Oosight microscope enables embryonic stem cell breakthrough

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A noninvasive, polarized light microscope invented at the Marine Biological Laboratory (MBL) played a crucial role in a recent breakthrough in embryonic stem-cell research aimed at developing medical therapies.

A team led by Shoukhrat Mitalipov, Ph.D., of Oregon Health & Science University reported the successful derivation of stem cells from cloned monkey embryos in the November 22 issue of *Nature*. While embryonic stem cells have been made from cloned embryos in a mouse, this is the first time they have been produced in a primate.

In humans, this method for deriving stem cells is a potential way to make "custom" tissues that are genetically identical to a patient, which would avoid rejection by the patient's immune system. Stem cells, in theory, can be induced to become any type of cell, tissue or organ. However, in recent years, some investigators had claimed it wouldn't be technically possible to make embryonic stem cells from monkeys or humans using this method (somatic cell nuclear transfer, or therapeutic cloning).

Mitalipov's stem-cell derivation succeeded, he says, largely due to the Oosight ™ microscope system developed by Cambridge Research & Instrumentation Inc. (CRi) of Woburn Mass., using technology invented at the MBL by senior scientist Rudolf Oldenbourg, Ph.D., and research associate Guang Mei, Ph.D. Former MBL research scientists David L. Keefe, M.D., and Lin Liu, Ph.D., both of whom teach in the MBL's Frontiers in Reproduction course, worked with Oldenbourg to adapt the

technology for somatic cell nuclear transfer and embryology.

“The use of the Oosight was one of the major modifications we made in our present work,” Mitalipov says.

The Oosight allowed Mitalipov’s team to clearly see and remove the meiotic spindle (and the attached genetic material) from 304 female rhesus monkey eggs. This is the first step in therapeutic cloning, called enucleation. Next, they inserted the genetic material from the skin cells of an adult male rhesus monkey into the eggs and allowed them to grow to the blastocyst stage. From these cloned embryos, the researchers obtained two viable stem cell lines that are genetically identical to the adult male monkey.

“We are thrilled that the Oosight worked for enucleating monkey eggs,” says Keefe. “We already had shown at the MBL, in 2000, that the technology developed by Dr. Oldenbourg facilitated noninvasive enucleation of mice eggs.” At that time, Keefe operated a research lab at the MBL and directed the Division of Reproductive Medicine and Infertility at Women & Infants Hospital in Providence, R.I. Keefe is presently professor and chair of the Department of Obstetrics and Gynecology at the University of South Florida College of Medicine.

“Before, the problem was always that you could not see the spindle in the egg,” Mitalipov says. “The only way to see it was to stain it with dyes. And that, we found, was very detrimental for egg quality.” The Oosight uses liquid-crystal polarized light technology to image the spindle noninvasively, with high contrast and quality.

“You can actually look in the Oosight microscope and see the spindle with your eyes, not frozen as a computer screen image,” says Mitalipov, which is critical for the next step: taking the spindle out of the egg. “You can’t manipulate the egg while looking at a computer screen. You have to

look at the egg. The Oosight, plus very skilled micromanipulations of the eggs, gave us a 100% success rate with enucleating."

The Oosight is based on technology that is the result of decades of MBL research pioneered by Distinguished Scientist Shinya Inoué. In the 1950s, Inoué was one of the first cytologists to make extensive use of the polarized light microscope to observe birefringent components of the cell, which led to his landmark discovery in 1951 of the meiotic spindle fibers in living cells. He later showed that dynamic disassembly of the spindle fibers can produce force that moves the chromosomes toward the poles of the cell during mitosis or meiosis. In 1957, Inoué added a polarization rectifier to his custom-built microscope, which dramatically decreased distortion and improved the contrast of the image. Rudolf Oldenbourg further improved the polarizing microscope in the mid-1990s, adding liquid crystals with electro-optical controls and software. This version, called the LC-PolScope, allows one to simultaneously measure the birefringence in every resolved specimen point across the entire viewing field of the microscope; traditional models could only measure a single point of the specimen at a time. The LC-PolScope continues to be refined and expanded for live cell imaging at the MBL. Its technology is being adapted to different application areas by Cambridge Research & Instrumentation and is sold under trademarks such as Oosight and Abrio.

In the mid-1990s, David Keefe set up a lab at the MBL in order to collaborate with Oldenbourg and Inoué. Keefe was interested in finding out if the noninvasive LC-PolScope could be used in clinical settings to evaluate the quality of human eggs prior to in vitro fertilization procedures, and thus reduce the number of nonviable embryos created; and to improve the efficiency of therapeutic cloning in animals.

"When I was training as an ob-gyn at Yale in the mid 1980s, we were one of the first places to use ultrasound to assess the health of babies in

utero," Keefe says. "As a student in the MBL Physiology Course, I learned of Drs. Inoué's and Oldenbourg's work, and realized we could use the polarizing microscope to assess health even earlier during development — back to the beginning, at the egg stage — without hurting the egg," Keefe says.

Using the LC-PolScope, Keefe, Oldenbourg and collaborators reported for the first time on layers of birefringence in the egg's zona pellucida in 1997. In 2000, Lin Liu, Keefe, Oldenbourg and collaborators reported that the polarized light microscope also helped enucleate mouse eggs, the critical step during somatic cell nuclear transfer, and proposed that this approach would improve the efficiency and safety of therapeutic cloning (Nature Biotech. 18: 223-225).

The LC-PolScope was commercialized first as the SpindleView in 1999, later as the Oosight in 2005, with improvements for fast viewing contributed by Michael Shribak, Ph.D., associate research scientist at the MBL. The Oosight has the same optical components and the algorithms as the LC-PolScope, but it is simplified for routine operation. While polarized light microscopy is now used throughout the world in infertility clinics, and was proposed as an aid to cloning research, its actual application to cloning research was not appreciated until the recent Nature paper by Mitalipov et al.

"In spirit and in application, the Oosight really expands on what Shinya Inoué started in the 1950s," says Oldenbourg. "And it was David Keefe's vision that the LC-PolScope could be used for in vitro fertilization or for enucleation, since it can be used to visualize the spindle without staining it with dyes."

"It's very significant that the Oosight and its applications were developed at the MBL," says Keefe. "The MBL is such a special and unique place. We had Shinya Inoué, Rudolf Oldenbourg, and our group of mammalian

embryologists and clinicians, all working literally 25 feet from each other's labs. That's why this work was accomplished."

Source: Marine Biological Laboratory