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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.
Progress towards genetic and pharmacological therapies for keratin genodermatoses: current perspective and future promise

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Abstract: Hereditary keratin disorders of the skin and its appendages comprise a large group of clinically heterogeneous disfiguring blistering and ichthyotic diseases, primarily characterized by the loss of tissue integrity, blistering and hyperkeratosis in severely affected tissues. Pathogenic mutations in keratins cause these afflictions. Typically, these mutations in concert with characteristic features have formed the basis for improved disease diagnosis, prognosis and most recently therapy development. Examples include epidermolysis bullosa simplex, keratinopathic ichthyosis, pachyonychia congenita and several other tissue-specific hereditary keratinopathies. Understanding the molecular and genetic events underlying skin dysfunction has initiated alternative treatment approaches that may provide novel therapeutic opportunities for affected patients. Animal and in vitro disease modelling studies have shed more light on molecular pathogenesis, further defining the role of keratins in disease processes and promoting the translational development of new gene and pharmacological therapeutic strategies. Given that the molecular basis for these monogenic disorders is well established, gene therapy and drug discovery targeting pharmacological compounds with the ability to reinforce the compromised cytoskeleton may lead to promising new therapeutic strategies for treating hereditary keratinopathies. In this review, we will summarize and discuss recent advances in the preclinical and clinical modelling and development of gene, natural product, pharmacological and protein-based therapies for these disorders, highlighting the feasibility of new approaches for translational clinical therapy.

Abbreviations: PC, pachyonychia congenita; EBS, epidermolysis bullosa simplex; SEI, superficial epidermolytic ichthyosis; KPI, keratinopathic ichthyosis; EI, epidermolytic ichthyosis; MAPK, mitogen-activated protein kinase; Hsp/c, heat shock protein/cognate; SF, sulforaphane.

Key words: animal models of disease – genetic skin diseases – in vitro disease models – keratin gene mutation – keratin genodermatoses – pharmacologic and molecular therapies – tissue-engineered human skin models

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Introduction

The cytoskeleton of keratinocytes is basically composed of a keratin intermediate filament (KIF) network, which provides major cytoskeleton resilience and mechanical integrity. These polymeric KIFs are highly susceptible to pathogenic mutations, and mutations in several keratin genes lead to diverse heritable skin fragility disorders. Ever since the early 1990s when there was the first indication that mutations in KIFs (K5 and K14) caused genetic skin fragility disorders, combined efforts by researchers worldwide have profoundly enhanced our knowledge of the molecular basis of a wide range of distinct genodermatoses (Table S1). These culminated in the availability of prenatal and postnatal genetic testing, counselling, improved disease diagnosis, reclassification and most recently promising therapy development (reviewed in 1–3). Three typical examples of these disorders to be emphasized in this review include the following: (i) the basal epidermolysis bullosa simplex (EBS; K5 and K14 mutations), (ii) the suprabasal keratinopathic ichthyoses (KPI; formerly called bullous congenital ichthyosiform erythroderma) such as superficial epidermolytic ichthyosis (SEI; formerly called ichthyosis bullosa of Siemens or IBS; K2 mutations) and epidermolytic ichthyosis (EI; formerly called epidermolytic hyperkeratosis; K1 and K10 mutations) (4) and (iii) pachyonychia congenita (PC; K6a, K6b, K16 and K17 mutations) (Fig. 1a–c). Both the basal and suprabasal epidermolytic keratinopathies are characterized by intra-epidermal tissue fragility, while PC is characterized by painful palmoplantar keratoderma, hypertrophic nail dystrophy, and often mucosal leukokeratosis (reviewed in 3). PC-related keratins; K6a, K6b, K16 and K17 are normally expressed in the nail, hair follicle, and epidermis of palmoplantar skin, but not otherwise in the interfollicular epidermis, unless induced during wound healing or hyperproliferative skin diseases such as psoriasis. Other types of keratins, such as K75, K81, K83, K85 and K86, are expressed only in the hair shaft and follicles, and are responsible for keratodermas of the hair and nails (see www.interfil.org; 1–3). The severity of the clinical phenotypes of these major keratinopathic genodermatoses can vary quite widely both among individuals and within families, with some demonstrated variation in the degrees of involvement of tissue-specific associated mutant keratins (reviewed in 3, 5, 6).

The majorities of disease-related keratin mutations are associated with heterozygous missense mutations or small insertion or deletion mutations and are mostly inherited as autosomal-dominant traits. However, autosomal recessive and mosaic cases have been reported (reviewed in 3). The recessive mutations range from splice site dominant-negative mutations, most often predicted to lead to nonsense-mediated mRNA decay (NMD), and loss of mutated allele expression (7–9). Analogous to K5 stop codon/
disease models, high-throughput drug screening and gene silencing represent novel approaches to improve therapeutic outcomes (20), that enhance tissue integrity and positively modify diseases, antisense DNA for selective silencing of gene expression and drugs – drug-targeted therapies. Most therapeutic goals have been challenged set point for the future development of gene-targeted and mutation hotspot codons have been identified, which is a useful diagnosis and therapy (16). Generally, EBS-, EI- and PC-associated cutaneous keratin disorders has had a significant impact on their skin care in the different disorders.

Environmental factors that are associated with patient lifestyle and postulated to likely be a combination of genetic, epigenetic and variability in disease/clinical phenotype are not known, but are shown in normal keratin network in control keratinocytes (d) and mutant keratin aggregates in a severe keratin-5 mutant keratinocyte cell line EB11.

truncation mutations in EBS (10, 11), heterozygous nonsense mutations predicted to lead to the expression of a truncated dominant-negative tissue-specific keratin protein that escapes NMD have been reported as well in these disorders (9, 12). Premature termination codon mutations have been reported in dominant keratinopathies, including K5 and K14 mutations in Dowling–Degos (13, 14) and Naegeli–Francescetti–Jadassohn syndromes (15), and K16 in PC (5). The reasons behind the variability in disease clinical phenotype are not known, but are postulated to likely be a combination of genetic, epigenetic and environmental factors that are associated with patient lifestyle and skin care in the different disorders.

New knowledge about the genetic basis of several monogenetic cutaneous keratin disorders has had a significant impact on their diagnosis and therapy (16). Generally, EBS-, EI- and PC-associated mutation hotspot codons have been identified, which is a useful set point for the future development of gene-targeted and drug-targeted therapies. Most therapeutic goals have been challenging to achieve, in part because of the great complexity of genotype-phenotype relationships, than anticipated previously (17–19). The skin is an ideal target for both gene and pharmacological therapy approaches, with the potential for easy accessibility, and cost-effective monitoring of treatment outcomes. The delivery of agents such as small interfering RNA (siRNA), Spliceosome-Mediated RNA Trans-Splicing technology (SMarT), ribozymes and antisense DNA for selective silencing of gene expression and drugs that enhance tissue integrity and positively modify diseases, represent novel approaches to improve therapeutic outcomes (20, 21). Recent technological innovations in animal and in vitro disease models, high-throughput drug screening and gene silencing, promise to extended the proof-of-concept efficacy of gene-based therapy recently demonstrated in reversing the plantar keratoderma of PC (21). Several of these novel approaches are discussed below.

Animal and in vitro disease modelling as tools for therapy development

Genetically modified mice, animal, in vitro tissue culture and bioengineering models have greatly enhanced knowledge of the molecular biology of several genodermatoses. These models faithfully recapitulate the features of human diseases at the clinico-pathological, ultrastructural and molecular genetics levels and represent valuable tools towards the rapid development and preclinical testing of novel gene and pharmacological therapies (reviewed in 22).

Animal models for cutaneous keratin disorders

Similar to humans, several naturally occurring disease-causing mutations that lead to blistering have been identified in animals, such as dogs, horses and sheep. These models have enhanced our understanding of disease pathogenesis (reviewed in 23–25). In addition, genetically engineered mouse models have been generated for the major forms of keratinopathic genodermatoses and have significantly contributed to our current understanding of the pathomechanisms of their different forms and also provided insights into the complex secondary effects mediated by signalling pathways and other systems that modify disease phenotypes (reviewed in 22, 23, 26). Despite the fact that murine models for human diseases often recapitulate the human phenotype, limitations are there that they are time-consuming, labour intensive, less cost-effective, have lack of corresponding human genes in their genome and are often associated with embryonic lethality, which in concert have led to the quest for alternative strategies (23).

In vitro models of cutaneous keratin disorders

Ever since the first cultivation of keratinocytes by Rheinwald and Green (27, 28) and its application in dermatological research, several advances have optimized tissue culture systems suitable for gene and pharmaco-toxicological studies. Isogenic, primary and immortalized cell lines derived from patients with keratin genodermatoses and controls as well as transgenic animals have been invaluable in devising therapies. Such models revealed clumped KIF in severely keratin-defective cells (Fig 1d,e) and have helped uncover disease pathomechanisms, variations in their gene expression profiles and promote therapy development (29) and (reviewed in 3). Interestingly, in vitro cell culture and tissue-engineered human skin disease models that mimic in vivo disease phenotypes (e.g. induction of keratin-clumping and cytolysis) have been successfully established through testing the impact of cellular insults, including heat, osmotic and mechanical stresses, on keratin-defective patient-derived cells (reviewed in 3).

These equivalent techniques have now been successfully extended to bio-engineer normal and keratin-defective skin (30–34), with the recent development and application of a humanized murine skin model (21, 31).

Humanized murine skin model for preclinical modelling and therapy

Long-term studies of orthotopic and non-orthotopic skin grafting being problematic owing to graft degeneration (35) have recently been revolutionized using an optimized surgical strategy termed ‘skin-humanized mouse model’ which utilizes the orthotopic
bioengineered skin grafting strategy (36, 37). This model allows the generation of a large number of engrafted mice within a relatively short time frame and consists of systematic deconstruction–reconstruction starting with cells isolated from affected patients’ skin, propagated and assembled into organotypic skin equivalents, followed by grafting onto immunodeficient mice. This strategy has been utilized to faithfully regenerate normal disease-free (38) and diseased humanized skin in vivo using patient-derived or gene-modified human keratinocytes (37, 39), such as PC (31) and Junctional-EB cells (40, 41), followed by grafting to immunodeficient mice.

Roles of molecular chaperones, ubiquitin–proteasome and MAP kinases in the pathogenesis of keratin genodermatoses

Details of disease modelling and exacerbating conditions are intricate steps towards refining therapy development. A common feature of hereditary keratinopathic skin and eye disorders is the occurrence of cytoplasmic keratin aggregates (clumps) in severe forms or when induced by trauma. These abnormal KIFs aggregates are cytotoxic and further compromise cytoskeletal integrity. In several human disorders, chaperones and the ubiquitin–proteasome system have been noted to modulate disease severity, providing novel therapeutic targets. Furthermore, keratinopathic genodermatoses have been proposed to be protein misfolding disorders (34, 42) in which keratin aggregates are reminiscent of those in other protein misfolding disorders (43, 44). In fact, keratin aggregates have been shown to interact with activated mitogen-activated protein (MAP) kinases, molecular chaperones and components of the ubiquitin–proteasome system, as in protein folding disorders (33, 45–51).

Native and activated keratin states are achieved in part by the specific activities of Hsp70 (HspA1A) and Hsp90 (HspC1), mediated by chaperone cofactors Hip (Hsp70-interacting protein) and Hop (Hsp70–Hsp90-organizing protein) (Fig. 2; pathway 1), and/or link it to the ubiquitin machinery which colocalizes with aggregated keratin and Hsp70 in the cytosol. CHIP can also divert mutant keratins for degradation via the ubiquitin–proteasomal pathway (53), as the turnover of keratin is also regulated by the ubiquitin–proteasome system (Fig. 2; pathway 2). In the epidermolytic keratin disorders EBS and EI, proteasomal inhibition resulted in the upregulation of stress-induced Hsp70 and Hsp90 in cell cultures and in murine model (33, 44, 54). Moreover, overexpression of the ubiquitin ligase CHIP significantly accelerated the degradation of mutant keratin in an Hsc70 (HspA8) or Hsc/p70-dependent mechanism, positing that Hsc/p70 chaperone machinery prime mutant keratins to the ubiquitin–proteasome system for degradation (46). So, activated MAP kinases colocalize with mutant keratin aggregates and Hsp70 in stressed cells compared with control cells, further suggesting a critical role in disease pathogenesis (33, 50). A p38-inhibitor augmented the proportion of keratin aggregate-positive cells in the severe EBS (EB11) cell line, corresponding to the requirement of phosphorylation of keratin aggregates prior to their degradation (45). Several lines of evidence have implicated the involvement of MAP kinases in modelling keratin genodermatoses including the correlation of stress-induced cell death with ERK and JNK activation (55, 56).

Current translational therapeutic approaches

Several in vivo and in vitro model systems have provided useful molecular details, which now represent focal points for small molecule discovery. These include the following: (i) modulation of the inflammatory response, (ii) gene modifier effects on keratin mutations, (iii) the role of molecular chaperones, ubiquitin–proteasome and MAP kinase systems in disease modification and (iv) induction of compensatory molecules for protein supplementation therapies.

Modulation of inflammation

Pathogenic mutations in EBS and related disorders are known to cause not only tissue cell fragility, but also local inflammation via the stress responses, as exemplified by a significant upregulation of proinflammatory cytokines in K5 knockout murine skin (57). Physiological concentrations of doxycycline, one of several small molecule-based strategies for EBS, increased the survival of K5-deficient mice and reduced inflammation as demonstrated by a decrease in the inflammatory cytokine IL-1β levels (26, 57). Moreover, doxycycline downregulated the activity of matrix metalloproteinase 13 and reduced bulla formation, suggesting EBS can be ameliorated by reducing local inflammation (26, 57).

Small molecule-based modification of disease phenotypes

Intriguingly novel approaches have been developed based on small molecule drugs with the propensity to activate compensatory keratins such as K6, K16 and K17 without injury, oxidative stress or UV light and functionally supplement defective keratins. This approach has been exemplified by two recent strategies for EBS and PC.

In EBS (with K14 mutation), the isothiocyanate sulforaphane (SF) was shown to induce K16 and K17 that are complementary to K14. The effects in K14-mutant mouse models of EBS resulted in reprogramming of keratin synthesis, ameliorated skin blistering and thus restored murine skin integrity (58). SF is a small molecule naturally occurring in precursor form in broccoli sprouts and other cruciferous vegetables (59), with known cancer chemopreventive/therapeutic potential and the ability to enhance Nrf2-dependent transcription (60). SF-mediated K16 induction was shown to depend partly on activation of the Nrf2 transcription factor, whereas that of K17 is related to glutathione levels in mouse epidermis; both presumably involving MAP kinases (61). Interestingly, bioactive SF in broccoli sprout extract have cleared phase I clinical trials (62–64) and are now considered safe for use in human skin (65). In PC, K6a is the most commonly mutated gene, suggesting that small molecules that can specifically downregulate K6a expression could be therapeutic. In a small molecule library screening, cholesterol-lowering statins downregulated the expression of K6a and a subset of other keratins (66), through the isoprenylation pathway, which is downstream of HMG-CoA reductase (enzyme inhibited by statins in the cholesterol biosynthesis pathway). As discussed previously, K6a expression is also less effectively and less specifically inhibited by retinoids, compared with statins; which is the basis of current consideration of the combination of low-dose retinoid and statins as a therapeutic approach by the patient with PC advocacy organization PC Project (www.pachyonychia.org). Such strategy might be beneficial for other epidermolytic keratinizing diseases such as EI. Other drug discovery strategies are based on discoveries about biological changes in keratinopathies and could include screens for drugs...
The misfolded keratin aggregates are targeted and cleared by the ubiquitin–ubiquitin ligase (UBL) system, whereas CHIP binds to the carboxyl terminus and acts as a chaperone-associated ubiquitin ligase to mediate the attachment of a polyubiquitin chain to the misfolded keratin aggregates upon induction. Bag-1/STUB1 associate with the ATPase domain of Hsp70 and mediate interaction of Hsp70 with the proteasome via its ubiquitin-like domain. This modulates molecular chaperones in favor of keratin refolding or degradation by the proteasomal pathways. Finally, the interruption or removal of mutant keratin leads to cell fragility.

By interference-related approaches at the synthesis phase (e.g., by RNAi and SmarT), and development of various small chemical molecules that can modulate intrinsic biochemical pathways such as the molecular chaperone, the ubiquitin–proteasome and MAP kinase machineries. By employing diverse cell stress models (reviewed in 3), recent studies offer a novel chemical chaperone [trimethylamine N-Oxide (TMA-N-Oxide)] that can: (i) reverse the enhanced propensity of keratin-defective keratinocytes towards apoptosis, as demonstrated by the reversal of mitochondrial defects and cardiac dysfunction in desmin-null mice upon cardiac overexpression of the anti-apoptotic Bcl-2 (67); (ii) selectively downregulate K6a expression in human keratinocytes as demonstrated by the macrolide sirolimus or rapamycin and statins (68, 69); (iii) upregulate partner keratins to reinforce the compromised cytoskeletal network; and (iv) slightly downregulate normal wild-type keratins but strongly inducing compensatory keratins. Further, high-throughput screening of chemical libraries may uncover new drugs that address these possibilities for intervention.

In an effort to reinforce the compromised cytoskeleton resilience, alternative strategies now explore the potential to modulate intrinsic biochemical pathways such as the molecular chaperone, the ubiquitin–proteasome and MAP kinase machineries. By employing diverse cell stress models (reviewed in 3), recent studies offer a novel chemical chaperone [trimethylamine N-Oxide (TMA-N-Oxide)].
Progress towards genetic and pharmacological therapies for keratin genodermatoses

(TMAO) and 4-phenylbutyrate (4-PBA]-based therapy approach for keratin genodermatoses such as EBS and EI (32–34, 42, 50). These small molecules are known to modify other neurodegenerative protein misfolding disorders (70–74) and have similarly been shown to attenuate keratin aggregation and modify keratinopathic genodermatoses in vitro (34, 42). Moreover, 4-PBA also (i) reduces the mislocalization or aggregation, and stabilizes proteins associated with other human diseases (75–79); (ii) remodels chromatin (80, 81); (iii) modulates heat shock protein, especially Hsp70 family members and MAP kinases (50); and (iv) degrades mutant keratin aggregates via ubiquitin–proteasome (33, 46) in addition to its other gene modifying effects (70, 82). Intriguingly, 4-PBA is an approved drug in clinical use for the treatment of urea cycle disorders (83), sickle cell disease and thalassaemia (74, 84). Furthermore, our recent data suggest that 4-PBA and TMAO-induced increases in Hsp70, which co-localized with K1 (EI) or phosphorylated K5 (p-K5 in EBS), could possibly prime and facilitate the turnover of chaperone-mediated degradation of mutated keratins as suggested in Fig. 2. In conformity with this concept, similar studies have delineated specific effects of chemical chaperones on protein trafficking in conjunction with altered Hsp70 expression in other disease models (85, 86). In fact, TMAO did not only affect HSPs, but was also shown to activate and modulate members of the MAP Kinase signalling pathways in EBS, suggesting a critical protective effect that involves MAP Kinases (50). Colocalization of keratin aggregates with phosphorylated p38 (45), suggested the association of TMAO-induced keratin aggregates reduction and the activation of both Hsp70 and phospho-p38 (see Fig. 2). In this light, pharmacological or other interventions aimed to accelerate the turnover of aggregates may be therapeutically beneficial in hereditary keratin disorders (87).

Small molecules development for clinical therapies

Other small molecule-based topical and or systemic therapies include corticosteroids, cyproheptadine and antibiotics (88–90). Similar to doxycycline discussed earlier, there are anecdotal reports of successful treatment with another anti-inflammatory agent, tetracycline (91, 92), which may prevent tumor necrosis factor alpha release or modulate the activity of metalloproteinases, to reduce bulla formation. However, a randomized trial did not reveal any difference between antibiotic and placebo-treated groups (93). Aminoglycosides family antimicrobials may lead to translation of proteins in individuals with premature termination codon mutations by transcript read through. Patients with PC treated with rapamycin have resulted in symptomatic improvement of painful plantar calluses (reduced pains and callus), suggesting oral or perhaps topical rapamycin or its derivatives as a therapeutic option (69).

Botulinum toxin type A (BTX-A) therapy

Although topical antiperspirants, such as aluminium chloride and glutaraldehyde (94, 95) initially seemed promising for the hyperhidrosis of localized patients with EBS (94, 95), later studies showed no benefit (96, 97). The commercially available BTX-A, is currently being evaluated as a potential therapy option in inherited epidermolysis keratin genodermatoses such as in EBS and PC, because patients with prominent blisters and plantar calllosity often have plantar hyperhidrosis (see Fig. 1c). Moreover, disease manifestations are often exacerbated during hot and humid conditions, plantar sweating, prolonged walking and other traumatic physical activity (98). Intradermal injection of BTX is a more effective remedy for focal hyperhidrosis because it blocks peripheral cholinergic nerve terminals (synapses), inhibits the release of acetylcholine and blocks autonomic cholinergic junctions (neuromuscular junction) of the postganglionic sympathetic fibres to the eccrine sweat glands, further decreasing hyperhidrosis (99, 100). Thus, patients with PC experienced dryness and remarkable reduction in pain associated with walking when treated with intracutaneous plantar injections of BTX-A (101). Later, plantar injection of BTX was also suggested as efficacious and safe treatment to reduce painful blistering and calllosities in EBS (101, 102). Recently, a retrospective study including more than 14 patients with EBS and PC reported remarkable reduction in plantar pain and blistering upon local injection of BTX, without any adverse effects except that mild anticholinergic side effects were observed in two patients (103). A large population study is needed to ascertain the prospects of this line of investigation.

Retinoids and their analogues in the treatment of keratin genodermatoses

Retinoids are known regulators of keratinocytes biology (reviewed in 104). Some topical or oral synthetic retinoids are beneficial in managing certain hereditary cutaneous keratinopathies including EI, PC and PPK. In hyperkeratotic keratinopathies, alternative treatment options include emollients containing glycerine, lactic acid, urea, and other topical and systemic retinoids. While some patients respond well to synthetic retinoids and calcipotriol (a vitamin D3-analogue; 105, 106), orally administered etretinate has been more successful in reducing thickening, but less effective at decreasing erythroderma. Importantly, several keratin gene promoters such as the KRT16a gene contain retinoic acid response elements (66), implying retinoids can downregulate the expression of these genes. A proof-of-concept study has demonstrated the upregulation of K4 with concomitant decrease in K2 levels in retinoid-treated EI epidermis in vivo (106). Although retinoids are broad spectrum in their action, evidence suggests that retinoid therapy could be more beneficial to EI patients with K10 rather than those with K1 mutations (106). Recently, using a 3D epidermal model, retinoic acid receptor-α (RAR) agonists were reported to be potent modulators of keratin 1, 2, 4 and 13 expressions (107), suggesting their use for patients with K1 and K2 mutations, the latter seen in patients with superficial KPI (SEI; formerly called ichthyosis bullosa of Siemens). Their efficacy for EI therapy might be achieved via a mechanism that involves the upregulation of compensatory K4 and/or K13 proteins which may functionally replace the mutated keratin molecules (107). It is important to investigate the effects of retinoids with affinities for different RARs on different tissue-specific keratins that might correlate with the effect on keratin expression reported in reconstructed epidermal equivalents (107). In fact, exogenous retinoid acid was shown to covalently bind K16 and K10 in mouse skin (108), raising the possibility that the binding of retinoid to keratins may be involved in the retinoid-induced consolidation of keratin cytoskeleton resilience in the epidermis; this possibility can be tested by treating murine skin with potent RAR agonists that have an affinity for the different -isoforms. Although the pathomechanisms differ, their phenotypic expressions might share a common
signalling pathway. Retinoids are known to inhibit epidermal differentiation program causing skin irritation, fragility and tenderness, and most patients do not tolerate long-term retinoid therapy. This has led to the need for newer, more specific drugs that mimic the effect of retinoids with minimal or no side effects or teratogenicity as exemplified by retinoic acid metabolism blocking agents (RAMBAs).

**Retinoic acid metabolism blocking agents**

RAMBAs are a new group of retinoid-like drugs that block intracellular breakdown of endogenous RA, thus mimicking the effects of exogenous retinoic acid (RA). The mechanism of action is largely through inhibiting CYP26, the enzyme responsible for the degradation of RA (109). Upon inhibition by RAMBAs, the intracellular all-trans-RA concentration rises to therapeutic values, but primarily in the skin where all-trans-RA is metabolized by the CYP-dependent 4-hydroxylase. Therefore, RAMBAs are able to provide retinoid-like effects in skin with less systemic side effects and post-treatment teratogenicity (110) and have proven clinically promising in a limited number of patients with keratinization disorders (109, 111). One such agent, liarozole, which inhibits CYP-dependent hydroxylation of all-trans-RA, has been granted orphan drug status for congenital ichthyosis. In patients with ichthyosis, clinical trial of liarozole versus acitretin (synthetic retinoid) showed mostly mild to moderate RA-related adverse effects that tended to occur less frequently in the liarozole-treated group, suggesting that liarozole is equally effective for ichthyosis as acitretin but showed a trend towards a more favourable tolerability profile (109).

**Corrective gene therapy approaches**

Owing to the dominant-negative effect most keratin gene mutations exert in these diseases, a conventional gene therapy approach would likely be ineffective. Although increasing the ratio of normal keratin protein to mutant protein ameliorates the keratin network appearance and decreases cell fragility (19), most, strategies have focused on selective inhibition of mutant alleles (112–114). Technologies, such as short inhibitory RNAs (siRNAs) and spliceosome-mediated RNA trans-splicing (SMaRT), are the most promising approaches that offer the best chances and enable inhibition of expression of the mutant alleles without affecting wild-type gene expression (17, 20, 112, 113, 115–118).

**siRNA technology**

Small or short inhibitory RNA (siRNA) technology was effectively used to downregulate mutant K6a (116) and K14 (119) allele expressions in cultured PC and EBS cells respectively. The mutant-specific siRNAs led to the formation of normal keratin filaments, reflecting the selective inhibition of mutant K6a in a cell culture model for PC (113). Owing to its accessibility to therapeutic agents, the skin represents an attractive target for nucleic acid-based therapies (120). Using an organotypic model, Hickerson et al. (30) showed enhanced delivery of siRNA to inhibit gene expression. Moreover, missense point mutations in the affected alleles of keratinopathies were silenced with siRNA, allowing expression of functional wild-type allele (112, 113, 116). The development and optimization of this mutation-specific siRNA therapy approach has now progressed to an efficacious small-scale human clinical trial, resulting in the first-in-human siRNA treatment of a skin disorder (21). Although the strategy is not currently in clinical practice, this study, in which increasing concentrations of siRNA directed against a K6a mutation were injected into the plantar skin of a patient with PC, showed an excellent result with a significant decrease in plantar keratoderma (21).

An alternative approach relies on exploring the functional redundancy of keratins in tissues affected by keratin mutation (121), including EBS, EI and PC. Mouse knockout studies on KRT6A null mice, have shown that KRT6B compensate for decreased KRT6A, suggesting that complete silencing of the defective keratin, could be an alternative approach that has more global therapeutic application than the development of siRNAs that target individual point mutations (122, 123). Based on this concept, gene-specific siRNA has been developed and will shortly be in clinical trials for treating the most common form of PC (related to KRT6A mutations; 114).

**SMaRT technology**

Spliceosome-mediated RNA trans-splicing (SMaRT) is another silencing technique and uses the endogenous spliceosome machinery to effectively excise mutant exons, knocking out the mutant protein in affected cells. Wally et al. (124) reported gene expression repair by SMaRT, resulting in replacement of a mutation of the plectin gene in EBS, a strategy that has also been used to successfully correct collagen XVII (125) mutations. Recently, SMaRT was used to specifically replace the first seven exons of the K14 gene in an EBS-DM patient cell line (20). Thus, this approach appears a promising tool for gene therapy of several autosomal-dominant blistering genodermatoses because it clears the mutation while increases normal allele expression. Efforts are underway to increase the efficiency of SMaRT therapy for clinical application (126).

**Protein supplementation and cell-based therapy approaches**

A decade ago, scientists considered upregulating the expression of another intermediate filament (IF) protein to compensate for keratin gene mutations. In cultured keratinocytes with dominant-negative K5 and K14 mutations, transfection with desmin, an IF normally expressed in muscle cells, restored the wild-type response to stress (127, 128). The ectopic expression of desmin in transgenic mouse epidermis resulted in the formation of a parallel and normal desmin filament network, although it did not rescue the K5 null mutant phenotype (129).

Furthermore, mouse models of EBS and EI demonstrated that a 50% reduction in mutant allele expression prevents blistering and normalizes skin function (19, 130). Despite the possibility that the therapeutic threshold could be greater in humans, these findings suggested the potential benefit of suppression of a mutant allele without increasing normal gene expression. Recessive EI disease provides evidence that knockdown to 50% of normal levels does not cause disease (e.g. no haploinsufficiency) as in the normal skin phenotype in heterozygous carriers of keratin gene defects (131, 132). The normal phenotype in KRT6A null mice, however, suggests that KRT6B compensates for KRT6A’s absence, likewise, the observation of milder disease phenotype upon overexpression of wild-type K15 in a kindred with recessive EBS because of ablation of K14 (133). Most recently, it has been demonstrated using K14-null mutant cells that the stress responses associated with keratin disorders can be ameliorated when cells were rescued by the addition of wild-type keratin protein (134). Little is known about the effect of replacing wild-type protein in cells with dominant-negative KRT mutations.
Induced pluripotent stem cells (iPSCs) and Revertant Mosaicism therapies

The emergence of regenerative medicine technological innovation where cells, such as dermal fibroblasts, are converted to induced pluripotent stem cells (iPSCs), with potential to later differentiate to any cell type in the body has opened new avenues of therapeutic approaches (reviewed in 23). This strategy is potentially able to develop patient-specific multiremedy strategies for genodermatoses. It mainly rely on ectopic expression of a limited number of transcription factors that can reprogramme somatic cells into a transient embryonic stem cell state. These transient cells express embryonic stem cell markers and possess both unlimited proliferative capacity and the ability to differentiate into the ectoderm, mesoderm and endoderm germ layers.

The patient-specific iPSCs (PS-iPSCs) have now been generated from several human diseases including heritable skin disorders (reviewed in 23). It has been demonstrated that both normal and patient-specific (PS) iPSCs can be differentiated directly into functional keratinocytes, with specific surface markers being used to purify iPSC-derived keratinocytes and allowing for enrichment of keratinocyte lineage cells (135). Further availability of reliable and mutation free non-viral integration of transcription factors will provide relevance prior to clinical application (23). Therefore, there is great promise that iPSCs may provide unprecedented sources of cells for heritable skin keratinopathies as well.

Revertant Mosaicism (RM) or ‘natural gene therapy’, is a naturally occurring phenomenon whereby a subpopulation of somatic cells spontaneous reverts to the wild-type phenotype via crossover or conversion which alleviates the requirement of further gene correction(136, 137). RM is now well known to not being a rare event and has been observed in several patients, including several types of EB (137). In heritable keratinopathies as revertant skin is visible and easily accessible, RM has been reported in cases of ichthyosis with confetti because of autosomal-dominant KRT14 mutation (138); EBS because of autosomal recessive KRT14 mutation (139) and in severe EBS-DM (EBS-Dowling-Meara) with autosomal-dominant KRT14 mutation (140). This therefore holds promise as it may serve as a natural, patient-specific source of gene-corrected cells for its usage of revertant keratinocytes for revertant cell therapy or iPSC to provide autologous regenerative source.

Nanotechnology for treating keratin genodermatoses

Several hurdles exist in developing novel agents including determining their optimal therapeutic combinations and effective delivery of agents to target tissue at concentrations that achieve efficacy without toxicity.

Nanotechnology is a relatively new and rapidly evolving field that may be applicable for the treatment of keratin genodermatoses, as it may enhance the delivery, bioavailability and also limit any unwanted toxicity of therapeutic agents. The structure and tunable surface functionality of nanoparticulate systems allows them to encapsulate/conjugate single or multiple agents either in the core or on the surface, rendering them ideal carriers for various drugs. Recently, we employed nanotechnology in the field of cancer to improve the outcome of chemoprevention also known as ‘nanochemoprevention’ (141). Nanotechnology may also be exploitable as an efficient delivery system for delivering siRNA and small molecules for the treatment of keratin genodermatoses. Most drugs or gene correction agents poorly penetrate cellular membranes have low bioavailability and are formulated in undesirable solvents. The use of nanocarriers permits the preparation of low water soluble medications as solid or liquid formulations which should be useful for treating keratin genodermatoses. So far no natural, or synthetic agents excepted have been nano-formulated for genodermatoses. However, siRNA gene correction agents are currently being nano-formulated for genodermatoses. It is hoped that the development and implementation of nanotechnology will greatly enhance and advance the treatment of genodermatoses and are being tested in cell and animal models.

Detailed overview of this growing field of nanotechnology/nanomedicine and their therapeutic applications in dermatology is beyond the scope of this review. Considerable effort has been generated in determining therapeutic efficacy and potential negative side effects. It is thought that in healthy skin, nanoparticles deliver pose minimal health risk because they mostly utilize biodegradable particles (142, 143). Even as the stability and low toxicity of some nanoparticles have been exploited in several setting, the current knowledge of their toxicity effects is comparatively inadequately documented (144–147). However, available data suggest nanoparticles can bypass the protective barriers, being distributed and accumulate in several organs, with documented toxicity in several organ systems excluding the skin (144, 148).

The mechanic adjuvant and other effects for skin barrier-defective diseases are yet to be addressed as there is limited data on nanoparticle penetration, transport and their interactions with diseased skin. Much emphasis on quantitative studies in relation to dose-exposure to penetration and therapeutic efficacy, as well as consistency in detection sensitivity of techniques used is needed. A plethora of unanswered questions emphasizes the technical challenges needed for translation of nanotechnology to clinical dermatology, thus pointing to the great opportunity for further investigative studies in this arena.

Conclusions and future prospects

This review reflects the research progress made in molecular genetics and pharmacological therapy for treating inherited cutaneous keratin disorders. Despite vigorous worldwide research efforts that have uncovered the pathophysiology of keratin genodermatoses, many treatment questions remain unanswered. Progress in successful therapy development has been challenging and prospects are improving, but patient management remains essentially symptomatic and palliative (97). These remedies mainly involve prevention of secondary bacterial infections, management of blisters and wounds, and minimization of trauma and pain with techniques mostly relying on the individual clinician’s personal experience. Novel strategies include gene therapy, protein replacement, iPSCs and pharmacological therapies, some of which have already entered the clinical arena of dermatology. The combination of animal, in vitro and bioengineered humanized skin murine approaches are relevant pathophysiological models that are helping scientists to address new therapeutic strategies (38, 149), including options that attempted to neutralize the cytotoxic effects of keratin aggregates by focusing on ameliorating tissue-specific damage, while maintaining normal skin function. Pharmacological agents, for example, 4-PBA approved for clinical use in other human diseases, and statins, approved cholesterol-lowering drugs are promising candidate drugs from chemical
libraries for keratin disorders. Moreover, gene therapy using RNA interfering agents, such as siRNA, SMaRT, ribozymes, and antisense DNA, may improve outcomes either alone or in combination with drug therapy. Despite the exciting proof-of-concept studies showing the potential value of siRNA technology for keratin gene disorders, major technical hurdles persist such as the development of safe, effective pain-free techniques for routine delivery into the epidermis. The current goal is to explore other potential delivery systems including chemical modification of the siRNAs, modulation of the chemistry of topical formulation, microneedle arrays and nanoparticles technology approaches.

The field of nanomedicine is pursuing new strategies to deliver oligonucleotides topically in nanoparticle emulsions or micelles that can traverse the epidermal barrier and improve pharmacokinetics and reduce associated side effects. Major advances in stem cell technology, particularly iPSCs, hoped that should allow lifelong expression of introduced genes. Future approaches will combine an understanding of the molecular basis of genodermatoses with advanced technologies such as iPSCs, siRNA and nanomedicine. Ultimately, personalized medicine through specific correction of an individual's keratin gene defects is the optimal way to correct the problematic skin fragility and uncomfortable thickening, thus, improving the quality of life of patients with keratin genodermatoses.

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Conflict of interest

The authors declare no conflicts of interest with respect to the preparation and publication of this article.

References
Progress towards genetic and pharmacological therapies for keratin genodermatoses

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Table S1. Human Heritable Cutaneous Keratin disorders: Keratin genes mutated and their associated clinical phenotypes.

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