Honoris causa Shinya Yamanaka por la Universidad de Salamanca

"Recent Progress in iPS Cell Research and Application"

Comunicación Universidad de Salamanca / 14/09/2023

Shinya Yamanaka

Center for iPS Cell Research and Application (CiRA), Kyoto University
Gladstone Institute for Cardiovascular Disease
Public Interest Incorporated Foundation, CiRA Foundation

Abstract

Induced pluripotent stem cells (iPSCs) can proliferate almost indefinitely and differentiate into multiple lineages, giving them wide medical applications. As a result, they are being used for new cell-based therapies, disease models, and drug development around the world. We are proceeding with an iPSC stock project in which clinical-grade iPSC clones are being established from healthy donors with homologous HLA haplotypes to lower the risk of transplant rejection. We started distributing the iPSC stock to organizations in Japan, and related clinical studies have begun for age-related macular degeneration (AMD), Parkinson's disease, corneal epithelial stem cell deficiency, cancer immunotherapy, and other diseases, giving expectation that iPSC-based regenerative medicine will be widely used in the future. However, donors with HLA homozygous are rare. Genome editing technology could be used as an alternative to reduce the transplant rejection risk. Indeed, we reported HLA gene-edited iPSCs that could expand the range of patients who benefit from iPSC therapies faster than the homologous HLA haplotype strategy. This technology also has the potential to prevent or treat genetic diseases and gives great hope to patients. Finally, we are automating iPS cell production to reduce costs for autologous transplantation to deliver better regenerative medicine.

The genome is the code for our entire body. It decides the color of our hair, the size of our nose, and whether we look more like our mother or our father. It is now recognized that a single cell in one's body has all the genes required to produce an entire body, but this was not always the case. Scientific experiments have suggested that some genes were lost or permanently inactivated with development so that cells could only access the genes necessary for their function. However, in 1962, a landmark study by John Gurdon would show otherwise. By inserting the nucleus of a frog's intestine cell into an enucleated egg, Gurdon successfully grew a normally developing tadpole, demonstrating that all the genetic code in a single intestinal cell was enough[1)].

Decades later, scientists would report embryonic stem (ES) cells[2)]. These cells are extraordinary but also controversial. Extraordinary because they show incredible developmental potential, with the ability to differentiate into just about any cell type in the adult body[2)]. They therefore have excited the scientific and medical community for the opportunities they provide to study human development and disease. The existence of ES cells has greatly advanced scientific understanding of how the brain, heart, and other organs develop from an embryo. Controversial because they are procured from human embryos, a process that requires the destruction of the embryo. In response, national laws and international guidelines have restricted the use of ES cells compared with other human cell types.

Considering Gurdon's work, scientists hypothesized that any cell could be transformed into an ES cell-like state if the appropriate portions of the genome were activated and inactivated. The question had long remained, however, which parts? While it was certain that not all several billion base pairs in the human genome would need manipulation, narrowing down the precise combination was viewed a daunting task. And it was, but the answer turned out to be far simpler than anticipated.

We showed that by transiently activating just four genes (Yamanaka factors) in adult cells is sufficient to reprogram them into induced pluripotent stem (iPS) cells[3)]. Adding to the amazement is that the same four genes successfully produced iPS cells from the adult cells of a number of species[4)]. The degree to which iPS cells behave as ES cells is remarkable, and years of study from laboratories around the world have confirmed that iPS cells and ES cells are functionally equivalent. From an ethical perspective, the impact of this research is profound, because we can acquire iPS cells from the very same blood samples one may donate to the Red Cross rather than an embryo[5)].

From a medical perspective, iPS cells have two attractive features. First, as mentioned above about ES cells, is their ability to differentiate into many cell types. Second, again like ES cells, is their ability to proliferate. The embryo is a rapidly growing structure, in which cells are replicating and developing constantly. iPS cells share this feature. Thus,

from a relatively small sample of human blood, we can generate a massive number of iPS cells from which we can prepare an assortment of different cell types.

Regenerative medicine using iPS cells

Human iPS cells were first reported in 2007. Yet within seven years, cell products made from them were already being used as regenerative medicine in human trials, and many more clinical trials have since begun.

The first-in-human transplantation was selected both because of remarkable scientific accomplishments and the relative safety of the treatment. Masayo Takahashi and her research team prepared retinal epithelial sheets prepared from iPS cells that they transplanted into one eye of a patient suffering from age-related macular degeneration (Fig. 1)[6].

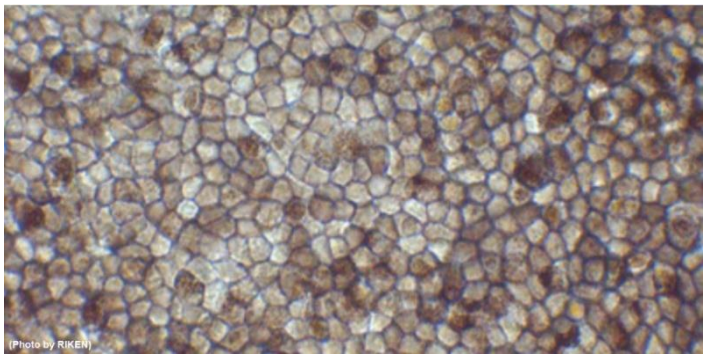

Fig. 1. Images of the retinal epithelial sheets used in the first in-human iPS cell trial.

This condition can be treated symptomatically with drugs, but the transplantation was expected to retard the degeneration if not stop it completely. Indeed, several years later, the patient's vision has stabilized, and no serious complications have occurred.

This study is an example of autologous cell therapy because the iPS cells were reprogrammed patient cells. However, it was obvious from this work that current reprogramming technology is inadequate for widescale iPS cell-based autologous therapies. The time and cost to reprogram the iPS cells, validate their safety, and finally prepare the cell product for the therapy (in this case, retinal epithelial sheets) are too high. Particularly for degenerative diseases such as age-related macular degeneration, time is precious to the patient.

In response, the manufacturing of clinical-grade iPS cell stocks has been investigated. However, like any transplantation, immune rejection is always a concern. Therefore, to prepare stocks that serves a wide population, in Japan, the CiRA Foundation has been cooperating with the Japanese Red Cross Society (JRCS) to recruit HLA homozygous donors. Compared to HLA heterozygous donors, cells from these donors have a higher probability of immune matching (Fig. 2).

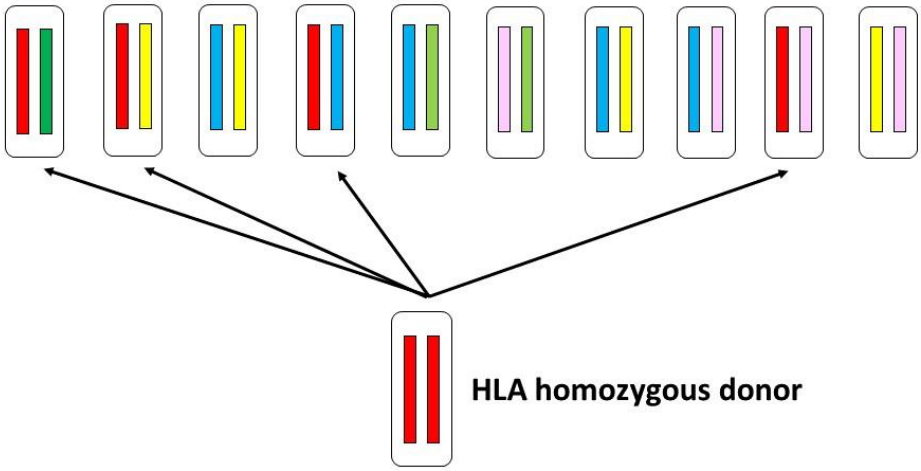

Fig. 2. Whereas an HLA heterozygous donor must match both HLA haplotypes of the patient, an HLA homozygous donor need to match only one haplotype to minimize the risk of immune rejection.

This strategy does not completely eliminate the risk, which is why almost all iPS cell-based transplantation therapies will be accompanied by immune suppressants, but for now it is perceived to be the best choice when adding the considerations of time and cost. Along with the JRCS, the CiRA Foundation is working with cord blood banks to find more HLA homozygous donors.

Once finding these donors, the CiRA Foundation reprograms the blood cells into iPS cells while assuring homogeneously high quality. To date, the CiRA Foundation has manufactured and distributed the cell stock from HLA homozygous donors for 9 ongoing clinical trials, with many more planned (Fig. 3). Furthermore, the stock is being distributed to institutes across the world that aim to develop iPS cell-based therapies. Even the aforementioned age-related macular degeneration project has shifted to this allogeneic iPS cell strategy by using iPS cells provided by the CiRA Foundation.

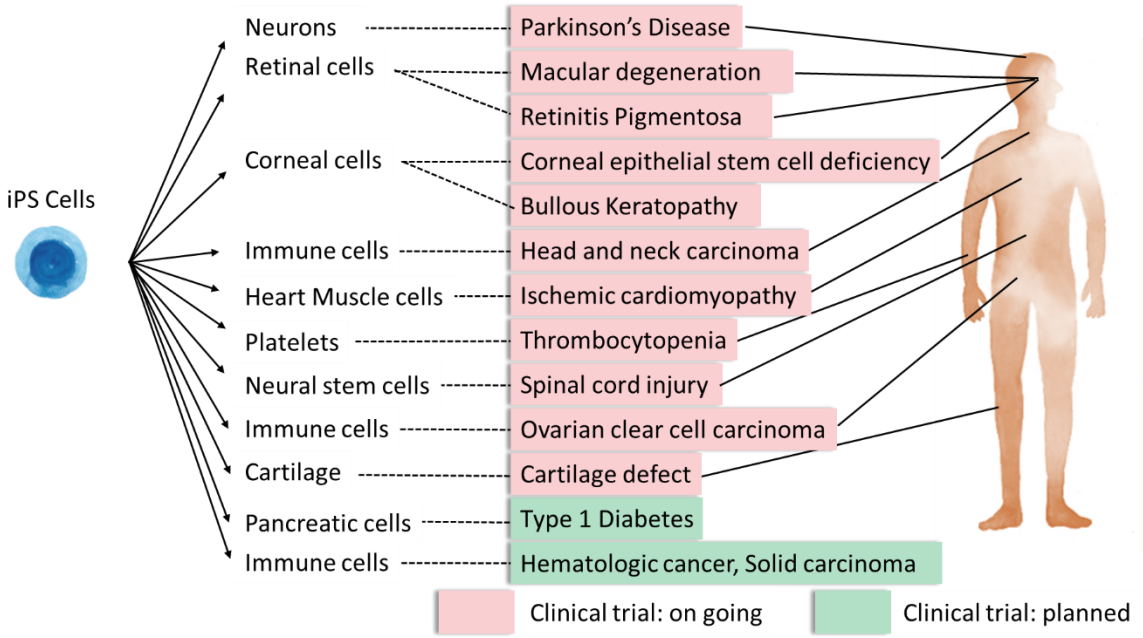

Fig. 3. A list of all iPS cell-based regenerative medicine in Japan as of May 2022. All projects are using iPS cells prepared from the CiRA Foundation except head and neck carcinoma.

Degenerative diseases are not the only targets for iPS cell-based regenerative medicine. Koji Eto and his team have developed technology to produce a cell product as an alternative to current platelet transfusions[7]. Depending on the country, several million platelet transfusions are performed in a year[8]. All of these transfusions depend on donors, but with aging populations across the world, many nations are anticipating a serious donor shortage. Adding to the challenge is that platelets are particularly difficult to store because they only have a shelf life of a few days. Therefore, a steady supply of donors is required in the current scheme. As a practical solution, Dr. Eto and colleagues are mimicking natural thrombopoiesis to produce platelets from iPS cells[7]. Following their iPS cell scheme, progenitor cells can be manufactured from the iPS cell stocks from HLA homozygous donors and stored for many months, and only when they are needed the platelets are produced from the stored progenitors. An in-human trial using these platelets is ongoing.

Another example is cancer. The latest generation of cancer therapy, following chemotherapy and radiotherapy, is immunotherapy, where a patient's immune cells are processed and transplanted. There have been many encouraging results from immunotherapy studies, but ultimately the condition of the patient is a major factor in the outcome. As a solution, Dr. Shin Kaneko and his team are combining iPS cell and CAR (chimeric antigen receptor) technologies to manufacture anticancer immune cells for the next generation of immunotherapy[9]. Here, CAR technology determines the effectiveness of the treatment by specifically attacking cancer cells but remaining unresponsive to healthy cells, while iPS cell technology assures the quality and abundance of the cells. Having adopted CAR that targets glypican-3 (GPC3), the research

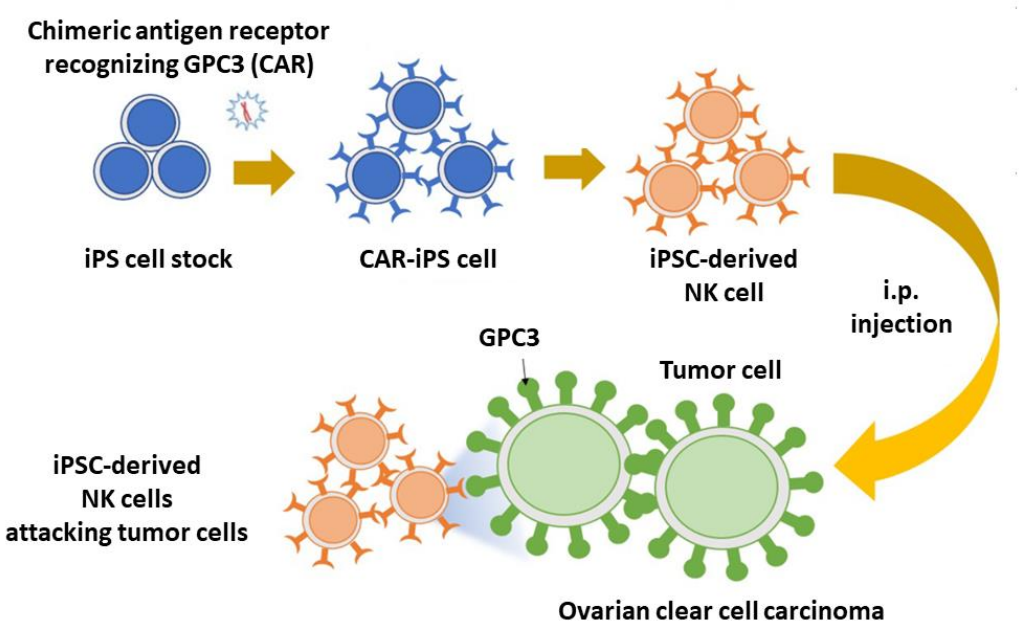

Fig. 4. New immunotherapy that combines iPS cell and CAR technologies. Because iPS cells are easier to proliferate than other cell types, the CAR is genetically incorporated into them. In this example, iPS cells with CAR targeting GPC3 are then differentiated into anti-cancer cells (in this case, NK cells) for immunotherapy against ovarian clear cell carcinoma.

team is now scheduled to begin a therapy for ovarian clear cell carcinoma (Fig. 4).

Other iPS cells for clinical use

Currently, the iPS cell stock at the CiRA Foundation can match approximately 40% of the Japanese population, praiseworthy progress in just several years. However, to provide stock that matches the entire population and then the world's entire population, the

task becomes exponentially more difficult, and finding donors for extremely rare HLA haplotypes is almost impossible.
One solution may come in the form of gene editing technology, namely, CRISPR-Cas. Rather than trying to recruit HLA homozygous haplotype donors, some scientists are editing the HLA genes to increase the percentage of the population for which iPS cell products are available. Here, HLA-A and HLA-B are knocked out, but HLA-C is preserved so as to avoid an innate immune response. In addition to manipulating these HLA class I genes, we are knocking out C2TA trans co-activator to suppress all HLA class II genes[10] (Fig. 5). If successful, it has been estimated that only 10 iPS cell lines made through this genome editing approach will sufficiently cover most of the world's population.

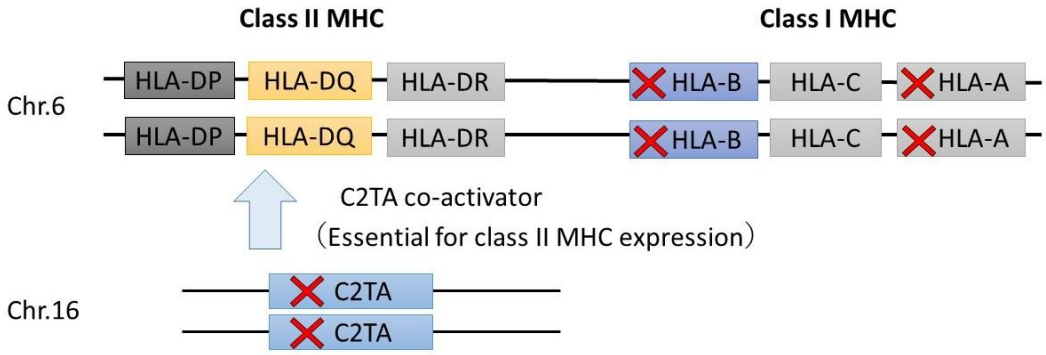

Fig. 5. CRISPR-Cas gene editing of HLA to enhance the probability of donor-patient matching. HLA class 1 genes are deleted in chromosome 6, and C2TA is deleted in chromosome 16.

Finally, while autologous iPS cells are currently unfeasible, they are ideal, since one's own cells have the lowest risk of triggering an immune response. Cost (around US$ 400,000 per iPS cell line), however, prohibits this option. Automation is one way to reduce cost by at least a magnitude. While automation is a worthwhile consideration in any manufacturing, the infancy of iPS cell technology leaves many unknowns that require further study. Therefore, an equal or greater effort must be devoted to basic research before a full commitment to manufacturing. Nevertheless, the CiRA Foundation is pushing forward with the "my iPS" project, which aims to reduce the cost of one iPS cell line to just US$ 10,000. Optimistically, this service will open beginning 2025.

In summary then, to expand the number of patients who can benefit from iPS cells, three parallel tracts for generating clinical-grade iPS cells are being pursued. The first and most progressed is the reprogramming of cells from HLA homozygous donors. The second involves the gene editing of HLA and related genes. The final is a system in which individuals can reprogram and store their own cells for future autologous treatments.

References

1. Gurdon, J. B. (1962). The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *J. Embryol. Exp. Morphol.* 10: 622–640.
2. Thomson, JA., Itskovitz-Eldor, J., Shapiro, SS., Waknitz, MA., J. Swiegiel, JJ., Marshall,VS. and Jones, LM. (1998) Embryonic stem cell lines derived from human blastocysts. *Science* 282, 1145-7
3. Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., and Yamanaka, S. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131, 861-872.
4. Takahashi, K. & Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663–676.
5. Okita, K., Yamakawa, T., Matsumura, Y., Sato, Y., Amano, N., Watanabe, A., Goshima, N., and Yamanaka, S. (2013) An Efficient Non-viral Method to Generate Integration-Free Human iPS Cells from Cord Blood and Peripheral Blood Cells. *Stem Cells* 31, 458-466.
6. Masayo ENJM Mandai, M., Watanabe, A., Kurimoto, Y., Hirami, Y., Morinaga, C., Daimon, T., Fujihara, M., Akimaru, H., Sakai, N., Shibata, Y., Terada, M., Nomiya, Y., Tanishima, S., Nakamura, M., Kamao, H., Sugita, S., Onishi, A., Ito, T., Fujita, K., Kawamata, S., Go, M.J., Shinohara, C., Hata, K., Sawada, M., Yamamoto, M., Ohta, S., Ohara, Y., Yoshida, K., Kuwahara, J., Kitano, Y., Amano, N., Umekage, M., Kitaoka, F., Tanaka, A., Okada, C., Takasu, N., Ogawa, S., Yamanaka, S., and Takahashi, M. (2017) First-in-human Clinical Study of Transplantation of Autologous iPSC-Retinal Pigment Epithelial Cell Sheet for Wet Age Related Macular Degeneration. *The New England Journal of Medicine* 376, 038-1046
7. Ito Y, Nakamura S, Sugimoto N, Shigemori T, Kato Y, Ohno M, Sakuma S, Ito K, Kumon H, Hirose H, Okamoto H, Nogawa M, Iwasaki M, Kihara S, Fujio K, Matsumoto T, Higashi N, Hashimoto K, Sawaguchi A, Harimoto KI, Nakagawa M, Yamamoto T, Handa M, Watanabe N, Nishi E, Arai F, Nishimura S, Eto K. (2018) Turbulence activates platelet biogenesis to enable clinical scale ex vivo production. *Cell*, 174, 636-648.
8. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6986537/
9. Ueda T, Kumagai A, Iriguchi S, Yasui Y, Miyasaka T, Nakagoshi K, Nakane K, Saito K, Takahashi M, Sasaki A, Yoshida S, Takasu N, Seno H, Uemura Y, Tamada K, Nakatsura T, Kaneko S. (2020) Non-clinical efficacy, safety, and stable clinical cell processing of iPSC-derived anti-GPC3 CAR-expressing NK/ILC cells. *Cancer Science*, 111, 1478-1490.
10. Xu H, Wang B, Ono M, Kagita A, Fujii K, Sasakawa N, Ueda T, Gee P, Nishikawa M, Nomura M, Kitaoka F, Takahashi T, Okita K, Yoshida Y, Kaneko S, and Hotta A. (2019) Targeted disruption of HLA genes via CRISPR-Cas9 generates iPSCs with enhanced immune compatibility. *Cell Stem Cell*, 24, 566-578.e7.

Edificio Rectorado (2ª planta)
Patio de Escuelas Mayores s/n
37008 Salamanca (España)

saladeprensa.usal.es
comunicacion@usal.es
+34 923 294 412