NEWS AND VIEWS

Progress in targeted delivery of therapeutic siRNAs

Three recent studies have demonstrated rapid advancements in overcoming the challenges facing application of RNA interference based therapeutics. Researchers at SiRNA Therapeutics (Boulder, USA) and Caltech and Children`s Hopsital (Los Angeles) have shown that packaging in colloidal particles significantly enhances the activity of siRNAs delivered intravenously to mice. These reports suggest that systemic administration of siRNAs may soon be ready for human trials.

Before siRNAs can be employed therapeutically, researchers must overcome the problems of *in vivo* stability, tissue specific targeting and unwanted immune system activation. Encapsulating siRNAs in lipid vesicles offers both protection from specific degradation by RNases and limits the interaction of the naked nucleic acid with immune system receptors that recognise pathogenic RNAs, offering to overcome at least two of these challenges. Previous experiments using chemically modified siRNAs against a mouse model of hepatitis B virus (HBV) required clinically unrealistic doses (~30mg/kg) to produce satisfactory reduction in serum levels of HBV siRNA (Morrisey et al, 2005). By encoating unmodified siRNA in a lipid bilayer to improve cellular uptake, and a polyethylene- glycol-lipid coating to slow systemic clearance, the required dosage for 95% reduction in HBV serum titre is reduced to 1-5mg/kg. Targeting of such vesicles has become the next focus of development. Whilst delivery to the liver is "relatively straight forward" according to SiRNA Therpaeutics Senior Director of Biology, David Morrisey, specific delivery to other tissues and avoidance of the liver mediated serum clearance will require some functionalisation of the lipid vesicles.

In attempting to tackle tissue specific delivery, Hu-Lieskovan et al (2005), in collaboration with Colando (Duarte, CA) describe cyclodextrin-containing polycationic colloidal particles tagged with transferrin protein that targets siRNA to tumour cells expressing a transferrin receptor in a murine model of metastatic Ewing`s sarcoma. The particles also contain polyethylene glycol to enhance serum lifetime. The formulation self-assembles once mixed and is suitable for the intravenous delivery of single and double-stranded nucleic acids at low concentration (~2.5mg/kg). Although unable to cross the blood-brain barrier, and so ineffective for brain tumours, no increase in serum indicators of immune response was observed and no functional or pathological defects were observed in the major organs. In addition, cyclodextrin-mediated delivery also largely eliminates the interferon response for known immunostimulatory siRNA motifs. An advantage of this methodology is that the delivery system may easily tuned to different cell surface receptors, so allowing for delivery to a range of tissues and organs.

Further to these colloidal delivery techniques, Song et al (2005) have described an antibody based delivery system which is effective at delivering siRNAs to hard-to-transfect HIV-infected primary T cells in culture. Further to this, *in vivo* studies in mice indicated that intratumoral or intravenous injection of antibody complexed siRNAs targeting c-myc, MDM2 and VEGF specifically inhibited subcutaneous B16 tumors expressing the HIV envelope – but not normal tissues or B16 cells not expressing envelope. An ErbB2 single-chain antibody fused with protamine also delivered siRNAs specifically into ErbB2-expressing cancer cells. This highly specific and readily customizable delivery system offers the potential for systemic, cell-type specific siRNA delivery.

The studies provide evidence that siRNAs can be can be delivered by a route of administration and at a dose that is acceptable for use in humans. SiRNA Therapeutics are currently testing their lipid vesicle-delivery in primate models of HCV, and hope to move to Phase I clinical trials in 2006, whilst Calando (Duarte, CA) are confident that the

cyclodextrin-containing polymer system will advance beyond the preclinical stage.

Taken together, these reports show the incremental improvements that are being made to siRNA delivery. Coupled with advances in selecting active siRNAs and, to some extent chemical modifications that enhance lifetime and/or potency, it seems that useful siRNA based drugs might be clinically available in the very near future.

REFERENCES

Morrisey DV et al. 2005. Nat Biotechnol, 23, 1002-1007.
Bradbury J. 2005. Drug Discovery Today, 10, 1266-67.
Hu-Lieskovan S et al. 2005. Cancer Res, 65, 8984-8992.
Song E et al. 2005. Nat Biotechnol, 23, 709-717.