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Revertant mosaicism in skin: natural gene therapy

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Abstract

Revertant mosaicism is a naturally occurring phenomenon involving spontaneous correction of a pathogenic mutation in a somatic cell. Recent studies suggest that it is not a rare event and that it could be clinically relevant to phenotypic expression and patient treatment. Indeed, revertant cell therapy represents a potential “natural gene therapy” because *in vivo* reversion obviates the need for further genetic correction. Revertant mosaicism has been observed in several inherited conditions, including epidermolysis bullosa, a heterogeneous group of blistering skin disorders. These diseases provide a useful model for studying revertant mosaicism because of the visual and accessible nature of skin. This overview highlights the latest developments in revertant mosaicism and the translational implications germane to heritable skin disorders.

Revertant mosaicism

Somatic reversion of a mutant phenotype was first identified in Lesch-Nyhan syndrome in 1988 [1]. This phenomenon has since been reported in other hematological conditions, such as primary immunodeficiency diseases and Fanconi's anemia, and nonhematological disorders, including epidermolysis bullosa (EB), Duchenne muscular dystrophy and tyrosinemia [2]. Revertant somatic mosaicism, a term popularized by Jonkman *et al.* [3], is characterized by the spontaneous partial or complete reversal of an affected somatic cell or cells to a wild-type phenotype [4]. It is not uncommon, occurring in up to 11% (30/272 cases) of patients with Wiskott-Aldrich syndrome [5], 18% (5/28 cases) of individuals with Fanconi's anemia [6] and 35% (7/20 cases) of subjects with nonHerlitz junctional EB [4]. The reasons for the relatively high frequency are unclear and could reflect a selective advantage of the revertant cell over its mutant counterpart, as well as high mutation rates and DNA polymerase errors [7]. With regards to revertant phenotypes, the skin provides a unique opportunity to investigate clinical patterns of disease expression such as mosaicism.

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For diseases such as EB, the key proteins underlying skin blistering and in which revertant mosaicism has been implicated are shown in Figure 1. Revertant mosaicism can occur in the germline or in somatic cells. Germline revertant mosaicism has previously been described in myotonic dystrophy wherein the size of the CTG repeats in two unrelated healthy individuals, born to clinically affected parents, was normal despite having inherited the myotonic dystrophy DNA-marker haplotype [8]. Somatic revertant mosaicism occurs secondarily to a spontaneous correction of a deleterious mutation during mitosis, resulting in a corrected cell population within a predominantly mutant population.

Mechanisms of *in vivo* reversion

Although originally thought to be rare single events, revertant mosaicism can occur as multiple independent events within the same patient [2,4,9,10]. In addition, *in vivo* reversion can involve multiple cell lineages [10] or be limited to a particular cell clone [2,11]. For instance, in Wiskott-Aldrich syndrome, revertant mosaicism appears to predominantly involve CD8 positive T cells [11,12], although other cell types have been implicated including natural killer cells and B lymphocytes [10], but not myeloid progenitor cells [2].

The corrective mechanisms implicated in revertant somatic mosaicism include back mutation, gene conversion, intragenic recombination and second-site mutation [4]. A schematic illustration of these mechanisms is shown in Figure 2. Back or reverse mutation occurs when the pathogenic mutation changes to a wild-type sequence, thereby restoring translation of the wild-type protein. This probably occurs randomly or reflects an increased mutation rate, as evidenced by its occurrence in disorders characterized by genomic instability, such as Bloom syndrome, or in conditions that hypothetically might be influenced by environmental exposure such as ultraviolet light, as for example in skin disorders [13]. Gene conversion and intragenic recombination both involve homologous recombination and cannot be dismissed as potential reversion mechanisms in compound heterozygotes [13]. Gene conversion involves the unidirectional and nonreciprocal transfer of genetic material from a donor sequence to a highly homologous acceptor sequence. This results in the acceptor being replaced wholly or in part by a sequence that is derived from the donor, while the donor sequence remains unchanged (for review, see [14]). Intragenic crossover is a further mechanism of homologous recombination involving the reciprocal transfer of genetic material between the donor and acceptor sequences.

Second-site mutation refers to the presence of a spontaneous compensating mutation either up- or downstream of a pathogenic frameshift, resulting in restoration of the reading frame. Second-site mutations can be intronic and act as splice enhancers [15]. Occasionally, the translated protein is abnormal because an aberrant sequence is present between the pathogenic and the second-site mutation, leading to partial reversion despite the restoration of the reading frame. Other less characterized reversion mechanisms include retrotransposons [16] and DNA slippage [17]. Retrotransposons are mobile genetic elements that were originally shown to modify gene expression in maize [18]. These controlling elements have since been shown to induce revertant mosaicism in mammals [19,20]. However, there is no evidence to implicate retrotransposons in revertant mosaicism in a human disorder, although it has been postulated as a possible reversion mechanism in Duchenne muscular dystrophy [16]. DNA slippage has been proposed as a potential mechanism in a case of Wiskott-Aldrich syndrome in which there was a spontaneous deletion of a six base pair insertion in the *WASP* gene in the patient's lymphocytes [17]. DNA slippage has previously been implicated to explain insertion and deletion of DNA repeats in both germline and somatic cells, particularly in regions of a high GC content [17]. Revertant mosaicism has been reported in several human disorders. Understanding the

involved mechanisms provides insight into how phenotypic expression of disease could be modified or manipulated.

Clinical manifestations of revertant mosaicism

In vivo reversion of somatic cells tends to predominantly involve tissues with high cell proliferation rates including the skin, liver and the hematopoietic system [21]. For instance, revertant mosaicism has been described in several types of immunodeficiency syndromes including Wiskott-Aldrich syndrome [7,10,11,17,21-26], Omenn syndrome [27], adenosine deaminase deficiency [28,29], X-linked severe combined immunodeficiency [30], X-linked ectodermal dysplasia and immunodeficiency [31], T cell immunodeficiency [32] and leukocyte adhesion deficiency type 1 [33,34]. Revertant mosaicism oftentimes leads to phenotypic improvement but can modify the phenotypic expression leading to atypical presentations of disease. For instance, *in vivo* reversion of a case of X-linked severe combined immunodeficiency, in which a second-site suppressor mutation was responsible for revertant T cells that infiltrated the skin, resulted in a clinical presentation resembling Omenn syndrome [35]. The timing of reversion during development can also influence the extent of revertant mosaicism and the severity of the condition. In the skin, early reversion events during embryonic development are more likely to result in larger revertant patches [9]. In addition, revertant cells that express wild-type protein might have a selective advantage *in vivo* [7], possibly reflecting the functional importance of the reverted protein in the cells [36]. Selective advantage, however, might not always occur, as evidenced by unchanging revertant patches in several patients with mosaic nonHerlitz junctional EB [9]. Moreover, phenotypic improvement might not always result from revertant mosaicism. Other mechanisms such as in-frame exon skipping of the exon bearing the pathogenic mutation or containing a premature termination codon can eliminate the disease-causing mutation and rescue protein translation [37-40].

Revertant mosaicism in heritable skin disorders

In the skin, revertant mosaicism can manifest as patches of seemingly normal skin (Figure 3). Suspicion of revertant mosaicism in the clinic can then be easily investigated through skin biopsy analysis, typically using immunohistochemistry and/or transmission electron microscopy (Figures 3). A summary of revertant mosaicism in inherited skin diseases is provided in Table 1. The first case of cutaneous revertant mosaicism was described in a 28-year old female with generalized atrophic benign epidermolysis bullosa (GABEB) [3]. This report demonstrated for the first time the role of mitotic gene conversion as a corrective mechanism in a human genetic disorder [3]. GABEB, now known as nonHerlitz junctional EB [41], is characterized by generalized skin blistering from birth, dental abnormalities, universal alopecia and nail dystrophy [42]. The proband was a compound heterozygote for two *COL17A1* mutations, namely a maternally-inherited frameshift mutation, c.1706delA, and a paternally-inherited nonsense mutation, p.Arg1226X. Examination showed multiple clinically unaffected patches distributed symmetrically over the extensor surface of hands and upper arms. Type XVII collagen was not detected in her affected skin whereas in the revertant skin, a mosaic pattern for type XVII collagen was observed by immunofluorescence microscopy. Sequence analysis of DNA extracted from these cells revealed the presence of the paternally-inherited mutation but the absence of the maternally-inherited mutation, suggesting that a reverse mutation abolished the maternal mutation in these keratinocytes. Further analysis identified a conversion tract spanning at least 381 base pairs in the maternal DNA.

Revertant mosaicism resulting from multiple different corrective mechanisms has been identified in the *COL17A1* gene in two unrelated subjects with GABEB [9]. This

observation dispelled the notion that revertant mosaicism results from a single or preferred mechanism [9]. The first patient was a 75-year old Dutch male with the germline *COL17A1* mutations denoted p.Arg1226X in exon 51 and c.4424_4425insC in exon 54. Since birth, he suffered from generalized trauma-induced skin blistering, universal alopecia, rudimentary nails and enamel hypoplasia. Close examination revealed a circular nonenlarging patch of normal-looking skin measuring ~2 cm² on his right middle finger. Evaluation of type XVII collagen expression in the reverted skin biopsies by immunofluorescence microscopy revealed a mosaic pattern with positive areas having similar intensity to normal control skin. By contrast, type XVII collagen was not detected in nonreverted skin. Sequencing of genomic DNA extracted from type XVII collagen positive keratinocytes from skin sections from a right middle finger biopsy revealed an additional *COL17A1* mutation denoted c.4463-1G>A at the intron 54-exon 55 border on the same allele as the original c.4424_4425insC mutation. This second-site mutation changes the consensus sequence of the 3' splice site with the invariant guanine (G) being replaced by adenine. Because exon 55 starts with a G, a novel splice site can be created one nucleotide downstream. Sequence analysis of cDNA synthesized from these type XVII collagen positive keratinocytes subsequently revealed a deletion of G at position c.4463, resulting in frameshift correction. Furthermore, additional studies in skin obtained from a clinically nonreverted piece of skin from the right upper shoulder in this patient revealed that ~25% of basal keratinocytes stained positively for type XVII collagen. Reversion in these keratinocytes resulted unexpectedly from a mechanism that eliminated the nonsense mutation, p.Arg1226X. To investigate the corrective mechanism in these revertant keratinocytes, single nucleotide polymorphisms (SNPs) around *COL17A1* exon 51, which contained the nonsense mutation, were investigated for loss of heterozygosity. In this case, loss of heterozygosity was not detected, suggesting that the underlying mechanism was a true back mutation.

The second case of revertant mosaicism resulting from multiple corrective mechanisms in the *COL17A1* gene was a 45-year old female with GABEB. In 1997 she was tested for revertant mosaicism, and a gene conversion event that led to loss of the maternal deletion was found [3]. She was re-evaluated to assess whether multiple corrective mechanisms might also occur within this individual. Type XVII collagen, detected by immunofluorescence labeling of skin biopsy from the reverted patch, was present in the whole basement membrane zone. Sequence analysis of extracted DNA from the revertant keratinocytes revealed the presence of both inherited mutations and an additional transversion, c.3782G>C, one nucleotide downstream of the nonsense mutation, which thereby was converted into a missense mutation. Furthermore, another corrective mutation was detected in the biopsy specimens from the forearm and upper arm, namely loss of the maternal c.1706delA insertion, resulting from a large gene conversion event [3].

Revertant mosaicism resulting from multiple second-site mutations was described subsequently in two unrelated individuals with nonHerlitz junctional EB caused by mutations in the *LAMB3* gene, which encodes the $\beta 3$ chain of laminin-332 [43]. The first case was a 46-year old male who was a compound heterozygote for a nonsense mutation, p.Arg635X, and a missense mutation, p.Glu210Lys. He had experienced generalized trauma-induced blistering since birth but spontaneously developed normal looking skin on his left lower leg within an area of chronic skin erosions. A stretch of about 25 basal keratinocytes was found on affected skin with normal laminin-332 staining amidst low intensity laminin-332 labeling. In addition, predominantly normal laminin-332 labeling was found in the revertant skin of his left lower leg, whereas another separate biopsy from reverted skin displayed completely normal immunolabeling. Sequence analysis of genomic DNA from peripheral blood lymphocytes revealed germline *LAMB3* mutations, namely p.Arg635X and p.Glu210Lys mutations. To investigate the reversion mechanism, DNA was extracted from laser-microdissected affected and revertant keratinocytes. Sequence analysis

of DNA from the revertant keratinocytes revealed the presence of a second-site mutation, denoted c.628+42G>A. This corrective mutation was, however, not identified in another revertant patch where a different nucleotide change, denoted c.596G>C, was identified. The same group also reported multiple second-site mutations in a second individual with nonHerlitz junctional EB with the germline *LAMB3* mutation p.Glu210Lys (c.628G>A). This individual reported several patches of clinically unaffected skin on his arms, shoulders and chest. Sequence analysis of DNA extracted from several revertant patches revealed distinct second-site mutations in different specimens.

The first case of revertant mosaicism in the recessive dystrophic subtype of epidermolysis bullosa (RDEB) was recently reported [44]. The proband was a 41-year old white British man who had germline *COL7A1* compound heterozygous mutations, namely a maternally-inherited nonsense mutation, p.Arg578X in exon 13, and a paternally-inherited frameshift mutation denoted c.7786delG in exon 104. The patient had large areas of fragile skin and erosions, mutilating scars and a history of recurrent cutaneous squamous cell carcinomas. However, two longstanding and non-expanding normal-looking patches of skin were present on his left wrist and right shin, respectively. Sequencing of genomic DNA extracted from both reverted patches revealed the presence of both mutations; however, sequencing of his cDNA derived from the reverted skin from his wrist revealed the presence of the maternal mutation but the absence of the paternal mutation. Further analysis using long-range PCR disclosed that in the reverted patches, the wild-type sequence for exon 104 occurred on the same allele bearing a paternal nucleotide for the exon 21 polymorphism, suggesting that an intragenic crossover had occurred between exons 21 and 104 [44]. Soon afterwards, a second case of RDEB and revertant mosaicism was reported in a 42-year old Spanish woman who had a blister-free patch of skin on her left forearm. That patch had developed three years earlier and had not extended [45]. The reverted skin showed similar intensity of type VII collagen staining compared to normal control skin, whereas little to no signal was detected in the unreverted skin. Sequence analysis revealed that she carried a homozygous frameshift mutation in the *COL7A1* gene, denoted c.6527insC, resulting in a premature termination codon both in the germline as well as in the reverted and unreverted skin cells. However, an additional mutation one nucleotide downstream of the inherited frameshift mutation was only present in the reverted keratinocytes, leading to frameshift correction and the formation of a normal basement membrane with type VII collagen.

Revertant mosaicism involving an autosomal dominant skin disorder has been reported in a Scottish female with Dowling-Meara EB simplex [46]. This condition is caused by dominant-negative mutations in the keratin genes, *KRT5* or *KRT14*, and is characterized by herpetiform blistering from birth. The proband had a heterozygous germline mutation in *KRT14*, p.Arg125Cys, but noted significant clinical improvement in her skin blistering from her early teens, particularly on her trunk. A skin biopsy taken from unaffected skin of her lower abdomen showed a normal keratin filament network in the majority of cultured keratinocytes. The p.Arg125Cys was barely detectable by sequence analysis of full-length *KRT14* cDNA derived from these cells and low levels of overlapping sequences were identified. These overlapping traces contained a heterozygous one base pair insertion upstream of p.Arg125Cys. This insertion, c.242insG, resulted in a premature termination codon and was present on the same allele as p.Arg125Cys, thereby nullifying the dominant-negative effect of the missense mutation.

Revertant mosaicism has recently been reported in another autosomal dominant disorder, ichthyosis with confetti (IWC) [47]. This case is noteworthy since the elucidation of the genetic basis of revertant mosaicism in the confetti patches led to speculation that the gene responsible for IWC is present within the area of loss of heterozygosity. IWC is characterized by generalized skin redness from birth, prominent scale and thickening of the

palms and soles. Early in life, hundreds to thousands of pale confetti-like spots appear on the skin and gradually increase in size [48]. Analysis of the confetti spots from several kindreds revealed normal skin histology. Genotyping of several revertant spots showed a single large segment of copy-neutral loss of heterozygosity (LOH) on chromosome 17q. This result is consistent with mitotic recombination as mechanism of LOH. Further assessment of the LOH led to the localization of the IWC disease locus to an approximately 3 Mb interval on 17q. This interval contains a gene cluster that encodes 28 type 1 keratins and 24 keratin-associated proteins. Illumina sequencing of a parent-offspring trio subsequently revealed that the affected subject had a single *de novo* mutation in *KRT10*, encoding keratin 10. The mutation was absent in the revertant spots. IWC is thus characterized by a high frequency of spontaneous reversion, which has not previously been observed in other disorders in which revertant mosaicism has been reported. Revertant mosaicism generally leads to phenotypic amelioration in the skin resulting from a single or a combination of reversion mechanisms. Clinically, physicians looking after patients with inherited skin disorders should look specifically for potential areas of reversion in the skin.

Partial revertant mosaicism in heritable skin disorders

Partial revertant mosaicism is a phenomenon in which the revertant protein is immunohistochemically reactive but nonfunctional, resulting in no clinical improvement [49]. Partial revertant mosaicism caused by the incomplete correction of a pathogenic deletion by a frame-restoring mutation has been reported in a 56-year old Austrian female with GABEB [50]. The affected individual harbored the homozygous *COL17A1* germline deletion, c.4003delTC. Although she did not report areas of normal skin, focal staining for type XVII collagen was present in the skin biopsies taken from her left lower back and her right upper arm. Sequence analysis of the DNA extracted from the revertant keratinocytes revealed that these cells harbored two mutant *COL17A1* alleles; one contained only the original deletion (c.4003delTC) and the second contained the deletion (c.4003delTC) as well as an insertion mutation, c.4080insGG. This insertion restored the reading frame just prior to the premature termination codon created by the original deletion, countered nonsense-mediated decay and led to the production of type XVII collagen. The resulting protein, however, which was predicted to contain 25 missense amino acids between the deletion and the insertion, was deemed nonfunctional because the skin remained clinically fragile.

Partial revertant mosaicism was also identified in a 67-year old female with recessive epidermolysis bullosa simplex. She was homozygous for a c.526-2A>C acceptor splice site mutation in the *KRT14* gene [49]. Previous immunostaining of her lesional skin and isolated keratinocytes had not detected keratin 14. Staining on a repeat biopsy from her clinically affected skin several years later showed spontaneous re-expression of keratin 14 in some basal keratinocytes and, in culture, a mixture of keratin 14-positive and negative keratinocytes were observed. Transmission electron microscopy revealed the re-expression of tonofilament bundles in the basal keratinocytes of her revertant skin, although they were reduced in number. Immunoblotting of revertant keratinocyte-derived lysate revealed the presence of protein corresponding to the expected molecular weight of the keratin 14 polypeptide. DNA analysis revealed no additional mutations in the *KRT14* sequence, but analysis of mRNA isolated from mosaic skin keratinocytes revealed the presence of an additional in-frame transcript (528T>G, 529Δ6), encoding abnormal keratin 14 with a two residue deletion and one amino acid change, resulting in the expression of a revertant but abnormal keratin 14 polypeptide. This finding thus provided an explanation for the positive antibody staining pattern and the appearance of semifunctional intermediate filaments that did not revert the clinical phenotype. This case is noteworthy because the additional mutation was present only in the mRNA, suggesting that an as yet unidentified modulating factor affected mutant *KRT14* pre-mRNA processing [49]. Partial revertant mosaicism thus

does not always lead to phenotypic improvement and can be serendipitously observed when skin or skin-derived cells are screened for other purposes.

Revertant mosaicism as “natural gene therapy”

The feasibility of *ex vivo* gene therapy for genetic skin disorders was demonstrated by the successful engraftment of *LAMB3* cDNA retrovirally-corrected epidermal sheets and maintenance of a functional epidermis in a 36-year old male with nonHerlitz junctional EB [51]. However, concerns remain regarding the safety of this approach as there is a potential for genotoxic risk if the viral long terminal repeats insert into the human genome or in a manner that might promote carcinogenesis [52-54]. By contrast, the natural occurrence of revertant mosaicism creates a unique opportunity for therapy in patients because the presence of reverted cells circumvents the need for retroviral vectors. Whatever the corrective process in this “natural gene therapy”, the occurrence of revertant mosaicism provides opportunities for translational research. To date, the only attempt to utilize revertant cell therapy in a clinical setting was in an individual with revertant mosaic nonHerlitz junctional EB associated with mutations in *COL17A1* [55]. This subject was also the first case in whom revertant mosaicism was reported [3]. In this case, revertant keratinocytes were isolated and expanded into epidermal sheets that were subsequently grafted back onto the patient. Although ~30% of keratinocytes were revertant in culture, fewer than 3% of the cells remained corrected in the graft and there were no clinically useful outcomes [55]. This study provides proof of principle that revertant therapy is feasible but further optimization is required for successful clinical application.

Concluding remarks

Over the past two decades, considerable research energy has focused on gene discovery and understanding of disease pathophysiology. Although basic research remains paramount to scientific advancement and optimal clinical translation, there is also a need to focus on the creation and optimization of therapeutic strategies that might ameliorate disease. While revertant mosaicism is a relatively rare event, the unraveling of the molecular mechanisms in this phenomenon may offer hope of personalized therapy in patients with inherited skin diseases. Several important questions, however, remain unanswered (Box 1). Clarification of these issues will help translate revertant mosaicism to the clinic. In the skin, revertant mosaicism generally is focal, leading to isolated patches of healthy skin. In contrast, revertant mosaicism in other tissues such as hematopoietic tissue appears to be more global, leading to overall phenotypic improvement. Perhaps in the context of revertant mosaicism, nature is indirectly offering us a window to develop strategies for natural gene therapy, opportunities that could provide therapeutic hope for thousands of people living with inherited skin diseases.

Box 1. Outstanding questions

- Why does revertant mosaicism occur?
- What are the molecular events underlying the various mechanisms of *in vivo* reversion?
- Are there epigenetic mechanisms involved in revertant mosaicism?
- How do culture conditions influence the phenotype of cells with revertant mosaicism?
- Does the revertant skin expand with time?

- Can therapy using reverted cells create and sustain clinical benefits for patients?

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Glossary

Epidermolysis bullosa

is a group of inherited skin fragility syndromes the hallmark of which is trauma-induced blistering. Mutations in genes encoding various macromolecules that normally provide structural integrity to keratinocytes or adhesion between epidermis and the dermis underlie this disease

Hemidesmosomes

are cell-extracellular matrix adhesion complexes that offer mechanical resilience to tissues. In skin they are located in basal

	keratinocytes and secure adhesion to epidermal basement membrane.
Tonofilament bundles	consist of polymerized keratin intermediate filaments. The keratin filaments provide the cytoskeleton to maintain keratinocyte shape and structural resilience.
Keratins 5 and 14	are the major keratins in the basal layer of the skin. In epidermolysis bullosa simplex caused by <i>KRT5</i> or <i>KRT14</i> mutations keratin filament integrity is compromised and blistering occurs in basal keratinocytes
Keratins 1 and 10	are present in the suprabasal layers of the epidermis. As keratinocytes differentiate, expression of keratins 5 and 14 is downregulated while the expression of keratins 1 and 10 is upregulated
Anchoring fibrils	are adhesion structures that provide integrity between epidermal basement membrane and dermal collagen; they mainly consist of type VII collagen.

Figure 1.

The molecular basis of inherited skin blistering involving hemidesmosome-associated proteins. (a) Light microscopy image of the skin; the boxed area indicates a dermal-epidermal junction (The section is stained with hematoxylin & eosin; scale bar=50 μ m); (b) Transmission electron microscopy image of a dermal-epidermal junction; hemidesmosome attachment complexes are boxed (scale bar=0.1 μ m); (c) A schematic representation of the protein organization of dermal-epidermal attachment complexes, the intrinsic proteins and the genes encoding them, and the associated genetic diseases. Revertant mosaicism has been reported for keratin 14 (*KRT14*), laminin-332 (*LAMB3*), type XVII collagen (*COL17A1*) and type VII collagen (*COL7A1*). In addition, revertant mosaicism has been demonstrated in IWC due to mutations in *KRT10*, encoding the suprabasal keratin 10 (not illustrated) Abbreviations: IC, intracellular (basal keratinocyte); PM, plasma membrane; LL, lamina lucida; LD, lamina densa; EC, extracellular (upper dermis); IWC – ichthyosis with confetti

Figure 2. Possible mechanisms underlying revertant mosaicism. Four main mechanisms of *in vivo* reversion have been reported. (a) A schematic showing back mutation, intragenic cross-over (single and double) and gene conversion. Unlike intragenic cross-over, gene conversion involves the unidirectional and nonreciprocal transfer of genetic material between a donor and an acceptor sequence. In this schematic, the paternal chromosome is shown in light blue with the pathogenic mutation illustrated in dark blue and the maternal chromosome is indicated in green with the mutation in orange. Asterisks represent the chromosome in which the mutation has been corrected. (b) Second-site mutation is a mechanism of correction for a pathogenic frameshift mutation. It can occur either upstream or downstream of the frameshift mutation and even in the intron, resulting in complete or partial restoration of protein translation.

Figure 3.

Revertant mosaicism in RDEB. (a) Clinical evidence of reverted (RS) and unreverted (nonRS) skin; note the severe blistering phenotype in the nonRS area. (b) Immunofluorescence image showing Type VII collagen expression shows bright linear labeling at the dermal-epidermal junction in normal human skin (NHS) and in the RS but no signal is detected in the nonRS sample (dashed line indicates dermal-epidermal junction; asterisk depicts subepidermal blistering, scale bar=50 μ m). (c) Transmission electron microscopy images showing anchoring fibrils beneath the lamina densa in NHS and also in RS (arrows) but not in nonRS samples. There is also blistering beneath the lamina densa in nonRS sample (asterisk). Scale bar=0.2 μ m

Table 1

Summary of revertant mosaicism in inherited skin disorders

Diagnosis	Reversion (partial or complete)	Reversion mechanism	Location and expansion of patches	Clinical implications of reversion	Reference
nonHerlitz junctional epidermolysis bullosa (AR; <i>COL17A1</i>) p.Arg1226X/c.1706delA	Complete	Mitotic gene conversion	Extensor surface of hands and upper arms; expansion of these patches was variable – some expanded gradually while others remain static.	Clinically healthy patches of skin in a phylloid distribution. Blistering could not be evoked.	[3]
nonHerlitz junctional RB (AR; <i>COL17A1</i>) c.2342delG/c.3781C>T	Complete	Second site mutation, c.2368+2C>T, on allele containing c.2342delG	N/D	N/D	[4]
nonHerlitz junctional RB (AR; <i>COL17A1</i>) c.2342delG/c.3781C>T	Complete	Second-site mutation (c.2342insG) or gene conversion on allele containing c.2342delG	N/D	N/D	[4]
nonHerlitz junctional RB (AR; <i>COL17A1</i>) c.2342delG/c.2342delG	Complete	Second site mutation on 1 allele (c.2344C>T)	N/D	N/D	[4]
nonHerlitz junctional EB (<i>COL17A1</i>) p.Arg1226X/c.4424_5insC	Complete	Multiple different correcting events 1 arm - back mutation which corrected the p.Arg1226X mutation (c.3781T>C) 2 right middle finger – second site mutation (c.4463-1G>A)	Right middle finger; no extension with time	Patch of healthy skin measuring 2 cm ² that has never blistered	[9]
nonHerlitz junctional EB (AR; <i>COL17A1</i>) p.Arg1226X/c.1706delA	Complete	Multiple different correcting events 1 Left hand – gene conversion 2 Ankle – second site mutation (c.3782G>C on 1 allele) 3 Forearm and upper arm – gene conversion	Left hand, forearms, upper arms and lower legs; no extension with time.	10% of total body skin was clinically normal and not prone to blistering	[9]
nonHerlitz junctional epidermolysis bullosa (AR; <i>COL17A1</i>) 4003delTC/4003delTC	Partial	Second-site mutation on 1 allele c.4080insGG	N/D	No improvement in skin blistering	[50]
nonHerlitz junctional EB (AR; <i>LAMB3</i>) p.Arg635X/p.Glu210Lys	Complete	Multiple correcting second-site mutations in different biopsies c.628+42G>A c.596G>C	Left lower leg; extension with time	Clinically unaffected skin that was no longer susceptible to trauma-induced blistering	[43]
nonHerlitz junctional EB (AR; <i>LAMB3</i>) c.628G>A/c.628G>A	Complete	Multiple correcting second-site mutations in different biopsies c.565-3T>C c.619A>C p.Lys207Gln c.629-1G>A	Arm, shoulder and chest; no extension with time	Clinically unaffected skin that was blister-free	[43]
Epidermolysis bullosa simplex Dowling-Meara (AD; <i>KRT14</i>) p.Arg125Cys	Complete	Second-site mutation c.242insG	Trunk; extension over time not reported	Improvement on skin blistering. Rarely gets blisters which also tend to heal quickly.	[46]

Diagnosis	Reversion (partial or complete)	Reversion mechanism	Location and expansion of patches	Clinical implications of reversion	Reference
Epidermolysis bullosa simplex (AR; <i>KRT14</i>) c.526-2A>C/ c.526-2A>C	Partial	Second-site mutation c.528T>G, c.529A6	N/D	No improvement in skin blistering	[49]
Kindler syndrome (AR; <i>KIND1</i>) No mutation analysis	Complete	N/D	Dorsal feet, left palm and neck. On the left palm, a 4 cm diameter patch of normal skin was present. Expansion was not documented	Clinically healthy skin with normal histology	[56]
Recessive dystrophic epidermolysis bullosa (AR; <i>COL7A1</i>) p.Arg578X/ c.7786delG	Complete	Intragenic cross over	Left wrist and right shin; no extension over time	Patches of skin measure 8 × 5 cm and do not blister despite repeated trauma	[44]
Recessive dystrophic epidermolysis bullosa (AR; <i>COL7A1</i>) c.6527insC/ c.6527insC	Complete	Second site mutation (c.6528delT on 1 allele)	Left forearm; no significant extension with time	Patch of skin measures 8 × 4.5 cm present for 3 years. Never blistered unlike the rest of her skin	[45]
Ichthyosis with confetti (AD; <i>KRT10</i>) 7 different mutations detected in 7 unrelated individuals, all affecting exon 7	Complete	Mitotic recombination	Limbs, trunk and back; expansion with time.	Hundreds to thousands of confetti-like lesions up to 4 cm in diameter with normal skin histology	[47]

N/D – not documented; AR – autosomal recessive; AD – autosomal dominant.