ADVANCES IN GENE THERAPIES FOR INTRACTABLE DISEASES

NEW TECHNOLOGIES TO OVERCOME A GENE-THERAPY BOTTLENECK AND USE ORGANOIDS CLINICALLY could result in fresh treatments for debilitating neurodegenerative and digestive diseases.

Researchers in Tokyo have broken ground in a gene therapy delivery method for diseases of the brain and central nervous system, and regenerative medicine for the intestinal tract.

For more than a decade a gene therapy involving short strings of base pairs, known as antisense oligonucleotides (ASOs), has been used to block the production of certain proteins by our body's RNA, explains Takanori Yokota, a professor in the Department of Neurology and Neurological Science at Tokyo Medical and Dental University (TMDU), in Japan.

However, ASOs bring with them a number of challenges, including unwanted side effects and problems with delivery, he says. So, while a number of ASO therapies have been approved by the Food and Drug Administration in the United States and the European Medicines Agency, for example, they have often been overshadowed by other treatment modes.

Yokota's team studies ways to improve ASOs targeting of neurodegenerative conditions, such as Alzheimer's and Parkinson's disease, and central nervous system disorders, which can result in the loss of motor neurons and progressive muscle wasting.

TARGETED GENE EXPRESSION REDUCTION WAS GREATEST IN NEURONS AND MICROGLIAL CELLS.

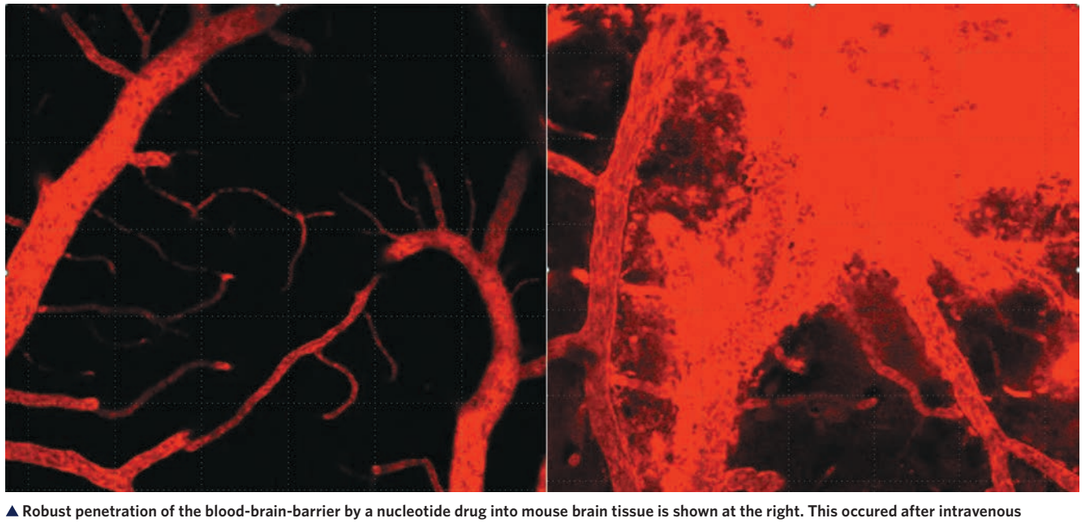
ASOs comprise a single strand of a few dozen base pairs arranged in the reverse of the order of a target RNA to allow them to bind to it, explains Yokota. But getting ASOs past the brain and spinal cord's natural barriers and into the central nervous system has been hard. The blood-brain barrier lining the capillaries, for instance, largely keeps the highly polar molecules at bay.

STRENGTH AND EFFECT

Yokota leads a team that in 2015[1] developed a new technique to address limitations around ASO delivery and strength of effect, using molecules called heteroduplex oligonucleotides (HDOs).

HDOs consist of ASO DNA and complementary RNA strands. The complementary strand is bound to cholesterol or tocopherol — lipophilic structures that are thought to assist HDO movement through cell membranes. In 2021, Yokota's team showed that the complementary strand is then cleaved[2] once inside the cells, releasing the parent ASO strand, which can block the protein expression of target RNAs.

The same year Yokota's team published a paper on the effectiveness of HDOs bound to cholesterol[3].



▲ Robust penetration of the blood-brain-barrier by a nucleotide drug into mouse brain tissue is shown at the right. This occured after intravenous injection of a heteroduplex oligonucleotide (HDO, right) drug, but not by antisense oligonucleotide (ASO, left) drug, in these images of live mouse brains.

"In experimental animals, we found that cholesterol bound HDO, unlike cholesterol bound ASO, efficiently reached the central nervous system by crossing blood-brain-barrier following a subcutaneous or intravenous injection," he says.

The animal research showed that the HDOs could distribute throughout the brain, spinal cord and nearby tissues and suppress the expression of four target genes by up to 90%, whereas single-stranded ASOs linked to cholesterol had limited effectiveness. Targeted gene expression reduction was greatest in neurons and microglial cells, resident cells that act as active immune defences in the central nervous system.

While high dose intravenous administration brings risks of side effects — such as lowered blood platelet count and localized brain necrosis — these issues can be negated by dividing the injections into two sets or using subcutaneous injection, says Yokota. Furthermore, the researchers confirmed that this outcome did not undermine the blood-brain barrier's integrity.

The team's plan is to improve the basic design of HDOs and apply the technique to more diseases, Yokota says. "We have been working with a number of big pharmaceutical companies to apply HDOs to Alzheimer's and Parkinson's, and also to cancer, infectious diseases, prion disease and psychiatric conditions, such as schizophrenia."

MINI ORGAN TRANSPLANT

Earlier in 2022, another team at TMDU performed what they believe may be the first case-study organoid transplant on a patient with ulcerative colitis, a disease that causes chronic inflammation of the intestine.

▲ Takanori Yokota (top right) is working to improve nucleotide drug delivery. Ryuichi Okamoto (top left and bottom centre) is developing organoids to help repair the large intestine.

Researchers cultured organoids — tiny, self-organizing, three-dimensional tissue cultures derived from stem cells — from healthy regions of the patient's own intestine, and then re-transplanted them to diseased regions where the organoids could generate fresh tissue.

For several decades, experts in the field of regenerative medicine have been focused on developing organoids, explains lead researcher, Ryuichi Okamoto, a professor in the Department of Gastroenterology and Hepatology. Despite their small size, organoids can replicate much of the complexity of an organ within the lab. "But this is the first clinical application of organoids that we are aware of," he says.

A significant challenge in treating some diseases of the digestive tract is the healing of the inner lining of the intestine, explains Okamoto. His team sees organoids as a possible solution.

For the case study, the team collected intestinal stem cells from the patient's healthy colonic mucosa and cultured them for about one month. They formed ball-shaped organoids just 0.1 to 0.2 millimetres across. The organoids were then cultured inside a type of jelly, placed in culture dishes and kept in a sterile lab.

An organoid was then transplanted into the colon of the patient using an endoscope — a minimally invasive procedure. In previous experiments using this method in mice, the team confirmed that the mucous membranes of the intestine regenerated in about a month[4]. However, comprehensive evidence from human subjects is yet to be collected.

Okamoto's team is now trialling the transplantation of organoids into a further eight patients. With luck, this may result in projects to develop new treatments for ulcerative colitis, and could potentially lead to organoid-based treatments for other gastrointestinal diseases, such as Crohn's disease.

"We are now on the cusp of a new era," Okamoto says. "In 10 to 20 years, maybe we could even replace entire organs." ■

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