The generation of ips cells from somatic cells critical thinking examples

Science, Genetics

\n[toc title="Table of Contents"]\n

\n \t

\n[/toc]\n \n

Compare and Contrast On IPS Papers

All the four papers Takahashi-miPS, Takahashi-hiPS, Park-hiPS and Yu-hiPS respectively induced the four genes (OCT4, SOX2, KLF4 and MYC) and reprogramming the differentiated somatic cells to produce IPS pluripotency cells. In the first paper, the authors found a combination of factors to make the IPS cells. The authors selected the 24 genes for factors that induce pluripotency in somatic cells. Afterwards, all the 24 genes were inserted into the mouse embryonic fibroblast (MEFs) by using retroviral transduction. The cells were cultured on STO feeder cells in ES cell medium containing G418. The authors obtained 24 colonies, and then the authors determined which of the 24 candidates were critical by examined the effected of the withdrawal of individual factors from the pool of transduced gene on the formation of G418- resistant colonies. The authors identified 12, 10 and finally 4 factors. Therefore, the result indicated that OCT4, SOX2, KIF4 and c-MYC were playing the important roles in the generation of IPS cells from MEFs cells.

Takahashi-hiPS, the authors started from the end of the first paper by using Human Adult Fibroblast cells instead of mouse cells. The optimized the expression of the GFP florescent by adding the mouse receptors Sic7aL for the retroviruses to the human cells. After introducing the retroviruses, which contain human OCT4, SOX2, KIF4 and c-MYC into HDF-Sic7aL modified cells, the authors found distinct type of colonies that were similar to the human embryonic cell colonies. Park-hiPS, the authors induced four factors in Adult Dermal Fibroblast cells, Primary Fetal Tissue, Neonatal Fibroblast and MSCs cells to produce IPS cells by using retroviruses. Yu-hiPS, the authors induced four factors (OCT4, SOX2, Nanog and LIN28) to reprogram human somatic cells and producing pluripotent stem cells that exhibit the essential characteristics of Embryonic Stem cell (ES cells). The authors used lentivirus. Line 28 and Nanog were not necessary but the authors still help.

Gene Expression of the IPS cells:

Takahashi-miPS, the authors performed RT-PCR to examine whether ES cell markers were expressed in IPS cells. The authors found that IPS clone expressed the majority of marker genes including NANOG, SOX2, OCT4 and Fgf4. By doing chromatin immunoprecipitation analysis, the result showed that the promoters of OCT4, and Nanog had increased acetylation of histone 3 and decreased demethylation of lysine 9 of histone 3. The combination of three factors (oct4, sox2 and c-myc) without Kif4 or (kif4, Sox2 and c-myc) without oct4 will not generate any colony. In addition, in the absence of Sox2, there were fewer colonies but still there were IPS cells. However, in the absence of c-myc, there were colonies but there were not IPS cells.

Takahashi-hiPS, the authors did RT-PCR to examine the expression of the genes. The authors found that human IPS cells expressed many undifferentiated ES cell-markers such as OCT3, SOX2, C-Myc and Nanog. Also, by doing western blotting to determine the level of the proteins. The authors found that the proteins levels of OCT3, SOX2, Nanog and SALL4, E-cad-herin and hTERT were similar in human IPS cells and hES cells. Park-hiPS, the authors used quantitative real-time PCR to examine the expression of factors. The authors found that the key pluripotency factors (oct4, sox2, nanog and klf4) were expressed in ESC. However, the authors lost by the third week of the differentiation whereas the expression of MYC was persisted.

Global Gene Expression, DNA Microarry:

Takahashi-miPS, the authors did global gene –expression profiles by DNA microarray of the EC cells and IPS cells. The authors found that IPS cells were clustered closely with ES cells but separately from fibroblast and their derivative. The authors are similar but not identical to each other. In the second paper, DNA microarray analysis showed that the global gene expression. The authors found that Oct4, Sox2 and Nanog were clustered closely between ES and IPS. However, OCT4, SOX2 and Nanog were separated between HDF and IPS. Park-hiPS, DNA microarray analysis showed that a tighter correlation between reprogrammed somatic cells (dHIf-IPS-3 and MRC5-ips2) and human ES cells(H1-OGN) or (dHicfi6). Therefore, the result showed that the cells reprogrammed from somatic sources are highly similar to embryo derived human ES cells but not identical. Yu-hiPS, the

result stated that gene expression of the ips colonies confirmed a similarity to five human ES cell lines (H1, H7, H9, H13 and H14) and dissimilarity to IMR90 cells.

The formation of teratoma:

The papers stated that on injecting the mouse by IPS cells, the authors observed the formation of teratoma that has all three primary embryonic germ layers Takahashi-miPS, IPS-MEF4 Wt-4 colonies differentiated into three germ layers including neural tissue, cartilage and epithelium cells. Park-hiPS, the authors did RT-PCR of the differentiated cells, the authors showed marker genes (GATA4, NCAM and RUNX1) expression for all three embryonic germ layers.

The differentiation ability of IPS cells:

Immunocytochemistry detected the cells positive of Biii-tublin, glial fibrillary acidic protein, alfa- smooth muscle actin and desmin. Takahashi-miPS, the authors induced the four selected factors into adult mouse-tail tip fibroblast (TTFs) to get ES. The authors found that these cells were morphologically indistinguishable from ES cells. In addition, the authors used RT-PCR for these cells to examine the expression. The authors found that IPS cells expressed the majority of ES cells marker genes. Takahashi-hiPS, the authors did RT-PCR as well, and found that these cells expressed FOXA2, AFP, SRY-box and BRACHYURY. However, the expression of OCT4, Nanog and SOX2 were decreased markedly. Thus, these data stated that the IPS cells could differentiate into three germ layers in vitro. Park-hiPS, during the

differentiation of IPS, RT-PCR stated that the expression of OCT4, SOX2 and Nanog were decreased, but the expression of C-MYC did not decrease.

Bisulfite Genomic Sequencing Analysis:

Takahashi-miPS, the result stated that the promoter of FbX and Nanog were demethylated in IPS cells. However, OCT4 promoter remained methylated in these cells. Takahashi-hiPS, the authors analyzed the status of the cytosine guanine dinucliotides (CpG) in the promoter regions in pluripotencey region such as OCT4, SOX2 and Nanog. The authors found that the authors were highly unmethylated in IPS and ES cells. However, CpG were mythylated in HDF cells. Park-hiPS, the OCT4 and Nanog were unmethylated in H1-OGN, dH1f-isp-1 and dH1cf32-ips2 cells. However, OCT4 and Nanog were methylated in Hdif and MRC5 cells.