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Production of Induced Pluripotent Stem Cells by Reprogramming of Blood Cells

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Abstract: Blood cells are the simple, efficient and economical source for the production of induced pluripotent cells. The discovery of induced pluripotent cells was not novel; it was pedestal on the scientific principals and technologies which have been developed over last six decades. These are nuclear transfer and the cloning of Animals, Pluripotent cell lines and fusion hybrids and Transcription Factors and lineage switching. The use of human embryonic stem cells in regenerative medicines was a breakthrough but make use of these cells arise ethical issues as they are obtained from human embryos. An alternative advancement using induced pluripotent stem cells, which mimics the embryonic stem cells has the significant gain that they replaced the embryonic stem cells. The pluripotent cells can be induced from terminally differentiated somatic cells by the Induction of only four defined factors including *c-Myc*, *klf4*, *Oct4* and *Sox2* which are enough to alter the fate of cell.

Key words: Fibroblasts, *C-Myc*, dedifferentiation, iPSCs, blood cells, induced pluripotency, proliferation, reprogramming, transcription factors

INTRODUCTION

Stem cell biology based on the scrutiny of cells that are functioning in bodies and maintaining the adult bodies. The body undergoes regeneration of numerous cells like skin cells, blood cells and gastrointestinal cells for the stability of body.

The regenerative ability of cells is crucial for survival. According to recent developments a number of human somatic cells types have been reported to generate induced pluripotent stem cells including fibroblasts, Keratinocytes and blood cells. Reprogramming of blood cells has three major rewards: Blood is the easiest, most available source of cells, because you would rather have 20 mm of blood drawn than have a punch biopsy taken to get skin cells (Judith Streak *et al*.2010). Secondly blood collection and storage is well established. Thirdly "it is more convenient and less invasive to obtain blood than dermal fibroblasts and Keratinocytes, in which several weeks are required for primary cell culture from skin biopsy" (Bin-Kaun Chou *et al*., 2011). Blood is composed of red cells that carry oxygen thought the body, white cells that are part of immune system and platelets that clot the blood after an injury. Not all of the blood cells in the blood sample can be converted to iPSCs. Because red cells and platelets lack the nuclei containing DNA, The only white cells converted to iPSCs. Further developments are being made for the treatment of different dieses and for the establishment of patient-specific analogue cells for regenerative medicines by reprogramming of blood cells. All the research going nowadays and in past on the reprogramming of somatic cells to stem like cells is based on the study of Takashi and Yamanaka (2006) who proved that only the combination of four transcription factors are enough to induced the pluripotency into the differentiated cell.

METHODOLOGY

Reprogramming of blood cells: To prove the ability of blood cells to dedifferentiate, (Yuin Han *et al*., 2009) devised an elegant plan of direct reprogramming of mobilized human CD34+peripheral blood cells. They first cultured the CD34+ cells in vitro with a combination of hSCF, hFlt3L and iL-3 cytokines which result in proliferation and expansion of CD34+cells by several magnitudes. They used the combination of four transcription factors *Oct4*, *Sox2*, *Klf4 and c-Myc* and obtained the human embryonic stem cells like colonies after 18 passages of the scheme they followed. Motohito *et al*. (2009) develop an well-designed arrangement of direct reprogramming of murine hematopoietic cells with no rearranged genes. They use the definitive genetic marker CD45 (which is a Protein Tyrosine Phosphatase (PTP) located in hematopoietic cells except ethrocytes and platelets. CD45 is also called the common leukocyte antigen.). They transduced the HSPCs population with cocktail of retroviral vectors

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harboring each of the iPSC factor genes *Oct4*, *Sox2*, *Klf4* and *c-Myc*. They obtained typical embryonic stem cell like colonies in approximately 21 to 28 days. Zhaohui *et al*. (2009) reported that "improved methods that achieve reprogramming of human fibroblasts without permanent genome alteration will probably be applicable to that of human blood cells as well". In their study they used the CD34+ cells of cord blood, adult blood and bone marrow with same arrangement of transcription factors. Methew *et al*. (2010) described derivation of "iPSCs from small, clinically advantageous volumes of non-mobilized human peripheral blood (T-cells) which can be harvested from large number of donors in a minimally invasive manner and cultured via well established protocols, using the combination of four transcription factors". They showed that TiPS have similar characteristics and differentiation potential as hESCs lines. (Yuin-Han *et al*., 2010) successfully reprogrammed cells from peripheral blood sources including samples obtained through routine venipucture. To induce reprogramming of enriched CD34+ blood cells, they infected with lenti-viruses expressing *Oct4*, *Sox2*, *Klf4* and *c-Myc* reprogramming factor. Colonies with well defined hESCs like morphology were first observed 21 days after transduction. Tomohisa *et al*. (2010) examined T cells that had been transfected with SeV vectors carrying *OCT3/4*, *SOX2*, *KLF4* and *c-MYC* were plated onto mitomycin C-treated SNL feeder cells at 5 3104 cells per 10 cm dish. Around day 25 after blood sampling, the number of ALP-positive hESC-like colonies was observed.

RESULTS AND DISCUSSION

Potential of blood cells: Dedifferentiation of blood cells reported in different research papers indicated that blood cells are the unique source of obtaining the induced pluripotent stem cells. The technology which is called as induced pluripotency revolutionized the present and future of medicinal sciences. Because through this technique, we not only obtain the desired cells for further studies; but also able to understand the myth of cellular differentiation and periodic changes happened in it.

Although many somatic cells can be reprogrammed to stem cell stage but due to sparking features of blood cells these are becoming the site of consideration for scientists. The technology to derive iPSCs from blood provides opportunity to generate histocampatible stem cells for many persons because of large collections in blood banks. If we compared the reprogrammed blood cells with patient-derived fibroblasts and Keratinocytes that takes weeks to establish, isolated CD34+ cells from blood or marrow just need to be cultured for 2 to 4 days before being reprogrammed by standard protocols.

The established induced pluripotent cells form blood cells are similar to embryonic stem cells in their morphology, proliferation, feeder dependence surface markers, gene expression and Teratoma formation.

Role of transcription factors: The transcription factors involved and reported by almost all research papers showed that each factor is determining the pluripotent stage in the cells. The function of *Oct4* and *Sox2* regulate the "stemness" genes by suppressing the differentiated-associated genes in human and murine cells (Boyer *et al*.2005, Loh *et al*., 2006; Wang *et al*., 2006). These factors can not bind their targeted genes in differentiated cells due to DNA methylation and histone modifications. The *c-Myc* and *Klf4* altered the chromatin structure for the binding of *Oct2* and *Klf4* on their target sites (Yamanaka *et al*., 2007).

Instead of these four transcriptional factors the studies are going on to check the efficiency of other transcriptional factors. The *Nanog* and *Oct4* was selected for reactivation of essential pluripotency genes. The resulted iPCSs were molecularly and functionally more closely resembled to ESCs (Maherali *et al*., 2007; Okita *et al*., 2007; Wernig *et al*., 2007)

Another report suggests that *c-Myc* act as a autonomous factor during the early stages of reprogramming, including the cellular proliferation and driving a concomitant switch towards an energy metabolism typical of cancer cells (Mikkelsen *et al*., 2008). Thus the cocktail of four transcription factors is enough for altering the fate of differentiated cells into pluripotent stem cells. The review of all related articles to induced pluripotency in blood cells opened a new avenue to generate the desired model cells in short time.

CONCLUSION

The induced pluripotent cells have the ability treat the chronic diseases like diabetes, Parkinson's disease, Alzheimer disease, Sickle cell anemia and other heart disease (Timothy *et al*., 2010). The most important and recent application of this technique is to establish the Cellular models of diseases. These models provide the understanding of pathophysiology and validation of drugs. We now have an excellent technology for relatively uncomplicated production of embryonic stem cells using blood cells. Development of iPSCs will undoubtedly spread through use of blood cells particularly for the preparation of disease specific iPS cell lines. In future, it is possible that every person will have their own iPS cell lines, prepared when they were still healthy, for future applications in clinical examination or therapy.

RECOMMENDATION

Although, the technical advancement has made the direction to replace the ESCs by iPSCs but still relatively little is known about their functional and molecular similarity with ESCs. There is need to careful analysis of genomic, morphological reliability of iPSCs. There should be specific and sensitive differentiation protocols and consistent assays to analyze the functionality and equivalence of iPSCs.

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