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RESEARCH ARTICLE

Non-Invasive Intravital Imaging of siRNA-Mediated Mutant Keratin Gene Repression in Skin

Robyn P. Hickerson,[1,6] Tycho J. Speaker,[1] Maria Fernanda Lara,[1,7] Emilio González-González,[2,3,8] Manuel A. Flores,[1] Christopher H. Contag,[2,3,4,5] Roger L. Kaspar[1]

[1]*TransDerm Inc., 2161 Delaware Ave., Santa Cruz, CA, 95060, USA*
[2]*Molecular Imaging Program at Stanford (MIPS), Stanford University School of Medicine, Stanford, CA, USA*
[3]*Department of Pediatrics, Stanford University School of Medicine, Stanford, CA, USA*
[4]*Department of Radiology, Stanford University School of Medicine, Stanford, CA, USA*
[5]*Departments of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA, USA*
[6]*Centre for Dermatology and Genetic Medicine, University of Dundee, Dundee, UK*
[7]*Urology Research Unit Virgen de la Victoria and Regional Hospital, Malaga, Spain*
[8]*Canvax Biotech S.L., Technological Park, Cordoba, Spain*

Abstract

Purpose: Small interfering RNAs (siRNAs) specifically and potently inhibit target gene expression. Pachyonychia congenita (PC) is a skin disorder caused by mutations in genes encoding keratin (K) 6a/b, K16, and K17, resulting in faulty intermediate filaments. A siRNA targeting a single nucleotide, PC-relevant mutation inhibits K6a expression and has been evaluated in the clinic with encouraging results.

Procedures: To better understand the pathophysiology of PC, and develop a model system to study siRNA delivery and visualize efficacy in skin, wild type (WT) and mutant K6a complementary DNAs (cDNAs) were fused to either enhanced green fluorescent protein or tandem tomato fluorescent protein cDNA to allow covisualization of mutant and WT K6a expression in mouse footpad skin using a dual fluorescence *in vivo* confocal imaging system equipped with 488 and 532 nm lasers.

Results: Expression of mutant K6a/reporter resulted in visualization of keratin aggregates, while expression of WT K6a/reporter led to incorporation into filaments. Addition of mutant K6a-specific siRNA resulted in inhibition of mutant, but not WT, K6a/reporter expression.

Conclusions: Intravital imaging offers subcellular resolution for tracking functional activity of siRNA in real time and enables detailed analyses of therapeutic effects in individual mice to facilitate development of nucleic acid-based therapeutics for skin disorders.

Key words: Genodermatosis, Gene therapy, Gene regulation, *In vivo* confocal fluorescence microscopy

Introduction

The combination of potency and specificity of small interfering RNAs (siRNAs) to degrade targeted messenger RNAs (mRNAs) has made this class of inhibitors very

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Correspondence to: Roger Kaspar; *e-mail:* roger.kaspar@transderminc.com