Shoukhrat Mitalipov and Masahito Tachibana’s Mitochondrial Gene Replacement in Primate Offspring and Embryonic Stem Cells (2009) [1]

By: Lee, Giselle Keywords: Spindle Transfer [2]

In 2009, at Oregon National Primate Research Center in Beaverton, Oregon, Shoukhrat Mitalipov, Masahito Tachibana, and their team of researchers replaced the mitochondrial genes [3] of primate [4] embryonic stem cells via spindle transfer. Spindle replacement, also called spindle transfer, is the process of removing the genetic material found in the nucleus [5] of one egg [6] cell, or oocyte [7], and placing it in another egg [6] that had its nucleus [5] removed. Mitochondria are organelles found in all cells and contain some of the cell’s genetic material. Mutations in the mitochondrial DNA can lead to neurodegenerative and muscle diseases. Mitalipov and Tachibana used spindle replacement to produce healthy offspring from an egg [6] with mutated mitochondria in rhesus macaques (Macaca mulatta). The experiment showed that spindle transfer eliminated the chance of transmission of mitochondrial diseases from the affected primates to their offspring, offering the potential to eliminate mitochondrial diseases in humans [8].

The experiment focused on mitochondria, which are energy-producing organelles found in all eukaryotic cells that are important to normal development. The mitochondria contain thirty-seven genes [3] responsible for energy production in cells. Occasionally, the genetic material found in the mitochondria contains mutations. Mitochondria are maternally inherited, meaning they are passed from mother to offspring. Mitochondria contain approximately 0.01 percent of a cell’s genetic material, which is called mitochondrial DNA. Each mitochondrion contains between two and ten copies of mitochondrial DNA. Each cell, containing many mitochondria, can contain thousands of copies of mitochondrial DNA. Because of the large number of copies of mitochondrial DNA, mutations occur in the mitochondrial DNA ten times more often than in the nuclear genome [8], the DNA of the cell’s nucleus [5]. Researchers associate many mutations of mitochondrial DNA with specific disorders, including myopathies, or muscular disorders, neurodegenerative diseases, and diabetes.

To identify mitochondrial DNA mutations within a cell, researchers analyze the genetic material of mitochondria from that cell. A cell normally contains many copies of the same mitochondrial DNA, called homoplasy. When a cell contains two or more types of mitochondrial DNA, called heteroplasy, researchers are alerted that mutated mitochondria are present. When enough cells contain mitochondrial mutations, different tissues can be affected, which can result in mitochondrial diseases.

In 2009, Mitalipov and Tachibana studied rhesus macaque eggs with mitochondrial DNA mutations. The researchers used the technique of spindle transfer to successfully transfer the nuclear DNA in the affected egg [6] into a donor egg [6] containing healthy mitochondrial DNA. Mitalipov and Tachibana hypothesized that nuclear DNA from an egg [6] with mutated mitochondrial DNA could be transplanted into an egg [6] with healthy mitochondrial DNA through the use of spindle transfer technique, which would eliminate the possibility of passing the mutated mitochondrial DNA from mother to offspring. When using spindle transfer, researchers first remove the genetic material from an egg [6] cell that contains heteroplasmic, or a mix of healthy and mutated mitochondrial DNA. Then the researchers transplant that egg [6] cell’s genetic material into a donor egg [6] cell whose nucleus [5] has been removed and whose mitochondrial DNA is undamaged. Following the transfer of genetic information to the egg [6] and after a sperm [10] has fertilized the egg [6] cell, the resulting embryo will develop with healthy mitochondria.

To begin, Mitalipov and Tachibana investigated the distribution of mitochondria around the cell’s cytoplasm within mature rhesus monkey [11] eggs to prevent transferring over mutated mitochondria. They found that mitochondria were evenly distributed across the cytoplasm. They also noted that chromosomes, which contain nuclear DNA, and spindle fibers that separate the chromosomes, lacked mitochondria in the cytoplasm that surrounds them. Those observations led Tachibana and Mitalipov to predict that they could transfer the spindle and chromosomes of a cell without also transferring the cytoplasm containing the damaged mitochondrial DNA. That would enable them to avoid transferring mutated mitochondria into the donor egg [6]. To test their prediction they removed the spindle-chromosomal complex surrounded by a small amount of cytoplasm, a complex called the karyoplast. Tachibana and Mitalipov calculated that only 1.5 percent of the cell’s cytoplasm was included in the karyoplast samples, a percentage they determined to be negligible. The researchers concluded that the small amount of mutated mitochondria contained in the 1.5 percent was not enough to affect the outcome of the egg [6] cell.

Next, Mitalipov and Tachibana investigated the process of transplanting the karyoplast into donor cells with their nucleus [5] removed. The team inserted the karyoplast into the egg [6] cell. After placement of the karyoplast in the egg [6] cell, the investigators attempted to facilitate the fusion of the karyoplast to the cell’s cytoplasm. To do that, they applied electrical stimulation to the egg [6] cell to encourage permeability, the ability of objects to pass through the cell’s membrane. After the
Sources

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