siRNA goes skin deep

By Tim Fulmer, Senior Writer

A University of California, Santa Barbara team has identified a peptide carrier that transports small interfering RNA across the skin of mice.¹ The researchers believe the peptide could be useful for topical delivery of siRNAs to treat a variety of skin diseases.

To date, siRNA delivery formulations primarily have involved packaging the molecules in soluble nanocarriers for systemic delivery to internal organs and tissues. Researchers have shied away from topical siRNA formulations because of the difficulty in transporting the molecules through the skin.

A key hurdle was the need to penetrate two barriers: the stratum corneum, which is the skin's outermost surface layer and consists of dead cells, and the cell membrane of the skin cells situated immediately below the stratum corneum.

The UCSB team, led by professor of chemical engineering Samir Mitragotri, looked for a way to link the siRNA to a carrier molecule that could cross both.

Building on studies by other labs that identified peptides able to cross either the stratum corneum or the cell membrane, Mitragotri and colleagues set up an *in vitro* phage display screen to identify peptides capable of crossing both barriers.

That screen identified a single peptide that penetrated porcine and human stratum corneum samples as well as the cell membranes of multiple human cell types, including keratinocytes, fibroblasts and epithelial cells.

The next question was whether the peptide could deliver functional siRNA to the interior of cells. Indeed, in cultured epithelial cells expressing GFP, a peptide-conjugated siRNA targeting GFP led to knockdown of the protein in about 60% of cells compared with less than 10% for siRNA alone.

Finally, the researchers looked for proof that the peptide-siRNA conjugate could hit its target *in vivo*. In healthy mice, topical application of a 10 μ M solution of peptide-siRNA conjugate targeting IL-10, a cytokine associated with atopic dermatitis, led to significantly greater

knockdown of IL-10 24 hours after administration than application of siRNA alone ($p < 0.01$).

The findings were published in the *Proceedings of the National Academy of Sciences*.

To confirm the therapeutic applications of the peptide-siRNA conjugates, the UCSB group should study "the effects of the method in a variety of murine disease models, such as hapten-induced contact hypersensitivity, atopic dermatitis and allogeneic skin transplantation," said Miyuki Azuma.

Azuma, a professor of molecular immunology at the Tokyo Medical and Dental University, published in *Molecular Therapy* in 2008 that a topically applied siRNA targeting CD86 (B7-2) decreased dendritic cell (DC)-mediated inflammation in a mouse model of atopic dermatitis.²

In that study the siRNA did not require a carrier because it targeted CD86 on peripheral DCs, which are confined to the outermost layers of skin.

Mitragotri said his next steps include studying the peptide-siRNA conjugate's dose response, efficacy and toxicity in animal models of undisclosed dermatological disorders.

Tracey Zimmermann, associate director of research at RNAi biotech Alnylam Pharmaceuticals Inc., said it will be necessary to optimize a formulation of the peptide-siRNA conjugate that is compatible with topical administration and maintains its ability to silence a disease-associated mRNA target.

Alnylam is developing multiple strategies for the direct and systemic delivery of RNAi molecules, including the use of conjugates, antibodies, liposomes and peptides. Last week at the Oligonucleotide Therapeutics Society meeting in Copenhagen, the company presented data on the *in vivo* characterization of some of its liposome-mediated delivery strategies.

The UCSB findings are patented and available for licensing.

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Contact: Samir Mitragotri, University of California, Santa Barbara, Calif.
e-mail: samir@engineering.ucsb.edu
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COMPANIES AND INSTITUTIONS MENTIONED

Alnylam Pharmaceuticals Inc. (NASDAQ:ALNY), Cambridge, Mass.
University of California, Santa Barbara, Calif.
Tokyo Medical and Dental University, Tokyo, Japan