Cryptogenic liver disease ? Inflammatory bowel disorder (IBD)
	K9	Palmoplantar epidermis	EPPK
	K19	Simple epithelia; hair follicle bulge cells; some basal cells in orogenital epithelia	?
	K20	Gastrointestinal epithelia; Merkel cells; uroepithelium	?
K6irs1, K6irs2	IRSa1–IRSa3	Inner root sheath of the hair follicle	?
Hb1–Hb6	Ha1–Ha9	Hair keratins; hard keratin sites (hair shaft, nails, tongue filiform papillae)	Monilethrix

[a]References in [50] (see also http://www.interfil.org).
[b]Single keratins listed denotes that this is an additional type I or type II keratin to the predominant keratin pair.

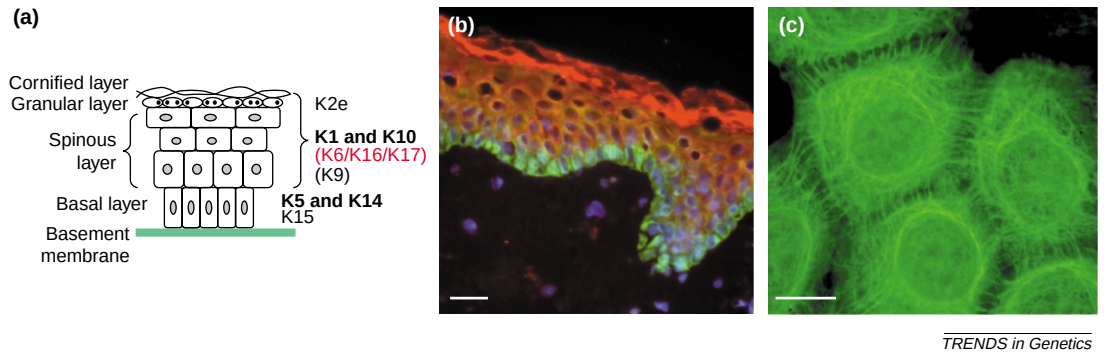

Fig. 1. Keratin expression in the epidermis. **(a)** The stratified epithelium of the epidermis. The basal layer of keratinocytes in contact with the basement membrane are actively dividing and express keratin K5 (type II) and K14 (type I) as the predominant keratin pair. Another type I keratin, K15, is also expressed in most basal cells. When the basal cells become committed to terminal differentiation they stop dividing and move into the suprabasal compartment. They then express K1 (type II) and K10 (type I) as the predominant keratin pair. These cells are also known as the spinous layer owing to the typically stellate appearance of the massive keratin bundles in these cells when visualized by light microscopy. As they differentiate further, the keratinocytes flatten out to form the granular layer, characterized by keratohyalin granules. The upper spinous layers and granular layer express an additional type II keratin, K2e. Finally, the keratinocyte becomes cornified – crosslinking of membrane proteins, such as loricrin, with the keratins to form an envelope – the nucleus breaks down and the cells die forming the cornified layer – a watertight, impermeable barrier. In wounded epidermis, the suprabasal layers switch-on the expression of K6, K16 and K17 in preference to K1 and K10. K6, K16 and K17 are normally confined to the appendages (hair follicle and nail bed) of the skin and to the thicker palmoplantar epidermis (soles and palms). K9 is unique to the palmoplantar epidermis. **(b)** Differentiation-specific expression of keratins as shown by immunofluorescence of the epidermis, with antibodies to K5 staining the basal cells (green), and antibodies to K10 staining the suprabasal cells (red). Scale bar: 20 μm. **(c)** Keratin intermediate filaments of oral keratinocytes (TR146 cells) in culture, stained with a keratin-specific antibody. Scale bar 10 μm. Another version of this micrograph originally appeared in Ref. [72].

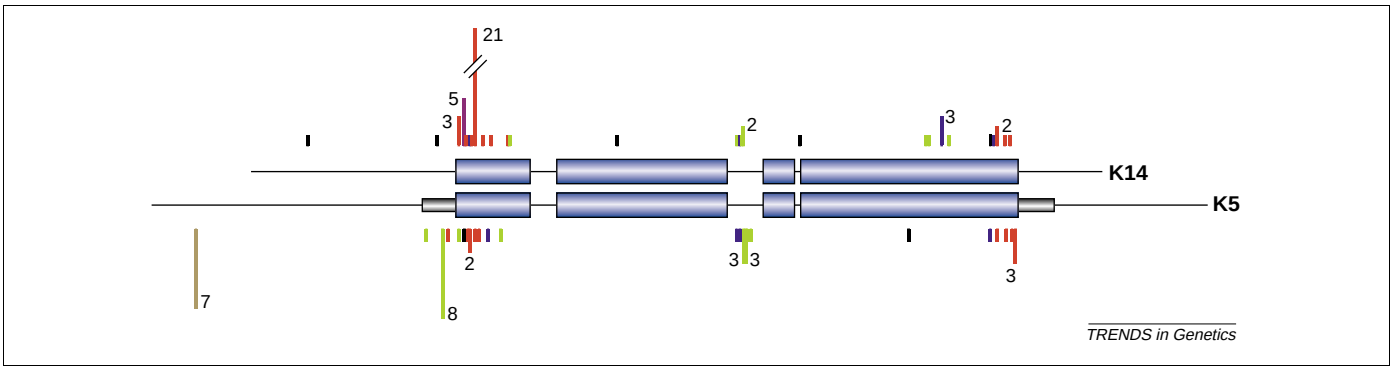

Fig. 2. Keratin mutations causing epidermolysis bullosa simplex (EBS). The position and frequency of 101 published mutations causing EBS are indicated above K14 and below K5 by colour coded bars. The shortest bars represent a single mutation. Coloured bars depict the following: red, EBS Dowling–Meara; blue, EBS Köbner; green, EBS Weber–Cockayne; black, homozygous mutations and knockouts; purple, all three phenotypes resulting from different amino acid substitutions; brown, EBS with mottled pigmentation. Note how the severe Dowling–Meara mutations are concentrated in the end domains of the central helical rod domain. Over 20% of mutations causing EBS trigger an amino acid substitution of R125 in K14. The most common mutations in K5 cause an amino acid substitution of I161 (8% of EBS cases reported) or P25 (7%; associated with mottled pigmentation). Figure based on mutations published to-date (see http://www.interfil.org).

and termination motifs and in the non-helical H1 domain; a milder form of the disease has been associated with mutations in the L12 linker or rod domain mutations outside the helix boundary motifs, similar to the mild EBS mutations [8–10]. Phenotypic variations in disorders associated with mutations in K1 and K10 have been studied by DiGiovanna and Bale [11]. However, in general, phenotypic variations in BCIE are less clearly linked to the nature of the mutations than those of EBS disorders. The same is also true of other suprabasal (secondary) keratins. The reason for this might be the reinforcement of secondary keratins that is provided by residual K5 and K14.

Pachyonychia congenita

The total of identifiable keratin-associated disorders is now high (Table 1). However, a further group of disorders that deserves special mention is the pachyonychia congenital and steatocystoma multiplex cluster, as these involve keratins K6, K16 and K17, which are rapidly induced by stress and wounding. Mutations in K6a, K6b, K16 and K17 cause thickened nails and non-epidermolytic keratoderma [12–15] in addition to either oral lesions with K6a and K16 mutations, or pili torti (twisted hairs) and pilosebaceous cysts with K6b and K17 mutations. As these keratins have overlapping expression patterns, the differences in phenotype between the keratin mutations is taken to reflect their relative abundance or importance in the affected tissues. Despite the well-documented induction of K6 or K16 in disease and wounding [16,17], no defect in wound healing is detectable in patients with pachyonychia congenita mutations, and little or no delay is seen in wound healing in K6a and K6a/b double-knockout mice [18,19]. However, slight differences must not be dismissed too lightly; in the timescale of mammalian evolution, a small genetic advantage in wound healing would be quickly selected for in a physically harsh environment.

Mutations suggesting functional differences in head- and tail-domains

Most pathogenic keratin mutations are found within the rod domains or in the H1 and linker regions, and all lead to cell fragility in tissues where they are expressed as major keratins. These mutations have thus confirmed a structural role for keratin intermediate filaments that is essential for tissue integrity. Conversely, the apparent lack of pathogenic mutations in the head- and tail-domains was initially thought to indicate that either of these domains were of less importance in keratin intermediate filament function and that any sequence variations occurring here would be asymptomatic. However, extended screening over many years along with the increasing use of mouse models is now beginning to reveal informative effects of less-common mutations in these end-domains. There might be individual functions for different keratin genes such that their diversity probably does not simply reflect redundant gene duplication.

The head- and tail-domains, especially the variable V1 and V2 subdomains, are responsible for the greatest sequence variation between different keratins. It is therefore possible that they confer unique (tissue specific?) properties to the individual members of the keratin family. The first mutation discovered in a V1 domain was found in K1 [20]. Unlike other mutations in K1 that cause BCIE with epidermolytic palmoplantar keratoderma, this mutation leads to non-epidermolytic palmoplantar keratoderma, a markedly different disease with no cytolysis (cell breakdown). Biochemical cross-linking experiments indicated that this K1 mutation altered an important lysine residue involved in interactions with loricrin and involucrin, two major proteins of the cornified envelope [21]. This residue is conserved in other type II keratins of the epidermis but not in simple epithelial keratins. Recently, several more mutations in the V1 and V2 domains have been identified. The most dramatic was a case of mutilating palmoplantar keratoderma diagnosed as ichthyosis hystrix of Curth-Macklin [22]. The mutation caused a frame-shift leading to a shortened V2 region and replacement of the many glycine residues with alanine. Although the mutation did not appear to affect filament assembly *in vitro*, cellular defects *in vivo* included retraction of the keratin filaments from the nucleus to form a dense disorganized cytoplasmic shell of filamentous material, and failure of loricrin to distribute correctly at the desmosomes, preventing the proper formation of the cornified envelope. It was thought that the mutant

hydrophobic V2 domain was probably interfering with normal interactions between K1 and loricrin [22].

Another mutation repeatedly found in the V1 domain of K5 is P25L, which has been consistently linked to EBS with mottled pigmentation [23–26]. Affected individuals show EBS-like blistering, pigment changes and, later in life, nail dystrophy with palmoplantar keratoderma. The P25L mutation is in an SxxSxxxPxxxR motif, highly conserved in type II keratins and other intermediate filaments. The blistering and clumping of keratin filaments observed in these patients again indicates a role for the head domain of K5 in filament assembly, which is supported by *in vitro* assembly studies of deletion mutants of K5 or K14 [27]. The consistent feature of mottled pigmentation (discrete pigmented macules of 2–5 mm diameter) associated with this particular mutation remains puzzling; it suggests that the keratin network influences melanin distribution in the epidermis in a way not yet understood.

The head- and tail-domains contain all the phosphorylation sites of keratins that have been identified to-date. Phosphorylation of intermediate filaments favours the depolymerized state in the assembly equilibrium. Phosphorylation of K18 affects filament organization and might play a role in filament reorganization during cell stress and mitosis. In addition, K8 and K18 phosphorylation might play a role in protecting hepatocytes from drug-induced liver injury [28]. To test the function of a major phosphorylation site, serine-52 in K18, mice overexpressing either wild-type human K18 or human K18 with a serine-52-alanine mutation were generated. Despite the mouse wild-type K18 still being present, the overexpression of the human serine-52-alanine mutant caused dramatic hypertoxicity to griseofulvin compared with the wild-type human K18 controls [28].

Another phosphorylation site common to the head domain of type II keratins K5, K6 and K8, lies within the conserved motif LL(T/S)PL [29]. This site is phosphorylated in mitosis and in cell-stress conditions. Either destroying this phosphorylation site in K5 [30] or creating a new one in K1 [31] can lead to cell fragility.

Thus, mutations of the head- and tail-domains suggest tissue-specific functions for these domains in some keratins, involving interactions with tissue-specific components. They also support a role for the head domain of keratins in filament assembly (as suggested by previous *in vitro* assembly assays) that could be modulated by cell signalling events leading to phosphorylation of serine.

Knockouts reveal some functional overlap

Most of the keratin disorders identified in humans are caused by dominant missense mutations. However, five cases of recessive EBS have now been reported in humans (5% of all reported EBS mutations; see http://www.interfil.org), which are all caused by functional knockout of the type I keratin K14, that is, premature termination mutations associated with no detectable expression of K14. Most recessive EBS patients were severely affected by blistering, and the basal keratinocytes had reduced filaments suggesting that the other type I basal keratin, K15, is not capable of fully compensating for the loss of K14. It will be interesting to discover whether K15 is functionally redundant. The recessive case with the shortest expressible K14 sequence to-date was predicted to be truncated after only 30 amino acids [32]; this is the closest to a true human knockout so far, but the patient suffered from only a mild clinical phenotype. As none of the recessive patients were diagnosed as severe Dowling–Meara cases, it seems that complete absence of a keratin is less detrimental than the disrupted filament assembly and aggregate formation caused by some dominant-negative missense mutations [33]. This has been supported by experimental K10 knockout mice, which showed no evidence of blistering, unlike dominant mutations in K10, which cause severe blistering phenotypes [34]. However, not all knockouts are so benign. K5 apparently has no functional substitute as the K5 knockout mouse dies at birth and no K5-null human patients have ever been identified. K5 is the only constitutive type II keratin in basal epidermal keratinocytes, and it appears that in cases where ablation of a keratin results in complete absence of filaments in cells, the consequences are more detrimental [35].

Evidence for compensation between keratins has also been identified in studies of the simple epithelial keratins. These keratins are mostly expressed in internal one-layered epithelia (see Table 1). K18 knockout mice were found to be viable, fertile and have a normal lifespan [36]. This was unexpected because of the early expression of the K8/K18 pair in the embryo. The K8 knockout showed embryonic lethality as expected, but even in this case survival was possible in the FVB/N strain of mice [37,38]. It was hypothesized that K19 and K7, which begin to be expressed shortly after K8/K18, could compensate for the loss of K18 or K8, respectively. To test this, K18 knockout mice were bred to homozygosity with K19 knockout mice so that no type I keratin would be present in the early embryo; early embryonic lethality was indeed observed in these double knockouts [39].

An unexpected observation in K8 and K18 knockout mice was that normal liver development could take place in the absence of keratin filaments; hepatocytes only express K8 and K18, therefore no compensation by other keratins is possible. However, in K18 knockout mice, Mallory bodies [aggregates of K8 plus other proteins such as ubiquitin and M_M120-1 (a Mallory body-specific protein)] develop with age [36]. As liver pathology occurs without Mallory body formation in drug-induced liver injury of K8 knockout mice, Mallory bodies are clearly not the cause of liver damage [40]. Rather, it appears that Mallory bodies have a role in protection against toxic injury, and that their formation is a function-specific to K8.

Studies of keratin knockouts in mice, therefore, have not only revealed some functional overlap, but also some individual functions for specific keratins. This is also supported by additional experiments in mice where one keratin is replaced with another or is ectopically expressed (see Table 2).

Different mutations associated with disorders of simple epithelia

Two groups of keratins were notable by their absence from the growing list of mutations for the keratin disorders.

Table 2. Keratin deficiencies generated in mice and their consequences

Keratin	Type of mutation	Phenotype	Refs
hK1	Dominant-negative (truncated protein)	Cytolysis of keratinocytes in neonates; hyperkeratosis in older mice	[51]
K1/K10	Ectopic expression of K1, K10 or K1 and K10 in pancreas	Diabetes caused by K1, or K1 and K10 together, but not K10	[52]
K4	Knockout	White sponge naevus	[53]
K5	Knockout	Cytolysis of basal layer of epidermis	[35]
	Replacement with hamster desmin	Ineffective at preventing cytolysis	[54]
K6a	Dominant-negative	Blistering in spinous layer; alopecia	[55]
	Knockout	Delayed wound healing	[18]
K6a/K6b	Double knockout	Fragility of tongue epithelium	[19]
K8	Knockout	C57BL/6 mice: embryonic lethal	[37]
		FVB/N mice: colorectal hyperplasia	[38]
K8/K19	Double knockout	Embryonic lethal owing to defective placenta	[56]
K10	Dominant-negative (truncated chimaera)	Epidermal blistering in suprabasal layer	[57]
	Dominant-negative (truncated protein)	Homozygotes: skin fragility in suprabasal layer Heterozygotes: epidermal thickening and/or hyperkeratosis	[58]
	Knockout	Very mild phenotype with no cytolysis	[34]
	Inducible dominant-negative	Blistering and hyperkeratosis at site of induction	[59]
	Ectopic expression in basal epidermis	Hypoplastic epidermis	[60]
	Ectopic expression under bovine K6β promoter	Severe abnormalities of the tongue and palate	[61]
K12	Knockout	Corneal fragility	[62]
K14	Dominant-negative	Epidermal blistering	[63]
K14	Knockout	Cytolysis of the basal layer of the epidermis; lesions in internal organs increase with age	[64]
	Inducible dominant-negative	Blistering at site of induction	[65]
hK16	Overexpression in skin	Acanthosis and aberrant keratinization	[66]
	Ectopic expression in basal layer	Epidermal thickening and reduced hair follicles	[67]
	Expression in $K14^{-/-}$ mice	Rescued blistering phenotype but alopecia and ulcers develop with age	[68]
hK16/hK14	Ectopic expression in basal layer	Normal	[67]
Chimaera	Expression in $K14^{-/-}$ mice	Rescued blistering phenotype; mild alopecia develops	[68]
K17	Knockout	Temporary alopecia	[69]
K18	Knockout	Liver pathology in older mice	[36]
	Expression in $K14^{-/-}$ mice	Could not fully rescue blistering phenotype	[70]
hK18	Dominant-negative mutation (R89C)	Liver inflammation and necrosis	[41]
	Overexpression of mutation (S52A)	Increased hepatotoxity of griseofulvin	[28]
	Overexpression of mutation (S33A)	Altered filament organization in pancreatic acinar cells; redistribution of 14–3-3 proteins; limited hepatocyte mitotic arrest	[71]
K18/K19	Double knockout	Die E9.5; cytolysis of trophoblast giant cells	[39]
K19	Knockout	No phenotype	[56]

These were the simple epithelial keratins and the hair keratins. For the simple epithelial keratins, this was thought to be because they were expressed in vital organs and at early stages of development, hence defects in these keratins result in embryonic lethality. Another possible explanation is that simple epithelial keratins are not as important as epidermal keratins because internal tissues are not subject to such stresses and therefore mutations in these keratins might not be pathogenic. However, evidence from mouse knockouts [36,38,40,41] suggested that a search for keratin mutations associated with liver or gut disease might be productive. Mutations in K8 and K18 have now been found in the liver of patients with cryptogenic cirrhosis [42,43]. These mutations led to amino acid changes in the H1 or L1 domains, but not in the α-helical rod domain. Intriguingly, an identical mutation to one of these – K8 G62C – has been found in patients with inflammatory bowel disorder (D.W. Owens *et al.*, unpublished observations). Both cryptogenic liver disease and inflammatory bowel disorder are late-onset diseases of polygenic etiology, and are therefore probably subject to environmental influences as well.

Why the types of mutations associated with K8 and K18 should be different from those of other epithelial keratins is intriguing. Possibly, K8/K18 mutations in the end domains of the α-helix, which, by analogy with the epidermal fragility disorders, would be highly disruptive mutations, would lead to embryonic lethality in humans. However, a mouse with an experimental mutation in the helix initiation motif of K18 was viable to adulthood, with a phenotype restricted to liver pathology [41]. One of the problems with investigating disorders in internal epithelia is the inaccessibility of the tissue for observation, making it more difficult to prove that keratin mutations are the primary cause of the pathology. In support of the involvement of K8/18 mutations in gastrointestinal disease, it has been shown that epithelial cells deficient in K8 and K18 are 100-times more sensitive to cell death induced by tumour necrosis factor-α (TNF-α) [44]. TNF-α is an inflammatory cytokine that is elevated in inflammatory bowel disorders and is also involved in liver apoptosis. K8- and K18-knockout mice are highly sensitive to TNF-α-induced apoptotic liver damage, and both K8 and K18 can bind to the TNF-α receptor. The interplay between the cytoskeleton and the TNF family members is not entirely clear; another group has shown that K8-null hepatocytes are more sensitive to Fas-mediated apoptosis, rather than TNF-α-mediated apoptosis [45].

Concluding remarks

The study of keratin disorders has enhanced our understanding of intermediate filament function in epithelia but, undoubtedly, more is still to be revealed. The one function common to all keratins is the structural function. How important this structural function is varies according to the tissue function and to the number of keratin genes expressed in that tissue. It appears that the structural role is less central to the function of keratins in simple epithelia than in the epidermis, and so the structural diseases caused by mutations of epidermal keratins might not occur in simple epithelia. In addition to carrying a mutation in a keratin, the simple epithelial tissue might have to be abnormally stressed (e.g. chemical assault, infection or inflammation) for a phenotype to manifest itself. Such mutations could thus contribute to complex disorders involving mutations in more than one gene. In the skin, the phenotype resulting from a pathogenic keratin mutation is clearly a result of the enormous physical stress the tissue is under, as seen in cultured keratinocytes from EBS patients where there is no indication of keratin abnormality until the cells are stressed [46]. Similarly, K14 with a mutation that causes Dowling–Meara EBS can form filaments *in vitro* [47] but rheological tests show that a mutation in the same amino acid can cause loss of elasticity of the filaments [48].

One reason why so many keratins exist might be to provide intracellular scaffolding with different physical properties in different cell types, although K8 and K18 filaments perform very similarly to K5 and K14 filaments when subjected to rheological-strain assays [49]. Another possibility is that all keratins form essentially similar filament scaffolds, but differ in their response to cellular signalling. Supporting this idea, the phosphorylation of differentiation-associated K1 differs from both K5 and K8 [29]. Maybe differences in the ability of keratins to respond to cell signalling are what functionally separate the keratin family members. Clearly, in proliferating cells, which must constantly reorganize their cytoskeleton, keratins must be able to respond to signalling very rapidly, whereas in differentiating cells the emphasis is on stabilizing and reinforcing the network. Similarly, cells in wounded epidermis must be able to remodel their cytoskeleton in response to signals from the environment, which might be the reason for rapidly expressing a new keratin pair (K6/K16). This might not have anything to do with cell proliferation or with increasing the speed of migration. Wound healing is a very complex process and the timing of its different components must be integrated yet flexible.

Just like the other components of the cytoskeleton, the intermediate filament network needs a way of adapting to change and must therefore be able to respond to cellular signals. Thus, maintaining the fidelity of the tissue-specific expression of keratin intermediate filaments could be essential, not only because of the different physical requirements of the tissue, but also because of the complexity of the local regulatory mechanisms.

Acknowledgements

We would like to thank Cancer Research UK, the Wellcome Trust and DebRA for supporting the work of our laboratory and some of the studies cited in this review. We sincerely apologize to researchers and colleagues whose work we were not able to cite owing to space restrictions.

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