SHORT COMMUNICATION

A Novel Method for Reprogramming Somatic Cells into ‘Embryonic’ Stem Cells

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Stem cells are the body’s ‘master’ cells, they are pluripotent cells, and in theory, they can potentially be differentiated to any tissue-type in the body. Until today, stem cells have been derived from the totipotent cells of the inner cell mass (ICM) of mouse and human blastocysts. The ability of a differentiated somatic cell to switch into a pluripotent cell has puzzled researchers for several years. It was not until exactly ten years ago, when Dolly the sheep was born (1) that scientists accepted the fact that somatic cells can be reprogrammed into an embryonic totipotent state.

Dolly was created after the transfer of a differentiated mammary gland cell nucleus into a donor oocyte which reversed the somatic program into an embryonic one, and developed to give rise to a live offspring.

Ten years after Dolly, an embryo-free alternative method for the derivation of stem cells has now been proven possible. In recent original research by Shinya Yamanaka of Kyoto University, mouse skin cells were de-differentiated into an embryonic state by a simple retroviral introduction of four genes that code for specific proteins known as transcription factors, namely Oct3/4, Sox2, c-Myc and Klf4 (2). The resulting cells were termed induced pluripotent cells or iPS cells. They carry analogous expressed markers (Oct4, Nanog, Sox2 and Fbx15) and chromatin modifications (DNA CpG dinucleotides, histone H3 lysine 4 and histone H3 lysine 27 methylation). In addition, iPS cells can multiply indefinitely in culture, while maintaining their pluripotent state, similarly to existing stem cells derived from embryos. In addition, iPS cells were selected according to their Nanog expression, which is a target of Oct3/4 and Sox2.

The selected cells were tested for their ability to produce adult chimaeric mice by injecting them into blastocysts, which were then transplanted into the uteri of pseudopregnant surrogates. Simple sequence length polymorphism (SSLP) was used to demonstrate the contribution of these cells to various organs including the testes. The germ line transmission was then tested by crossing the resulting chimaeric mice for a second generation with limited success.

The work by Yamanaka’s group could have come under severe scrutiny after the irreproducible (later fraudulent) work by Woo Suk Hwang claiming to have innovatively derived embryonic stem cells from human cloned embryos. However, the replication of Yamanaka’s results by Rudolf Jaenisch and his team (3) from the Whitehead Institute of Biomedical Research in Cambridge, Massachusetts, as well as the joint effort (4) between Konrad Hochedlinger of the Harvard Stem Cell Institute in Boston, Massachusetts and Kathrin Path of the University of California, Los Angeles, corroborated the work of the Japanese team.
The derivation of stem cells, without the need for eggs, sperm, or embryos, is the great prize for stem cell research. In theory, one can speculate that individual’s iPS cells can be propagated in the laboratory and differentiated into any type of cell in the body. But of course, the method is still far from perfect still, and there is a high dose of scepticism surrounding it. The reprogramming of cells is inefficient, with only less than 0.1% of skin cells that will be fully reprogrammed. As in most gene knock-in experiments, antibiotic resistance genes are inserted along the genes of interest. The cells are then supplemented with antibiotics to destroy non-transfected cells.

The major drawback of these experiments is the increased activation of tumour causing genes such as the reactivation of c-Myc in the current studies. This deregulation of certain factors can be the result of the utilization of retroviruses as a method of transfecting cells, which introduce genes at random locations in the genome. A thorough understanding about the molecular pathways that dictate this reprogramming is needed. Alternative methods have are already been suggested such as the replacement of the retrovirus by an adenovirus system for transient expression, and as Alan Trounson of Monash University said ‘I can think of a dozen experiments right now – and they’re all good ones’.

These findings have been particularly welcomed in the quarters where the use of embryos for research is ethically opposed. Moreover, fellow researchers and the public are already speculating about the application of these methods for human cell therapy and treatment of diseases and disabilities like Parkinson’s, Alzheimer, Type 1 diabetes, strokes, and spinal cord injury. Therefore, whether embryonic or somatic, the race is still on for the ultimate method and the ultimate cells that will be employed in clinical medicine.

REFERENCES