GENE DELIVERY

Cell specific therapy on target

A polymer–lipid nanoparticle with a low molecular weight can preferentially deliver small interfering RNA to endothelial cells, offering an opportunity to treat many diseases.

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The use of genes to treat or prevent diseases (also known as gene therapy) has been explored for over 40 years. Yet, few techniques – be it to replace a mutated gene with a healthy one, suppress a dysfunctional gene using small interfering RNA (siRNA) molecules, or introducing a new gene into the body – have made it to the clinic. Although current clinical trials show encouraging outcomes[1-3], achieving cell-specific delivery remains challenging. Nanoparticles have been used to deliver genes but due to their size and charge they are often deposited in the liver, and can cause undesirable off-target effects. Writing in *Nature Nanotechnology*, Daniel Anderson and colleagues[4] at Massachusetts Institute of Technology, Alnylam Pharmaceuticals, Harvard Medical School, University of Colorado and Technion Israel Institute of Technology now report a low-molecular-weight polymeric nanoparticle that preferentially delivers siRNA to endothelial cells, which are implicated in many diseases.

Inder Verma of the Salk Institute for Biological Studies famously opined that the three major challenges to gene therapy are “delivery, delivery, and delivery”. In RNA interference, to achieve the desired therapeutic effect, nanoparticles carrying siRNA molecules that inhibit the expression of certain genes would ideally be designed such that they are delivered only to the cells of interest. Many methods have been used to target nanoparticles to the desired cells. For example, by decorating nanoparticles with single-chain antibodies or ligands such as transferrin or folate[2] that are expressed on a variety of cancer cells. These methods generally result in improved uptake of nanoparticles in target tissues but getting the siRNA molecule to silence the relevant genes *in vivo* to achieve therapeutic outcomes remains difficult[5].

Anderson and co-workers developed a library of lipid-polymer particles formulated with siRNA and tested each of the particles for their ability to reduce gene expression

in cells. From the library and screening process, they identified one compound (called 7C1) that preferentially delivers siRNAs to endothelial cells *in vivo* (Fig. 1). The compound, which is a combination of epoxide-terminated lipids and polyethyleneimine, forms multilamellar vesicles that are between 35 and 60 nm in diameter. The nanoparticle formulation could reduce 90% of gene expression in endothelial cells found in the lung using a dose of just 0.1 mg per kg body weight. Silencing genes in endothelial cells of the heart and kidneys was also possible.

Unlike other lipid or lipid-like nanoparticles, this formulation does not significantly reduce gene expression in liver or immune cells, even at the dose necessary for silencing genes in endothelial cells. The implications are that common side effects such as toxicity and non-specific activation of the immune system will be minimized.

The nanoparticles were tested in a variety of *in vivo* disease models, including emphysema, primary tumour growth, and metastasis. In lung vasculature, 7C1 was able to simultaneously silence up to five genes that are specific to endothelial cells at a low dose of 0.25 mg per kg, whereas 2 mg per kg of a control formulation did not alter gene expression in endothelial cells. This opens up the possibility of targeting multiple genes at once. The reason these particles preferentially target endothelial cells is not yet clear. However, it is suggested that the preference may be due to a unique interaction between the lipid and certain serum proteins.

Polyethyleneimine alone has long been used for gene delivery but various versions of the polymer with differing molecular weight have been associated with off-target effects, toxicity or poor delivery. The low-molecular-weight lipid-polyethyleneimine variety described by Anderson and co-workers was well-tolerated and non-toxic even at 10 times the therapeutic doses in both acute and chronic settings. Silencing lasted 21 days in lung endothelial cells, with a less long-lived effect in renal and cardiovascular tissues. The latter may be the result of less efficient delivery to these tissues, or different cell turnover rates that might dilute the RNA interference effect. This length of silencing matches the results of Alnylam's recent clinical trial using PCSK9 RNA interference that targets the liver[6].

So far, efforts have mainly focused on improving the siRNA rather than the nanoparticle used for delivery. siRNAs that operate in the low picomolar range[3] *in vivo* are currently available and with a nanoparticle system such as 7C1 that delivers to a particular subset of cells, achieving both good delivery and therapeutic outcome

may soon be possible. Furthermore, unlike previous nanoparticles, 7C1 does not seem to rely on the enhanced permeability retention effect for delivery (that is, the size and charge of the nanoparticle did not determine the delivery location in this case). This means that non-specific delivery to tissues such as liver and spleen are limited.

The next step is to move 7C1 into clinical trials to see whether its specificity for endothelial cells can be translated to treating complex diseases such as emphysema, hypertension and arteriosclerosis, all of which are associated with dysfunctional endothelial cells[7].

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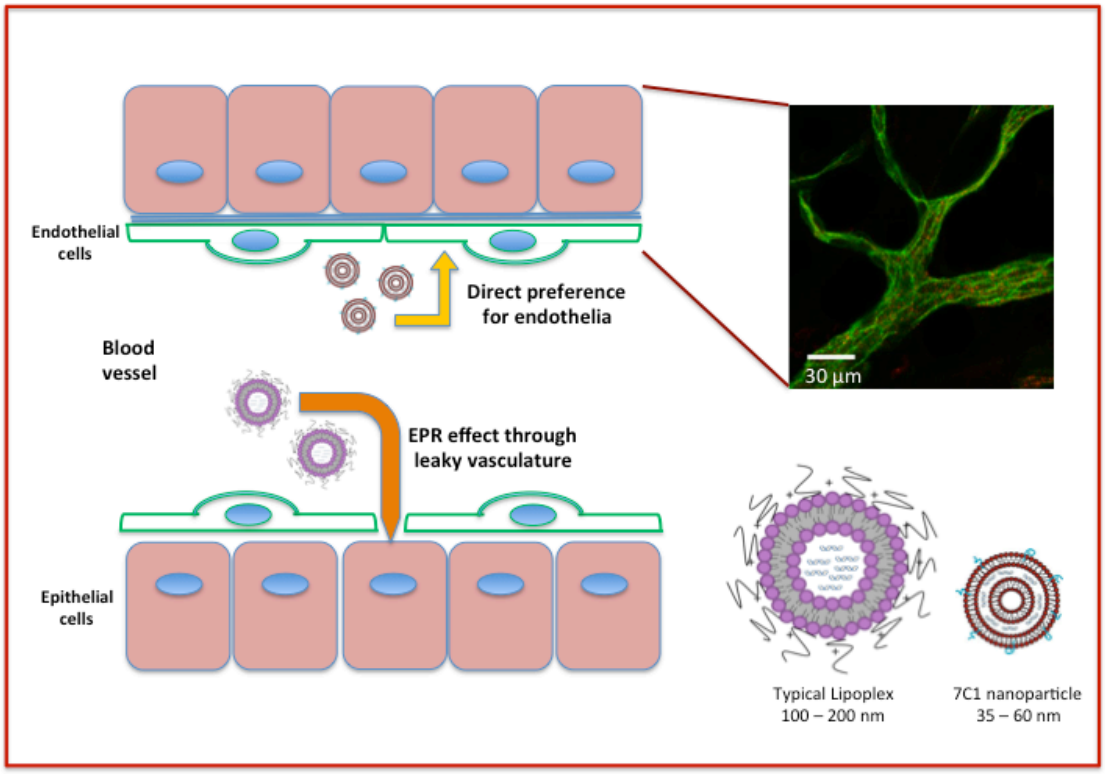

Figure 1: Small, multilamellar 7C1 nanoformulations selectively deliver siRNA to endothelial cells *in vivo***.** Typical lipoplexes (purple circles) ranging from 100 to 200 nm in diameter normally enter tissues through leaky blood vessels by the enhanced permeability and retention (EPR) effect, whereby the size and charge of the nanoparticle determine where it goes. Instead of relying on the EPR effect, 7C1 nanoparticles (red circles) bind preferentially to endothelial cells. Inset: Confocal fluorescence microscope image of skin tissue showing colocalization of intravenously administered 7C1 encapsulating siRNA (red) with endothelial cells (green)[4].