Science

Stimulus-Triggered Acquisition of Pluripotency (STAP) Stem Cells: Promise and Controversy

In an unexpected and astounding discovery, scientists at the RIKEN Center for Developmental Biology in Kobe, Japan reported that they were able to reprogram specialized but immature mouse cells into pluripotent stem cells by simply exposing them to stress, either by bathing them in acid or by squeezing them.[1] The researchers called their new reprogramming approach stimulus-triggered acquisition of pluripotency (STAP). In one STAP protocol, the scientists lowered the pH of the cells' environment from a pH of just above 7—the normal pH of human blood—to a mildly acidic pH of 5.7 for twenty five minutes. This incredibly simple process triggered an increase in the expression of the pluripotency gene *OCT4* in a subset of the treated cells, so-called STAP cells, suggesting that they had reverted to a primitive stem cell state resembling embryonic stem cells.

When these STAP cells were injected into mouse embryos, they were able to contribute to all the tissues of the adult organism such that the animal was chimeric (i.e., composed of cells originating from the host embryo and from the STAP cells). This ability to generate chimeric animals is a hallmark characteristic of induced pluripotent stem cells and of embryonic stem cells. When the STAP cells were grown in laboratory conditions used to grow pluripotent stem cells, they began to grow and to divide, acquiring morphological and structural features associated with embryonic stem cells. Surprisingly, the STAP cells were also able to contribute to the developing

[1] Haruko Obokata et al., "Stimulus-Triggered Fate Conversion of Somatic Cells into Pluripotency," *Nature* 505.7485 (January 30, 2014): 641–647; and Haruko Obokata et al., "Bidirectional Developmental Potential in Reprogrammed Cells with Acquired Pluripotency," *Nature* 505.7485 (January 30, 2014): 676–680.

placenta, unlike previously characterized pluripotent stem cells, suggesting that they may represent a very primitive developmental state preceding the specialization of the cells that give rise to the embryo proper. Indeed, some have suggested that STAP cells are totipotent cells comparable to bona fide embryos. In my view, this claim involves a fundamental misunderstanding of developmental biology. Totipotent cells are cells that can give rise to the cell types that make up not only the mature organism but also the extra-embryonic tissues including the placenta. Embryos are totipotent cells that not only give rise to both the embryonic and extra-embryonic cell types but also do so in an organized and step-wise manner. Though this has not yet been explicitly tested in the lab, the data described in the two published papers suggest that it is unlikely that STAP cells can do what embryos can do. The new STAP protocol to generate pluripotent stem cells from specialized mammalian cells without the need for genetic manipulation would revolutionize stem cell science.

However, at the time of this writing (April 10, 2014), no one has been able to replicate the initial discovery. In fact, since they were published, it has become clear that there are methodological problems with the original two *Nature* papers describing the STAP protocol.[2] Significantly, a formal investigation launched by the RIKEN Center for Developmental Biology has concluded that the Japanese scientist who pioneered these STAP studies engaged in research misconduct that has undermined the trustworthiness of her data.[3]

Other Stem Cell Developments

Three research papers published this past quarter report novel developments in stem cell technology. First, a group of scientists from the Centre for Genomic Regulation in Barcelona, Spain has discovered a more efficient mechanism for reprogramming induced pluripotent stem cells.[4] This protocol, which reprograms a kind of blood cell called B cells using a specific gene called C/EBPα, decreases the time frame needed for cell reprogramming from several weeks to a few days. The C/EBPα factor opens the genetic regions of the B cells that contain the genes involved in reprogramming cells to pluripotency.

Next, Daisuke Nakada and his colleagues from the University of Texas Southwestern Medical Center and the Baylor College of Medicine in Texas report that stem cells in the blood-forming tissues of a female mouse's body react differently to hormones—specifically estrogen—than the corresponding stem cells from a male.[5]

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[2] For details see the following representative blog post: "Are STAP Stem Cell Nature Papers Compromised?," *Knoepfler Lab Stem Cell Blog*, February 23, 2014, http://www.ipscell.com/2014/02/are-stap-stem-cell-nature-papers-compromised/.

[3] Elaine Kurtenbach, "Japan Laboratory Says Probe Finds Stem Cell Research Falsified," *US News and World Report*, April 2, 2014, http://www.usnews.com/news/business/articles/2014/04/01/japan-lab-says-stem-cell-research-falsified.

[4] Anna B. Di Stefano et al., "C/EBPα Poises B Cells for Rapid Reprogramming into Induced Pluripotent Stem Cells," *Nature* 506.7487 (February 13, 2014): 235–239.

[5] Daisuke Nakada et al., "Oestrogen Increases Haematopoietic Stem-Cell Self-Renewal in Females and During Pregnancy," *Nature* 506.7484 (January 23, 2014): 555–558.

Female blood-forming stem cells divided more frequently than male stem cells when they were bathed in estrogen, explaining why red blood cell production is enhanced during pregnancy.

Finally, stem cell biologists often assume that the process of cell specialization in a mammalian organism is an irreversible process akin to a log falling off a waterfall or to a digital clock progressing from yesterday to tomorrow. The reverse process, called dedifferentiation, had not been identified in a physiological context. Until now. A team from the Massachusetts General Hospital in Boston reports that they have observed dedifferentiation in the mammalian airway.[6] When the MGH scientists destroyed the basal stem cells found in the airway, they discovered that other specialized cells in the region began to proliferate and to dedifferentiate into stem cells filling the lacuna left by the destruction of the original stem cells. This discovery reveals that the processes of regeneration in normal tissue and in its diseased cancer counterparts are more complex than previously anticipated.

Understanding Fertility and Treating Infertility in Men and Women

What makes a fertile male? Scientists have recently shown that two genes, and only two genes, on the Y chromosome are enough to make a mouse a fertile male.[7] They generated reproductive germ cells from males with only two Y chromosome genes, the testis determinant factor Sry (sex-determining region Y) and the spermatogonial proliferation factor, Eif2s3y. Sry is the gene that transforms the primordial embryonic gonads into testes rather than into ovaries, and Eif2s3y is apparently the only gene needed for sperm formation. Using artificial reproductive technologies, the scientists were able to obtain live offspring from these two-gene males. The authors conclude that their findings are "relevant, but not directly translatable, to human male infertility cases."

Women who are treated with chemotherapy or radiation therapy are often faced with the prospect of infertility because of egg depletion. A team from Cornell University has discovered that the genes for checkpoint kinase 2, either Chk2 or Chek2, are responsible for eliminating the damaged oocytes that exist in the ovary.[8] Checkpoint kinase 2 (CHK2) is a molecular machine that plays a critical role in recognizing and repairing DNA damage. Deleting the genes of checkpoint kinase 2 in female mice prevented their eggs from being eliminated after radiation therapy. This opens up the possibility that a drug that blocks CHK2 function would protect a cancer patient's eggs from radiation-induced self-destruction.

Moving to a tissue-based solution to drug or to radiation induced infertility, the Practice Committee of the American Society for Reproductive Medicine has

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[6] Purushothama Rao Tata et al., "Dedifferentiation of Committed Epithelial Cells into Stem Cells In Vivo," *Nature* 503.7475 (November 14, 2013): 218–223.

[7] Yasuhiro Yamauchi et al., "Two Y Genes Can Replace the Entire Y Chromosome for Assisted Reproduction in the Mouse," *Science* 343.6166 (February 7, 2014): 69–72.

[8] Ewelina Bolcun-Filas et al., "Reversal of Female Infertility by Chk2 Ablation Reveals the Oocyte DNA Damage Checkpoint Pathway," *Science* 343.6171 (February 7, 2014): 533–536.

published a recent state-of-the-science review of ovarian tissue cryopreservation.[9] The committee points out that ovarian tissue cryopreservation, both ovarian cortical tissue and whole ovary cryopreservation—though, strictly speaking, they are still experimental or investigational approaches—may be the only option available to pre-pubertal girls undergoing aggressive chemotherapy and radiotherapy. Thus, the committee goes ahead and makes clinical recommendations for the use of this technology in hospitals today.

Finally, and involving a more radical solution to some forms of infertility, surgeons in Sweden have performed live-donor uterus transplants on nine infertile women with absolute uterine-factor infertility (AUFI). After six months, seven women retained their uteri with regular menstrual periods.[10] In one case, the transplanted uterus had to be removed because of blood clots, and in another, a local infection not fully treatable with antibiotics doomed the transplanted organ. Mild rejection episodes were successfully managed with corticoid steroids. From a Catholic perspective, it is important to note that the uterine transplants involved donors who were both pre- and post-menopausal. Moreover, five of the donors were the recipients' mothers while the remaining four were close relatives. Lastly, a news release from the University of Gothenburg and the Sahlgrenska University Hospital has announced that embryos conceived from the women's own eggs using IVF will be transplanted into their donated wombs.[11] The publication of this preliminary report raises numerous ethical questions. How are we to evaluate the morality of a uterine transplantation (UTx)? Briefly, in my view, the removal of a uterus from a living donor—even from a post-menopausal woman—would involve an act of sterilization that is illicit. It robs a woman of her functional and reproductive integrity. However, it should be morally acceptable to transplant a uterus obtained from a post-mortem donation, if such a procedure is medically feasible.

Neural Circuits for Appetite and for Mate Choice

Two recent papers describe the identification of specific neural circuits for appetite and for mate choice. First, a team from the University of Washington has identified a group of nerve cells in the region of the brain called the parabrachial nucleus associated with appetite.[12] In experimental trials, hungry mice lost their appetite when the investigators selectively activated these nerve cells with a laser. They resumed eating as soon as the laser was turned off. If they identify the parallel

[9] Practice Committee of the American Society for Reproductive Medicine, "Ovarian Tissue Cryopreservation: A Committee Report," *Fertility and Sterility* (e-pub March 28, 2014).

[10] Mats Brännström et al., "The First Clinical Uterus Transplantation Trial: A Six-Month Report," *Fertility and Sterility* (e-pub February 28, 2014).

[11] Krister Svahn, "Next Step in Live-Donor Uterus Transplant Project," *Sahlgrenska Academy News*, March 3, 2014, http://www.sahlgrenska.gu.se/english/news_and_events/news/News_Detail/next-step-in-live-donor-uterus-transplant-project.cid1208775.

[12] Matthew E. Carter et al., "Genetic Identification of a Neural Circuit that Suppresses Appetite," *Nature* 503.7474 (November 7, 2013): 111–114.

circuitry in human patients, researchers hope to design therapies that can increase or decrease appetite.

Next, a Japanese group of scientists have found the nerve cells in the Japanese rice fish, also known as the medaka or Japanese killifish, that are associated with a female's inclination to mate with a male that she knows.[13] These nerve cells are called terminal-nerve gonadotropin-releasing hormone 3 (TN-GnRH3) neurons. They remained inactive when the female was surrounded by unfamiliar male fish. However, when she sees a male she knows, they are stimulated, releasing chemical signals that make the female more open to mating. Once again, these findings, and other data from other model organisms, suggest that it is likely that neural circuitry involving male choice exist in our species as well.

These two papers highlight an emerging research program to identify the neural circuits involved in the behaviors of vertebrate animals, including those fundamental species-specific behaviors associated with *Homo sapiens.* With the advent of optogenetics—the ability to control neural behavior with light—scientists hope to actually be able to manipulate and control these neural circuits to enhance or to suppress behavior. The bioethical questions raised by the realization of this research program in human patients will be legion.

Rev. Nicanor Pier Giorgio Austriaco, OP

[13] Teruhiro Okuyama et al., "A Neural Mechanism Underlying Mating Preferences for Familiar Individuals in Medaka Fish," *Science* 343.6166 (February 7, 2014): 91–94.