Science

Trans-differentiation Nuclear Reprogramming

For the last fifteen years, much has been made about stem cell technologies—both embryonic pluripotent stem cells and induced pluripotent stem (iPS) cells—and their potential for therapeutic advance. Indeed, this year's Nobel Prize in Physiology or Medicine was awarded to Sir John B. Gurdon and Shinya Yamanaka for the seminal discovery that mature cells can become reprogrammed to become pluripotent. More recently, however, as I have occasionally noted over the past two years in these pages, there has been a concerted effort to directly reprogram one mature cell type into another type without going through an intermediate pluripotent stem cell stage. This process is sometimes called trans-differentiation.

Three papers published recently illustrate this theme. First, Michael Ginsberg and colleagues describe a novel method using three genes, *ETV2*, *FLI1*, and *ERG1*, to obtain vascular epithelial cells (the cells that normally line the blood circulatory system) by directly reprogramming cells taken from amniotic fluid.[1] As part of their study, the research team at Cornell's Weill Medical College was also able to show that these reprogrammed human endothelial cells could be introduced into mice and form functional blood vessels within injured livers. In time, these reprogrammed vascular cells could be used to treat a wide range of disorders linked to damaged or dysfunctional blood vessels in human patients. Strikingly, these results also suggest that amniotic cells—the same cells routinely obtained during prenatal testing—could be reprogrammed into every organ cell type including liver, heart, kidney, and brain cells, completely bypassing the need for pluripotent stem cells.

---

[1] Michael Ginsburg et al., "Efficient Direct Reprogramming of Mature Amniotic Cells into Endothelial Cells by ETS Factors and TGFβ Suppression," *Cell* 151.3 (October 26, 2012): 559–575.

Next, another research team has used direct reprogramming technology to take one type of adult cell in human brains—non-neuronal cells called pericytes—and to transform them into human neurons.[2] These induced neurons were able to generate their own action potentials, the electrical signals transmitted within the nervous system, and to make functional connections with other neurons. These neurons could eventually be used to treat patients with neurodegenerative diseases such as Alezehimer's and Parkinson's—again without the use of any pluripotent stem cells.

Finally, in what will now seem to be a repeat of the same leitmotif, scientists from the Whitehead Institute and MIT have turned skin cells into immature Sertoli-like cells.[3] Sertoli cells are the cells that support the development and maturation of sperm cells. In the laboratory, the induced Sertoli cells were able to form the seminiferous tubular structures found in testes. When injected into mice, they were also able to migrate to the testes and to integrate themselves into the animal's endogenous tubules. The team was also able to show that these cells were able to sustain the viability of other cells in culture. They speculate that these cells could be used to sustain other cell types in the human body, like ailing neurons in a patient with a neurodegenerative disease like Lou Gehrig's disease.

The three papers suggest that the trans-differentiation approach may lead to therapies much sooner than the stem cell differentiation approach. The Cornell team has estimated that it may take just four years to get their directly reprogrammed vascular cells to human clinical trials! It is ironic that pluripotent stem cells may be ceding their time in the therapeutic spotlight so soon after Nobel recognition.

Stem Cell–Based Reprogramming

Stem cell research continues to evolve. Several papers this quarter better reveal the inner workings of cell reprogramming. For instance, a Harvard research team has uncovered a molecular roadmap for the reprogramming that is used to generate iPS cells.[4] Recall that the initial discoveries of Yamanaka that earned him a Nobel Prize had involved introducing four genes (c-Myc, Klf4, Oct4, and Sox2) into adult cells to reprogram them back into the pluripotent state. The Harvard team has now discovered that these four genes work in two separate waves of gene activity. The first wave driven by the genes c-Myc and Klf4 is initiated early, while the second wave driven by the genes Oct4, Sox2, and Klf4 is initiated late in cellular reprogramming. The team discovered that cells that are unable to reprogram properly were not able to initiate the second wave of gene activity, which is the wave responsible for the stable establishment of the pluripotent state. This data will now allow scientists to identify those genes that act as roadblocks to reprogramming so that the efficiency of iPS production can be increased.

[2] Marisa Karow et al., "Reprogramming of Pericyte-Derived Cells of the Adult Human Brain into Induced Neuronal Cells," *Cell Stem Cell* 11.4 (October 5, 2012): 471–476.

[3] Yosef Buganim et al., "Direct Reprogramming of Fibroblasts into Embryonic Sertoli-like Cells by Defined Factors," *Cell Stem Cell* 11.3 (September 7, 2012): 373–386.

[4] Jose M. Polo et al., "A Molecular Roadmap of Reprogramming Somatic Cells into iPS Cells," *Cell* 151.7 (December 21, 2012): 1617–1632.

Next, in an unexpected discovery, the Crooke Laboratory at Stanford University showed that exposing cells to viral material to stimulate their innate immune response enhances nuclear reprogramming to the pluripotent cell state.[5] Further investigation showed that stimulating the innate immune response in these cells via a molecule called TLR3 allowed the reprogramming factors to better find and bind to their targets on the cellular DNA. More specifically, this happens because TLR3 activation "opens up" and relaxes the winding of the usually compact DNA that is highly organized in cells.

Finally, researchers from the University of Pennsylvania have uncovered large tracts of the human genomes of adult skin cells that do not bind well to the four reprogramming factors needed to make iPS cells.[6] These regions marked by a special DNA modification called H3K9me3 included genes important for reprogramming. When the scientists were able to decrease the amounts of H3K9me3 in cells, the cells became more reprogrammable. The process of reprogramming also sped up twenty- to fifty-fold, suggesting that the H3K9me9 modification of DNA was indeed a major impediment to the process of generating iPS cells. Together, these three discoveries only promise to accelerate the protocol for and improve the efficiency of nuclear reprogramming in the future.

Of particular interest to Catholic bioethicists, the International Society for Stem Cell Research (ISSCR) has published a summary of the presentations and post-presentation discussions of a focus session of the 2012 ISSCR Annual Meeting in Yokohama, Japan, which focused on the ethical and policy issues raised by attempts to take stem cells to the patient bed side.[7] As the published meeting report itself noted, the session addressed both preclinical barriers to translation (preclinical data and transfer and sharing of materials) and clinical barriers to human trials (trial design and comparators and perception and communication of risk to patients, families, and patient groups).

Creating Gametes in the Laboratory

Three scientific reports portend significant developments in the field of reproductive biology. Most spectacularly, scientists at Kyoto University in Japan have discovered a protocol to make eggs from pluripotent stem cells.[8] The same Japanese scientists had produced healthy mouse pups by using sperm derived from pluripotent stem cells in 2011. The team began by taking either human embryonic stem cells or iPS cells and culturing them in a molecular cocktail that transformed them into the

[5] Jiun Lee et al., "Activation of Innate Immunity Is Required for Efficient Nuclear Reprogramming," *Cell* 151.3 (October 26, 2012): 547–558.

[6] Abdenour Soufi, Greg Danahue, and Kenneth S. Zaret, "Facilitators and Impediments of the Pluripotency Reprogramming Factors' Initial Engagement with the Genome," *Cell* 151.5 (November 15, 2012): 994–1004.

[7] Kazuto Kato et al., "Ethical and Policy Issues in the Clinical Translation of Stem Cells: Report of a Focus Session at the ISSCR Tenth Annual Meeting," *Cell Stem Cell* 11.6 (December 7, 2012): 765–767.

[8] Katsuhiko Hayachi et al., "Offspring from Oocytes Derived from In Vitro Primordial Germ Cell–like Cells in Mice," *Science* 338.6109 (November 16, 2012): 971–975.

precursor cells, primordial germ cell-like cells, which in the body would eventually become either sperm or eggs. They then mixed these primordial cells with fetal ovarian cells obtained from healthy mice to reconstitute ovarian tissue that was then grafted onto the natural ovaries within living mice. A month later, the primordial germ cell-like cells had become eggs, which were harvested and fertilized in a petri dish. The resulting embryos were implanted into surrogate mothers, who delivered healthy mice three weeks later. The transformative potential of this technology may not be immediately evident but if this protocol works with human pluripotent stem cells, it would give people the ability to make eggs not only from infertile women, but from men, from children, from centenarians, indeed from any source of human tissue—even biopsy samples. The ethical issues will be thorny!

Spermatogonial stem cells (SSCs) are the stem cells that inhabit the testes in order to generate sperm throughout a man's life. A team from the University of Texas at San Antonio has demonstrated that it is possible to remove these testicular stem cells from a monkey prior to chemotherapy, to freeze them, and to return them to the animal after the completion of chemotherapy where they restarted sperm production.[9] This was the first time the procedure has been accomplished in a primate animal model, paving the way for human clinical trials that would seek either to preserve or to restore the fertility of male cancer patients.

Finally, in a related line of research, another team from Kyoto University has taken mouse spermatogonial stem cells, mixed them with support cells, and discovered that they were able to reconstitute the testicular environment of these stem cells.[10] This is the first time that anyone has been able to recreate the testicular stem cell environment in the laboratory. If transferrable to the human system, which is likely, it could be used to identify some of the root causes of human infertility at the cellular and molecular level.

Genomic and Proteomic Analysis of Individual Organisms and Individual Cells

The human genome published twelve years ago is the DNA sequence of a generic human person compiled from the sequences of many actual real people. It took billions of dollars, many years, and international teams of scientists to complete. Our sequencing technology has so improved, however, that in recent years, the complete genomes of individual persons have now been described.

For example, the genome of an extinct individual of the hominin species called the Denisovans has been determined from a fossil finger.[11] The sequence revealed that the Denisovans had interbred with the human ancestors of modern day individuals

[9] Brian P. Hermann et al., "Spermatogonial Stem Cell Transplantation into Rhesus Testes Regenerates Spermatogenesis Producing Functional Sperm," *Cell Stem Cell* 11.5 (November 2, 2012): 715–726.

[10] Mito Kanatsu-Shinohara et al., "Reconstitution of Mouse Spermatogonial Stem Cell Niches in Culture," *Cell Stem Cell* 11.4 (October 5, 2012): 567–578.

[11] Matthias Meyer et al. "A High-Coverage Genome Sequence from an Archaic Denisovan Individual," *Science* 338.6104 (October 12, 2012): 222–226.

from Southeast Asia and Oceania. For instance, 6 percent of the genomes of contemporary Papuans from Papua New Guinea is derived from the Denisovans.

The technology to characterize the genome of an individual human cell has also been realized. Using an amplification technique called polymerase chain reaction, or PCR, a team from Harvard has been able to determine the sequence of individual human cells with up to 70 percent accuracy.[12] Now a technique called multiple annealing and looping-based amplification cycles sequencing has now been used to sequence the DNA of individual human sperm.[13] In time, the technology should be used to determine the genomes of individual cancer cells within a tumor or of individual cells left behind at a crime scene.

In the not too distant future, with the accelerating developments in sequencing technologies, the genomes of every single man, woman, and child will be routinely sequenced. How will we use this information? How will it change the way we think about ourselves and how we marry and how we have our children? How will we prevent it from being exploited? These will become increasingly pressing questions for all bioethicists.

A Biological Basis for Homosexuality?

In a recent paper that made headlines in the mainstream media and on the internet, a group of biologists have suggested that they may have found a biological explanation for the apparent hereditability of homosexuality.[14] The team hypothesizes that homosexuality is caused by the faulty reprogramming of the genome during embryonic and fetal development. In their view, epigenetic marks—molecular modifications of DNA that can influence whether genes are turned on or off—that are known to influence sexual development would be improperly erased in certain people. These marks would then inappropriately lead to the turning on or off of genes that are involved in shaping the sexual preference of the individual. Such a mechanism would explain how homosexuality could be heritable without there being any specific genes linked to the condition.

At this point, however, it needs to be emphasized that this proposal remains a hypothesis that has not yet been confirmed in any human individuals. If it is indeed confirmed, it would suggest that homosexuality is fundamentally a maladaptive trait that emerges from faulty epigenetic programming, countering recent attempts of several evolutionary biologists to argue that homosexuality is a beneficial trait. Moreover, it would open up the real but certainly controversial possibility that parents may one day choose to utilize drugs that would alter epigenetic marks to prevent any transmission of homosexuality to their children.

---

[12] Chenghang Zong et al., "Genome-Wide Detection of Single-Nucleotide and Copy-Number Variations of a Single Human Cell," *Science* 338.6114 (December 21, 2012): 1622–1626.

[13] Sijia Lu et al., "Probing Meiotic Recombination and Aneuploidy of Single Sperm Cells by Whole-Genome Sequencing," *Science* 338.6114 (December 21, 2012): 1627–1630.

[14] William R. Rice, Urban Friberg, and Sergey Gavrilcts, "Homosexuality as a Consequence of Epigenetically Canalized Sexual Development," *Quarterly Review of Biology* 87.4 (December 2012): 343-368.

How Successful Is Sex Reassignment Surgery?

Finally, I raise a very politically incorrect but important question: should sex reassignment surgery be the standard of care for individuals who struggle with gender identity disorder or transsexualism? Many assume that it should be because many assume that "matching" the patient's sex and gender will "cure" the individual and allow him or her to lead a happy life. This assumption, however, has now been put into question by a paper published by investigators at the Karolinska Institutet in Sweden.[15] In this study, the Swedish research team assessed the mortality, psychiatric morbidity, and psychological integration expressed in criminal behavior in all transsexuals who underwent a sex change operation between 1973 and 2003 in Sweden. This cohort of 324 transsexual persons was compared with randomly selected population controls matched by birth year and sex (birth sex or reassigned sex, respectively). This study constitutes the first nationwide population-based, long-term, follow-up of sexually reassigned transsexual persons. As the authors note, the most striking result was the high mortality rate in both male-to-female and female-to-male transsexuals, compared to the general population. Moreover, mortality from suicide was high among transsexual persons even after adjustment for prior psychiatric morbidity, supporting previous observations that transsexualism, even after sex reassignment surgery, is a strong risk factor for suicide. Inpatient care for psychiatric disorders was also significantly more common among this cohort, both before and after sex reassignment. Numerous studies had shown that transsexuals have more psychiatric ill-health than the general population prior to sex reassignment, but this paper shows that the increased risk for psychiatric hospitalization *persisted* after surgery. The authors conclude, "Even though surgery and hormonal therapy alleviates gender dysphoria, it is apparently not sufficient to remedy the high rates of morbidity and mortality found among transsexual persons."

Importantly, the paper notes that there are published studies prior to theirs that had not found an increased mortality rate after sex reassignment. However, they contend—effectively, in my view—that these previous clinical studies may have been biased, because people who regard their sex reassignment as a failure are more likely than their peers to be lost to follow-up. Population-based register studies that examine every individual in a population in a given period of time, like this study, do not have that bias. I have to acknowledge that the authors claimed (perhaps to be politically correct?) that their study does not address whether sex reassignment is an effective treatment or not. However, their data can be used to address this taboo even if the investigators themselves refuse to do so. The simple question is this: can any reasonable individual label a medical intervention effective if the rates of death and of ill health remain unchanged before and after the treatment?

Rev. Nicanor Pier Giorgio Austriaco, OP

[15] Cecilia Dhejne et al., "Long-Term Follow-up of Transsexual Persons Undergoing Sex Reassignment Surgery: Cohort Study in Sweden," *PLoS One* 6.2 (February 22, 2011): e16885.