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RNAi Therapy

Antibodies guide the way

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In a recent issue of *Nature Biotechnology,* Song *et al*[1] showed that small interfering RNAs (siRNAs) can be delivered into a target cell population through antibody-mediated endocytosis.

Several diseases such as cancers are a difficult target for any therapeutic strategy; therefore, there is a continuous search for new approaches. The use of siRNAs to target mRNAs for degradation through the biologically conserved RNA interference (RNAi) pathway is one such approach that is receiving increasing attention.[2–4] However, clinical use of siRNAs entails at least two critical steps: delivery of siRNAs to the appropriate cells and subsequent maintenance and expression.

A key goal for those seeking to develop therapeutic strategies for diseases such as cancer is to deliver drugs to the target cells only.[5,6] The advantages of being able to avoid delivering such agents to healthy cells are obvious. For example, antibodies and peptide-mediated delivery of therapeutics to tumour cells could alleviate the problem of side effects because high concentration of the drug within tumours could be attained without affecting normal tissues.[6,7]

The authors approach to targeting siRNAs to the right cells uses endocytosis, a major cellular mechanism for the uptake of macromolecules from the environment.[8] Ligands that recognize cell-specific receptors preferentially or specifically expressed by the target cell can be conjugated to delivery agents (e.g. liposomes) to promote specific cellular uptake via receptor-mediated endocytosis.[8,9] The use of monoclonal antibodies to direct the delivery of siRNAs carried inside liposomes to specific cell types had already been demonstrated *in vivo*.[10] However, most approaches to receptor-mediated gene transfer use a ligand moiety that is chemically conjugated or mixed to agents that confer DNA-binding capacity. By contrast, in this new work, the authors used engineered antibodies that combine in a single fusion protein the function required to target cell recognition, nucleic acid binding, and cellular internalization to deliver siRNAs into a defined cell population.[11,12] Specifically, the antibody heavy chain sequences were fused to protamine as a nucleic acid-binding domain (Figure 1a) and used to target siRNAs to cells expressing human immunodeficiency virus (HIV) envelope glycoprotein (gp120). The siRNA–antibody complexes are taken up by the cell via receptor-mediated endocytosis and dissociated in the endosomes, leading to the release of siRNA molecules into the cytoplasm where they can enter the RNAi pathway and guide the sequence-specific mRNA degradation (Figure 1b).

The success of this targeting strategy was impressive: siRNA against various genes delivered through an anti-gp120 antibody silenced gene expression only in cells expressing gp120. The authors also demonstrated that anti-ErbB2 single-chain antibody fused to protamine could deliver siRNAs specifically to a breast cancer cell line expressing the ErbB2 receptor, a member of the epidermal growth factor receptor (EGFR) family, which plays an important role in the development of human malignancies.[13] More importantly, a cocktail of siRNAs against the pro-oncogene *c-myc*, *MDM2* and vascular endothelial growth factors when delivered via the protamine-Fab antibody inhibited tumour growth in mice. Similar to *in vitro* data, only tumours expressing gp120 and treated with the siRNA/protamine antibody complexes exhibited reduced growth.

Taken together, these results are an exciting advance in siRNA delivery, as they provide a conclusive example of the use of engineered antibodies to deliver siRNAs into cells. Therefore, in future, siRNAs could be specifically directed against general vital target genes in tumour cells, without affecting normal cells. Although still not as efficient as viral vectors, the strategy has several advantages. These include high target cell specificity, lack of infection risk, nonactivation of innate immunity (despite the localization of the siRNA within the endosomes) and potential activation of endosomal Toll-like receptors (TLRs).[14] Nonetheless, these experiments were performed in nonrelevant immune cells, so additional studies are needed to evaluate the immunostimulatory capacity of antibody-mediated siRNAs.

For systemic application, antibody-targeting strategies should have the following characteristics: (i) stability in blood with a prolonged half-life; (ii) transport across capillary endothelial wall; (iii) specific binding to cell plasma membrane; (iv) active endocytosis in the cell; and (v) siRNA activity in the targeted cells with the desired biological end point. The preferential accumulation of the delivered siRNAs in specific cells in the body is dependent upon the interplay between these processes. During the first three steps, siRNAs must maintain binding to protamine until they reach the target cells. Suppression of tumour growth in mice indicates the ability of the targeted antibody to carry siRNAs, extravasate, home to and kill the targeted tumour cells. A key issue regarding the potential utility of the method is whether it would be effective in larger, more clinically relevant animals. Additionally, the relatively high concentration of siRNA required to inhibit gene expression is not tenable in patients, so the method requires further improvements to address this problem.

One of the major requirements imposed by targeting methods is the availability of specific receptors on a particular cell type for successful delivery entry. Several studies point to the heterogeneous nature of cancer cells.[15] Therefore, the question is whether a targeting receptor will be expressed in all metastases in a particular patient. For example, if in breast cancer the lung metastases do not express the targeted receptor (e.g. ErbB2), then it is doubtful that the targeting strategy will benefit

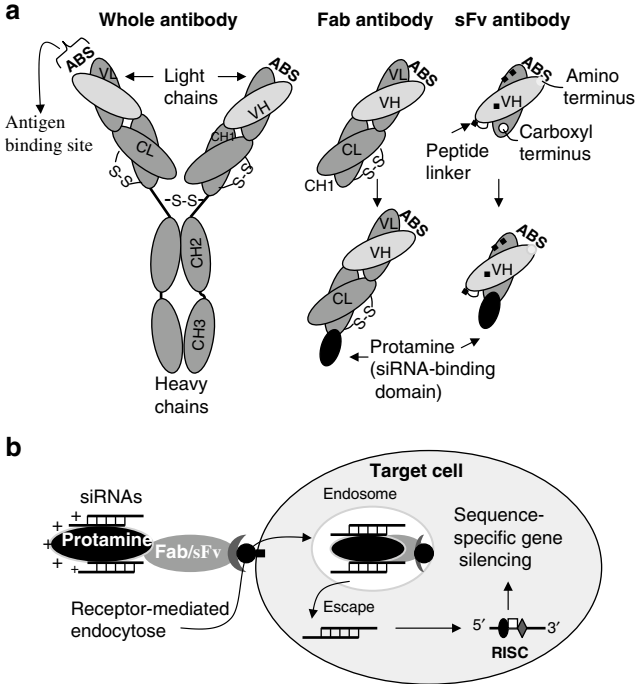

Figure 1 Antibody-mediated siRNA targeting. (**a**) Schematic structure of a whole IgG antibody, monovalent Fab antibody and a single-chain antibody (sFv) is shown. IgG antibody is composed of four chains: two heavy and two light chains. Each chain is composed of two domains: a variable region (VL or VH) and constant region (CL, CH1, CH2, and CH3). The variable region of one light chain pairs with the variable region of one heavy chain to form the overall antigen-binding site (ABS). In the whole antibody, the chains are joined via disulphide bonds. However, in scFv, the heavy and light chains are covalently linked by a flexible peptide linker. The human cationic peptide protamine was fused to the C-terminus of the engineered antibody. (**b**) The antibody recognizes its antigen receptor expressed on the cell surface, and then the complex of antibody and siRNA is internalized via endocytosis. Subsequent to endosomal escape, the siRNA is released into the cytoplasm and then incorporated into the RNAi targeting complex known as RNA-induced silencing complex (RISC) that uses the antisense siRNA strand to recognize and cleave the targeted mRNA.

patients. Additionally, most antibodies have low tumour penetrance and the ability of the targeted siRNAs to gain access to all tumour cells *in vivo* is questionable. Thus, there is an urgent need for combination therapy.

A weakness of the fusion proteins used by the authors is that purification and refolding steps are needed for each recombinant fusion protein. This might be addressed by the production of soluble antibodies with phage display technology.[16] Despite the limitations mentioned above, there is no doubt that antibody targeting is an attractive strategy for the selective delivery of siRNAs. The potential of the method is by no means limited to synthetic siRNAs and may be extended to gene-encoding hairpin siRNAs, which are potentially useful for maintaining sustained expression of siRNAs.

Transcriptional targeting, whereby regulatory sequences from tissue-specific genes are used to control the expression of siRNAs, should represent an additional viable strategy. For example, it may be possible to restrict siRNA expression to cancer cells by placing the siRNA under the control of a promoter taken from a gene selectively expressed in cancer cells. The successful delivery or expression of siRNAs in a desired cell population will undoubtedly have great therapeutic applications in treating various human diseases. ■

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