Treatment of Sickle Cell Anemia Mouse Model with iPS Cells Generated from Autologous Skin

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Abstract

It has recently been demonstrated that mouse and human fibroblasts can be reprogrammed into an embryonic stem cell-like state by introducing combinations of four transcription factors. However, the therapeutic potential of such induced pluripotent stem (iPS) cells remained undefined. By using a humanized sickle cell anemia mouse model, we show that mice can be rescued after transplantation with hematopoietic progenitors obtained in vitro from autologous iPS cells. This was achieved after correction of the human sickle hemoglobin allele by gene-specific targeting. Our results provide proof of principle for using transcription factor-induced reprogramming combined with gene and cell therapy for disease treatment in mice. The problems associated with using retroviruses and oncogenes for reprogramming need to be resolved before iPS cells can be considered for human therapy.

One of the greatest hopes for iPS technology is that it will be usable for patient specific treatments. If iPS cells are made from the patient's own tissue, there will be no immune rejection issues. In this paper Rudolf Jaenisch and colleagues treated mice carrying a mutation that causes sickle cell anemia with iPS cells generated from their own fibroblasts cells.

Sickle cell anemia is caused by a mutation in hemoglobin, the molecule that carries oxygen in red blood cells. The mutation causes the red blood cells to be sickle shaped and inflexible, clogging capillaries and restricting blood flow causing pain and even potentially damaging tissue. The mutation is partially dominant. **Heterozygous** mutations impart a resistance to malaria. **Homozygous** mutations cause sickle cell anemia. The red blood cells originate from **hematopoietic stem cells**, which also give rise to immune system cells. Hematopoietic cells reside in the bone marrow. Bone marrow transplants replace a patient's hematopoietic stem cells with the stem cells from a donor. This is a risky procedure as the recipient may develop a

condition called **Graft Versus Host Disease**. The new immune system cells from the transplanted stem cells may identify the recipient's cells as foreign and attack all over the body. This condition can be fatal.

The researchers began their experiments on normal mice. They transfected fibroblasts with retroviruses carrying the four Yamanaka factors. Similarly to the original Yamanaka technique, they used a mouse that had a gene for drug (neomycin) resistance in place of a pluripotency gene. When the cell tries to express the pluripotency gene it becomes resistant to neomycin. 20 days after transfection they selected 12 cells that were resistant to the drug. Ten out of the 12 eventually grew into cells lines that resembled embryonic stem cells and expressed several pluripotency markers. These lines were then transfected with a gene called HoxB4, which has been shown to cause hematopoietic differentiation, as well as the marker protein Green Fluorescent Protein (GFP). The cells expressed GFP and were determined to be hematopoietic precursor cells.

Next the hematopoietic precursor cells (differentiated from the iPS cells) were injected into the blood stream of mice that had their hematopoietic stem cells destroyed by irradiation. Irradiation without replacing the hematopoietic stem cells is lethal. The mice survived for up to 20 weeks following the procedure, indicating the transplant had been successful. Multiple cell types originating from hematopoietic stem cells were found to express GFP, meaning they originated from the iPS cells that had been transfected with HoxB4 and GFP. IPS cells were therefore able to be differentiated in hematopoetic stem cells and can effectively be used for transplantation.

They next tested whether this procedure could be used to treat mice that have a mutation that gives them a human-like form of sickle cell anemia. Fibroblasts were isolated from these mice and formed into iPS cells. Before these cells could be made into hematopoetic stem cells and transplanted they had to fix the genetic mutation causing the sickle cell anemia. The researchers used a technique called **homologous recombination**. They inserted DNA into the cell that carried a correct gene and a gene for drug (hygromyocin and gancyclovir) resistance. Surrounding these two genes are sequences of DNA that perfectly match DNA in the mouse cells. These matching (or homologous) regions of DNA from both the inserted DNA and the cellular DNA bind to each other. Through events that will not be explained in detail here, the inserted DNA and the cellular DNA can flip places. The inserted DNA with the functional hemoglobin gene and the drug resistance would now be in the place of the mutated gene, and the mutated gene had been removed. To identify cells where homologous recombination had taken place they selected cells that were resistant to the drug. However, since mice have two copies of the mutant gene and homologous recombination is rare, most drug resistant cells are likely heterozygous for the mutation.

These cells were then transplanted into the irradiated mutant mice that the skin cells were originally derived from. Not only did the mice survive the procedure, the researchers found that

the symptoms of sickle cell anemia were dramatically reduced. Testing did show that the animals were effectively heterozygous for the mutation.

The authors conclude that they have successfully generated iPS cells from mutant animals, differentiated the iPS cells into hematopoetic stem cells, and corrected the mutation to successfully transplant into mice to treat sickle cell anemia. They note however that there are several issues that need to be solved before such treatments could be used on humans. First, a replacement for cancer causing gene c-Myc needs to be used. Secondly, it is necessary that something other than a retrovirus is found to introduce the Yamanaka factors, as the retroviruses could cause harmful mutations. It is also necessary to develop reliable and effective procedure for differentiating the iPS cells into the desired cell types.