The First Direct Reprogramming of Adult Human Fibroblasts

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In recent issues of *Cell* and *Science*, Yamanaka and colleagues (Takahashi et al., 2007) and Yu and colleagues (Yu et al., 2007) demonstrate that expression of four specific transcription factors gives adult human fibroblasts many of the characteristics of human embryonic stem cells. Refinements of this procedure will make it possible to produce pluripotent human cell lines without use of an embryo. There are profound scientific and social implications of this research.

In an earlier seminal experiment, Yamanaka and his colleagues showed that substantial reprogramming of adult mouse cells toward embryo stem cells could be achieved by induced expression of just four transcription factors, namely Oct3/4, Sox2, Klf4, and c-Myc (Takahashi and Yamanaka, 2006). The cells were selected based on expression of *Fbx15*, a gene present early in development and in embryo stem cells but which is not essential for normal development or pluripotency. The resulting cells, known as induced pluripotent stem (iPS) cells, had many of the characteristics of mouse ES cells, yet they failed to contribute to chimeras at term and showed distinct differences in gene expression and chromatin organization when compared with ES cells.

Earlier this year, the authors extended their findings and revealed that selection for Nanog expression after transduction of the four original factors yielded a population more similar to ES cells (Okita et al., 2007). Indeed, in some cases, the iPS cells were able to contribute to germline chimeras, the gold standard criterion for a pluripotent stem cell population. Similar results were confirmed by others working independently (Maherali et al., 2007; Wernig et al., 2007). In addition, it was found that it was possible to select only on the basis of morphology and growth characteristics (Blelloch et al., 2007; Meissner et al., 2007).

The question then immediately arose as to whether these approaches might be effective using adult human cells. Now this has been shown to be the case, with the generation of human iPS cells. Yamanaka and his colleagues (Takahashi et al., 2007) introduced a mouse receptor for retrovirus into human cells to increase the frequency of transduction by amphotropic retrovirus. The fact that 60% of cells exposed to the retrovirus expressed a reporter gene at a level similar to that in murine fibroblasts confirmed that this adaptation provided an effective transduction protocol.

They then introduced the same four genes into adult human fibroblasts and selected the first human iPS cells on the basis of their morphology and growth characteristics. They note that every iPS clone carried between three and six retroviral integrations for each factor and point out that this may result in damaging mutations. The human iPS cells were very similar to human ES cells in many regards, including morphology, proliferation, expression of cell-surface markers, gene expression, chromatin organization at specific gene promoters, and telomerase expression. In addition, human iPS cells were able to form tissues of all three major lineages both in tissue culture and after transplantation into immune-deficient mice.

The molecular mechanisms that bring about this direct reprogramming are not known. The authors note that Oct3/4 and Sox2 may upregulate expression of core genes associated with pluripotency and speculate that c-Myc and Klf4 act to modify chromatin structure to allow Oct3/4 and Sox2 access to these key target genes. In this experiment, as in the earlier reports, the proportion of cells that were reprogrammed was very small, being on the order of 1 in 1000. A number of explanations are offered to account for this low proportion. It may be that in a heterogeneous culture only a subset of cells with a particular chromatin organization is susceptible to the treatment. Alternatively, reprogramming may depend upon viral integration into a particular site (or sites). Finally, it may be a matter of chance variation in expression of the transgenes because their integration into unselected sites results in different levels of expression.

Additional insight as to the mechanisms that induce pluripotency in human fibroblasts may be provided by the report of a second group, led by James Thomson (Yu et al., 2007), who have also succeeded in the direct reprogramming of human somatic cells. Published in *Science* the same day as the Yamanaka report, this second example of human iPS cells utilized a different cocktail of factors, replacing Klf4 and c-Myc with NANOG and LIN28. The publication of comparable findings using similar—but distinct—methods underscores the validity of the human iPS cells and removes any doubt that their isolation might be irreproducible. Furthermore, a comparison of the relative contributions of the varied factors used in each system may lead to improved efficiencies and possibly a virus-free method to induce pluripotency.

No doubt publication of these results will lead to demands that no more stem cell lines be derived from

human embryos, but they will be premature, as has been argued previously by others (Hyun et al., 2007). Methods for reprogramming that do not depend upon the use of viral vectors must first be established, perhaps by use of small molecules able to induce the expression of key genes. Extensive tests will then be required to confirm that the resulting cell lines are equivalent to those derived from embryos. In the meantime, it is essential that research continues to improve procedures for derivation and maintenance of human embryonic stem cells (hESCs). The cells produced in these studies will provide the basis for comparison in assessing cells derived by reprogramming. In the meantime, hESCs will also be an invaluable resource for research and for use in therapy in the clinical trials that are already being planned. It is likely that hESC research will also provide information that will be useful in the refinement of procedures for reprogramming adult cells.

The ability to derive pluripotent cells from adult human tissues opens important opportunities in research and therapy. Cell lines derived in this manner are often referred to as "patient specific," with the implication that cell lines would be produced for each of us as the need arises. In practice, such a labor-intensive response would impose an extraordinary burden upon health providers. Moreover, in some cases, a personalized therapy would be unnecessary and indeed inappropriate—for example, for autoimmune conditions or when an immediate treatment need cannot accommodate the several weeks required to obtain sufficient cells of confirmed quality. By contrast, the ability to derive cells from selected adults would facilitate the building of libraries of lines with known genotypes, offering almost everyone cell lines of a comparatively close genetic match and therefore requiring minimal immunosuppressive treatment.

In our haste to consider use of such cell lines for therapy, we overlook their enormous potential in research and drug discovery. A significant portion of drug development cost arises from the late identification of those drugs that cause unacceptable side effects in select patients. Many of these cases reflect differences in clearance of the drug by the liver. Having human hepatocytes of varied genotypes available in the laboratory is expected to provide an earlier means of identifying these compounds with an enormous cost savings. Suitable hepatocytes could be produced from iPS cells derived from individuals known to have the critical alleles for key metabolic enzymes.

In addition, it will become possible to study cells in the laboratory that are equivalent to those in a patient with an inherited disease, even if the causative mutation has not been identified. These cells may be used to identify the molecular mechanisms that cause a particular disease and also lead to the development of high-throughput drug screens.

In principle, nuclear transfer represents an alternate approach for the production of stem cells from a patient. Following the publication of the first success of therapeutic cloning in primates (Byrne et al., 2007), it is now expected that research in this area will make rapid progress. However, in the long term, there seems to be little doubt that direct reprogramming will be the more valuable and effective procedure. Reprogramming does not require oocytes, which for the foreseeable future must be obtained from donors by laparoscopic recovery after hormone stimulation. In addition, direct reprogramming offers a means of producing pluripotent stem cells that will be more ethically acceptable in that the process does not make use of an embryo.

The identification of human iPS cells provides a pleasing illustration of scientific progress in that the success of the cloning experiments that led to the birth of Dolly prompted many people to investigate the possibility of reprogramming cells directly. Just 11 years later, the techniques developed by this new line of research will soon make somatic cell nuclear transfer unnecessary, for this purpose at least.

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