

Shoukhrat Mitalipov and Masahito Tachibana's Mitochondrial Gene Replacement Therapy Technique

In 2009, Shoukhrat Mitalipov, Masahito Tachibana, and their team of researchers developed the technology of mitochondrial gene replacement therapy to prevent the transmission of a mitochondrial disease from mother to offspring in primates. Mitochondria contain some of the body's genetic material, called mitochondrial DNA. Occasionally, the mitochondrial DNA possesses mutations. Mitalipov and Tachibana, researchers at the Oregon National Primate Research Center in Beaverton, Oregon, developed a technique to remove the nucleus of the mother and place it in a donor oocyte, or immature egg cell, with healthy mitochondria. The resulting offspring contain the genetic material of three separate individuals and do not have the disease. Mitalipov and Tachibana's technology of mitochondrial gene replacement built on decades of research by different scientists and enables researchers to prevent the transmission of human mitochondrial diseases from mother to offspring.

Mitochondrial gene replacement therapy enables researchers to remove mutated mitochondria. Mitochondria are the organelles found in cells that provide energy for cells to function. Mitochondria contain a small portion of the cell's genetic material, called the mitochondrial DNA, or mtDNA. Mitochondrial DNA is comprised of thirty-seven genes responsible for energy production and cell function. Mitochondria are passed down from mother to offspring, meaning that offspring receives maternal mitochondrial DNA. Occasionally, the mother's mitochondrial DNA contains mutations, which often leads to mitochondrial disease. Because there are no known treatments for mitochondrial diseases, researchers experimented with ways to prevent the transmission of mutated mitochondrial DNA to offspring.

When developing the technology of mitochondrial gene replacement therapy, Mitalipov, Tachibana, and their team of researchers built off of the research of other scientists, and used many of the same techniques in his own work. In 1952, Robert Briggs and Thomas King transplanted nuclei into frogs' (*Rana pipiens* and *Rana catesbeiana*) eggs, which was the first successful instance of transferring of DNA into a complex organism. Briggs and King transplanted the nucleus of the embryonic cell of a tadpole into a frog oocyte from which the nucleus had been removed, or enucleated. Embryonic cells are cells found in the early stages of embryonic development. The nucleus contains most of the genetic material of a cell and dictates cell function. The oocytes with the embryonic nuclei resulted in healthy tadpoles that later developed into frogs. Briggs and King's experiment showed that nuclear transfer could be successful in more complex organisms than researchers had previously used.

The next instance of successful nuclear transfer was in John Gurdon's 1962 experiment. Gurdon used the nucleus of an intestinal cell from a tadpole (*Xenopus laevis*) and transplanted it into an enucleated oocyte of the same species of frog. Intestinal cells are differentiated somatic cells. Differentiated cells are cells that have begun functioning as a specific type of cell. Somatic cells are those of the body and are not involved in reproduction. When Gurdon transferred the intestinal cells into oocytes, the resulting tadpole was a genetic match to the intestinal cell donor tadpole. Researchers had thought nuclear transplantation of differentiated cells was impossible because the cells were already functioning as a certain tissue. The experiment was the first to demonstrate somatic cell nuclear transfer, which is the use of somatic differentiated cells in nuclear transfer rather than embryonic cells that have not differentiated.

In 1975, Derek Bromhall in Oxford, England, experimented with the embryos of rabbits using Briggs and King's method of nuclear transfer. He noted that Briggs and King's method used in amphibians damaged mammalian oocytes, causing them to die. Bromhall developed his own method that

involved using a glass pipette to penetrate the rabbit oocyte, extract the nucleus, and transplant the nucleus into the enucleated donor oocyte. He then fertilized the oocyte. Within a few days, an embryo began to form from the oocyte, demonstrating that mammalian oocytes could be used in nuclear transfer.

The use of nuclear transfer in sheep in 1986 continued the development of nuclear transfer techniques and the development of Mitalipov and Tachibana's mitochondrial gene therapy. At the Institute of Animal Physiology in Babraham Institute in Babraham, England, Steen Willadsen removed one of eight cells of a sheep's (*Ovis aries*) embryo. He removed an oocyte from another sheep and removed its nucleus. Applying a small electrical shock to the donor oocyte and the embryonic cell, a technique he developed, he fused the embryonic cell into an enucleated oocyte. The fused oocyte and cell began to divide into multiple cells. He implanted the oocyte into the womb of an adult female sheep, resulting in the birth of three lambs. The experiment demonstrated that nuclear transfer could be successfully demonstrated using more complex mammalian oocytes.

In 1988, Douglas Wallace described the existence of mitochondrial DNA disease. Wallace was a researcher at Emory University School of Medicine in Atlanta, Georgia. In an article he published, Wallace detailed a specific mitochondrial DNA mutation that was associated with the disease Leber's hereditary optic neuropathy, a degenerative disease that attacks the eyes. Wallace examined the hereditary nature of mitochondrial diseases and found that a single mutation in the mitochondrial DNA could lead to a mitochondrial disease.

In 1996, Ian Wilmut and Keith Campbell used somatic cell nuclear transfer, a form of nuclear transfer involving developed cells, to clone a sheep (*Ovis aries*). In the experiment, the team used the somatic cell of an adult sheep. They removed the nucleus of the somatic cell and transplanted it into an enucleated donor oocyte. Wilmut and Campbell's experiment differed from Willadsen's in that they used a differentiated cell from an adult sheep as opposed to an embryonic, undifferentiated cell. After the team fused the somatic nucleus to the donor oocyte, they implanted it into the womb of an adult sheep and allowed it to develop. Wilmut and Campbell, after many attempts, successfully transplanted the oocyte into a donor sheep and produced a healthy lamb named Dolly. Dolly contained the exact genetic code of the sheep that donated the somatic cell. The process of somatic cell nuclear transfer, using differentiated somatic cells, became known as a kind of cloning.

Wilmut and Campbell found that differentiated cells can turn off the genes associated with pluripotency. Pluripotency is the ability of a cell to function and grow into cell types that make up tissues of the body. Pluripotency is present in embryonic stem cells, cells of the early embryo. When a cell differentiates in an embryo, Wilmut and Campbell observed, it deactivates the genes in the nucleus responsible for the functioning of other tissue types. Wilmut and Campbell observed that after transplanting a somatic sheep cell's nucleus into a donor oocyte, the cytoplasm in the oocyte transformed the differentiated nucleus, or somatic cell nucleus, into a pluripotent one. Cytoplasmic factors had turned the nuclear genes responsible for pluripotency back on. That enabled an adult cell to be transformed back into a pluripotent embryonic cell.

In 2009, Mitalipov and Tachibana developed the technology of mitochondrial gene replacement therapy at the Oregon National Primate Research Center in Beaverton, Oregon. Mitalipov was the first to successfully apply the technology in 2009 and documented the results in his 2009 article "Mitochondrial Gene Replacement in Primate Offspring and Embryonic Stem Cells." Prior to that article, no other researchers had successfully performed mitochondrial replacement therapy in *Macaca mullata*, or rhesus macaque monkeys.

Mitalipov and Tachibana, in their research, found two ways in which mitochondrial diseases can arise. The researchers argued that the first way that a mitochondrial disease can arise is when the mother passes highly mutated mitochondria to her offspring, though she herself may not express a mitochondrial disease yet. An individual can possess a high ratio of mutated mitochondria to healthy mitochondria but may not develop a disease until later in life. A mix of healthy and mutated mitochondria is referred to as heteroplasmy. The development of a mitochondrial disease later in life occurs because the ability to repair damaged mitochondria diminishes as a person ages. As a result, mutations accumulate over time. A mother may possess a higher percentage of mutated mitochondrial compared to healthy mitochondria. The accumulation of mitochondrial mutations

could add onto her existing ones and allow the disease to develop later in life.

Mitalipov and Tachibana claimed that the second way a mitochondrial disease can arise in an individual is through the transmission from mother to offspring when the mother has already developed and is expressing a mitochondrial disease. A mother will pass on a mitochondrial disease to her offspring if all or nearly all of her mitochondria are mutated, a condition known as homoplasmy. A mother with homoplasmy is guaranteed to pass on the mitochondrial disease to her offspring. Because there were no existing treatments or cures for the disease, the researchers determined a way to prevent the transmission of the disease, rather than focusing on a treatment or cure. The first step required in Mitalipov and Tachibana's mitochondrial gene replacement therapy technique involves the retrieval of the donor oocyte. The female body only matures one oocyte at a time, making a collection of many oocytes problematic for researchers requiring multiple oocytes for experimentation. To stimulate the production of multiple oocytes, doctors administer a series of hormones to induce the over-production of oocytes. Once the oocytes are mature, they are removed from the donor all at once. The nuclei are removed to allow room for the mother's genetic material to be implanted.

At that point in the process, there are two paths researchers can take. Mitalipov and Tachibana developed two types of mitochondrial gene replacement therapy that differ slightly. The two are called pronuclear transfer and maternal spindle transfer.

According to the researchers, the first type of mitochondrial gene replacement therapy is pronuclear transfer. In pronuclear transfer, the researchers remove the affected mother's oocyte. Immediately upon removal, they fertilize the oocyte with the sperm of the father. Once the oocyte has been fertilized, a pronucleus begins to form. The pronucleus is the fusion of the sperm's nucleus and the oocyte's nucleus and forms the genetic material of the offspring. After the formation of the pronucleus, researchers remove the entire pronucleus and transplant it into the donor oocyte whose nucleus was removed. The oocyte that forms has the nuclear genetic material of the mother and father with the healthy mitochondrial DNA of the donor.

In the second type of mitochondrial gene replacement therapy that Mitalipov and Tachibana developed, known as maternal spindle transfer, the affected mother's oocyte is removed. The researchers remove the spindle fibers that are surrounded by cytoplasm. The spindle fibers contain the genetic information of the nucleus. The spindle fibers and the cytoplasm that surround them are called the karyoplast. The karyoplast is transplanted into the donor oocyte whose spindle fibers were removed. After the karyoplast successfully transplants, researchers then fertilize the oocyte with sperm and implant the fertilized oocyte back into the woman with mutated mitochondria. The resulting offspring has the nuclear genome of the mother and sperm donor, but has the mitochondrial genome of the donor woman. However, the offspring lacks the mitochondrial disease that its mother possesses.

Mitalipov and Tachibana's development of the technique encountered debate within the scientific community and in the general public. Francoise Baylis, an ethicist, argued that the manipulation of the human genome, no matter how small, alters what she considers the normal development of that individual. She questioned whether doctors should be allowed to change the genetic make-up of an individual before they are born to consent to such a change. Another area of concern that Baylis noted was the rarity of mitochondrial diseases. She argued that there are much more common diseases that effect much larger numbers of the population that deserve funding. Baylis questioned whether we are saving a few lives at the expense of thousands of others.

Others, such as Mitalipov and his colleague Don Wolf, argued that with plenty of testing, the therapy has the potential to save many lives and reduce human suffering. They argued that nonhuman primates serve as a strong background of research. The researchers claimed that the scientific community is ready to begin trials on human subjects.

Although the technology has not been approved for use in humans in the United States, as of 2017, it has been approved in the United Kingdom.

Sources

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