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Cutting-edge trends on regenerative therapy: Induced embryonic and pluripotential stem cells, angiogenic growth factor and gene therapy

Actualidad en terapia regenerativa: Células madre embrionarias y pluripotenciales inducidas, factor de crecimiento angiogénico y terapia génica

José R. Hidalgo Díaz[1]✉, MD; Juan C. Chachques[2], MD; and Ángel Paredes Cordero[3], MD

[1] Universidad de Ciencias Médicas, Havana, Cuba, and Hospital Militar Dr. Alejandro Dávila Bolaños, Managua, Nicaragua. Cardiac Bioassist Association. Paris, France.
[2] Pierre et Marie Curie University and Georges-Pompidou European Hospital. Paris, France.
[3] Department of Cardiovascular Surgery. Instituto de Cardiología y Cirugía Cardiovascular. Havana, Cuba.

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Abbreviations
iPS: induced pluripotent stem cells
SC: stem cells

There is no doubt that the most recent discovery of Regenerative Medicine is iPS (Induced Pluripotent Stem cells), also called Reprogrammable Adult Stem Cells (SC), "third way cells" or Induced Pluripotency Stem Cells (**Figure 1**)[1]. John Gurdon from the UK and Shinya Yamanaka from Kyoto University in Japan were awarded the Nobel Prize for Medicine precisely for their research on Stem Cells[1,2].

Gurdon successfully reprogrammed cells from an adult frog. The nucleus was extracted from a live frog egg and replaced by a tadpole cell. The modified egg became a normal tadpole. He undoubtedly managed to reprogram a cell[2]. Yamanaka went much further and discovered that adult cells could be reprogrammed to create polyvalent cells, that is, pluripotent cells just like embryonic SC. He engineered four genes from a mouse embryo and then transferred them into cells taken from the skin of another mouse. Once reprogrammed, they became "polyvalent" cells that were able to continue developing into adulthood without risk of rejection[3].

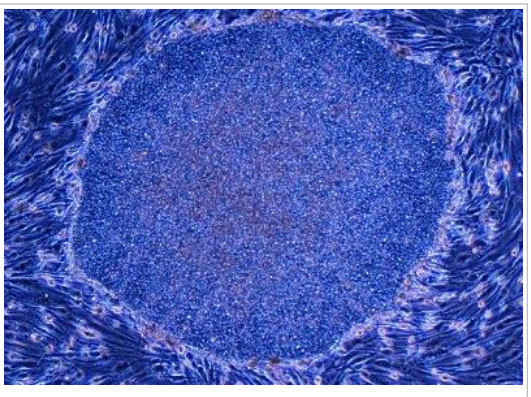

Figure 1. Reprogrammed adult cell (iPS) with polyvalent function. Taken from: Hidalgo Díaz, ContraPunto, 2019[1].

These polyvalent cells (iPS), which are ultimately pluripotent, can be used in

✉ *JR Hidalgo Díaz*
Del Complejo Policial (Seguridad Personal) Cristhian Mungía. 1 Cuadra Arriba, 2 al Lago, Casa # 77 Bloque XXVlll. Managua, Nicaragua.
E-mail address:
hidalgodiaz2003@yahoo.com

humans to grow heart, nerve and liver cells, among other cell lines. So, far from being mere fiction, the stunning truth is that somatic cells from humans can be reprogrammed and used in human pathological models[4].

But iPS cells also have their drawbacks since the cells extracted from the skin and eventually reprogrammed preserve within their genome certain "latent memory" of their adult origin and that "mark" persists in any organ or tissue that is grown from them; an issue that must be addressed before using them on a large scale in the treatment of some illnesses such as neurodegenerative disorders (Parkinson and Alzheimer), diabetes, ischemic heart disease and many others. In fact, there is a specific line of research aimed at preventing these cells from ever degenerating into cancerous forms throughout their evolution course (oncogenesis)[5].

Now then, iPS cells are obtained by "slowing down" the biological clock, that is, by reprogramming simple skin or hair cells of a patient: But they are so versatile and carry such a flexible power of transdifferentiation that their difference with embryonic SCs is practically non-existent. It will no longer be necessary to use SC extracted from the bone marrow, whether mononuclear or mesenchymal, nor will techniques such as Ficoll, apheresis, or others as controversial as the use of modified peripheral blood be used in the future. Much less will any scientist or pseudo-scientist think of grafting SC (say, from animals such as sheep) to humans; a procedure that has been practiced in clinics in the third world, using the inhabitants of these villages as guinea pigs. These cells, besides not being autologous, do not share the same human lineage[6].

Joseph Ecker, Head of the Genomic Analysis Laboratory at the Salk Institute for Biological Studies in California, USA, has come across a completely unexpected problem. As stated above, the cellular reprogramming of skin cells that eventually transforms them into iPS does not wholly erase the genetic history that led to adulthood. The hot spots of the genome still persist in their adult state and surprisingly remain so after they are reprogrammed into iPS and in turn converted into cells of other tissue types suitable for transplantation. It is as if the “hard drive” does not completely erase their record[1,4].

James Thomson, the famous embryologist from the University of Wisconsin and world-renowned for obtaining the first human embryonic stem cells, has also come across this pitfall in his research, which is the persistence within iPS cells of a small segment of the "genetic history" belonging to the previous cells that originated them. Therefore, although iPS cells are quite promising, the research lines closest to a definitive application in regenerative therapy place embryonic stem cells as a strategically important counterpart option[4].

The serious ethical, religious, political and even judicial problem involved in the use of iPS cells in regenerative therapy for the purpose of human procreation is well known, although the therapeutic use of embryonic stem cells is no less so, since it means the destruction of two-week old human embryos that are frozen in fertility centers in the USA, Western Europe and Asian countries that are highly developed in regenerative therapy technologies[7].

In contrast, iPS cells do not have that problem but do have the difficulty described above when reprogrammed to create them. This problem is related to the most important area of research in Biomedicine: epigenetics. All cells during their embryonic development are assigned to a destination according to their position. They must "memorize" it as they move and proliferate, but that memory is not in their DNA sequence but in other molecules that adhere on top of them; that is why this type of biomedical research is called, epigenetics, which means above the genes[8].

The epigenetic molecules found are histones, which are proteins, and the methyl radical-CH3, which strongly adheres to both histones and DNA. The process of genetic methylation causes the gene in a given cell to become inactive, thus producing the inactivation of all the cells that come from it, since they also retain memory[4].

According to Hidalgo[4], Ecker and his team (Salk Institute, California, USA) were the ones who first examined methylomas, which are the genome methylation profiles for five human iPS cell lines maintained in culture, and compared the former with embryonic SC (telomeres) and those from other areas (centromeres) that are essential for distributing the genetic material equally between two daughter cells. Technically, according to the reprogramming method of the Kyoto University, only four genes are added to the skin cells or the five proteins produced by those genes. This is quite simple for a well-trained team (**Figure 2**) and such a regenerative therapy technique should always be done in this way if it is actually intended to be used on a large scale in human clinical practice, always in accordance to the provisions of the Declaration of Helsinki for medical research involving human subjects. However, there

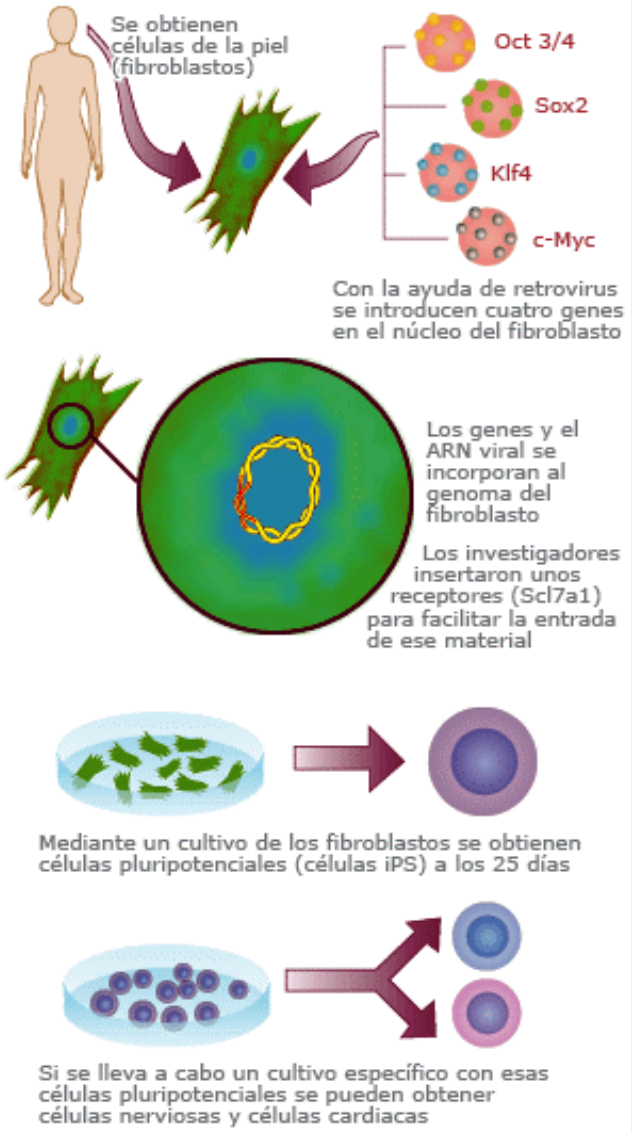

Figure 2. Reprogramming method to grow iPS (induced pluripotent stem cells). Taken and modified from: elmundo.es (https://www.elmundo.es/elmundosalud/2007/11/20/biociencia/1195572777.html).

are still loose ends as science advances and the problems of iPS cells in their methylation profiles or the epigenetic state of the human genome need to be solved.

It is clear that the reprogramming process is imperfect and some areas of the human genome that are methylated in the original skin cells remain methylated in the iPS cells reprogrammed from them. This does not occur in embryonic stem cells and is the most important difference found between these two types of cells, so further research is needed as conservative sectors of society constantly press against the use of embryos and therefore embryonic stem cells[4].

According to Hidalgo[4] both the Salk Institute of California and the Center for Regenerative Medicine in Barcelona are already using retroviruses to generate iPS cells, but these cells still remain in culture for a long time and are therefore "stressed".

The implantation of these cells in cardiac surgery can be by epicardial (**Figure 3**) or intracoronary route, but transventricular is also useful, and even type-1 collagen tissue or matrix may be used to create a bioartificial myocardium, as described in the MAGNUM study (Myocardial Assistance by Grafting a New Bioartificial Upgraded Myocardium)[9], since iPS cells can be seeded in the matrix mentioned above, prior to definitive implantation on the surface of the infarcted ventricle, as is done with autologous bone marrow cells[9,10]. Professor JC Chachques of the European hospital Georges Pompidou in Paris, France, is currently developing a new project for "biomimetic" treatment using tissue engineering,

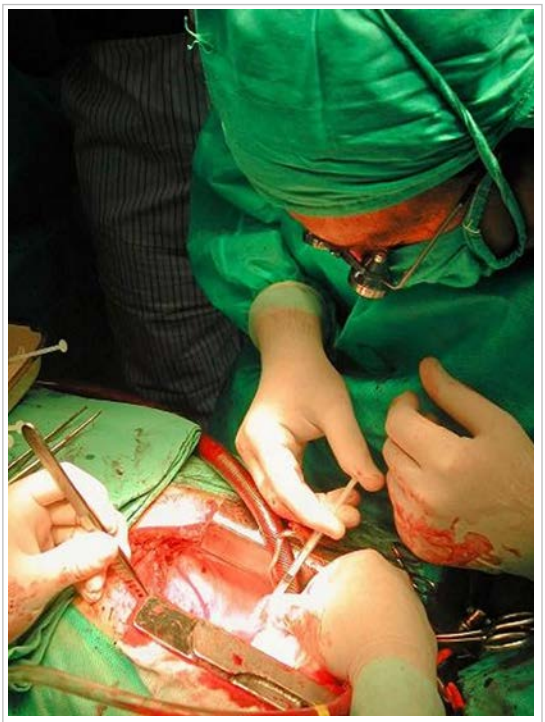

Figure 3. Dr. José R. Hidalgo Díaz implants stem cells directly (surgically, on February 24, 2004, at the Instituto de Cardiología y Cirugía Cardiovascular from Havana, Cuba) into the heart of a patient who had suffered a myocardial infarction. Taken from: Hidalgo Diaz[7]. CorSalud 2018;10:47-51.

where collagen is replaced by polycaprolactone (PCL), an elastomer that slowly degrades within two years[11]. SC implanted in the PCL matrix could become in the future, a bioartificial myocardium[9-11].

For their part, embryonic stem cells also have their drawbacks: a) they accumulate mutations when manipulated and cultivated, b) they are immunologically incompatible with the patient to whom they are implanted, which is not the case with iPS stem cells, and c) as already mentioned, there are serious ethical controversies for their use and countless judicial problems in the control of research[4].

Mesenchymal and umbilical cord cells are also used, but they are less likely to couple to the electromechanical system of the heart although there is no doubt about their myogenic and angiogenic power that causes thickening of the heart's fibrous eschar, achieves an "anti-remodeling" effect on the heart muscle and prevents other problems that occur in patients after a myocardial infarction. The same applies for mononuclear cells, CD-34+obtained by the Ficoll method. The echocardiogram (colorkinesis) has satisfactorily demonstrated the effect of these cells on myocardial regeneration[7]. In our opinion, other methods used, such as peripheral blood collection and its association with cell growth factors, lack any future perspective.

In addition to their use in Cardiology and Cardiovascular Surgery, and in cardiac bioassistance, SC are used for the treatment of diseases in several specialties, for example: corneal regeneration (Ophthalmology), chondrocyte implantation for joint defects (Orthopedics), islet of Langerhans transplant in diabetes mellitus (Endocrinology), Huntington and Parkinson diseases, spinal cord regeneration, Alzheimer, senile dementia, Duchenne muscular dystrophy (Neurology), hepatocytes as a bridge to liver transplantation (Gastroenterology), implantation of keratinocytes in burn patients (Dermatology and Caumatology), chronic lower limb ischemia (Angiology), chronic lymphocytic leukemia, aplastic anemia, immunodeficiencies and myeloma (Hematology).

The so-called qualitative method was initially used, but has become totally obsolete; therefore, flow cytometry is currently the appropriate method for identifying and counting the cells to be implanted. This is a quantitative, safe and effective method for such purposes, and is the one accepted by the International Association of Cardiac Bioassistance.

Regenerative therapy on extramedullary organs, first performed in Paris, France in 2000 with the first SC heart transplant, made it clear that the problem of many conditions originates at the cellular level and therefore that is where we must maneuver in order to solve them[12]. Cuba was one of the pioneers of regenerative therapy by using SC for the treatment of extramedullary diseases in humans. A team of scientists, led by Dr. José Hidalgo Díaz, transplanted SC obtained by the Ficoll method into an infarcted heart on February 27, 2004, at the Instituto de Cardiología y Cirugía Cardiovascular from Havana, Cuba; being in fact the first application of this procedure in our country, in Central America and the Caribbean. The members of this team were, in addition to Dr. Hidalgo who led it, Doctors Ángel Paredes, Consuelo Macías, Elvira Dorticós, José Manuel Ballester, Alberto Hernández Cañero and Porfirio Hernández, among others, all members of the Grupo de Terapia Regenerativa del Instituto de Cardiología y Cirugía Cardiovascular y el Instituto de Hematología de Cuba[7].

Some experimental tests on animals had been previously performed at the Broussais Hospital in Paris, France, under the direction of Professor J. C. Chachques, who was leading the most important project of Regenerative Therapy in the European Economic Community (*Contrat Commission Européenne* ERB 4001GT957737) related to the use of SC in humans[7]. The preliminary preclinical results were presented in the Intercontinental Cardiology journal in 2001[13], by Chachques, Hidalgo Díaz and other collaborators, from the Departments of Cardiovascular Surgery of the *Hôpital Georges Pompidou and Broussais*, Paris, France. This was the first time in America that there was talk of research on SC.

On the other hand, angiogenic or vascular endothelial growth factor (VEGF-cells) is proposed for patients with myocardial infarction by means of percutaneous or surgical revascularization[12-14]. This method has been studied in experimental animals at a number of institutions, one of which is the experimental surgery center of the European Georges Pompidou Hospital in Paris[8]. In one of its published works, an experimental myocardial infarction by ligation of two coronary arteries was induced. Three weeks later, the animals were randomized into four groups (control, autologous myoblast implant, VEGF-cells and both). The evaluation included an immunohistological study for quantitative analysis of capillaries three months after surgery, and concluded that there was improvement in the left ventricular ejection fraction, reduction of post-ischemic remod-

eling and angiogenesis in the group where VEGF-cells were used, thus paving the way for further in-depth research[8].

Author's note

With this article we make a special tribute to Dr. Abelardo Ramírez Márquez (*may he rest in peace*), who devoted all his strength in advancing this novel and futuristic therapy in Cuba since its beginning; and to Professor and Doctor Jesús Herrero (*may he rest in peace*), Head Director of the Fundación de Ingeniería Biomédica y Tecnologías Sanitarias de España.

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