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Topical antiviral siRNA

A practical siRNA microbicide?

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The idea of simple, cost-effective microbicides for preventing or treating sexually transmitted diseases has great appeal and importance. A recent publication by Palliser *et al*.[1] provides the first demonstration that sequence-specific nucleic acids in the form of small interfering RNAs (siRNAs) can be used for this purpose.

It is widely acknowledged that RNA interference (RNAi) is a powerful mechanism for targeted message destruction and consequent inhibition of gene expression. The triggers for RNAi are siRNAs, which are 21–23 nucleotide duplexes. The siRNAs are complexed with protein components of the RNAi machinery (RNA-induced silencing complex or RISC) and one of the two siRNA sequences is selected as the guide strand following cleavage of the passenger strand by the RISC-associated protein Argonaute 2.[2–4] The guide strand serves to identify the target message via base pairing, allowing Argonaute 2 to cleave the target mRNA, resulting in destruction of the message.[5] Given the efficiency of this process in inhibiting gene expression, there has been a great deal of interest in therapeutic applications of siRNAs, and viruses have been a major target.[5]

Early on, the most significant challenge to using siRNAs *in vivo* was delivery. In the past years, there have been several significant publications demonstrating that siRNAs can be effectively delivered to a variety of target cells *in vivo*.[6–10] Each of these approaches was directed at getting either systemic or tissue-specific delivery of siRNAs for therapeutic targets. The most recent addition to this list is a clever application by Judy Lieberman and her colleagues,[1] which used siRNAs as a vaginally applied microbicide to block HSV-2 infection in a murine model.

The goal of developing an siRNA microbicide was to harness the power of RNAi to provide protection against a sexually transmitted pathogen. To test the possibility of a topical siRNA application, they first demonstrated that fluorescently labeled siRNAs complexed with cationic lipids could be taken up by vaginal and ectocervical epithelium cells as well as the underlying lamina propria and stroma. They went on to ask whether or not siRNAs applied intravaginally could silence endogenous transcripts expressing green fluorescent protein (GFP) in a GFP transgenic mouse model. Amazingly, GFP expression was virtually completely silenced throughout the mouse vagina and cervix, and did not spread to other organs, demonstrating selective uptake of the lipid–siRNA complex within the vaginal-cervical site of application. Moreover, the silencing lasted for 9 days post-administration.

Palliser *et al*. proceeded to further examine this novel topical application in an infectious HSV-2 viral challenge. They first tested siRNAs targeting three different essential HSV-2 genes, UL5, UL27 and UL29, which encode essential viral proteins. Their cell culture results demonstrated that one of the siRNAs targeting the UL29 transcript encoding a DNA binding protein was the most potent. This siRNA was tested via intravaginal application in animals by applying the siRNA–lipid complex 2 h before and 4 h after viral challenge. The controls included a non-specific siRNA and a less potent siRNA targeting the HSV-2 UL27 gene transcript, which encodes the envelope glycoprotein-B. Animals that received 500 pmol of either of the anti-HSV-2 siRNAs had significant protective responses as assessed by a clinical disease scoring system or survival. In particular, mice treated with the anti-UL29 siRNA had a 75% survival rate as compared to a 25% survival rate among the control siRNA-treated animals. Viral shedding was also prevented or severely delayed in mice treated with the anti-HSV-2 siRNAs. The amount of virus shed by the anti-UL29 siRNA-treated mice was reduced over 150-fold relative to the control animals. Pathological examination of the anti-HSV-2 siRNA-treated mice showed markedly reduced signs of viral-induced inflammation or cell death.

An important issue to be addressed in using siRNAs as antiviral microbicides is whether or not they can be administered after viral infection and still be effective. To address this problem, they applied either 500 pmol of single targeting siRNAs or 250 pmol of each of the siRNAs targeting UL27 and UL29 viral sequences at 3 and 6 h intervals after HSV-2 challenge. Somewhat disappointingly, administration of the single siRNAs was no better than the control siRNA, but the combined siRNAs resulted in statistically significant protection and 5/6 of the treated animals survived the HSV-2-induced lethality. Thus, combination therapies might provide a mechanism for post-infection treatment within a specified window of application.

What are the future prospects for siRNA microbicides for sexually transmitted diseases? Palliser *et al*. calculated that scaling up the synthesis costs from the animal studies to humans would at current costs amount to approximately US$8.00 per treatment. Certainly, this is an acceptable cost in developed nations, but would it really be practical in third-world countries, where this cost could exceed the monthly incomes of some individuals? As the authors pointed out, the siRNAs that were reported in their study were in no way optimized for potency and longevity of function. Perhaps this cost could be reduced by an order of magnitude if the scale of siRNAs needed for efficacy could be reduced accordingly. Other viruses that are transmitted sexually are potential candidates for siRNA microbicides, such as HPV and HIV. In the case of HIV though, the target cells are primarily macrophages, dendritic cells and T-lymphocytes, which may be more difficult to target by simple lipid-based delivery. It is also possible that targeting viral mRNAs along with cellular transcripts encoding viral receptors could provide a synergy that would make dosing regimens more affordable. Bacterial

infections are also potentially targetable by the microbicide approach if the genes encoding their portal of entry can be inhibited. The work of Palliser *et al*. has provided a very elegant proof of principle for siRNA microbicides. This work should stimulate thinking about other viral and bacterial pathogens that can be targeted using vaginal or other mucosal tissue topical application of siRNAs. ■

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1 Palliser D, Chowdhury D, Wang QY, Lee SJ, Bronson RT, Knipe DM *et al*. An siRNA-based microbicide protects mice from lethal herpes simplex virus 2 infection. *Nature* 2006; **439**: 89–94.
2 Gregory RI, Chendrimada TP, Cooch N, Shiekhattar R. Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. *Cell* 2005; **123**: 631–640.
3 Matranga C, Tomari Y, Shin C, Bartel DP, Zamore PD. Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes. *Cell* 2005; **123**: 607–620.
4 Miyoshi K, Tsukumo H, Nagami T, Siomi H, Siomi MC. Slicer function of *Drosophila* Argonautes and its involvement in RISC formation. *Genes Dev* 2005; **19**: 2837–2848.
5 Hannon GJ, Rossi JJ. Unlocking the potential of the human genome with RNA interference. *Nature* 2004; **431**: 371–378.
6 Hu-Lieskovan S, Heidel JD, Bartlett DW, Davis ME, Triche TJ. Sequence-specific knockdown of EWS-FLI1 by targeted, nonviral delivery of small interfering RNA inhibits tumor growth in a murine model of metastatic Ewing's sarcoma. *Cancer Res* 2005; **65**: 8984–8992.
7 Morrissey DV, Lockridge JA, Shaw L, Blanchard K, Jensen K, Breen W *et al*. Potent and persistent *in vivo* anti-HBV activity of chemically modified siRNAs. *Nat Biotechnol* 2005; **23**: 1002–1007.
8 Song E, Zhu P, Lee SK, Chowdhury D, Kussman S, Dykxhoorn DM *et al*. Antibody mediated *in vivo* delivery of small interfering RNAs via cell-surface receptors. *Nat Biotechnol* 2005; **23**: 709–717.
9 Soutschek J, Akinc A, Bramlage B, Charisse K, Constien R, Donoghue M *et al*. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature* 2004; **432**: 173–178.
10 Zhang W, Yang H, Kong X, Mohapatra S, San Juan-Vergara H, Hellermann G *et al*. Inhibition of respiratory syncytial virus infection with intranasal siRNA nanoparticles targeting the viral NS1 gene. *Nat Med* 2005; **11**: 56–62.