DAVID PARKINS

Hacking the body's delivery service

Researchers are taking advantage of nature's extracellular-vesicle network to deliver RNA therapies. **By Amanda Keener**

When Lydia Alvarez-Erviti started her postdoctoral studies at the University of Oxford, UK, in 2008, her goal was to develop gene therapies for neurodegenerative diseases. She had identified her target — α-synuclein, a protein that accumulates in the brains of people with Parkinson's disease — and designed a short interfering RNA (siRNA) to reduce the amount of α-synuclein made in mice. But she needed to get the siRNA into the brain. The method would have to protect the RNA, cross the barrier between circulating blood and the brain, and be safe enough to use repeatedly. Fortuitously, a colleague had begun studying something that could work — naturally occurring, nano-sized vesicles called exosomes.

Exosomes are 30–100-nanometre-wide lipid spheres that are used by cells throughout the body to transfer small molecules such as microRNA (miRNA). Optimized to travel in the body without attracting undue attention from the immune system, each tiny package is "an ideal drug carrier", says Juliane Nguyen, a bioengineer at the University of North Carolina at Chapel Hill.

Around ten years ago, Alvarez-Erviti, who is now at the Center for Biomedical Research of La Rioja, Spain, and her colleagues proved exosomes' potential as drug carriers in a mouse model of Parkinson's disease[1]. Now, a large body of a work in animals, along with early studies in people, has demonstrated the proficiency and safety of exosome products.

Exosomes are expensive to isolate from other types of extracellular vesicle (EV), and they naturally carry diverse, often uncharacterized, material. In terms of safety and standardization, these complexities place exosome-based therapies somewhere between cell therapy and treatment with small-molecule drugs. But these challenges have not deterred Alvarez-Erviti's team or the other research groups and companies working to standardize and scale up EVs for use in people. "When you work with exosomes," she says, "you need to have to have a lot of gumption."

The natural alternative

For RNA and small-molecule drugs, getting inside cells is a major bottleneck for reaching targets. The body has measures in place to keep foreign material out of cells, including cell membranes and RNA-degrading enzymes.

Biotechnologists have come up with various workarounds. Synthetic nanoparticle carriers or empty viruses, for example, are often used to protect drugs from degradation and to promote their entry into cells. Among the most popular carriers are liposomes — spheres of lipid molecules, usually 100–200 nanometres in diameter, that can fuse with the cell membrane to deliver their cargo. But in high doses, liposomes can damage cells, and both liposomes and viral carriers can trigger immune reactions after repeated administration. These drawbacks have led many to consider exosomes as carriers — the RNA transport service that the body already has in place.

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Exosomes are regarded as safer than artificial vesicles because they already circulate through the body. Researchers have found that exosomes can be administered to cells in the lab without causing cell death, and repeatedly injected into mice without causing inflammation[2]. Alvarez-Erviti harvests exosomes from immature immune cells because vesicles from these cells don't have immune-activating molecules on their surfaces. Exosomes from mesenchymal stem cells are also popular because stem cells tend to avoid immune detection.

Like most nanoparticle drug carriers, exosomes accumulate mainly in the liver, lungs and spleen. But they also show an affinity for the tissues they were originally collected from. Bioengineer Ke Cheng at North Carolina State University in Raleigh found that when exosomes harvested from fibrosarcoma cells are injected into tumour-bearing mice, the vesicles are drawn to the tumours[3].

This homing characteristic means exosomes can deliver more of the drug to where it is needed, reducing the potential for side effects. Cheng's team reported that loading a liposome-based chemotherapy drug called doxorubicin into cancer-cell exosomes increased the amount of the drug that reached the tumours. Treatment with exosome-encased doxorubicin also shrank the tumours to a greater degree than did doxorubicin alone.

Vesicles from some non-cancerous cells also have useful homing abilities. According to Steven Stice, a stem-cell biologist at the University of Georgia, Athens, and co-founder of nearby biotechnology company Aruna Bio, exosomes from a human neuronal stem-cell line called AB126 cross the blood–brain barrier and home in on sites of injury. And some researchers are engineering exosomes to increase their retention in certain tissues. For example, Alvarez-Erviti's team genetically engineered cells to produce exosomes bearing rabies-virus proteins on their surface and that caused the vesicles to accumulate in the brain where the receptor for the protein is found.

Peptides that direct vesicles to desired tissues can also be chemically linked to exosome surface proteins or embedded into vesicle membranes — an approach that could speed up their preparation in clinical settings. Cheng's team, for example, used a commercially available phospholipid reagent to slip a peptide known to home to heart cells into exosome membranes. This increased exosome accumulation in the hearts of rats induced to have heart attacks[4].

Controlling the contents

When Alvarez-Erviti began to work with exosomes, she already had a therapeutic molecule for them to carry. But EVs are naturally filled with proteins, RNAs and lipids. Although their biological activity is largely uncharacterized, some seem to be therapeutic in their own right.

Researchers are working to identify the therapeutically active molecules inside exosomes and use them in new treatments. Cheng's team has found a human exosomal molecule, called miRNA-21-5p, that reduces the rate of heart-muscle cell death and improves blood-vessel growth and tissue repair after heart attacks in mice. The team's long-term goal is to generate exosomes with high levels of the miRNA and a cardiac cell homing peptide. These superexosomes, as Cheng calls them, would be administered through the bloodstream immediately after a heart attack.

One way to load EVs with therapeutic cargo is to disrupt vesicle membranes with electrical current or chemicals to allow drugs to enter. Another option is to genetically engineer vesicle-forming cells to make an RNA or protein drug before vesicle formation. However, there's no guarantee that an engineered cell will load the desired cargo into its vesicles. "The cells decide what to encapsulate," says Young Kwon, a biomedical and materials scientist at the University of California, Irvine.

Nguyen's team is studying how cells make those decisions, to find ways to ramp up exosome loading with artificial cargo. Researchers have identified strands of code common in natural exosomal RNAs that probably play a part in packaging the molecules. And Nguyen has found that copying some of these molecular codes onto other RNAs increases their loading into exosomes by up to 100-fold. She plans to use the technology to load breast cancer exosomes with miRNAs that block blood-vessel formation and cancer spread.

Another route to control vesicle content is to force their formation through physical or chemical manipulation of cells. Kwon's team chemically coaxes cells to pinch off membrane-bound pieces of themselves called blebs that, compared with naturally occurring EVs, are more homogeneous in size and content. Any RNA made by a cell should be distributed into the blebs randomly. Such cells can be made to produce ten times as many blebs as they can vesicles — and in hours instead of days[5].

A new biologic

EVs are challenging to turn into commercial products for the same reason that they have so many advantages — they have to come from living cells. Most companies are using a few well-characterized cell lines to produce all their exosomes. Stem cells are a natural choice, because they can be cultured for a long time and do not produce an immune response. Cells produce the most exosomes when grown in suspension rather than on a flat surface, says Jan Lötvall, who studies exosomes at the University of Gothenburg, Sweden. But stem cells must adhere to something to grow, so some companies use spherical microcarriers suspended in media — an approach that can increase exosome production 20-fold.

Firms also need to improve methods for purifying EVs from cell-growth media on a large scale — much bigger than in academic labs. Lötvall says that manufacturing issues such as these are surmountable, but will make EVs an expensive option for delivering therapies. There is also no clear path to approval yet. Cheng says drug regulators such as the US Food and Drug Administration have yet to release guidance on how these vesicles can be tested for safety and potency. For now, researchers and companies test them batch by batch, each using different assays depending on the drug they're developing.

Creating artificial exosomes could sidestep these challenges. But researchers still need to work out how exosomes are made and why they are so effective at infiltrating cells and evading immune detection. Only after they answer these basic questions will this new mode of drug delivery be ready for clinical service.

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