EDITORIAL

RETURNING TO THE PAST: 60 YEARS IN THE UNDERSTANDING OF HOW CELLS AND ORGANISMS DEVELOP

"If you're really interested in something, keep going – don't give up." Professor John Gurdon.

Orietta Beltrán, MD, MSc[1]

[1]Médica, MSc Genética Humana. Profesora Asistente Universidad Militar Nueva Granada, Facultad de Medicina. Bogotá, Colombia.

Correspondencia: orietta.beltran@unimilitar.edu.co

The first attempt to reschedule a somatic cell into a pluripotent stem was performed in 1952 when Robert Briggs and Thomas King (1) successfully transferred nuclei of *Rana pipiens* blastocysts to oocytes obtaining viable embryos. However, they inferred that the somatic cell nuclear transfer (SCNT) technique was not applicable to differentiated cells due to the irreversible character of differentiation processes. Subsequently, in 1962, the biologist John Gurdon, University of Cambridge, made a revolutionary discovery: he demonstrated the formation of embryos developed in *Xenopus laevis* tadpoles starting from transference of adult frogs' gut cells nuclei to oocytes thus evidencing that genomes of somatic cells maintain their ability to generate viable cloned organisms and that the differentiation process is a reversible mechanism through nuclear reschedule due to epigenetic modifications, regulated by the existence of –unidentified– factors which induce pluripotency. Additionally, another John Gurdon's observation was that genes didn't get lost but they had a differential expression (2,3,4). Three decades later, in 1996, the embryologist Ian Wilmut et al (5) achieved successful clonation in a mammal, Dolly the sheep, that was followed by cows, pigs, mice, goats, and cats clonation as well as less efficient clonation in rats, non human primates and dogs by several investigators (6,7,8).

Furthermore, in 1998, the biologist James Thomson, University of Wisconsin, managed to isolate and typify human embryonic stem cells (9) which pointed out the great possibility of using them for medical applications through obtaining in vitro cell lines from all lineages, for example, cardiomyocytes, osteoblasts, chondrocytes, hepatocytes and neurons. But ethical concerns around investigation with embryonic stem cells are controversial, because usually they are extracted through destruction of human embryos discarded by fertility clinics. Likewise, the SCNT technique has ethical implications due to the questionable need to use oocytes. Another technical disadvantage of SCNT is its low efficiency (1-4%) and confirmation of structural chromosomic abnormalities in some of the obtained cell lines which makes it a limited tool in the production of autologous cells or tissues for therapeutic use (6). However, it was a transcendental contribution of organisms through the SCNT which demonstrated that when a nucleus is transferred in the oocyte cytoplasm, changes in the chromatin structure are produced which regulate reversibly the differentiation, becoming an important test of developmental plasticity (6,8).

On the other hand, other investigators applied the fusion of somatic cells with embryonic stem cells technique in order to obtain pluripotent cells. By using this technique, Richard Miller and Frank Ruddle, in 1976, demonstrated that mice thymocytes gained pluripotency following cell fusion with embrionary carcinoma cells (9). One of the most interesting studies was that of Chad Cowan et al in 2005, who were able to merge human fibroblasts with embryonic stem cells, thus obtaining hybrid cells with morphological features and gene expression of typical patterns of embryonic stem cells as well as genes involved in pluripotency (Oct4, Nanog, TDGF1 and Rex1), with specific silencing of somatic status genes in the whole genome and evidence of epigenetic changes in the Oct4 promoter (10).

Nevertheless, a striking discovery took place in 2006 when Shinya Yamanaka and Kazutochi Takahashi, investigators of the Stem Cells Biology Department, Kyoto

University, Japan, determined that viral transfection of four genes (Oct4, Sox2 KLF4 yc-Myc, acronym OSKM) in adult mice fibroblasts generated cells with embryonic stem cells features which were named induced pluripotent stem cells (iPS) (11); besides, these same factors were required in the generation of human iPS from human fibroblasts (12). The resultant cell don't seem to be substantially different from embryonic stem cells and eventually can provide an appropriate source of different cell types specific for each patient (13).

John Gurdon and Shinya Yamanaka's scientific legacy in that cell differentiation is not an unidirectional and irreversible process has involved the progress in the knowledge of stem cells biology and epigenetic mechanisms regulation. The induction of pluripotency through specific factors, sometimes called "Yamanaka factors", has allowed a rapid transition in the use of induced pluripotent stem cells in the study of pathophysiological models, discovery of genes and drugs, embryonic development and differentiation studies as well as a potential source of cell therapy and specific disease cell lines to assess feasible targets and therapeutic agents.

Recently, the Nobel Prize Assembly announced that John Gurdon, along with Shinya Yamanaka had been awarded with the Nobel Prize of Physiology or Medicine in 2012 for demonstrating that adult differentiated cells can be rescheduled to become immature "stem" cells that are capable of developing any kind of body tissue (14).

The current challenge of investigators in the field of Stem Cells and Regenerative Medicine is to understand the molecular functioning of pluripotency both at the genetic and epigenetic level, and increase the efficiency and safety of nuclear reschedule techniques in the clinical application of iPS.

References

1. Briggs R, King TJ. *Transplantation of Living Nuclei From Blastula Cells into Enucleated Frogs' Eggs*. Proc Natl Acad Sci U S A. 1952 May;38(5):455-63.
2. Gurdon JB. *The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles*. J Embryol Exp Morphol. 1962 Dec;10:622-40.
3. Gurdon JB. *The transplantation of nuclei between two species of Xenopus*. Dev Biol. 1962 Aug;5:68-83
4. Gurdon JB. *Adult frogs derived from the nuclei of single somatic cells*. Dev Biol. 1962 Apr;4:256-73.
5. Campbell KH, McWhir J, Ritchie WA, Wilmut I. *Sheep cloned by nuclear transfer from a cultured cell line*. Nature. 1996 Mar 7;380(6569):64-6.
6. Wilmut I, Beaujean N, de Sousa PA, Dinnyes A, King TJ, Paterson LA, Wells DN, Young LE. *Somatic cell nuclear transfer*. Nature. 2002 Oct 10;419(6907):583-6.
7. Byrne JA, Pedersen DA, Clepper LL, Nelson M, Sanger WG, Gokhale S, Wolf DP, Mitalipov SM. *Producing primate embryonic stem cells by somatic cell nuclear transfer*. Nature. 2007 Nov 22;450(7169):497-502.
8. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. *Embryonic stem cell lines derived from human blastocysts*. Science. 1998 Nov 6;282(5391):1145-7. Erratum in: Science 1998 Dec 4;282(5395):1827.
9. Wilmut I, Paterson L. *Somatic cell nuclear transfer*. Oncol Res. 2003;13(6-10):303-7.
10. Miller RA, Ruddle FH. *Pluripotent teratocarcinoma-thymus somatic cell hybrids*. Cell. 1976 Sep;9(1):45-55.
11. Cowan CA, Atienza J, Melton DA, Eggan K. *Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells*. Science. 2005 Aug 26;309(5739):1369-73.
12. Takahashi K, Yamanaka S. *Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors*. Cell. 2006 Aug 25;126(4):663-76.
13. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. *Induction of pluripotent stem cells from adult human fibroblasts by defined factors*. Cell. 2007 Nov 30;131(5):861-72.
14. Gurdon JB, Melton DA. *Nuclear reprogramming in cells*. Science. 2008 Dec 19;322(5909):1811-5.
15. Vogel G, Normile D. Nobel Prize in physiology or medicine. *Reprogrammed cells earn biologists top honor*. Science. 2012 Oct 12;338(6104):178-9.