Review

Revertant mosaicism in skin: natural gene therapy

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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) (see Glossary), 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 regard to revertant phenotypes, the skin provides a unique opportunity to investigate clinical patterns of disease expression, such as mosaicism. 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

Glossary

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

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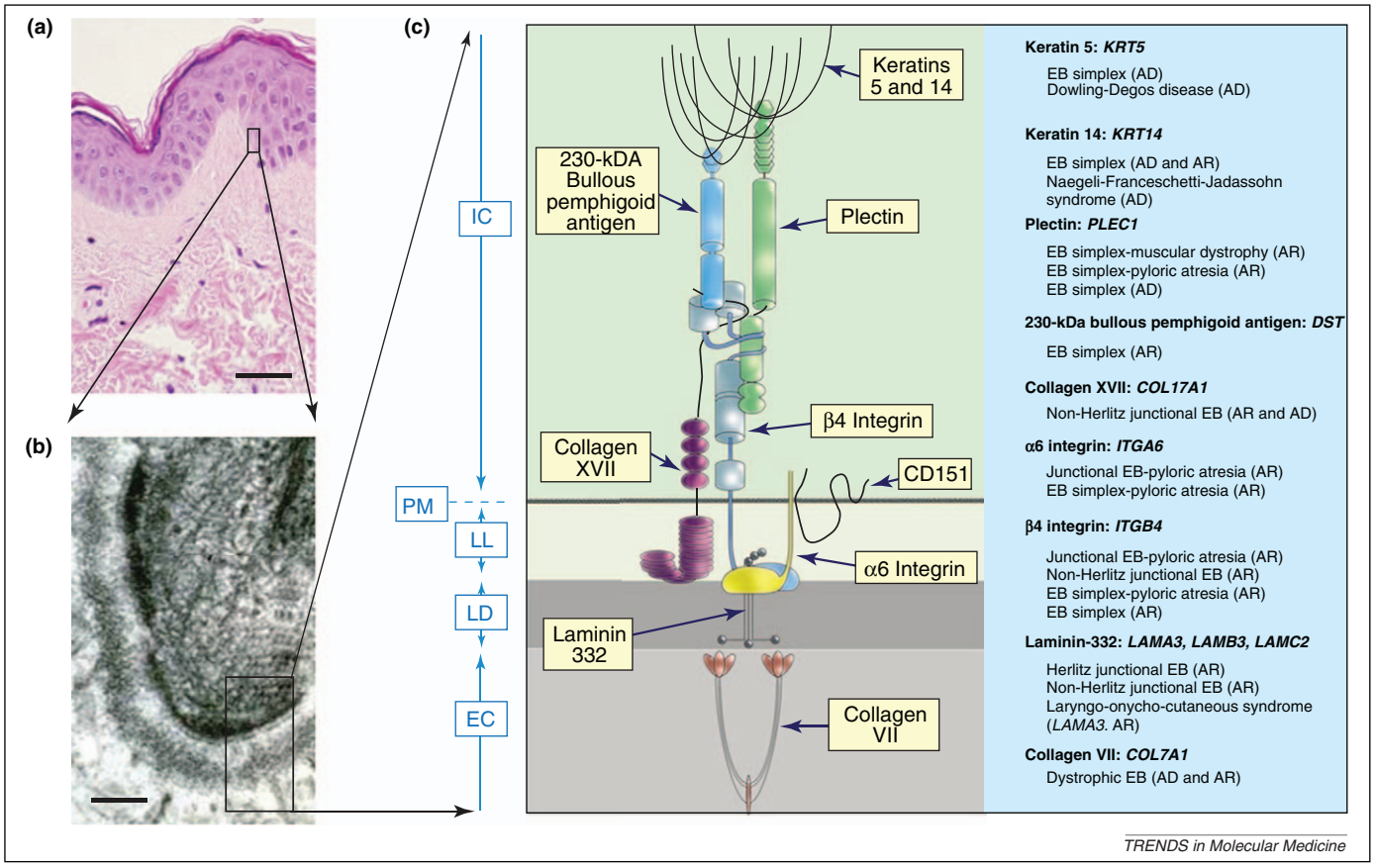

Figure 1. The molecular basis of inherited skin blistering involving hemidesmosome-associated proteins. **(a)** Light microscopy image of the skin; the boxed area indicates a dermal–epidermal junction (the section is stained with hematoxylin and eosin; scale bar = 50 μm). **(b)** Transmission electron microscopy image of a dermal–epidermal junction; hemidesmosome attachment complexes are boxed (scale bar = 0.1 μm). **(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 ichthyosis with confetti 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).

by genomic instability, such as Bloom syndrome, or in conditions that hypothetically might be influenced by environmental exposure such as ultraviolet light, 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 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, whereas 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 upstream 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 only 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].

Thus, collectively, 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, the liver and the hematopoietic system [21]. For instance, revertant mosaicism has been described in several types of immunodeficiency syndromes, including