MILESTONE 11

Targeted siRNA delivery in vivo

Advances in the characterisation of selective gene silencing through small interfering RNAs (siRNAs) increased the interest in using this approach as a therapeutic tool. The first proof-of-principle demonstration of the therapeutic potential of siRNAs was performed by Judy Lieberman's group in 2003 (MILESTONE 8). To silence gene expression in a mouse model, the group administered siRNA by hydrodynamic tail-vein injection, which has two major challenges for potential clinical application. First, it involves the exchange of a large proportion of the blood volume. Second, the method worked for liver cells but it was unclear if it could also be used to target other cells, for example, immune cells.

Both challenges were addressed by Lieberman and colleagues in a subsequent study published in 2005. To target specific cells in the body, the authors used an antibody fragment (F105) that recognised HIV-1 envelope protein, which is expressed on the surface of cells that are infected with HIV. They fused F105 with protamine, generating F105–P molecules. The fusion to protamine was necessary for binding siRNAs, because the antibody fragment itself does not bind them. F105–P had previously been shown to transport DNA into cells.

The researchers first confirmed that F105–P enabled the uptake of siRNA, and the selective and dose-dependent reduction of target mRNA levels only in cells expressing HIV-1 envelope. Then, they tested how F105–P works in primary T cells, which are challenging to transfect with conventional methods. F105–P loaded with siRNAs targeting the HIV-1 capsid gene *gag* not only decreased HIV replication in HIV-infected T cells, but also reduced the release of viral particles into the cell culture medium. This demonstrated that the approach enables the targeting of selected cell types based on cell surface proteins to silence or reduce mRNA expression.

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The next step was to show that F105–P-mediated gene silencing also works in vivo after systemic application of F105–P. For this, tumour cells expressing an HIV envelope protein were implanted into mice. Only these cells, but not surrounding cells, took up siRNA when F105–P was injected into the tumour cells or administered through intravenous injection. Furthermore, injection of F105–P molecules binding a mixture of siRNAs against tumour-growth-related genes reduced tumour growth, thereby demonstrating effective in vivo gene silencing by F105–P-mediated siRNA delivery.

The researchers also demonstrated the broader applicability of their approach by using an antibody against the receptor ERBB2 to silence Ku70 expression in *ERBB2*-expressing cells.

The major achievement of this work was the proof-of-principle demonstration of targeted siRNA delivery in vivo. A similar approach, published a year later, used RNA-based aptamers instead of antibodies, which are easier to produce and less immunogenic. Both methods laid the foundation for targeted siRNA delivery in vivo. Although some important issues, like the pharmacokinetics and a detailed safety and toxicity assessment, remained to be investigated in more detail, targeted siRNA uptake by selected cells was an important step towards developing siRNAs for clinical applications while minimising adverse side effects.

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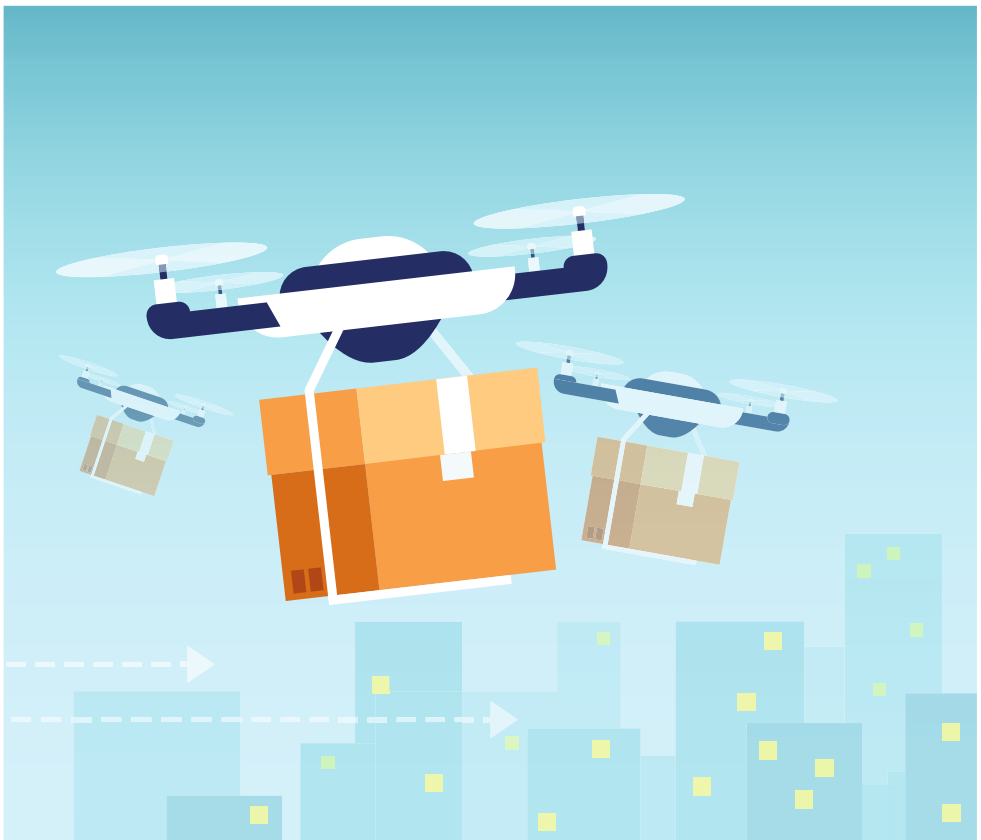

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MILESTONE STUDIES Song, E. et al. Antibody mediated in vivo delivery of small interfering RNAs via cell-surface receptors. *Nat. Biotechnol.* **23**, 709–717 (2005) | McNamara II, J. et al. Cell type–specific delivery of siRNAs with aptamer-siRNA chimeras. *Nat. Biotechnol.* **24**, 1005–1015 (2006).