Your Brain Under the Microscope: The Promise of Stem Cells

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Editor's Note: Until recently, scientists primarily worked with two kinds of stem cells from animals and humans: embryonic stem cells and non-embryonic "somatic" or "adult" stem cells. Scientists are just now beginning to improve their understanding of a third kind: induced pluripotent stem cells. Our authors describe how they were discovered, what they are, and why a growing number of researchers and clinicians believe that they may be one of the keys in helping address various brain disorders.

When the International Society for Stem Cell Research (ISSCR) first met in Washington, D.C. in 2003, a few hundred attendees participated in the discussions. In June 2013, just 10 years later, a record 4,000 researchers from all over the world attended the society's meeting in Boston. The ISSCR now has more than 3,000 members and three affiliated indexed journals, one of which has one of the highest impact factors in the field. In addition, the number of abstracts that utilized reprogramming technology increased exponentially from basically none to more than 220 in just 5 years (see graph). The ISSCR's rapid growth has run parallel with an unprecedented display of general interest on the part of researchers and clinicians from different backgrounds and levels of expertise. Both trends speak directly to the potential impact of stem cell research.

We all begin our lives with one major stem cell: a fertilized egg. That one stem cell then divides and forms new cells that, in turn, also divide. Even though these cells are identical in the beginning, they become increasingly varied over time. As a result of this process, which we call cell differentiation, our cells become specialized for their locations in the body. As we develop in the womb, our cells differentiate into nerves, muscles, and so on, and the organs begin to organize and function together.

Scientists long believed that a mature or specialized cell could not "reprogram," or return to an immature state. A few researchers challenged this view, however. In 1966, John Gurdon (Wellcome Trust/CRUK Gurdon Institute, Cambridge, UK) was the first to show that if you removed the nucleus containing the genetic material of a fertilized frog egg (stem cell) and replaced it with the nucleus of a fully differentiated intestine cell from a tadpole, the

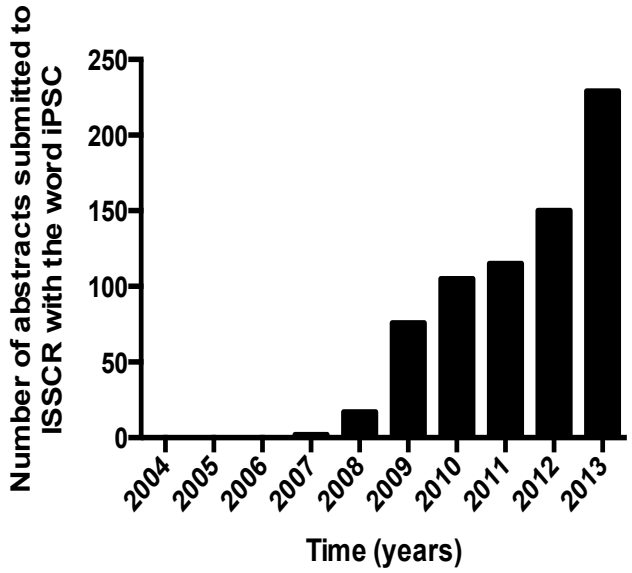
modified egg would grow into a normal frog with the same genetic material as the original egg.[1]

Gurdon's findings were confirmed by others, including Robert Briggs and Thomas King Jr., whose earlier works showed that normal hatched tadpoles could be obtained by transplanting the nucleus of a blastula cell to the enucleated eggs of a leopard frog *(Rana pipiens)*.[2] In 1997, Ian Wilmut electrofused (a technique used to fuse cells using electrical impulse) nuclei of cultured sheep adult mammary gland cells into enucleated sheep eggs and produced a single cloned sheep named Dolly.[3] These researchers sent the scientific community this message: it was now possible to reprogram adult cells to an immature state by exposing them to a yet-unknown combination of factors that were present inside enucleated eggs. These reprogrammed cells became pluripotent again, meaning they were capable of going through a new process of maturing and specializing.

Even though the pioneering researchers provided the proof of principle that reprogramming was possible, the cloning experiments they performed were very time-consuming, difficult to reproduce, extremely inefficient for mammalian cells, and ethically controversial when envisioned for human cells. In addition, an important piece of the puzzle was still missing: What made the reprogramming of adult cells possible? It was not until 2006 that Japanese researcher Shinya Yamanaka and his postdoc Kazutoshi Takahashi were able to answer this question.

The Reprogramming Pioneers

When Yamanaka presented his first reprogramming results at the 2006 ISSCR meeting, many scientists were skeptical. Yamanaka claimed that with the addition of only four factors that are master regulators of cell pluripotency, his team could induce an adult skin cell (fibroblast) to become a pluripotent stem cell (then called an induced pluripotent stem cell, or iPS cell) within only a month. Many thought his results were too good to be true, but later that year, when his procedure was published with a description of the four factors he used for reprogramming experiments, dozens of labs around the world (including ours) tried his protocol.[4, 5] To our complete astonishment, it worked in our lab the very first time—and it worked in many other labs as well.[6, 7] Yamanaka and Takahashi's research results played a major role in popularizing and disseminating stem cell research because by uncovering the basic factors and principles of the reprogramming process, they made it possible for researchers from other fields to work with pluripotent stem cells. The impact and potential of their stem cell research earned Yamanaka and Gurdon the Nobel Prize in Physiology or Medicine in 2012.



Using iPS Cells to Study Neurological Diseases

Human iPS cells, which can, in principle, form any cell in the body, could provide an attractive alternative when the traditional models for neurological diseases are inadequate. Nearly all of our current knowledge about human neurodevelopmental and neurodegenerative diseases at the cellular level is derived from studies in postmortem brain tissues. These samples often represent the end stage of a disease and therefore are not always informative representations of a disease's developmental path. Furthermore, the pathology observed in these tissues is potentially not the authentic disease cellular phenotype. Genetically modified ("transgenic") mice provide an alternative way to reproduce human genetic forms of neurodegenerative diseases to serve as models for observation as to their developmental course in a neurotrophic phrase. However, use of these models is limited to monogenetic (the origin of diverse individuals or kinds by descent from a single ancestral individual or kind) disorders in which the specific gene mutations are known—disorders that represent the minority of neurological diseases. And in some cases, mouse transgenic technology cannot adequately model neurological disorders with defined genes because of intrinsic differences between species.

For example, mice have much less of a complex brain architecture than humans; there are a number of brain structures present in humans that are not present in rodents. This suggests a need for advancement toward human models of disease. Currently, many subtypes of disease-relevant neurons can be developed from iPS cells using a combination of manual selection and the addition of mixtures of different neurotropic factors to the culture. Differentiation protocols can provide enriched populations of particular subtypes of

neurons that are relevant to specific diseases. These subtypes include dopaminergic neurons for Parkinson's disease, hippocampal and cholinergic neurons for Alzheimer's disease, motor neurons for amyotrophic lateral sclerosis (ALS, or Lou Gehrig's disease), and inhibitory interneurons for schizophrenia. [7-10]

To date, most experiments involving disease modeling for neurological diseases utilize iPS cells-derived neurons from patients with monogenetic disorders for which the gene mutation is defined and well characterized. The modeling of monogenic brain disorders has promoted rapid advancements in the field by helping to establish the basic tools for culturing functional human neurons. In addition, initial modeling research revealed meaningful neuronal phenotypes, such as differences in synaptogenesis, neuronal size and arborization complexity, and connectivity properties.[11, 12] Importantly, monogenic disorder modeling presents an opportunity to perform gain-of-function and loss-of-function studies and to confirm the specificity of the neuronal phenotypes observed. In addition, studying the in vitro phenotypic consequences of the mutation in specific genes can highlight molecular mechanisms responsible for subtle alterations in the nervous system, perhaps pointing to common mechanisms for more complex, multi-gene diseases.

Nonetheless, the vast majority of neurological disorders (for example, autism spectrum disorders, schizophrenia, Parkinson's disease, Alzheimer's disease, and Lou Gehrig's disease) are complex in nature and likely multifactorial: a combination of mutations in several genes and extrinsic factors (such as influence of neighboring cells in the neuronal niche and environment) is likely involved in the disease pathology course. Recently, scientists have made successful attempts to detect a specific neuronal phenotype using sporadic

neurological disease models. Hopefully there will be more advances in the near future as the technology becomes sensitive enough to detect more subtle phenotypes.[11-13]

Finding Clinically Relevant Drugs

Candidate compounds for treating central nervous system (CNS) deficiencies fail in clinical trials in more than 90 percent of cases because of poor targeting (the drug does not target the affected area of the brain efficiently), lack of efficacy, and unacceptable side effects.[14] Pluripotent stem cells derived from patients with CNS diseases offer a significant advantage, as researchers can take into consideration the patient's genetic background and the developmental course of the disease. Importantly, these stem cells allow for the generation of both genetic and sporadic forms of the disease.

Before developing a screening platform with the aim of discovering new drugs for a treatment, a consistent abnormal phenotype needs to be identified and reproduced on a large scale. Researchers are making progress in this process, and as large pharmaceutical companies move into stem cell-related drug research, more systematic progress is expected.[15-17] The best examples so far are coming from partnering between research organizations (universities and institutes) with industry and start-up companies that have scientists as advisors. A few months ago, a group from iPierian Inc. configured a high-content chemical screen using an indicator of ALS pathology in human motor neurons derived from iPS cells from patients with ALS. The group identified small molecule compounds (i.e. digoxin) that alleviated the disease-related phenotype in iPSC-derived patient neurons, thus demonstrating the feasibility of iPS cell–based disease modeling for

drug screening. The general strategy for drug screening is to identify a reliable disease-related phenotype and to develop high-throughput screening platforms to test bioactive compounds (such as proteins and small molecules) that protect the patient neurons from either developing or progressing through the disease course. After rigorous testing, these screenings will likely unearth therapeutic compounds that could benefit a group of patients.

Finally, iPSCs may also be used to assess developmental as well as cell-type-specific drug toxicities. Indeed, existing commercially available human iPS-derived hepatocytes, cardiomyocytes, and neural cells may provide the basis for humanized assays to detect off-target activity and side effects of drugs in a tissue-specific manner.[18] We firmly believe that reprogramming technology can be a valuable, additional tool for screening and validating CNS compounds for pharmaceutical companies in the near future, ultimately culminating in the discovery of new therapies.

Cautionary Notes

iPS cell lines and their derived progeny bear a significant intrinsic variability, as revealed by abnormal expression of imprinted genes, differential expression profiles, and inconsistent neuronal differentiation competence.[19-21] For that reason, researchers still need to conduct comparative experiments with well-established human embryonic stem (HES) cell lines as a benchmark for complete reprogramming and ideal differentiation protocols. It is our expectation that the use of HES cell lines may decrease over time, but studying reprogramming without them would be unconceivable at this point.

This variability can become a real hurdle for disease modeling, especially when comparing cells from patients with sporadic forms of diseases that have multifactorial etiologies. The

differences observed have been generally attributed to random integration of viral vectors causing potential insertional mutagenesis, reactivation of reprogramming transgenes, and persistency of donor cell gene expression.[22] New technology that promotes the delivery of reprogramming factors in a non-integrative way is available and becoming more popular among disease modeling groups.[23, 24] Reprogramming can also be achieved by using synthetic genes and small molecules, and further improvement of these methodologies will promote widespread use by the scientific community.[25] As more research groups use nonintegrative approaches, we anticipate that the iPS cell lines generated will have decreased intrinsic variability.

Identifying disease-relevant phenotypes requires researchers to compare experimental cells with "healthy" control cells. New gene-targeting technologies in iPS cells can enable more efficient and less variable rescue from monogenetic alterations. In addition, the generation of isogenic cell lines allows for more relevant controls that take into account the individual's genetic background. Examples of methods currently using iPS gene editing are zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR). [26-29]

For sporadic cases, alternative ways to decrease variability will include using neurotypical family members as controls or including groups of patients who present common clinical histories and/or respond to drugs in a similar manner. New high-throughput genomic tools, such as genomic deep sequencing, are beginning to reveal naturally occurring genetic variation that can help us to understand the differences between cell lines. When possible, reprogramming cells from genetically identical individuals, such as monogenetic twins who

are concordant or discordant for a specific neurological condition, will also help us to understand variability and to generate relevant disease hypotheses.

The Road Ahead

Reprogramming technology has opened the door for many new insights into the brain and brain-related conditions. The recapitulation of early stages of human neural development made possible by using iPS cells is an invaluable tool that can reveal the exact moment of the disease onset, thus fostering the generation of new diagnostic tools and potentially optimizing novel therapeutic interventions.

Although it has been only seven years since the introduction of somatic reprogramming technology to generate iPS cells, clinical studies that bring iPS cell–based therapy to patients are already underway. In August 2013, the Japanese Ministry of Health, Labour, and Welfare approved the first pilot clinical study using isogenic iPS cells for age-related macular degeneration (AMD). The study will be conducted mainly by the Takahashi group at the RIKEN Center for Developmental Biology in Kobe, Japan. They plan to transplant sheets of iPS cell–derived retinal cells into the subretinal space of AMD patients to rescue and restore the pigmented epithelium responsible for absorbing visual stimuli.[30] If Takahashi's study is conducted safely, it will be the first clinical demonstration of iPS cells for medical use and will undoubtedly impact the outlook regarding the safety and efficacy of iPS cell–based therapy. Advanced Cell Technology, an American biotechnology company, is applying for Federal Drug Administration approval for a less ambitious clinical trial of injecting human iPS cell–derived platelets as a potential treatment of coagulopathies. Because platelet cells lack a nucleus, scientists expect that the risks of tumors and tumor-associated immune

responses will decrease. Nonetheless, the main challenge in the field remains: Much more groundwork is needed to improve understanding of the biology of reprogrammed cells and their progenies. In addition, we need to be vigilant about avoiding the dissemination of unproven applications.

Incorporation of bioengineering techniques making the use of bio scaffolds to allow for cells to grow in three-dimensions will raise our level of understanding of the different brain structures and eventually begin to dissect out the birth of more complex neuronal networks. Earlier this year, an Austrian group led by Jürgen Knoblich assembled in vitro the first iPS cell–derived rudimentary brain.[31] The cerebral organoids produced by the researchers recapitulated early stages of human development (up to approximately nine weeks of pregnancy) and modeled for microcephaly, a neurological condition that is not efficiently modeled in rodents. More refinement of the technique will be required in order to maintain the cells as organoids or tissue in a viable and stable state for longer periods; nevertheless, the tissue-engineering approach is a very promising and powerful tool for understanding various aspects of human brain development. Neuroscientists in the past could not have predicted a scenario in which patient-derived, live functional neurons would be readily available for research, and researchers in the future will not be able to imagine a scenario without it.

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