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SPECIAL ARTICLE

Ten years since the discovery of iPS cells: The current state of their clinical application☆

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Abstract On the 10-year anniversary of the discovery of induced pluripotent stem cells, we review the main results from their various fields of application, the obstacles encountered during experimentation and the potential applications in clinical practice. The efficacy of induced pluripotent cells in clinical experimentation can be equated to that of human embryonic stem cells; however, unlike stem cells, induced pluripotent cells do not involve the severe ethical difficulties entailed by the need to destroy human embryos to obtain them. The finding of these cells, which was in its day a true scientific milestone worthy of a Nobel Prize in Medicine, is currently enveloped by light and shadow: high hopes for regenerative medicine versus the, as of yet, poorly controlled risks of unpredictable reactions, both in the processes of dedifferentiation and subsequent differentiation to the cell strains employed for therapeutic or experimentation goals.

PALABRAS CLAVE
Células iPS;
Células troncales embrionarias;
Medicina regenerativa;
Reprogramación celular

Diez años desde el descubrimiento de las células iPS: estado actual de su aplicación clínica

Resumen Al cumplirse 10 años del descubrimiento de las células pluripotenciales inducidas se revisan los principales resultados en sus distintos campos de aplicación, los obstáculos con los que se ha encontrado su experimentación, así como las posibles aplicaciones en la práctica clínica. La eficacia de las células pluripotenciales inducidas en la experimentación clínica puede equipararse a la de las células troncales embrionarias humanas, pero, a diferencia de estas, no presentan la grave dificultad ética que conlleva la necesidad de destruir embriones humanos

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para su obtención. El hallazgo de estas células, que constituyó en su día un verdadero hito científico merecedor de un Premio Nobel de Medicina, está hoy rodeado de luces y sombras: grandes esperanzas en la medicina regenerativa frente a riesgos aún no bien controlados de reacciones imprevisibles, tanto en los procesos de desdiferenciación como en la posterior diferenciación hacia las estirpes celulares empleadas con fines terapéuticos o de experimentación.

Background

Few biomedical discoveries in recent decades have raised so many expectations as the achievement of adult reprogrammed cells or induced pluripotent stem (iPS) cells.[1] Pluripotent cells are obtained from adult cells from various tissues that, after genetic reprogramming, can dedifferentiate to a pluripotency state similar to that of embryonic cells, which allows for subsequent differentiation into different cell strains (Fig. 1).[2,3]

In our opinion, this discovery is relevant not only to biomedical issues but also to ethical ones, given that iPS cells could replace human embryonic stem cells (whose use raises numerous ethical problems) in biomedical experimentation and in clinical practice. However, after the last 10 years, the use of iPS cells has still not been clarified. A number of expectations have been met, but other mainly clinical expectations are still far from being achieved.

Current research limitations with iPS cells

There is a notable low efficacy in the techniques employed for obtaining a sufficient proportion of iPS cells, which represents a difficulty in its clinical application.[4] Another limitation is the incomplete reprogramming, which depends on the type of cell employed,[5] and the problems of mutagenesis resulting from inserting exogenous transcription-factor coding genes, which can cause tumors in the employed cells used.[6] Recent studies aim to mitigate this effect.[7] A clinical trial for treating macular degeneration with retinal pigment epithelium cells derived from autologously obtained iPS cells has recently been halted.[8] After an initially successful experience with the first treated patient, the genetic sequencing of the iPS cells obtained from the second patient revealed mutations in 3 different genes, one of which was classified as oncogene in the Catalogue of Somatic Mutations in Cancer.

iPS or human embryonic stem cells

A highly debated topic in the scientific arena is whether iPS cells are similar to human embryonic stem cells. A recent study concluded that human embryonic stem cells and iPS cells are molecularly and functionally equivalent.[9] The experiment consisted of comparing (at the transcriptomic and epigenetic level) human iPS cells and human embryonic cells generated from the same individual, differentiating human embryonic cells in skin cells and reprogramming them to iPS cells to establish the comparison with the original embryonic cells. Although this study was based on genetically identical cells, the authors found no essential differences in the patterns of genetic expression, of genome methylation or in the differentiation capacity of the embryonic stem cells and the iPS cells derived from them. The authors therefore concluded that the cells were molecularly and functionally equivalent.

Fields for applying iPS cells

The use of iPS cells has focused on 2 objectives. The first objective is to obtain a relevant cell type for certain diseases that enables studies on its *in vitro* pathophysiological mechanisms as an alternative to performing biopsies and growing primary cultures of differentiated cell types.[10] Specific objectives have been achieved in this field, given that iPS cells have been obtained from somatic cells of patients with more than 30 different diseases, especially neurological, cardiac and hematological diseases; undoubtedly, experimental material of considerable quality.

The second challenge is deriving (from iPS cells) cells from various tissues that help the in-depth study of the pathogenesis and treatment of various diseases,[11,12] especially in the cardiology area.[13] In the preclinical area, experience has been gained with mice in the treatment of sickle cell anemia, correcting the altered somatic cells through gene therapy. Starting with the modified cells, iPS cells were generated in which the causal defect had been corrected, enabling the researchers to obtain hematopoietic progenitors, which were then injected into mice with sickle cell anemia, significantly improving their clinical condition.[14] Dopaminergic cells can also be produced from iPS cells to treat Parkinson's disease in mice,[15] and factor VIII-generating endothelial cells can be produced from iPS cells to treat hemophilia.[16]

Unfortunately, the clinical applications of iPS cells are still far from what was expected. In effect, only 1 clinical trial has been started with iPS cells, a study implemented by the RIKEN institute (Kobe, Japan), targeted at treating eye disease; in this case, age-related macular degeneration.[17] As stated previously, this trial was temporarily suspended due to the onset of potentially oncogenic mutations in the reprogrammed cells.[8] Additionally, regulatory changes have been introduced in Japan that have imposed new restrictions

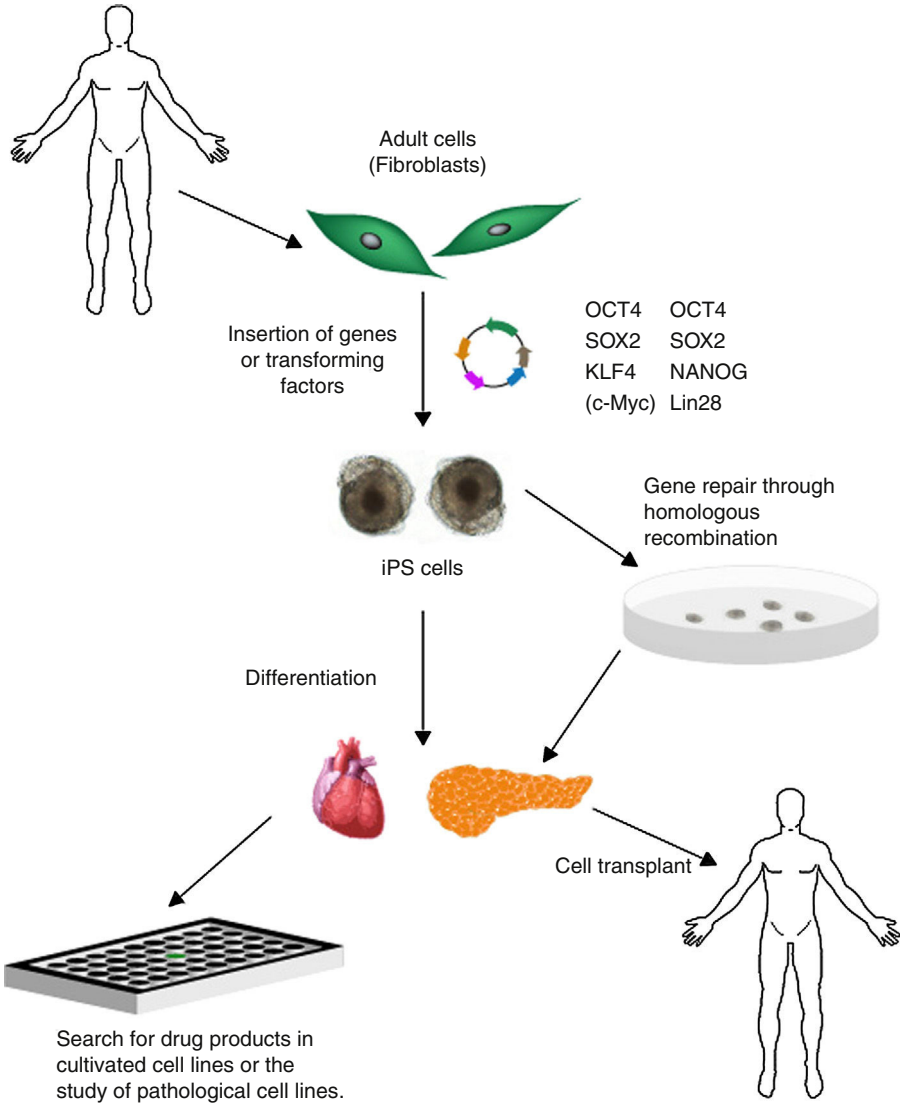

Figure 1 Diagram for obtaining iPS cells from adult cells.

on conducting clinical trials with pluripotent cells, which adds further barriers to the therapeutic use of iPS cells.

Despite the uncertainty, other avenues have opened up, which in the near future could offer new possibilities for using iPS cells. One example is the combination of cell reprogramming and genetic editing,[18,19] using a promising methodology known as clustered regularly interspaced short palindromic repeats (CRISPR-Cas9),[20] which helps, in an easier and more financial manner than previous procedures, modify the human genome to suppress disease-causing disorders. In this respect, techniques could be employed such as CRISPR-Cas9 to modify the genetic disorders that we wish to remedy in the iPS cells derived from the affected somatic cells. Thus, by correcting this disorder in the iPS cell, we proceed to the differentiation toward the cell strain that will be employed in the treatment; in this case, a cell strain free of the genetic anomaly present in the original somatic cell. As an alternative to the CRISP-Cas9 procedure, we can use restriction enzymes through the transcription activator-like effector nucleases (TALEN) system, based on modified nucleases, which have already been tested in a number of clinical trials.[21,22]

There are also new expectations concerning the production of human organs in animals, which could help solve the scarcity of transplants. This production has so far been achieved in rats, transplanting human embryonic stem cells in the mass of pluripotent cells of initial embryonic stages of other species (gastrula, blastocyst), so as to continue in their development to post-implantation stages.[23] However, this practice has ethical difficulties resulting from the use of human embryonic cells, as well as a number of safety problems in the trials.[24] These safety problems include the possibility that human neurons could form in the animal, creating nerve tissue with the hypothetical ability to create human consciousness, or that human sex cells are generated able to conceive a human embryo within a chimeric animal. On the 23rd of September 2015, the US National Institutes of Health stated that it would not finance this type of research, while it reconsiders its funding regulations regarding the ethical assessment of the proposed trials, the

object of controversy in this case.[25] A number of authors, however, have shown their support for continuing this research and have openly criticized the decision.[26]

To overcome the ethical difficulty resulting from destroying human embryos, the substitution of human embryonic cells with iPS cells has been proposed. The iPS cells could be used for creating organs not only in rats but also in pigs, which would bring its use closer to humans due to the size of the organs produced. However, these experiments also raise significant ethical problems due to the possibility that transplanted human iPS cells could produce tissues other than those intended by the research study, such as human nerve cells in the animals' brains. This possibility has meant that significant financial assistance for these types of experiments has been canceled by the corresponding US health authorities.[24]

Future prospects

Cell therapy using iPS cells constitutes a promising resource for regenerative medicine, which will require optimizing the processes of dedifferentiation and subsequent differentiation toward cell strains useful for treating a number of diseases. It is essential that we improve the performance and safety of this therapy and the tissue implantation process, given that they can give rise to limitations in cell integration and migration. The greatest therapeutic potential of iPS cells is based on obtaining them from the patient's own cells (autogenous) to prevent the risk of immunological rejection. There are proposals for creating iPS cell banks, for which the immunologic compatibility treatment would be analogous to that of current transplantations. It is essential that we further our understanding of the biological mechanisms of cellular reprogramming and other mechanisms that lead undifferentiated cells to transform into cells of different tissues.[26]

Conclusion

Since the discovery of iPS cells 10 years ago, there has been an assortment of achievements linked to the new possibilities in research on the pathogenesis of various diseases and their treatment. There have also been a fair number of apparent failures in its clinical application, which are delaying the promising use of iPS cells in regenerative medicine, but which do not detract from their immense possibilities in the field of experimental biomedicine.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126:663–76.
2. Wilson K, Wu J. Induced pluripotent stem cells. JAMA. 2015;313:1613–4.
3. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131:861–72.
4. Patsch C, Challet-Meylan L, Thoma E, Urich E, Heckel T, O'Sullivan J, et al. Generation of vascular endothelial and smooth muscle cells from human pluripotent stem cells. Nat Cell Biol. 2015;17:994–1003.
5. Kim K, Zhao R, Doi A, Ng K, Unternaehrer J, Cahan P, et al. Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. Nat Biotechnol. 2011;29:1117–9.
6. Selvaraj V, Plane J, Williams A, Deng W. Switching cell fate: the remarkable rise of iPS cells and lineage reprogramming technologies. Trends Biotechnol. 2011;28:214–23.
7. Ichida JK, Julia TCW, Williams LA, Carter AC, Shi Y, Moura MT, et al. Notch inhibition allows oncogene-independent generation of iPS cells. Nat Chem Biol. 2014;10:632–9.
8. Garber K. RIKEN suspends first clinical trial involving induced pluripotent stem cells. Nat Biotechnol. 2015;33:890–1.
9. Choi J, Lee S, Mallard W, Clement K, Tagliazucchi G, Lim H, et al. A comparison of genetically matched cell lines reveals the equivalence of human iPSCs and ESCs. Nat Biotechnol. 2015;33:1173–81.
10. McKernan R, Watt F. What is the point of large-scale collections of human induced pluripotent stem cells? Nat Biotechnol. 2013;31:875–7.
11. Tiscornia G, Vivas E, Belmonte J. Diseases in a dish: modeling human genetic disorders using induced pluripotent cells. Nat Med. 2011;17:1570–6.
12. Zhu H, Lensch M, Cahan P, Daley G. Investigating monogenic and complex diseases with pluripotent stem cells. Nat Rev Genet. 2011;12:266–75.
13. Birket M, Ribeiro M, Verkerk A, Ward D, Leitoguinho A, den Hartogh S, et al. Expansion and patterning of cardiovascular progenitors derived from human pluripotent stem cells. Nat Biotechnol. 2015;33:970–9.
14. Hanna J, Wernig M, Markoulaki S, Sun C, Meissner A, Cassady J, et al. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. Science. 2007;318:1920–3.
15. Wernig M, Zhao J, Pruszak J, Hedlund E, Fu D, Soldner F, et al. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc Natl Acad Sci U S A. 2008;105:5856–61.
16. Xu D, Alipio Z, Fink L, Adcock D, Yang J, Ward D, et al. Phenotypic correction of murine hemophilia A using an iPS cell-based therapy. Proc Natl Acad Sci U S A. 2009;106:808–13.
17. Kamao H, Mandai M, Okamoto S, Sakai N, Suga A, Sugita S, et al. Characterization of human induced pluripotent stem cell-derived retinal pigment epithelium cell sheets aiming for clinical application. Stem Cell Rep. 2014;2:205–18.
18. Sun N, Yazawa M, Liu J, Han L, Sanchez-Freire V, Abilez O, et al. Patient-specific induced pluripotent stem cells as a model for familial dilated cardiomyopathy. Sci Transl Med. 2012;4:130ra47.
19. Hotta A, Yamanaka S. From genomics to gene therapy: induced pluripotent stem cells meet genome editing. Annu Rev Genet. 2015;49:47–70.
20. Park C, Kim D, Son J, Sung J, Lee J, Bae S, et al. Functional correction of large factor VIII gene chromosomal inversions in hemophilia. A patient-derived iPSCs using CRISPR-Cas9. Cell Stem Cell. 2015;17:213–20.
21. Osborn MJ, Starker CG, McElroy AN, Webber BR, Riddle MJ, Xia L, et al. TALEN-based gene correction for epidermolysis bullosa. Mol Ther. 2013;21:1151–9.
22. Ma N, Liao B, Zhang H, Wang L, Shan Y, Xue Y, et al. Transcription activator-like effector nuclease (TALEN) mediated gene correction in integration-free β-thalassemia induced pluripotent stem cells. J Biol Chem. 2013;288:34671–9.

23. Wu J, Okamura D, Li M, Suzuki K, Luo C, Ma L, et al. An alternative pluripotent state confers interspecies chimaeric competency. Nature. 2015;521:316–21.
24. Vogel G. NIH debates human–animal chimeras. Science. 2015;350:261–2.
25. Sharma A, Sebastiano V, Scott CT, Magnus D, Koyano-Nakagawa N, Garry DJ, et al. Lift NIH restrictions on chimera research. Science. 2015;350:640.
26. Atala A, Murphy S. Regenerative medicine. JAMA. 2015;313: 1413–4.