REVIEW ARTICLE



# Nonsense Suppression Therapy: An Emerging Treatment for Hereditary Skin Diseases

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**Nonsense mutations cause the premature termination of protein translation via premature termination codons (PTCs), leading to the synthesis of incomplete functional proteins and causing large numbers of genetic disorders. The emergence of nonsense suppression therapy is considered to be an effective method for the treatment of hereditary diseases, but its application in hereditary skin diseases is relatively limited. This review summarizes the current research status of nonsense suppression therapy for hereditary skin diseases, and discusses the potential opportunities and challenges of applying new technologies related to nonsense suppression therapy to dermatology. Further research is needed into the possible use of nonsense suppression therapy as a strategy for the safer and specific treatment of hereditary skin diseases.**

*Key words:* readthrough; aminoglycosides; PTC124; nonsense-mediated decay; suppressor tRNA; hereditary skin diseases.

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## SIGNIFICANCE

Nonsense suppression therapy refers to an effective means of preventing protein translation termination and ultimately alleviating disease symptoms by promoting PTC-readthrough. For decades, this therapy has been successfully used in the clinical treatment of certain genetic diseases, but its attempts in hereditary skin diseases are still shallow. Here, we review the research status of the latest nonsense suppression treatments for hereditary skin diseases, and describes the readthrough mechanisms of classic aminoglycosides, the novel compound PTC124, NMD inhibitors, and suppressor tRNA. We also present future prospects for topical formulations, combination drugs, new compounds, emerging technologies in dermatology related to nonsense suppression therapy.

Almost all organisms use the same genetic code, consisting of 61 sense codons that code amino acids and 3 nonsense (or stop) codons that do not encode any amino acids. The process of protein translation begins with the recognition of the initiation codon and ends with the recognition of a stop codon, and involves the participation of mRNA, rRNA, tRNA, ribosomes, and a series of protein factors (1). A nonsense mutation refers to the mutation of a codon encoding a certain amino acid into a stop codon due to substitution of bases. A premature termination codon (PTC) has the same structure and function as a stop codon in the normal translation process, leading to premature termination of protein translation, resulting in an incomplete non-functional or harmful polypeptide chain (2). PTCs can trigger nonsense-mediated mRNA decay (NMD), which targets mRNAs containing PTCs for degradation, thereby preventing their translation and further reducing protein levels (3). Genetic diseases caused by nonsense mutations account for approximately 12% of all genetic diseases (4). In view of this high incidence, corresponding nonsense suppression therapy has great potential for therapeutic applications, which is a strategy aimed at restoring protein functional defects, reversing disease phenotypes, and improving the progression of genetic diseases.

Nonsense suppression treatments typically prevent the termination of protein translation via the PTC-readthrough. Specifically, there are various methods, such as classic aminoglycosides, NMD inhibitors, suppressor tRNA, RNA pseudouridylation, RNA editing, and the CRISPR/Cas9 system (3, 5). In eukaryotes, the termination process of protein translation involves 2 types of release factors (RF), eRF1 and eRF3. At the ribosome A site, eRF1 directly recognizes and interacts with 3 stop codons, resulting in the release of the peptide chain. The combination of eRF3 and eRF1 promotes translation termination activity, then tRNA, mRNA, and ribosomal subunits are dissociated (6) (**Fig. 1**). When eRF1 directly recognizes and binds to PTCs at the ribosome A site, if the adjacent homologous tRNA competes with eRF1 for binding to the PTC, the amino acid transported by the homologous tRNA will replace the original stop codon. Thus, protein translation can continue to form proteins of normal length or of similar functions, which is called PTC-readthrough. As long as there is no substitution of the necessary amino acid, or even when it contains a wrongly incorporated amino acid, the protein still has part of its normal activity.

As early as 1982, Temple et al. (7) proposed that, through specific anticodon mutations, a suppressor tRNA can inhibit nonsense mutations in β-thalassemia

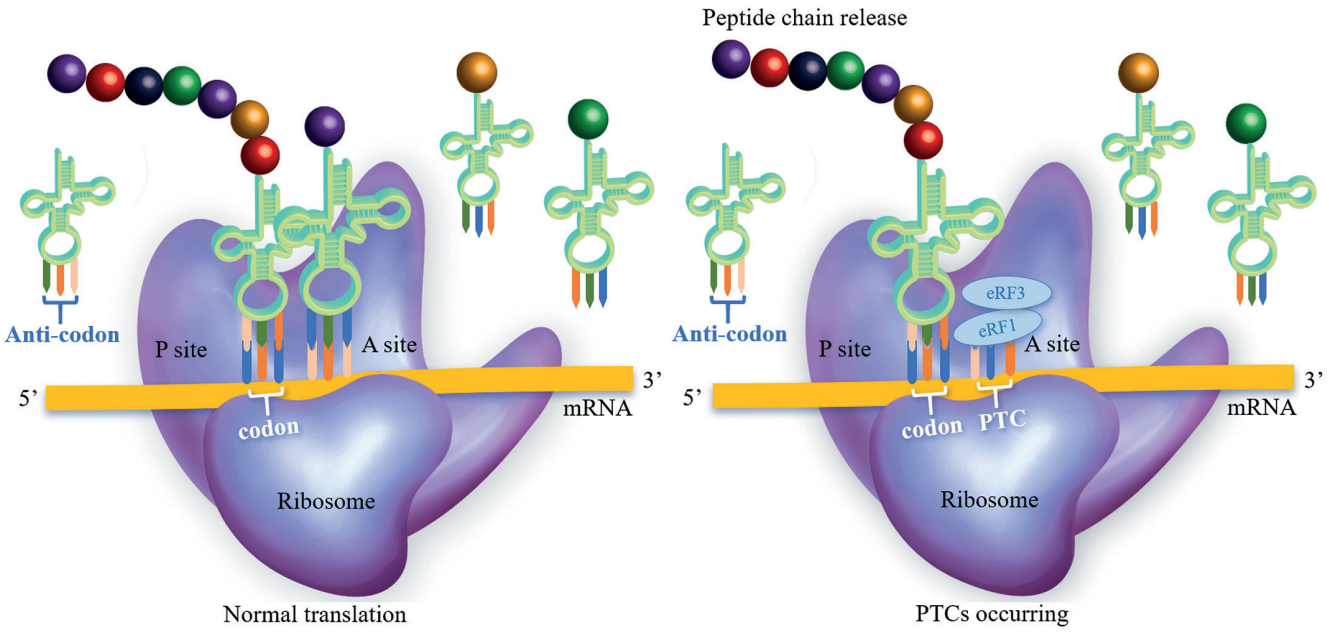


**Fig. 1. Normal translation of proteins (*left*) and termination of protein translation when a premature termination codon (PTC) occurs (*right*).**

mRNA and also provide a method for gene therapy of β-thalassemia caused by nonsense mutations. Similarly, Kiselev et al. (8) reported that the use of suppressor tRNA can produce dystrophin, and some Duchenne muscular dystrophy (DMD) cases are caused by nonsense mutations in the dystrophin gene. Howard et al. reported that treatment with aminoglycoside antibiotics can inhibit nonsense mutations in severe cystic fibrosis (CF) and promote the expression of functional proteins (9). With continuing research, nonsense suppression has gradually found applications in treating multiple disease types, such as thrombotic diseases, nervous system diseases, ocular diseases, and hereditary skin diseases (10–27) (**Table I**). For some recessive disorders, successful gene

**Table I. Nonsense mutation and nonsense suppression studies in other diseases**

| Disease | Gene | Mutation | Stop codon | Nonsense suppression therapy | Reference |
|---|---|---|---|---|---|
| CF | *CFTR* | G542X | UGA G | Gentamicin | (10) |
| DMD | *DMD* | mdx | UAA A | Gentamicin | (11) |
| SMA | *SMN1* | W102X | UAG T | Geneticin | (12) |
| Menkes syndrome | *ATP7A* | R201X | UGA | Copper | (13) |
| A-T | *ATM* | TAT51<br>AT153LA<br>AT187LA<br>AT185LA | UGA C<br>UGA A<br>UAA G<br>UAA G | Geneticin | (14) |
| CDKL5 syndrome | *CDKL5* | R59X<br>R134X<br>Q347X<br>E364X | UGA<br>UGA<br>UAG<br>UAA | Geneticin, gentamicin, PCT124 | (15) |
| Hurler syndrome | *IDUA* | Q70X<br>W402X | UAG C<br>UAG G | Gentamicin | (16) |
| Cystinosis | *CTNS* | 753G→A (W138X) | UGA | Gentamicin | (17) |
| XNDI | *AVPR2* | E242X | UAG C | Geneticin | (18) |
| XLRP | *RP2* | Arg120stop | UGA G | Gentamicin | (19) |
| Choroideremia | *CHM* | | | Gentamicin, paromomycin, PTC124, PTC-414 | (20) |
| | *CHM, NOI, GUP* | Q32X<br>R139X<br>Q524X | UAA<br>UGA<br>UAG | Gentamicin, paromomycin | (21) |
| USH1 | *PCDH15* | p.R3X<br>p.R643X<br>p.R929X<br>p.R245X | UGA C<br>UGA G<br>UGA A<br>UGA A | Geneticin, paromomycin, gentamicin, NB30 | (22) |
| RCS | *PAX2* | | | | (23) |
| CHARGE syndrome | *CDH7* | | | | (24) |
| HCS | *NOTCH2* | The last coding exon | | | (25) |
| β-thalassemia | $tRNA^{Lys}$ | | UAG | Suppressor tRNA | (7) |
| VWD | *VWF* | 2908del C in exon 22 | | | (26) |
| SDS | *SBDS* | K62X | UGA | PCT124 | (27) |

SMA: spinal muscular atrophy; A-T: ataxia-telangiectasia; XNDI: X-linked nephrogenic diabetes insipidus; XLRP: X-linked retinitis pigmentosa; USH1: type 1 Usher syndrome; RCS: Renal coloboma syndrome; HCS: Hajdu-Cheney syndrome; VWD: Von Willebrand disease; SDS: Shwachman-Diamond Syndrome.

therapy can be obtained by partial correction of translation because even a small amount of functional protein is sufficient to improve clinical symptoms (28).

In dermatology, previous clinical studies have shown that topical and systemic use of readthrough compounds can improve nonsense-mediated skin disease phenotypes. The aim of this paper is to review the mechanisms for controlling nonsense suppression, and its relevance to hereditary skin diseases, providing evidence for the application of nonsense suppression therapy as a viable treatment option for incurable hereditary skin diseases.

## NONSENSE SUPPRESSION THERAPY

### *Aminoglycosides*

Aminoglycosides are a kind of natural or semi-synthetic antibiotic. By directly targeting the bacterial ribosome A site to change its conformation, they lead to tRNA reading errors and interfere with protein synthesis (29). In eukaryotic cells, natural stop codons are surrounded by specific upstream and downstream sequences, and there are multiple cellular mechanisms that make it difficult to readthrough, with a probability of 0.001%–0.1% (30); however, PTCs usually lack the protection of these sequences, and aminoglycosides can promote the binding of nearly homologous tRNA to PTCs, replacing eRF1, allowing protein synthesis to continue, and suppressing nonsense mutations. Aminoglycosides have strong sequence specificity for PTC-readthrough therapy, which depends on the stop codon itself and the surrounding nucleotide sequence (31). Therefore, aminoglycosides act mainly on nonsense mutation sites (32). Even if the readthrough of the natural stop codon occurs, its effect is slight and will not have a significant impact.

Different aminoglycoside drugs have different nonsense mutation suppression activities (33). Different chemical structures determine the affinity of interactions with ribosomes and may influence the PTC-readthrough efficiency (34). Even different components of the same drug with very similar structures can have different nonsense mutation suppression activities. For example, gentamicin is a mixture of multiple compounds which have different nonsense mutation suppression activities. The main component is the C group, in addition to a small amount of gentamicin B, gentamicin B1 and so forth. Gentamicin B1 has the main nonsense mutation suppression activity, while the very close structural analog gentamicin B lacks readthrough activity. Thus, the amount of different ingredients of the same medicine may vary between formulations (35, 36). This explains why the same drug can have different therapeutic effects. Moreover, the core component, which has the main readthrough effect, can achieve an ideal nonsense suppression effect even at a low dose. Increasing the concentration of non-core components will not increase the readthrough activity or drug toxicity, but will reduce the readthrough activity of the core components (35, 37), suggesting that components with major nonsense mutation suppression activity may also have major drug toxicity.

Despite their proven clinical utility, these therapies also have many shortcomings, leading to potential side-effects, including ototoxicity and nephrotoxicity, as well as increased drug resistance. In certain diseases caused by nonsense mutations, they can restore only part of the protein function, and in many diseases they cannot restore any protein function (38). In addition, the binding efficiency of different drugs is not the same, which may lead to the synthesis of full-length or truncated proteins, and the function of these proteins is not yet known and may be harmful (37).

The application of aminoglycosides has opened the door to the treatment of nonsense mutations. Although clinical applications are limited by their toxicity and the low readthrough efficiency of the stop codon, the benefits are beyond doubt. In order to reduce drug toxicity, aminoglycoside derivatives have been developed (39). They are less toxic and have fewer accompanying negative effects. Therefore, they may be more beneficial to suppress nonsense mutations, although this potential advantage requires further evaluation *in vivo*.

### *Non-aminoglycosides*

Among the non-aminoglycoside drugs, the most promising drug at present is PTC124, also known as ataluren (brand name Translarna), which is a new type of small molecule drug that has been shown to promote nonsense suppression through high-throughput screening (40). It shares no structural similarity to aminoglycosides, and consequently, it lacks the associated toxic side-effects (41). It is more specific and selective for the PTC-readthrough without affecting the translation termination of natural stop codons (42). PTC-124 is tolerable orally and can be used at significantly lower dosages to obtain higher levels of readthrough compared with aminoglycosides (43). Its mechanism may be that it forms a stable complex with PTC-containing mRNA, preventing eRF1 from recognizing the PTC and inhibiting translation termination (44). Recent research shows that PTC124 is selective to the ribosomal A site and promotes the insertion of amino acids in the PTC site of homologous tRNAs to allow translation to continue (45). PTC124 has been approved for Phase 2 clinical trials for over 10 years and Phase 3 clinical trials for 5 years. It seems to have a positive effect on a variety of diseases, but has also been reported to be ineffective (46). This may be related to cell metabolism, drug solubility, compound permeability differences, the influence of other drugs, NMD levels, the significant difference in the activity of PCT124 *in vivo* and *in vitro*, or other factors (47). In addition to PTC124, there are many other small molecule compounds that can promote PTC-readthrough, such as RTC13, RTC14, GJ071, GJ072, RTC204, RTC219,

BZ6, BZ16, and clitocine (48). These compounds display reduced toxicity and have varying degrees of nonsense suppression activity (49). Clitocine works at the transcriptional level and promotes the decoding of nonsense codons (50). However, the specific mechanism of action of other compounds is still unclear. Many non-aminoglycoside antibiotics have also been shown to suppress PTCs. Negamycin is an antibiotic whose chemical structure is different from aminoglycosides, but its effect on ribosomal decoding is similar (51). In addition, tylosin, josamycin, spiramycin, erythromycin, azithromycin, and other macrolide antibiotics also have a certain PTC-readthrough effect. They probably promote PTC-readthrough by inhibiting the release of the peptide chain from the ribosome (52).

### *Suppression of nonsense-mediated mRNA decay*

NMD preserves normal physiological levels of transcription and translation, and allows the body to dynamically adjust to different environments (53). It plays a role in recognizing and degrading PTC-containing mRNA, thereby preventing the translation of mutant transcripts and further reducing the level of abnormal proteins (54). By inhibiting NMD, mutated mRNA transcripts are stabilized, and the content of peptides is increased, thereby enhancing the readthrough effect to alleviate certain disease phenotypes. The efficiency of NMD may be related to sequences upstream and downstream of the PTC, or it may vary for different mutations and different genes (47). NMD suppression therapy may also have potentially harmful side-effects. Because NMD is active in many cellular processes, in addition to regulating the expression of transcripts involving nonsense mutations, NMD also regulates the expression of other gene transcripts. It affects DNA replication, repair, and telomere integrity during the cell cycle, and thus maintains the stability of the genome. Excessive suppression of NMD may bring some negative effects (55). Therefore, the reasonable choice of NMD inhibitors in treatment is worthy of consideration.

### *Suppressor tRNA*

Suppressor tRNA refers to tRNA molecules with mutations in the anticodons that act by correcting nonsense mutations. They exist naturally and can also be obtained by editing the anticodons (56). Suppressor tRNA promotes amino acid substitutions of PTCs to generate full-length functional proteins and restore protein activity. The readthrough efficiency of suppressor tRNA will depend on its expression level and stability in target organs, the nature of amino acids substituted for nonsense codons, the contextual sequence of codons, the efficiency of translation bypass, and various cellular mechanisms (5, 7, 57). The degradation rate of tRNA is slow, hence the action time is long. The chemical structure of suppressor tRNA is similar to that of natural tRNA, and it has little toxicity and should rarely cause an immune response. However, since it may also cause the readthrough of natural stop codons, and the expression varies with different diseases, further experiments are needed to evaluate its safety and effectiveness.

## NONSENSE SUPPRESSION IN HEREDITARY SKIN DISEASES

Nonsense mutations have been shown to exist in some hereditary skin diseases, and the use of nonsense suppression therapy can be used to effectively treat them. **Table II** summarizes and lists the PTC-related hereditary skin diseases that have been studied.

### *Pseudoxanthoma elastica*

Pseudoxanthoma elastica (PXE) is caused by mutations in the *ABCC6* gene that encodes the transmembrane transport protein ABCC6. Approximately 35% of *ABCC6*

**Table II. Nonsense mutation and nonsense suppression studies in hereditary skin diseases**

| Disease | Gene | Mutation | Stop codon | Readthrough drug | Application method | Reference |
|---|---|---|---|---|---|---|
| PXE | *ABCC6* | p.R1141X | UGA | PTC124 | | (58) |
| NPPK | *SERPINB7* | c.796C>T | UGA | Gentamicin | Topical 0.1% gentamicin | (59) |
| RDEB | *COL7A1* | R578X/R578X<br>R613X/R1683X<br>R578X/V168GfsX12<br>R2814X/IVS17-2delA<br>R236X/IVS85-1G>A | | Gentamicin | Topical 0.1% gentamicin, intradermal injections | (61) |
| | | p.Q251X<br>p.R578X/p.Q906X<br>p.R2610X<br>p.G2073D/p.R578X | UAG<br>UGA/UAG<br>UGA<br>UGA | Gentamicin, amlexanox | | (62) |
| | | R578X/Q906X<br>Q251X/Q251X<br>R578X/R578X<br>R163X/R1683X | UGA/UAG<br>UAG | Geneticin, paromomycin, gentamicin | | (63) |
| HHD | *ATP2C1* | c.1402C>T | UGA | Gentamicin, paromomycin | Topical 0.1% gentamicin | (64) |
| XP | *XPC* | | UGA | Geneticin, gentamicin, PTC124, BZ16, RTC14 | | (65) |
| | *XPA* | | UGA | *Hargsup* $tRNA^{opal}$ | | (5) |
| HSS | *CDSN* | c.643C>T | UAG | Gentamicin | Topical 0.1% gentamicin | (67) |

gene mutations are nonsense mutations, leading to the synthesis of truncated, non-functional ABCC6 protein. A total of 25 different nonsense mutations have been found in the *ABCC6* gene of PXE patients, including the most common stop codon mutation, p.R1141X. Cells containing the mutant *ABCC6* gene have been treated with different concentrations of PTC124, which can induce the PTC-readthrough in a time-dependent manner. The optimal concentration is 5 mg/ml, and higher concentrations do not improve the readthrough. The efficiency is related to the context sequences of the PTC, which is consistent with previous evidence (58). It is worth noting that PTC124 only causes a partial correction of ABCC6 expression at this concentration, but a small amount of functional protein seems to be sufficient to alleviate the disease phenotype.

### *Nagashima-type palmoplantar keratosis*

Nagashima-type palmoplantar keratosis (NPPK) is caused by mutations in *SERPINB7*. Studies have confirmed that gentamicin has a significant readthrough effect in cells transduced with the mutant gene c.796C>T and in keratinocytes from NPPK patients (59). Subsequently, 0.1% gentamicin ointment has been externally applied to the affected areas of 5 patients with mutant c.796C>T for 4 weeks, and hyperkeratosis was ameliorated (59). In a recent randomized double-blind controlled clinical trial, 20 NPPK patients with nonsense mutations were given topical gentamicin ointment for 30 days (60). The symptoms of hyperkeratosis and the peculiar smell of the treatment group were significantly improved compared with the control group. The improvement effect of 0.3% gentamicin ointment was more obvious than that of 0.1%, although there was no statistical difference. However, erythema did not improve significantly, which is consistent with previous studies (60). This may be due to the fact that the level of restored functional protein is still insufficient, or the functional protein induced by gentamicin has not yet fully exerted its effect.

### *Recessive dystrophic epidermolysis bullosa*

The prevalence of nonsense mutations in recessive dystrophic epidermolysis bullosa (RDEB), in which the production of type VII collagen (COL7A1) is hindered, is close to 30%. A preliminary study involving 0.1% topical or intradermal injection of gentamicin in 5 RDEB patients with nonsense mutations has provided very encouraging results, clearly indicating the reconstitution of full-length COL7A1 expression and the formation of anchoring fibrils. The frequency of blistering was decreased, and wound healing was accelerated in the experimental group (61). Atanasova et al. (62) demonstrated in *in vitro* experiments that amlexanox, as a PTC-readthrough compound, induces the synthesis of full-length COL7A1 in fibroblasts and keratinocytes of RDEB patients. Gentamicin has also been shown to recover functional protein in RDEB cells (both keratinocytes and fibroblasts, which harbour COL7A1 mutation) (63).

### *Hailey-Hailey disease*

Hailey-Hailey disease (HHD), also known as chronic benign familial pemphigus, is linked to mutations in the *ATP2C1* gene encoding hSPCA1. More than 80 pathogenic *ATP2C1* mutations have been reported in HHD patients, 20% of which are caused by PTCs resulting in the synthesis of a truncated form of hSPCA1. It has been found that 0.1% topical gentamicin can reduce the erythema area and increase the healing rate in an HHD patient who carries a UGA nonsense mutation (R468X). In addition, *in vitro* cell experiments have shown that the full-length hSPCA1 protein is increased in the presence of paromomycin (64).

### *Xeroderma pigmentosum*

Fifteen percent of patients with xeroderma pigmentosum (XP) have been identified as carrying PTCs. XP is genetically divided into seven different types (XPA to XPG) and one variant (XPV). Studies have reported that treatment of patients' primary XP-C cells with NMD inhibitors can lead to a significant increase in *XPC* mRNA levels and XPC protein production; the same results can be obtained by treating cell lines with geneticin. Geneticin has higher readthrough efficiency and toxicity than other aminoglycosides, such as gentamicin (10). Treatment with non-aminoglycoside compounds, such as PTC124, BZ16, and RTC14, can also lead to increased levels of *XPC* mRNA. Although the protein yield is small, as little as 1% of the normal protein function after PTC-readthrough may be sufficient to restore a nearly normal or clinically less severe phenotype, and the toxicities of these compounds are lower than those of geneticin and gentamicin (47, 65). Interestingly, based on the above studies, the authors of one study (65) proposed that topical application of aminoglycoside drugs may also help prevent sun-induced skin cancer in XP patients, and at the same time can greatly reduce the toxic side-effects of the drug.

In another study focusing on XP-A cells in XP disease, suppressor tRNA was found to repair genetic defects caused by nonsense mutations and partially restore the *XPA* gene deficiency phenotype (5). Perhaps due to the low abundance of *XPA* transcripts and tRNAs, and multiple factors affecting the readthrough of nonsense suppression and the regulation of other biological mechanisms in the cell, the expression and inhibition efficiency of suppressor tRNAs are still low (66).

### *Hypotrichosis simplex of the scalp*

Hereditary hypotrichosis simplex of the scalp (HSS) is usually caused by a nonsense mutation in the *CDSN* gene

that encodes corneodesmosin. A recent study by Peled et al. (67) has found that gentamicin restores full-length corneodesmosin expression in cells transduced with the mutant gene c.643C>T and in primary keratinocytes from HSS patients, and local application of 0.1% gentamicin ointment to the scalp of 4 HSS patients relieved symptoms. This study further confirms the potential value of topical aminoglycoside drugs in the treatment of hereditary skin diseases.

In conclusion, the purpose of nonsense suppression therapy is typically to allow for PTC-readthrough in various ways to hinder the termination of protein translation and ultimately alleviate the disease phenotype. In this review, we introduced the readthrough mechanisms of classic aminoglycosides, the novel compound PTC124, NMD inhibitors, and suppressor tRNA, and summarized the research status of the latest nonsense suppression treatments for hereditary skin diseases. In general, nonsense suppression therapy has achieved positive effects in treating several hereditary skin diseases and may provide a new treatment regimen. But, to date, the application of nonsense suppression therapy in hereditary skin diseases is limited. Emerging technologies such as RNA pseudouridylation, RNA editing, and CRISPR/Cas9 technology have been clearly proven to have therapeutic effects in other non-dermatological genetic diseases (68), but they have not yet been applied to dermatology. Therefore, the above-mentioned technology has broad prospects in the treatment of hereditary skin diseases. In addition, due to the effectiveness of gentamicin topical preparations on skin lesion healing, if aminoglycosides are made into topical preparations, they are very safe under the premise that the blood concentration can be controlled. The development of new dressings or the preparation of other readthrough drugs into topical preparations may also be a direction for future treatments. The combined use of NMD inhibitory compounds and PTC-suppression drugs may improve the effects of nonsense suppression treatment by increasing the abundance of PTC-containing mRNA substrates (33). Some non-aminoglycoside drugs, such as PTC124, also show better human tolerance. The gradual and stable expression of suppressor tRNA will provide advantages for correcting hereditary skin diseases. As a small transcript, tRNA can be developed into nanoparticle materials for topical or systemic delivery. How to maximize the targeting of nonsense suppression therapy and reduce related side-effects will be the focus of future research.

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The authors confirm that this study complies with ethical standards.

*The authors have no conflicts of interest to declare.*

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