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Targeting the Palm: A Leap Forward Toward Treatment of Keratin Disorders

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Any rational therapy benefits from an understanding of basic biology and the simplicity of its strategy. Among keratinopathies, epidermolytic palmoplantar keratoderma stands out by virtue of hotspot mutations in the *KRT9* gene, exclusively expressed in the palmoplantar epidermis. In this issue, Leslie Pedrioli *et al.* report on the successful application of *KRT9*-specific siRNAs in cultured cells and in a mouse model. The study beautifully illustrates the potency of a thorough experimental approach and the challenges that remain, especially in its delivery.

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Efficacy, specificity, and potency of a drug represent the lynchpins of a successful therapy. In the case of genetic disorders, onset of disease and the cell type of origin mount additional hurdles to be overcome. Keratinopathies are caused mostly by dominant mutations in at least 23 of the 54 human keratin genes expressed as the "keratin pairs" that typify epithelial differentiation (Szeverenyi *et al.*, 2008; http://www.interfil.org). Therefore, sites of expression reveal the major site(s) of disease, despite the notion that most keratinocytes express 4–10 different keratin proteins. Further, there appears to be reasonable genotype–phenotype correlation, indicating that mutations severely compromising the cytoskeleton's integrity cause more severe disease phenotypes than those that do not. Although pathomechanisms of the keratinopathies are more complex than originally thought (Coulombe and Lee, 2012), one can reasonably argue that reducing the expression of the mutant allele in dominant keratin disorders should restore a more functional cytoskeleton from the intact allele, leading to greater tissue integrity. Proof of principle stemmed from mouse models in which the ratio of mutant and normal keratin alleles has been modified genetically (Cao *et al.*, 2001; Hesse *et al.*, 2007).

Among keratinopathies, epidermolytic palmoplantar keratoderma (EPPK) is unique for several reasons: the expression of the culprit, *KRT9*, is restricted to the upper strata of the palmoplantar epidermis, forming a cytoskeleton together with at least six additional keratins. The majority of EPPK patients suffer from a missense mutation in one of the three hotspot codons, giving rise to a focal epidermolytic keratoderma (http://www.interfil.org). This setting invited Leslie Pedrioli *et al.* (this issue, 2012) to develop an siRNA-based therapy approach, testing both generic and mutation-specific siRNAs directed against *KRT9*. The team first scanned all possible 19-mer siRNAs for the repression of KRT9, using transiently expressed luciferase reporters to monitor specificity and efficacy of siRNAs. Next, siRNAs that efficiently inhibited the two prominent KRT9 missense mutations M157V and R163Q were identified using a similar strategy. The best siRNAs were able to repress a mutant *KRT9* allele in the 50 pM range, without apparently affecting the expression of other keratins. Ultimately, siRNAs must be delivered *in situ*. Unfortunately, no mouse model for KRT9 is currently available. As a first step, Leslie Pedrioli *et al.* coinjected the most potent siRNA, siR163Q-13, together with a mutant KRT9-luciferase reporter carrying the same mutation, into mouse footpad epidermis. This delivery route had been previously approved in a phase Ib clinical trial for pachyonychia congenita (Leachman *et al.*, 2010). To test for specificity, a wild-type KRT9-luciferase reporter was applied together with the above siRNA in another footpad. Despite the limitations imposed by the nature of the delivery, i.e., injection, the data suggest that the siRNA was more specific in repressing the mutant compared with the normal allele.

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As one finds with any good study, Leslie Pedrioli and colleagues' data raise a number of issues that in the above setting relate to RNAi, keratin biology, and skin physiology. The specific and effective repression of mutant KRT9 alleles is supported by encouraging data in model systems for the related, dominant keratinopathies pachyonychia congenita, epidermolysis bullosa simplex, and Meesmann corneal dystrophy (Leachman *et al.*, 2010; Liao *et al.*, 2011; Coulombe and Lee, 2012). These studies imply that specifically targeting individual keratin mutations is feasible, although current bioinformatics approaches are unable to deliver reliable predictions. Further, siRNA complexed into stable nucleic acid particles appears to be stable for up to 2 weeks (Davidson and McCray, 2011). Whether the use of repeated cycles of siRNA that are necessary to treat chronic diseases will avoid immunological recognition through RIG and TLR receptors expressed in keratinocytes remains to be determined. The stability of the target mRNA and encoded protein(s) represents another challenge. The long half-life of keratin intermediate filament proteins and their mRNAs may indeed outlast most siRNA formulations. Therefore, including data from well-established three-dimensional keratinocyte culture models is of paramount importance in future studies. In combination with experiments on animal models, the question of how efficient siRNA-mediated repression of mutant keratins must be in order to eliminate dominant-negative effects remains to be answered.

In many epidermal keratinocytes, keratins represent some of the most abundant proteins. Current studies on treating keratinopathies with siRNAs assume that repressing a single keratin isotype, i.e., KRT9 as in the Leslie Pedrioli *et al.* (this issue, 2012) paper, is of little consequence for skin integrity and physiology in view of keratin's abundance and their complexity of expression. This may not be the case and is not well supported by *in vivo* data. The only supporting mouse studies are those of the functional replacement of KRT18 by KRT 19 and the partial replacement of KR14 by KRT18 (Hutton *et al.*, 1998; Magin *et al.*, 1998). More recent data, rather, point to non-overlapping keratin functions, spear-headed by KRT17's role in control of protein biosynthesis and inflammation (Coulombe and Lee, 2012). With these and additional data in mind, future studies should include more comprehensive assays to evaluate treatment success in the context of skin physiology.

> Delivery *in vivo* is the major limitation in applying siRNA technology to skin diseases.

In addition, the greatest hurdle for siRNA-based treatment of skin disorders remains delivery to the cell of origin. Recent advances in lipid-mediated nucleic acid delivery to the skin have considered trans- and intracellular, as well as transfollicular and transappendageal, routes to treat a range of genetic and non-genetic conditions (Geusens *et al.*, 2011). The truth is that the underlying mechanisms for successful delivery of nucleic acids (the basis of any *ex vivo* therapy into live keratinocytes, including stem cells) are not well known. As odd as it seems, there is no other way than back from bedside to the bench: are all keratinocytes equal in their ability to take up, transport, and process siRNAs? Which of the aforementioned routes are actually being taken by siRNA that is delivered to cells? How many stem cells are targeted, and does restoring their phenotype confer a selective advantage over their neighbors? These are some of the questions that must be answered before the exciting strides taken by Leslie Pedrioli *et al.* (this issue, 2012) find their way to the clinic.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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