

Inhibition of CD44 Gene Expression in Human Skin Models, Using Self-Delivery Short Interfering RNA Administered by Dissolvable Microneedle Arrays

Maria Fernanda Lara,^{1,2} Emilio González-González,² Tycho J. Speaker,¹ Robyn P. Hickerson,¹ Devin Leake,³ Leonard M. Milstone,⁴ Christopher H. Contag,^{2,5} and Roger L. Kaspar^{1,2}

Abstract

Treatment of skin disorders with short interfering RNA (siRNA)-based therapeutics requires the development of effective delivery methodologies that reach target cells in affected tissues. Successful delivery of functional siRNA to the epidermis requires (1) crossing the stratum corneum, (2) transfer across the keratinocyte membrane, followed by (3) incorporation into the RNA-induced silencing complex. We have previously demonstrated that treatment with microneedle arrays loaded with self-delivery siRNA (sd-siRNA) can achieve inhibition of reporter gene expression in a transgenic mouse model. Furthermore, treatment of human cultured epidermal equivalents with sd-siRNA resulted in inhibition of target gene expression. Here, we demonstrate inhibition of CD44, a gene that is uniformly expressed throughout the epidermis, by sd-siRNA both *in vitro* (cultured human epidermal skin equivalents) and *in vivo* (full-thickness human skin equivalents xenografted on immunocompromised mice). Treatment of human skin equivalents with CD44 sd-siRNA markedly decreased CD44 mRNA levels, which led to a reduction of the target protein as confirmed by immunodetection in epidermal equivalent sections with a CD44-specific antibody. Taken together, these results demonstrate that sd-siRNA, delivered by microneedle arrays, can reduce expression of a targeted endogenous gene in a human skin xenograft model.

Introduction

THE DISCOVERY OF RNA interference (RNAi), coupled with the development and synthesis of short interfering RNAs (siRNAs) with minimal off-target and immunostimulatory activities, has resulted in intense efforts to develop this new class of nucleic acid-based therapeutics. siRNAs have entered clinical trials for a number of indications (for reviews see Vaishnaw *et al.*, 2010; Burnett *et al.*, 2011; Chen and Zhaori, 2011), including skin (Leachman *et al.*, 2010). Skin represents an attractive target tissue for siRNA therapeutics because of its accessibility, the availability of rapid outcome measures, and the existence of a large number of dominant genodermatoses as well as skin cancers that could benefit from siRNA-based therapies (Pfutzner and Vogel, 2000; Khavari *et al.*, 2002; Leachman *et al.*, 2008; McLean and Moore, 2011; Ra *et al.*, 2011; Leslie Pedrioli *et al.*, 2012). However, difficulties in

delivering the siRNA across the outermost stratum corneum barrier and inefficient cellular uptake have hampered translation to the clinic (Leachman *et al.*, 2010). We have shown that a delivery method composed of dissolvable microneedle arrays loaded with self-delivery siRNA (sd-siRNA) cargo can largely overcome these barriers in a transgenic mouse model (Gonzalez-Gonzalez *et al.*, 2010b) and that sd-siRNA can inhibit mutant gene expression in an organotypic human epidermal model in the absence of transfection reagents such as cationic liposomes (Hickerson *et al.*, 2011a).

The CD44 family contains transmembrane proteins (Screaton *et al.*, 1992) that colocalize with hyaluronan throughout the epidermis (Wang *et al.*, 1992) and bind hyaluronan at the cell surface through a common amino-terminal domain (Aruffo *et al.*, 1990). More than 80% of CD44 in epidermis and cultured keratinocytes is expressed as epican, a heparan/chondroitin sulfate proteoglycan (Zhou

¹TransDerm, Santa Cruz, CA 95060.

²Department of Pediatrics and Program in Molecular Imaging, Stanford University, Stanford, CA 94305.

³Dharmacon Products/Thermo Fisher Scientific, Lafayette, CO 80026.

⁴Department of Dermatology, Yale University, New Haven, CT 06510.

⁵Department of Radiology and Department of Microbiology and Immunology Stanford University, Stanford, CA 94305.