Cholesterol Paves the Way for Topically Applied Viricides

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Sexually transmitted viral infections have the potential to be prevented and treated by topical viricides. Here, Wu et al. (2009) demonstrate that cholesterol-conjugated small interfering RNAs (siRNAs) targeting a cellular receptor combined with an antiviral siRNA when topically applied to mucosal tissue blocked lethal herpes virus infections in mice.

RNA interference has received a great deal of attention for its therapeutic potential (Kim and Rossi, 2007). In particular, small interfering RNA duplexes (siRNAs), which mimic the products of the RNase III enzyme Dicer have been shown to trigger potent, sequence-specific knockdown of gene expression in a wide variety of eukaryotic organisms (Hannon and Rossi, 2004). These siRNAs are engaged by Argonaute family members of the RNA-induced silencing complex (RISC), where they guide complementary base pairing with targeted mRNAs, resulting in cleavage and subsequent destruction of the targeted transcripts (Hall, 2005; Hammond, 2005). siRNAs can potentially be used to treat a variety of diseases ranging from hereditary disorders to viral infections. A key to successful therapeutic applications is the ability to deliver these macromolecules to the tissues of interest. A number of strategies for systemic delivery of siRNAs have been described, but only a small handful is useful for specific tissue delivery (Kim and Rossi, 2007).

One of the first clinical trials for siRNAs was directed at inhibition of respiratory syncytial virus (RSV) in which delivery of siRNAs was achieved using an unencapsulated siRNA in an intranasal spray, which results in trafficking of the siRNAs to lung epithelial cells, the site of RSV infection (DeVincenzo et al., 2008). For nonrespiratory viral infections the route of siRNA administration needs to coincide with the primary sites of viral infection. A number of viruses are transmitted sexually via infection of mucosal cells and/or invading immune cells. These include serious pathogens such as members of the herpes virus family, human immunodeficiency virus (HIV), and human papilloma virus (HPV). The use of topically applied viricides is very attractive as either a prophylaxis or treatment regimen immediately following exposure to the virus. The first demonstration that topically applied siRNAs could block a viral infection came from experiments in mice using murine herpes simplex virus 2 (HSV2) infection either preceded by or immediately followed by vaginal application of cationic lipid encapsulated siRNAs targeting the viral envelope glycoprotein UL27 and DNA-binding protein UL29 encoding transcripts. Compared with saline or vehicle controls, the siRNA treatments resulted in protection of mice from lethal infection (Palliser et al., 2006). The siRNAs were most effective when applied either immediately prior to or within 1 day post infection, and the protection was quite transient.

In efforts to improve upon the idea of an siRNA topical viricide, Wu et al. (2009) tested another approach, which used siRNAs conjugated to cholesterol for topical application. These investigators first observed that increasing the dosage of lipoplexed siRNAs did not result in improved inhibition of HSV2, and in fact increased amounts of lipid had the negative side effect of enhancing viral infectivity. This led them to explore other options for delivery. The choice of cholesterol was predicated upon earlier studies, which showed that cholesterol could be used for functional, systemic delivery of siRNAs (Soutschek et al., 2004) or antisense oligonucleotide antagonists of miRNAs (antagomirs) to a variety of tissues (Krutzfeldt et al., 2005). Aside from cholesterol, including an siRNA for the cellular target nectin-1, a receptor required for HSV2 entry, significantly improved protection (Figure 1). Knockdown of nectin-1 persisted for several days both in vitro and in vivo following a single administration of the siRNA. A combination of the nectin-1 siRNA along with the antiviral UL29 siRNA gave the most potent and durable inhibition. Protection from HSV2 induced lethality was achieved using this combination of cholesterol-conjugated siRNAs at a combined dose of 1 mg/kg. The authors looked for possible non-specific effects mediated by interferon (IFN) gene induction, but did not observe any increases in IFN gene expression relative to the controls.

Mechanistically, it is of interest to understand how the cholesterol assists in delivery of siRNAs to mucosal epithelial cells. Given that cholesterol has been shown to be a nontoxic and somewhat general delivery vehicle for oligonucleotides, it is of great interest to better understand how it facilitates intracellular delivery of siRNAs. Does cholesterol dock with a receptor and become internalized, or does it insert within the cell membrane and become pinocytosed? How does siRNA with a large cholesterol moiety enter RISC? Wu et al. (2009) observed that a single phosphorothioate (PS) modification on each strand of the siRNA made a significant difference in the efficacy of the siRNAs. Is this just a stabilizing property, or does the PS somehow facilitate the internalization of the siRNAs, as it does for PS backbone modified antisense oligonucleotides? Not to be overlooked, though, is the relatively high concentration of cholesterol-siRNA (1 mg/kg) used in these studies to obtain optimal inhibition. This would translate into over 100 mg for a single human application, so clearly cost will be an issue for future therapeutic development.

An interesting observation about the efficacy of siRNAs in the Wu et al. (2009) study was the discrepancy in the amounts of intracellular siRNAs targeting UL29

versus nectin-1, despite the fact that equal concentrations of material were applied to the cells and tissue. The authors observed that the kinetics of loss of the two siRNAs was similar, but the amount of detectable anti-nectin-1 siRNA was substantially greater than that of the anti-UL29 siRNA at all times tested. They point out that this is probably not an artifact of detection but could reflect the difference in stability between an siRNA that is readily loaded into RISC versus one that is poorly loaded. Once in RISC the siRNA would be stabilized, and its potency could be longer lasting. If this observation can be generalized, it could impact on the cost of treatment if lower concentrations can be effectively used in topical viricides.

Despite questions about the mechanism of delivery and the potential concerns about cost, the possibility of a topical, vaginally applied viricidal treatment for heterosexually transmitted HIV infection is worth considering. Similar to the HSV2 approach, cotargeting of a viral and cellular RNA should be considered. In the case of HIV, there are now known to be several hundred essential cellular genes required for HIV entry, integration, replication, maturation, and budding (Brass et al., 2008; Konig et al., 2008), providing a rich repository of potential targets for an HIV viricide. Since HIV infects immune cells, it will be of interest to determine whether or not cholesterol-conjugated siRNAs can be used in a topical viricidal formulation for prevention of HIV infection.

Overall, the studies of Palliser et al. and the improvements in delivery described by Wu et al. provide an exciting opportunity to explore the use of topically applied siRNAs for treatment of viral infections. The lack of toxicity and the long lasting antiviral activity of the combined cellular and viral targeting by siRNAs are provocative and clearly warrant further testing in both animals and ultimately in human clinical trials.

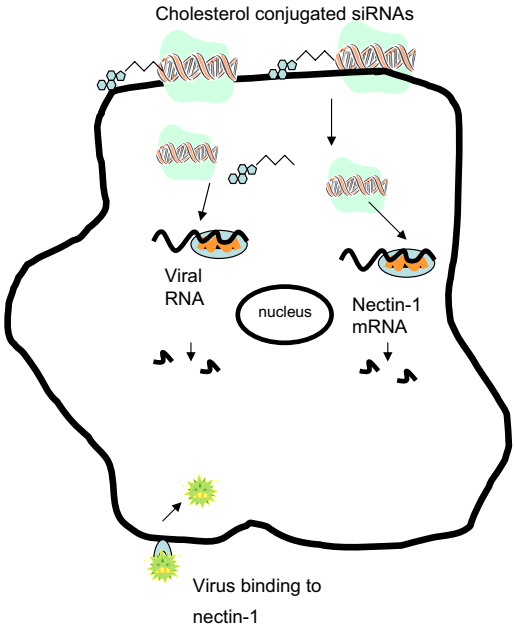

Figure 1. Topical siRNA Viricide Mechanism
Cholesterol-conjugated siRNAs dock to mucosal cell surface, siRNAs are internalized, and antisense guide strand is engaged in the RNA-induced silencing complex (RISC), which targets and degrades the viral RNAs and nectin-1 RNA. The net result of the dual targeting is inhibition of viral infection and prevention of pathogenicity.

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