Generation of Pluripotent Stem Cells from Adult Mouse Liver and Stomach Cells

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Abstract

Induced pluripotent stem (iPS) cells have been generated from mouse and human fibroblasts by the retroviral transduction of four transcription factors. However, the cell origins and molecular mechanisms of iPS cell induction remain elusive. This report describes the generation of iPS cells from adult mouse hepatocytes and gastric epithelial cells. These iPS cell clones appear to be equivalent to embryonic stem cells in gene expression and are competent to generate germline chimeras. Genetic lineage tracings show that liver-derived iPS cells are derived from albumin-expressing cells. No common retroviral integration sites are found among multiple clones. These data suggest that iPS cells are generated by direct reprogramming of lineage-committed somatic cells and that retroviral integration into specific sites is not required.

In 2006 Shinya Yamanaka and colleagues transfected mouse fibroblasts with four genes, Oct3/4, Sox2, Klf4 and c-Myc to create iPS cells. In this study the same group attempted to create iPS cells from mouse stomach and liver cells using the same procedure from their earlier work. Just like the original experiment they used mice that had the DNA for the pluripotency stem cell gene Fbx15 replaced with two genes; a gene that creates a blue color and another gene for drug resistance. If a somatic cell was turned into a stem cell-like cell when it was transfected with the four factors it turned blue and survived exposure to the drug.

The researchers were able to obtain cells resembling embryonic stem cells from both the stomach and liver cell by transfecting with retroviruses carrying all four factors. In order confirm the cells were iPS cells the performed tests normally applied embryonic stem cells.

1. They first examined the iPS cells generated from the stomach and liver cells for the expression several pluripotency genes. They not only found that the cells originating from stomach and liver expressed pluripotency marker, but they were expressed at higher levels than in the fibroblast iPS cells.

2. They next examined if the liver and stomach iPS cells could form teratomas. When the cells were injected into mice they formed teratomas, or tumors which contain all three germ layers. This is strong evidence that the stomach and liver iPS cells are pluripotent.
3. They next attempted to make chimeras from the stomach and liver iPS cells. Chimeras are animals that have cells of two or more genetic origins. The cells were injected into mouse blastocycts and transplanted into a surrogate female mouse. The pregnancies resulted in live animals which were found to have cells that originated from both the original blastocycts and the stomach and liver iPS cells. This shows that the iPS cells are pluripotent and can develop normally into many body tissues.

IPS cells have previously been shown to be likely to develop into cancer cells in chimeras. This is largely thought to be caused by c-Myc. The researches next examined the development of cancer in chimeras generated from the stomach and liver iPS cells in comparison to those developed from fibroblasts. About 30% of 46 chimeras generated from fibroblasts developed tumors within 30 weeks. By contrast, none of the 65 animals generated from stomach or liver cells developed tumors in the same time period.

Scientists also theorize that the use of retroviruses to transfect the four factors might contribute to cancer formation. Retroviruses insert their genetic information into the host cell's DNA. The insertions might interrupt normal gene sequences, and cause mutations that could lead to cancer. They found that there were fewer regions where the virus had inserted its genetic information into the cell's DNA in both the liver and stomach iPS cells than in the fibroblasts iPS cells.

The efficiency of the iPS procedure is very low. Only about 0.02% of transfected cells become iPS cells. The authors had previously theorized that a possible explanation for this was that there may have been a small percentage of more immature or adult stem cells in the skin cell population from which the fibroblasts were isolated and that it was these cells that were turning into iPS cells. Adult stem cells are more similar to embryonic stem cells than somatic cells are. In theory, they should be easier to turn into iPS cells. In the last experiment from this paper they examine this possibility. Mature liver cells express a protein called albumin. They engineered a DNA construct makes a cell permanently expresses the marker gene beta-galactosidase if albumin is turned on. They found that most liver iPS cells expressed the beta-galactosidase marker. This means that most of the liver cells started out as mature cells and not an immature or adult stem cell type.

In this study the authors showed that they were able to create iPS cells from liver and stomach cells. Interestingly these cells seem superior in some ways to those made from fibroblasts; they had stronger expression of pluripotency markers, there were fewer viral integration sights, and chimeras created with liver and stomach cells were less likely to develop cancer. They also have shown that at least in the liver most iPS cells originate as mature cells as opposed to an immature cell type.