Use of Self-Delivery siRNAs to Inhibit Gene Expression in an Organotypic Pachyonychia Congenita Model

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Although RNA interference offers therapeutic potential for treating skin disorders, delivery hurdles have hampered clinical translation. We have recently demonstrated that high pressure, resulting from intradermal injection of large liquid volumes, facilitated nucleic acid uptake by keratinocytes in mouse skin. Furthermore, similar intradermal injections of small interfering RNA (siRNA; TD101) into pachyonychia congenita (PC) patient foot lesions resulted in improvement. Unfortunately, the intense pain associated with hypodermic needle administration to PC lesions precludes this as a viable delivery option for this disorder. To investigate siRNA uptake by keratinocytes, an organotypic epidermal model, in which pre-existing endogenous gene or reporter gene expression can be readily monitored, was used to evaluate the effectiveness of "self-delivery" siRNA (i.e., siRNA chemically modified to enhance cellular uptake). In this model system, self-delivery siRNA treatment resulted in reduction of pre-existing fluorescent reporter gene expression under conditions in which unmodified controls had little or no effect. Additionally, treatment of PC epidermal equivalents with self-delivery "TD101" siRNA resulted in marked reduction of mutant keratin 6a mRNA with little or no effect on wild-type expression. These results indicate that chemical modification of siRNA may overcome certain limitations to transdermal delivery (specifically keratinocyte uptake) and may have clinical utility for inhibition of gene expression in the skin.

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INTRODUCTION

Since the discovery that small interfering RNAs (siRNAs) can effectively silence gene expression in a number of mammalian systems, there has been increasing interest in developing these inhibitors as therapeutics to treat a variety of diseases, including those of the skin. Although siRNA-based therapeutics are currently in clinical trials for a number of targets, including the liver, kidney, lung, skin, and eye, as recently reviewed (Vaishnaw *et al.*, 2010), a number of inherent physical barrier properties of the skin represent formidable hurdles to siRNA delivery and progress into the clinic has been impeded.

To develop effective siRNA-based therapeutics for treatment of skin disorders, it is necessary to first identify potent and specific inhibitors and then develop effective delivery methods for these nucleic acid inhibitors to target cells in the skin. For the treatment of pachyonychia congenita (PC), we have identified inhibitors that are both selective and potent (Hickerson *et al.*, 2008; Smith *et al.*, 2008). The first reported siRNA clinical trial (Phase 1b) in skin used one of these inhibitors (TD101) to treat this rare skin disorder, and some efficacy was noted (Leachman *et al.*, 2008, 2010). PC is a dominant negative skin disorder caused by mutations in the inducible keratin genes, including *KRT6A, KRT6B, KRT16,* or *KRT17* genes, which often result in thickened dystrophic nails, leukokeratosis, and acanthosis histologically with painful blistering (Leachman *et al.*, 2005; Smith *et al.*, 2006). Detailed clinical and genetic analysis of PC is reported by Wilson *et al.*, 2011, and will be reported by Eliason *et al.* (personal communications). TD101 siRNA (also known as K6a_513a.12 siRNA, see Hickerson *et al.*, 2008) specifically targets the keratin 6a (K6a) single C to A nucleotide mutation, which results in the K6a p.Asn171Lys amino acid mutation.

Delivery of functional siRNA to skin (specifically to the epidermal keratinocytes) involves (i) stratum corneum penetration and (ii) keratinocyte uptake followed by incorporation into the RNA-induced silencing complex. Although hydrodynamic intradermal injection of unmodified, standard siRNA (i.e., siRNA without the modifications that allow for "self-delivery") to mouse skin resulted in target inhibition (Gonzalez-Gonzalez *et al.*, 2009), treatment methods that do not involve generation of

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Abbreviations: EGFP, enhanced green fluorescent protein; HPEKp, human primary epidermal keratinocyte progenitors; K, keratin protein; KRT, keratin gene; PC, pachyonychia congenita; tdTOM, tandem tomato fluorescent protein

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