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We hope that making available the relevant information on Pachyonychia Congenita will be a means of furthering research to find effective therapies and a cure for PC.
Keratin disorders: from gene to therapy

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The term ‘keratin’ is generally accepted to refer to the epithelial keratins of soft and hard epithelial tissues such as: skin, cornea, hair and nail. Since their initial characterization, the total number of mammalian keratins has increased to 54, including 28 type I and 26 type II keratins. Inherited defects that weaken the keratin load-bearing cytoskeleton produce phenotypes characterized by fragility of specific subsets of epithelial tissues. The vast majority of mutations are either missense or small in-frame in-del mutations and disease severity often relates to the position of the mutation in relation to the rod domain. The most complex epithelial structure in humans, the hair follicle, contains trichocyte (‘hard’) keratin filaments and approximately half of the 54 functional human keratin genes are trichocyte keratins. So far, only four of these have been linked to human genetic disorders: monilethrix, hair–nail ectodermal dysplasia, pseudofolliculitis barbae and woolly hair, while the majority of the hair keratins remain unlinked to human phenotypes. Keratin disorders are a classical group of dominant-negative genetic disorders, representing a large healthcare burden, especially within dermatology. Recent advances in RNA interference therapeutics, particularly in the form of small-interfering RNAs, represent a potential therapy route for keratin disorders through selectively silencing the mutant allele. To date, mutant-specific siRNAs for epidermolysis bullosa simplex, pachyonychia congenita and Messmann epithelial corneal dystrophy-causing missense mutations have been developed and proven to have unprecedented specificity and potency. This could herald the dawn of a new era in translational medical research applied to genetics.

KERATINS: PRINCIPAL STRUCTURAL PROTEINS OF EPITHElia

Complex organisms possess many different types of epithelial tissues, which mainly function as barrier tissues found at the interfaces between the organism and its environment (1,2). For example, the epidermis forms the outmost protective layer of the skin; the anterior corneal epithelium is the outermost protective covering of the eye; and the gut is lined with so-called simple epithelial monolayers. The cytoplasm of all human cells contains a dense network of 10 nm intermediate filaments. In epithelial cells, this cytoskeleton is made up of various combinations of keratins. Humans possess 54 functional keratin genes and hundreds of pseudogenes (3). The active genes are organized into two dense gene clusters on chromosome 12q (all the type II keratins plus one type I keratin, K18) and 17q (the remaining type I keratins). The genes are compact and closely spaced ≈10 kb apart but despite this, they show beautifully specific patterns of gene expression within adjacent epithelial cell compartments. For this reason, monospecific antibodies against keratin proteins are routinely used to determine tissue-of-origin and/or differentiation state of tumours, ≈80% of which are epithelial in origin (4). Furthermore, the highly tissue-specific expression patterns of keratins, coupled with their compact genes, have led to the widespread use of keratin promoters to specifically target transgenes to particular epithelial tissues (5).

Keratin proteins form self-assembling heteropolymers (6,7). They are obligate heteropolymers where at least one type I (acidic) and type II (neutral-basic) protein must be present to allow assembly; however, in most epithelial cells more complex mixtures of keratins are expressed. Both keratin types possess a central alpha-helical rod domain of ≈300 amino acids within which are three flexible linker domains. The rod domain is flanked by non-helical head and tail domains, which vary in size and sequence between the individual keratin proteins. It is these variable domains that are thought to impart tissue-specific functions, such as interactions...
with other cytoplasmic proteins or allowing attachment to membranes or to organelles. Because keratins form polymers consisting of vast numbers of component subunits, they are very prone to dominant-negative interference when one allele carries a missense or small in-frame insertion/deletion mutation affecting the rod domain (8). In particular, the ends of the rod domain are involved in end-to-end overlap interactions in the elongation phase of filament assembly (9). Short, highly conserved sequence motifs located in these regions (the helix boundary motifs) are therefore exquisitely sensitive to dominant-negative mutations and associated with the most severe disease phenotypes (8). The primary function of the keratin intermediate filament cytoskeleton is to provide epithelial cells with structural resilience against mechanical trauma. Since epithelial cells tend to form barrier tissues, they have to resist some of the most severe physical stress levels experienced by any human tissue, for example, the trauma that the sole epidermis experiences during walking or running.

Thus, inherited defects that weaken, or in extreme cases, result in complete loss of this load-bearing cytoskeleton, produce phenotypes characterized by fragility of specific subsets of epithelial tissues. Typically, this is seen as macroscopic blistering of the tissue or in some cases, microscopic blisters occur (cytolysis). Often, the tissue tries to compensate for the fragility by overgrowing in response to the mechanical damage, leading to hyperkeratosis (gross thickening) of the affected tissue.

In addition to the type I and type II classification of keratin proteins, which relates to gene structure, chromosomal location, isoelectric point and importantly, ability to form heteropolymers with the opposite type, keratins fall into a further two categories (3). The term ‘keratin’ is nowadays generally accepted to refer to the epithelial keratins of soft epithelial tissues, such as the epidermis. In addition, hard epithelial tissues such as hair and nail also express a group of specialized type I and type II keratin proteins with exceptionally high-cysteine content—known as trichocyte or hair keratins. The rod domains of these proteins are highly similar to the epithelial keratins, but the head and tail domains are very cysteine rich, allowing an enormous degree of cross-linking between the keratins and keratin-associated proteins to make the exceptionally tough heterogeneous polymers of which hair and nail is composed (10). A further site of expression of trichocyte keratins is the tip of the filiform papillae on the tongue. Figure 1 demonstrates normal and mutant keratin expression.

### BASAL EPITHELIAL KERATIN DISORDERS: HEREDITARY SKIN BLISTERING

The archetypal keratin disorder is the hereditary skin blistering disease epidermolysis bullosa simplex (EBS), caused by dominant mutations in either of the genes encoding keratins K5 or K14 (11–13). This pair of type I and type II keratins (K14 and K5, respectively) are specifically expressed in a single layer of epithelial cells—the basal cell layer. This innermost proliferative cell compartment of the multilayered stratified epithelia is in contact with the basement membrane of the underlying stroma. Mutations in either K5 or K14 can produce clinically indistinguishable skin blistering due to fragility of the basal cell compartment. The phenomenon of phenocopy is a feature of many keratin disorders due to the expression of many keratin genes in type I/type II pairs within a given tissue (8). In EBS, like most keratin diseases, the vast majority of mutations are either missense or small in-frame in-del mutations. The reported human keratin mutations are recorded in Human Intermediate Filament Database, www.interfil.org (14). The position of the mutation within the rod domain correlates with disease severity, with mutations affecting the helix boundary motifs being associated with severe generalized skin blistering, whereas those located elsewhere in the molecule produce milder, site-specific skin blistering, limited to sites that experience high levels of mechanical stress, such as palms and soles. There are also less common recessive cases of EBS where there is homozgyosity or compound heterozygosity for premature termination codon (PTC) mutations in the KRT14 gene (15). In these cases completely lacking a K5/K14 cytoskeleton, the severity of skin blistering is comparable to the most severe dominant-negative mutations, illustrating that the latter completely compromise cytoskeletal function.

In recent years, dominant skin disorders which lack skin blistering but are associated with abnormal pigmentation and/or mild developmental defects of the skin, such as lack of fingerprints, have been found to be linked to particular mutations in K5 (Dowling-Degos disease; DDD) (16) and K14 (Nageli-Franchesetti-Jadassohn disease; NFJS) (17). The pathomechanism is not entirely clear in these dominant conditions, both of which result from 3′ nonsense or frameshift mutations, closely following the ATG codon of K5 or K14. When this class of mutation occurs more 3′ in the coding sequence of K14, for example, the allele is recessive and heterozygous individuals show no signs of NFJS. Because these PTC mutations are so close to ATG, it is possible that translation occurs from the next ATG and results in an N-terminally deleted keratin protein, as has been described for other genes (18). Figure 2 shows examples of human keratin disorders.

### DIFFERENTIATION-SPECIFIC EPITHELIAL KERATIN DISEASES: FRAGILITY AND/OR OVERGROWTH OF COMPLEX EPITHELIA

In the stratified epithelia such as the epidermis, cell proliferation is strictly limited to the basal cell compartment (1). When cells leave this layer, differentiate and migrate upwards, they exit cell cycle and switch on alternative keratins. In the epidermis, for example, the suprabasal cells cease transcription of K5 and K14 but instead express K1 and K10. In EBS, blistering wipes out the basal cells in the area of the blister and these are replaced by inward migration of basal cells from the blister margins. In the case of bullous ichthyosis (BI), caused by mutations in either K1 or K10, the fragile cells are above the proliferative compartment (19). In these situations rupturing suprabasal cells bathe the unaffected basal cells beneath with cytokines, leading to over-proliferation of the epithelium—known as hyperkeratosis. In the case of BI, this leads to the formation of a highly thickened epidermis made up of fragile cells. This thick, spongy
epidermis is highly prone to bacterial and fungal colonization and is highly disfiguring and debilitating for the patient.

Mutations in other suprabasal keratins lead to hyperkeratosis of specific epithelia. For example, mutations in K9, which is expressed only in palm and sole epidermis, lead to epidermolytic palmoplantar keratoderma (EPPK), where only the palm and sole epidermis is fragile and undergoes hyperkeratosis (20). Mutations in K2, a keratin expressed in the outermost layers of the epidermis, lead to ichthyosis bullosa of Siemens, a milder, site-restricted form of BI (21,22). Mutations in any of the site-specific keratins K6a, K6b, K6c, K16 or K17 lead to phenotypic variants of pachyonychia congenita (PC) (23–26). In PC, there are focal areas of extremely painful hyperkeratosis on the soles plus hyperkeratosis of a variety of other tissues where these keratins are expressed, including nail, oral mucosa and other sites.

The majority of the known keratin disorders, listed in Table 1, involve suprabasal keratins of differentiated epithelial tissues, including diseases of oral and anogenital mucosal tissues, the corneal epithelium of the eye and many of the soft epithelial layers that surround the hair follicle, sebaceous glands and the nail bed.

**DISORDERS OF THE CYSTEINE-RICH TRICHOCYTE KERATINS: FRAGILITY OF HAIR AND/OR NAIL**

In terms of tissue architecture and compartmentalized gene expression, the most complex epithelial structure in humans is the hair follicle (10). This mini-organ system consists of concentric layers of soft epithelial cells, each of which express different combinations of keratins (Fig. 3). The outermost layer, the outer root sheath, is continuous with the basal layer of the epidermis. Each successive deeper layer expresses a range of specific epithelial keratins and deeper still, varying combinations of trichocyte keratins that ultimately differentiate into the inert, exceedingly tough, chemically cross-linked heteropolymer of the hair shaft *per se*. Hair and nail are primarily made up of terminally differentiated cell remnants packed with trichocyte keratin filaments, which are embedded in a matrix of high-cysteine keratin-associated proteins. This material can be thought of as analogous to polyacrylamide—the keratins being the acrylamide- and the keratin-associated proteins acting as the bisacrylamide cross-linking agent. By varying the type and amount of these components, nature has produced a range of super-strong biopolymers for different applications, such as hair and nail. In non-human species, additional applications of this biotechnology include claw, horn and feathers.

About half of the 54 functional keratin genes that humans possess are trichocyte keratins (3). So far, only four of these have been linked to human genetic disorders of hair and/or nail. The first of these is monilethrix—an autosomal dominant hair fragility/aloepecia syndrome (27–29). A key characteristic, which gives the condition its name, is beaded hair resembling beads on a string. This is somewhat misleading as these hairs can be difficult to identify in many patients and hair fragility with or without varying degrees of alopecia is a more consistent finding. Mutations in either one of the type II trichocyte keratins KRT81, KRT83 or LRT86 lead to monilethrix. The type of mutations and their location within the keratin rod domain is highly analogous to the epithelial keratin disorders.

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**Figure 1.** (A) Immunofluorescence staining of human epidermis showing the expression of keratin K5 in the basal layer—the deepest cell compartment (red). The skin keratin-associated protein profilaggrin, which is involved in the terminal differentiation of the epidermis, is expressed in the outmost living cell layers (green). Nuclei are counterstained (blue). (B) Expression of wild-type keratin K12 in a transformed epithelial cell line (red). Nuclei counterstained (blue). Bundles of keratin filaments are seen throughout the cytoplasm. (C) Expression of a mutant K12, carrying the missense mutation p.Leu132Pro (red), showing complete collapse of the cytoskeleton into dense aggregates due to the strong dominant-negative effect of this typical mutant keratin.
More recently, mutations in K85 have been linked to hair–nail ectodermal dysplasia (HNED), a rare condition where there is complete alopecia and highly abnormal, primitive nails (30). This disorder suggests that K85 is more critically important for the structure of hair and nail than the proteins involved in monilethrix.

Epithelial keratins expressed in the outer, soft layers of the hair follicle have also been linked to human hair disorders. K75 is expressed in a single layer of cells between the outer and inner root sheath of the hair follicle (31). An ancestral missense mutation in K75 has been shown to be very common in the human population, particularly in African ancestral groups (32). This mutation is a strong genetic predisposing factor for pseudofolliculitis barbae (PB). PB is characterized by epidermal cysts, which are due to ingrown hairs, particularly on areas that are regularly shaved. Curly hair is also a risk factor. The K75 mutation confers an odds ratio of about 6. Combining this with curly hair, the odds ratio rises to about 50. The proposed pathomechanism is that fragility of the cell layer expressing K75, in combination with curly hair, means that a shaved hair is more likely to bend within its follicle and enter the weak root sheath, allowing it to in-grow (33). The epidermal cysts in PB indeed contain quite lengthy ingrown hairs. This is a classic example of a multi-factorial trait where genetic factors determining hair structure interact with an environmental stimulus (shaving) to produce the disorder.

Very recently, mutations in an epithelial keratin of the hair follicle inner root sheath have been linked to autosomal dominant woolly hair, again showing that a structural abnormality of the epithelial layers surrounding the hair shaft can lead to abnormal hair structure (34).

The majority of the hair keratins remain unlinked to human phenotypes. It may be that these are involved in very rare conditions such as HNED or it may be that sequence variants in some of these proteins may contribute to what we regard as the ‘normal’ range of hair strength, texture, curliness etc. observed in the general population.

RNA INTERFERENCE THERAPY STRATEGIES FOR DOMINANT-NEGATIVE KERATIN DISEASES

The study of rare families with recessive EBS has been useful in directing therapy development for keratin diseases. Specifically, heterozygous carriers of a PTC mutation in the KRT14 gene have perfectly normal skin and so there does not appear to be a haploinsufficiency issue in EBS at least (15). Thus, if a means could be found to silence the dominant-negative mutant allele without affecting the wild-type allele, one would predict this to be therapeutic. In recent years, RNA interference, particularly in the form of short-interfering RNA (siRNA), has emerged as a possible method to induce allele-specific gene silencing (35).
siRNA molecules are double-stranded RNA molecules consisting of 19 nucleotides with 2-nucleotide overhangs at the 3’ ends. Most keratin mutations are single-point mutations leading to missense changes and so there are 19 possible positions where the mutant base can be placed within a potential siRNA. High-throughput methods using reporter genes such as yellow fluorescent protein (36) or luciferase (37) allow for each of the possible 19 siRNAs to be tested for knockdown effects against the wild-type or mutant allele, over a range of siRNA concentrations. An example of such a siRNA sequence walk is shown in Figure 4A. Each of the individual graphs gives a measure of potency over the siRNA concentration range, as well as specificity for the mutant versus the wild-type allele. Promising inhibitors identified need to be tested in secondary assays, such as western blot to confirm specificity for the mutant allele. Surprisingly perhaps, it has proven possible to make highly potent, highly specific siRNA to essentially knock out the mutant allele at the protein level, with negligible effect on the normal allele as shown in Figure 4B.

Although a new siRNA has to be designed for each individual mutation, it is possible to target recurrent CpG-mediated...

### Table 1. Human keratin disorders to date: genes and associated phenotypes

<table>
<thead>
<tr>
<th>Protein</th>
<th>Disorder(s)</th>
<th>Phenotype(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epidermal keratins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K1</td>
<td>Bullous congenital ichthyosiform erythroderma (BCIE)</td>
<td>Blistering and erythroderma in infancy; widespread epidermolytic hyperkeratosis, including palms and soles</td>
</tr>
<tr>
<td></td>
<td>Epidermolytic palmoplantar keratoderma (EPPK)</td>
<td>Epidermolytic hyperkeratosis largely restricted to palms and soles</td>
</tr>
<tr>
<td></td>
<td>Striate palmoplantar keratoderma (SPPK)</td>
<td>Striate keratoderma on palms, focal or diffuse keratoderma on soles</td>
</tr>
<tr>
<td></td>
<td>Ichthyosis hystrix, Curth-Macklin type (IHC)</td>
<td>Severe, widespread ‘spiky’ hyperkeratosis; very severe, mutilating palmoplantar keratoderma</td>
</tr>
<tr>
<td>K2</td>
<td>Ichthyosis bullosa of Siemens (IBS)</td>
<td>Superficial blistering and mild epidermolytic hyperkeratosis largely limited to flexural skin; palms and soles spared. Some cases may resemble K10 phenotype</td>
</tr>
<tr>
<td><strong>K3</strong></td>
<td>MECD</td>
<td>Photophobia, ocular foreign body sensation, myriad corneal microcysts and grey lines visible by slit lamp within corneal epithelium</td>
</tr>
<tr>
<td><strong>K4</strong></td>
<td>White sponge nevus (WSN)</td>
<td>Benign oral leukokeratosis, primarily affecting the buccal mucosa. Occasional involvement of the anogenital mucosa</td>
</tr>
<tr>
<td><strong>K5</strong></td>
<td>EBS</td>
<td>Inherited skin blistering due to fragility of basal keratinocytes. Subtypes of varying severity</td>
</tr>
<tr>
<td></td>
<td>DDD</td>
<td>Pigmented lesions and benign hair follicle cysts</td>
</tr>
<tr>
<td><strong>K6a</strong></td>
<td>PC, K6a type (PC-6a)</td>
<td>Painful and debilitating focal keratoderma, nail dystrophy, hyperkeratosis of mucosal tissues, various forms of epidermal cysts</td>
</tr>
<tr>
<td><strong>K6b</strong></td>
<td>PC, K6b type (PC-6a)</td>
<td>Painful and debilitating focal keratoderma, nail dystrophy, hyperkeratosis of mucosal tissues, various forms of epidermal cysts</td>
</tr>
<tr>
<td><strong>K6c</strong></td>
<td>Focal palmoplantar keratoderma (FPPK)</td>
<td>Painful and debilitating focal keratoderma with minimal or absent nail dystrophy and minimal or absent mucosal involvement</td>
</tr>
<tr>
<td><strong>K8</strong></td>
<td>Cryptogenic cirrhosis (CC)</td>
<td>Rare variants suggested as a risk factor in late-onset cirrhosis</td>
</tr>
<tr>
<td></td>
<td>Inflammatory bowel disease (IBD)</td>
<td>Rare variants suggested as a risk factor in inflammatory bowel disease</td>
</tr>
<tr>
<td><strong>K9</strong></td>
<td>EPPK</td>
<td>Epidermolytic hyperkeratosis normally completely restricted to palms and soles, with a circumscribed red margin</td>
</tr>
<tr>
<td><strong>K10</strong></td>
<td>Bullous congenital ichthyosiform erythroderma (BCIE)</td>
<td>Blistering and erythroderma in infancy; widespread epidermolytic hyperkeratosis, with minimal or absent palm and sole involvement.</td>
</tr>
<tr>
<td><strong>K11</strong></td>
<td>MECD</td>
<td>Photophobia, ocular foreign body sensation, myriad corneal microcysts and grey lines visible by slit lamp within corneal epithelium</td>
</tr>
<tr>
<td><strong>K12</strong></td>
<td>White sponge nevus (WSN)</td>
<td>Benign oral leukokeratosis, primarily affecting the buccal mucosa. Occasional involvement of the anogenital mucosa</td>
</tr>
<tr>
<td><strong>K13</strong></td>
<td>EBS</td>
<td>Inherited skin blistering due to fragility of basal keratinocytes. Subtypes of varying severity</td>
</tr>
<tr>
<td></td>
<td>NFJS</td>
<td>Autosomal dominant absence of fingerprints with abnormal skin pigmentation</td>
</tr>
<tr>
<td><strong>K14</strong></td>
<td>PC, K16 type (PC-16)</td>
<td>Painful and debilitating focal keratoderma, nail dystrophy, hyperkeratosis of mucosal tissues, various forms of epidermal cysts</td>
</tr>
<tr>
<td><strong>Non-epidermolytic palmoplantar keratoderma (NEPPK)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>K15</strong></td>
<td>PC, K17 type (PC-17)</td>
<td>Painful and debilitating focal keratoderma, nail dystrophy, hyperkeratosis of mucosal tissues, various forms of epidermal cysts, particularly pilosebaceous cysts</td>
</tr>
<tr>
<td></td>
<td>Steatocystoma multiplex</td>
<td>Widespread pilosebaceous cysts with minimal or absent nail changes of other features of PC</td>
</tr>
<tr>
<td><strong>K18</strong></td>
<td>Cryptogenic cirrhosis (CC)</td>
<td>Rare variants shown to be an uncommon risk factor in inflammatory bowel disease</td>
</tr>
<tr>
<td></td>
<td>Inflammatory bowel disease (IBD)</td>
<td>Rare variants shown to be an uncommon risk factor in inflammatory bowel disease</td>
</tr>
<tr>
<td><strong>K19</strong></td>
<td>Autosomal dominant woolly hair (ADWH)</td>
<td>Severely defective scalp hair</td>
</tr>
<tr>
<td><strong>K20</strong></td>
<td>Pseudofolliculitis barbae (PB)</td>
<td>Strong genetic risk factor for ingrown hair cysts in response to shaving, particularly in certain ancestral groups</td>
</tr>
<tr>
<td><strong>Hair/nail keratins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>K21</strong></td>
<td>Monilethrix</td>
<td>Fragile hair; varying degrees of alopecia; beaded hair appearance</td>
</tr>
<tr>
<td><strong>K22</strong></td>
<td>Monilethrix</td>
<td>Fragile hair; varying degrees of alopecia; beaded hair appearance</td>
</tr>
<tr>
<td><strong>K23</strong></td>
<td>Monilethrix</td>
<td>Widespread and severe alopecia and nail fragility</td>
</tr>
<tr>
<td><strong>K24</strong></td>
<td>Monilethrix</td>
<td>Fragile hair; varying degrees of alopecia; beaded hair appearance</td>
</tr>
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</table>
‘hotspot’ mutations, of which there are many in the keratin disorders. Allele-specific siRNAs have been developed against key mutant alleles of the KRT6a gene in PC (36) and KRT5 gene in EBS (37). In the case of PC, proof of concept was demonstrated in a mouse model and following good manufacturing practice manufacture and the Food and Drug Administration (FDA) approval, a small Phase 1b clinical trial was recently carried out (38). In this double-blinded split body trial, matched hyperkeratotic lesions on the foot of a PC patient were injected with siRNA or vehicle alone. After some weeks, one lesion regressed and a small patch of normal skin appeared at the injection site, which, in stark contrast to the other foot, was no longer painful. Upon unmasking, it was the siRNA-treated foot that showed the improvement. This first-in-human study gives in vivo proof of concept for allele-specific siRNA therapy. The challenge now in the field is to find an effective and non-invasive means of delivering siRNA into the skin or other tissues. Avenues being explored currently are chemical modification of the siRNA, topical formulations and physical methods such as micro-needle arrays.

A particularly tractable keratin disorder for application of siRNA therapy is the ocular surface disease Meesmann epithelial corneal dystrophy (MECD). MECD is caused by a dominant-negative mutation in either K3 or K12, which are expressed only in the keratinocyte cells of the anterior corneal epithelium (39). The K3/K12 intermediate filament cytoskeleton imparts mechanical strength to these keratinocytes and dysfunction of this system leads to mechanical fragility of the anterior corneal epithelium. In addition, slit lamp observation of a MECD cornea often shows multitudinous microcysts within the anterior epithelium as shown in Figure 2D.

The cornea provides a highly attractive target tissue for proof of principle of siRNA therapy in terms of eye drop formulations for siRNA delivery and as a model for in vivo optimization of delivery strategies. The corneal epithelium is a moist, thin, four cell layer stratified epithelium and unlike epidermis, it is non-cornified, i.e. it has no equivalent of the stratum corneum. In addition, the cornea is easily accessible, disease status is easily monitored and the surface to be treated is small. The keratin aggregates are readily seen in the cornea by slit lamp or in vivo confocal examination and the keratin proteins K3 and K12 have a high turnover (half-life \(\approx 6\) h; unpublished data) and therefore therapeutic effects can be seen quickly. Validation of treatments is facilitated through the ability to treat one eye while using the opposite eye as an in vivo control. To date, allele-specific siRNAs have been successfully developed against the mutant allele of the KRT12 gene for a severe form of MECD (Liao H, MacEwen CJ, Weed KH, Porter L, Corden LD, Gibson AB, Moore JE, Moore CBT and McLean WHI, manuscript in preparation).
SMALL MOLECULE TREATMENT OF KERATIN DISORDERS

Recently, small molecule approaches have emerged that could be developed to treat keratinizing disorders. Currently, retinoids are the only class of drugs used clinically for treating the hyperkeratosis that arises from keratin mutations. The promoters of many keratin genes (and genes encoding most other epithelial structural molecules) contain retinoic acid response elements, such as the \textit{KRT6a} gene (40) and therefore, retinoids can down-regulate expression of these genes. Unfortunately, retinoids are too broad spectrum in their action. Although they indeed thin the hyperkeratosis, they do this by essentially shutting down the epidermal differentiation programme completely and the skin now blisters rather than overgrowing. Most patients cannot tolerate retinoid therapy in the longer term and so there is a great need for newer, more specific drugs.

The expression of some keratins, such as K6, K16 and K17, is induced in certain situations, for example, in response to wound healing, oxidative stress or UV light. It has been shown that these stress-response keratins have antioxidant response elements in their promoters. One possible therapeutic angle is to switch on these additional keratins in cells affected by a mutation in a different keratin, e.g. to activate K6, K16 and K17 in EBS, where there is a defect in K5/K14. Proof of concept for this has recently come from the K14 knockout mouse (41), which in some ways mimics recessive EBS (15). When these K14-deficient animals are treated with sulforaphane, a natural product found in broccoli, which induces antioxidant-responsive genes, the phenotype is ameliorated (42). Development of this class of molecule for human use is now on-going.

In PC, the most common gene mutated is K6a and so a therapeutic approach would be to look for small molecules that, like retinoids, can down-regulate K6a expression but perhaps in a more specific manner than retinoids. A small molecule library screen was recently performed and surprisingly, it emerged that the cholesterol-lowering statins can down-regulate the expression of K6a and a subset of keratins (40). This was shown to involve the isoprenylation pathway, which lies downstream of the enzyme inhibited by statins, HMG-coA reductase, within the cholesterol biosynthesis pathway. At the protein level, the K6a inhibitory effect of statins does not appear to be as strong as retinoids (40); however, it is possible that a combination of low-dose retinoid plus statins might be efficacious for some keratinizing disorders. Clinical trials of this type are on-going, co-ordinated by the PC patient advocacy organisation PC Project (www.pachyonychia.org).

CONCLUSIONS

Keratin disorders are a classical group of dominant-negative genetic disorders. Although individually rare, collectively...
they represent a large healthcare burden, particularly within dermatology. Like all other branches of genetics, therapy development has been slow to follow on from the initial genetic discoveries; however, recent advances in siRNA therapeutics and the fact that academic groups increasingly have access to small molecule libraries herald the dawn of a new era in translational medical research applied to genetics.

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