CLINICAL IMPLICATIONS OF BASIC RESEARCH

Targeting Specific Cell Types with Silencing RNA

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RNA interference, which is mediated by small, double-stranded fragments of RNA called short interfering RNA (siRNA), is now a popular method to silence the expression of specific genes. It exploits the ability of the short, double-stranded fragment to abrogate the expression of the gene that shares its sequence and is useful not only as a basic research tool but also for drug-target validation. But the greatest hope for RNA interference lies in the potential use of siRNA oligonucleotides as drugs. The issue that has long bedeviled oligonucleotide-based therapies is how to deliver these agents to specific cellular or tissue targets. Song and colleagues[1] recently described a way to do so.

It was already known that coupling protamine, a protein that binds DNA, to the Fab portion of an antibody enables the complex to deliver small pieces of DNA to a cell expressing the appropriate antigen on its surface. Song and colleagues substituted siRNA for DNA and fused protamine to a Fab directed against the human immunodeficiency virus type 1 (HIV-1) envelope protein. Conveniently, they were able to mix the siRNA with the fusion protein; chemical coupling of the two was not necessary. When the mixture was added to the target cells, the complex honed in on cells expressing the HIV-1 envelope antigen. Moreover, when the mixture included an siRNA targeted to the HIV-1 gag protein, viral replication was suppressed in infected primary T cells — a type of cell normally refractory to the delivery of oligonucleotides.

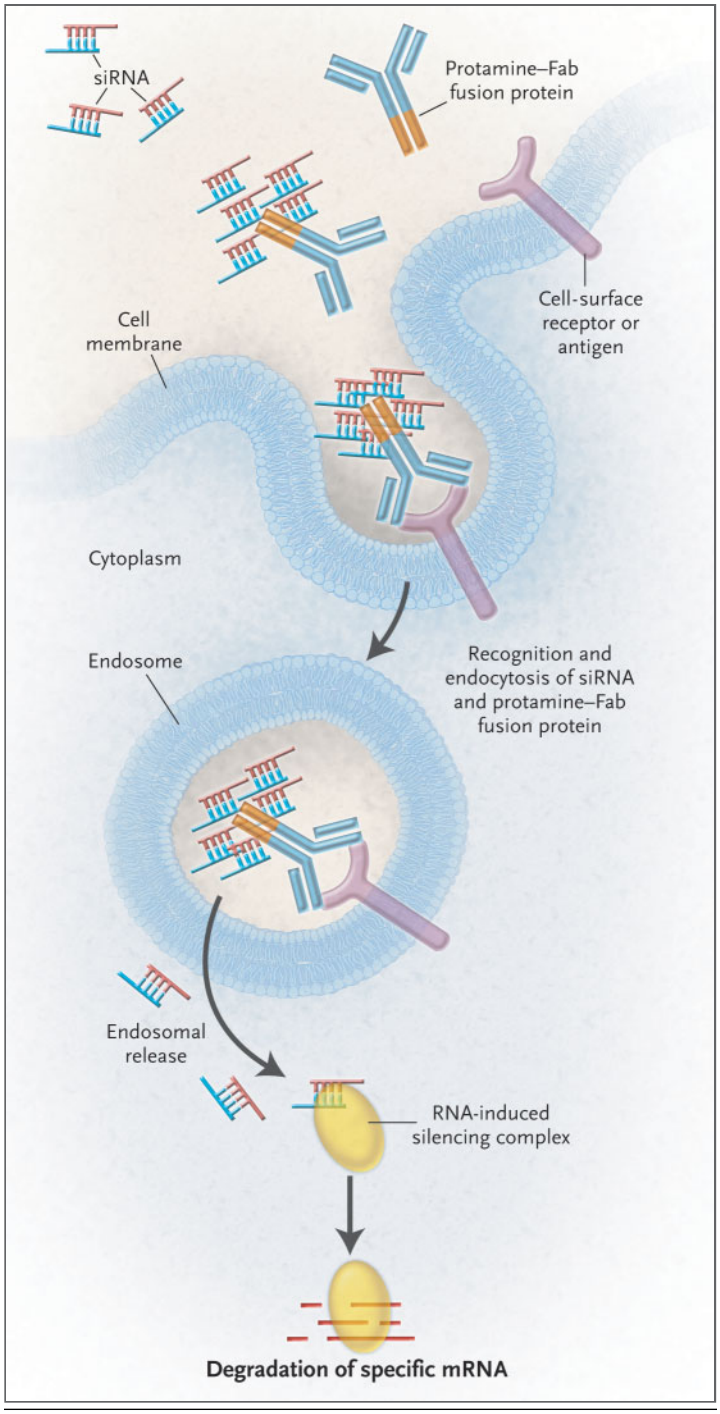
To test the efficacy of the antibody–protamine fusion proteins in vivo, Song et al. engineered mouse melanoma cells to express the GP160 protein of HIV-1 and implanted the cells into the flanks of syngeneic mice. They then injected the animals' flanks with a mixture of protamine and siRNA designed to target genes regulating the cell cycle (*c-myc*), apoptosis (*mdm2*), and angiogenesis (*vegf*). This cocktail significantly inhibited the establishment of tumors expressing GP160 but was ineffective against tumors that did not express GP160. Systemic delivery of the siRNA mixtures also inhibited the growth of established tumors, albeit less effectively than direct intratumoral injection.

To show that these results were not restricted to cells engineered to overexpress surface antigen, the authors tested the ability of a fusion product consisting of protamine and a single-chain antibody that binds the ErbB2 receptor (which is commonly expressed on breast-cancer cells) to deliver an siRNA designed to target an intracellular protein. The fusion product inhibited the expression of the protein in cell culture, albeit at a lower efficiency than was achieved with the GP160 antibody–protamine complex.

The beauty of the approach devised by Song et al. is its simplicity (Fig. 1). Its weak point is its requirement for an appropriate antigen–antibody combination that provides specificity, although, as noted by the authors, a cell-surface receptor ligand could be substituted for the antibody fragment. Such a ligand would have to be attached to the protamine fragment. Of concern, however, is the diminished activity of the complexes of the protamine fragment and single-chain ErbB2 antibody siRNA as compared with the full-length GP160 antibody–protamine complexes.

Questions also remain about possible nonspecific immunomodulatory activities of the siRNA–

Figure 1. Cell-Specific Targeting of Silencing RNA.
The expression of selective genes can be silenced by specific short interfering RNAs (siRNAs). These are usually introduced into cell cultures with the use of various transfection agents or injected intravenously either as naked nucleic acids or in a complex with lipids. However, large doses of siRNA are required for in vivo silencing, and nonspecific adverse effects can result from inadvertent tissue targeting. For example, to target siRNA specifically to HIV-1–infected melanoma cells, Song and colleagues mixed a protamine–antibody fusion protein directed against a cell-surface antigen with the siRNA. Once injected, the complex was bound by cell-surface receptors, internalized into endosomes, and subsequently released and incorporated into the cytoplasmic RNA-induced silencing complex, ensuring the degradation of a specific messenger RNA (mRNA).



protamine–antibody complexes. Although Song et al. provide evidence that the protamine–GP160 antibody–siRNA complexes do not trigger a nonspecific response of interferon from melanoma cells in vitro, the immunostimulatory motifs present in the siRNA used in their in vivo experiments may have triggered an inflammatory response leading to the activation of dendritic cells. Even though localized inflammation may not be such a bad outcome in a tumor-treatment setting, it would be best avoided in most clinical applications. This could be resolved by using an siRNA that does not contain the immunostimulatory uracil–guanidine dinucleotide and evaluating the ability of the various complexes to stimulate cytokine production in dendritic-cell cultures. If these issues and the potential toxicity of the approach described by Song et al. are addressed, the technique is limited only by the ability to select the appropriate target cell and its expressed antigen or receptor.

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1. Song E, Zhu P, Lee SK, et al. Antibody mediated in vivo delivery of small interfering RNAs via cell-surface receptors. Nat Biotechnol 2005;23:709-17.