Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts

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Abstract

Direct reprogramming of somatic cells provides an opportunity to generate patient- or disease-specific pluripotent stem cells. Such induced pluripotent stem (iPS) cells were generated from mouse fibroblasts by retroviral transduction of four transcription factors: Oct3/4, Sox2, Klf4 and c-Myc. Mouse iPS cells are indistinguishable from embryonic stem (ES) cells in many respects and produce germline-competent chimeras. Reactivation of the c-Myc retrovirus, however, increases tumorigenicity in the chimeras and progeny mice, hindering clinical applications. Here we describe a modified protocol for the generation of iPS cells that does not require the Myc retrovirus. With this protocol, we obtained significantly fewer non-iPS background cells, and the iPS cells generated were consistently of high quality. Mice derived from Myc(-) iPS cells did not develop tumors during the study period. The protocol also enabled efficient isolation of iPS cells without drug selection. Furthermore, we generated human iPS cells from adult dermal fibroblasts without MYC.

This study was performed by the same group headed by Shinya Yamanaka that originally discovered the procedure to create iPS cells. Creating iPS cells is extremely inefficient and technically challenging. The methods they used were very similar to the original study. Instead of using Fbx15 controlling a color and a drug resistance gene, they used the gene Nanog. Nanog is more critical for stem cell function and a better indicator that cells activating these genes are actually pluripotent.

In this study the authors were originally attempting to determine if other genes similar to the original four Yamanaka factors; c-Myc, Oct3/4, Klf4, and Sox2, could also be used to create iPS cells from mouse fibroblasts. They accidently discovered that one of the factors, c-Myc does not need to be transfected to create iPS cells. It takes approximately 7 days for iPS cells to form with all four factors. However, without c-Myc the process takes longer, approximately 14-21 days. It is also about half as efficient as using all four factors. Approximately 0.01% of cells transfected with three factors became iPS cells, compared to about 0.02% of cells using all four factors. This is an important finding because c-Myc has been shown to cause cancer.

To test whether the absence of c-Myc affected cancer formation the researchers made chimeras, or animals that have multiple genetic backgrounds. To compare both methods they injected iPS cells made with all four factors and iPS cells made without c-Myc into blastocysts. The

obtained 37 chimeras for from the blastocycts injected with iPS cells made with all four factors. In other words, the iPS cells became part of the embryo they were injected in and formed portions of the body. Six of the 37 died from tumors within 100 days. 26 chimeras were made using the iPS without c-Myc. None of these developed tumors in the 100 day time frame.

They also tested whether it was possible to create human iPS cells without c-Myc. With viral transfection with only Sox2, Oct3/4 and Klf4 they obtained human iPS cells. These cells looked like human embryonic stem cells, differentiated in culture, and expressed pluripotency genes. However, although the researchers were able to create human iPS cells without c-Myc, they were not able to consistently. About half of their experiments failed. The authors comment that more efficient methods are needed.

One of the biggest challenges in making iPS cells a tool to be used in therapy is to make sure they are safe. Cells that are likely to cause cancer cannot ethically be used. Removing the cancer causing gene c-Myc is a big step to making these cells safe to use in disease therapies.