The many ways to make an iPS cell

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Several new approaches for generating induced pluripotent stem cells reduce the risk of insertional mutagenesis.

This paper briefly reviews some of the techniques that scientists are using to make iPS cell lines.

IPS was first invented in 2006 by Shinya Yamanaka when he and colleagues used retroviral vector to transfect somatic cells with four transcription factors, Oct3/4, Sox2, Klf4, and c-Myc, to create embryonic stem cell-like cells. IPS is a very promising technique that might one day be able to replace the use of embryonic stem cells in cell transplant therapy. However there are many issues with iPS that need to be resolved before this can happen.

One of the major barriers that needs to be overcome is the use of retroviral vectors. Retroviruses insert their genetic information into the DNA of the host cell. This can cause mutations that can affect the normal functioning of the cell or even cause cancer.

One group attempted to utilize **adenoviruses** at the vector. These viruses do not insert their DNA into the host DNA. This would be advantageous for medical usage, except that it makes iPS more difficult. As the DNA of the four Yamanaka factors are not permanently inserted into the host DNA their expression is only temporary. The researchers had to repeatedly transfect the cells over the course of several days so the genes were consistently expressed. Using this method they were able to transfect mouse liver cells, but with an efficiency of 0.0006%. They were unable to obtain iPS cells from fibroblasts. (Note: Another group that published the same month as this paper was able to create iPS cells with fibroblasts, except the efficiency was less than 0.0001 %.)[1] Yamanaka originally achieved an efficiency of 0.02% with retroviral transfection in fibroblasts.

Another group attempted to create iPS cells without using any virus at all. They used circular pieces of DNA called **plasmids**. They engineered two plasmids; one containing the DNA sequence for three of the four genes, Oct4, Sox2, Klf4 and the other containing just the c-Myc sequence. Both of these plasmids were then transfected together into mouse embryonic fibroblasts. Similarly to the adenovirus procedure, the plasmid transfection was repeated several times over the period of seven days to maintain the expression level of the four genes. The efficiency of iPS formation was 0.0015%, over twice as effective as the adenovirus technique but still much less efficient that retroviral transfections. Plasmids can also randomly insert

themselves into host DNA. However, with plasmids this is a rare event, whereas it is a natural part of the retrovirus life-cycle and cannot be avoided.

Another group has attempted to replace one or more of the four factors with small molecules. In this method chemicals turn on the pluripotent program genes or alter the way genes are expressed by interacting with DNA in various ways. The researchers found that they could successfully transform fibroblasts into iPS cells with only two of the four factors, Sox2 and Oct4 if they included valproic acid. They also showed that the addition of valproic acid made retroviral transfection with the four factors more efficient.

None of these methods solves the problem of finding a replacement for retroviral transfection. These methods are too inefficient. It is possible that a combination of methods will be more successful. For example the addition of valproic acid to an adenovirus transfection might be more efficient than the adenovirus vectors alone. More research is needed to improve transfection techniques.

1. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. (2008) Induced pluripotent stem cells generated without viral integration. Science. Nov 7;322(5903):945-9.