

Induction of Pluripotency by Defined Factors

Induced pluripotent stem (iPS) cells were originally generated from mouse and human fibroblasts by retroviral introduction of *Oct3/4*, *Sox2*, *c-Myc*, and *Klf4*. iPS cells are similar to embryonic stem (ES) cells in morphology, proliferation, gene expression, and most importantly, pluripotency. Compared to ES cells, iPS cells have less ethical controversy and can be generated from various genetically identified individuals including disease patients or those having specific human leukocyte antigen (HLA) types. Patient-specific iPS cells provide unprecedented opportunities in disease research, drug screening, and toxicology. A bank of iPS cells constructed from HLA-homozygous donors would provide significant resources for stem cell therapy. However, recent reports of tumor formation following transplantation, and the large diversity between iPS cell clones highlight potential problems. Furthermore, the mechanism of reprogramming remains unclear.

In addition to fibroblasts, iPS cells can be generated from various somatic cells, such as hepatic cells, gastric epithelial cells, neural cells, dental pulp cells, peripheral blood cells, and cord blood cells. As alternatives to retroviral transduction, iPS cells can be generated by lentiviruses, adenoviruses, plasmids, transposons, recombinant proteins, or synthesized mRNA. Recently, we reported an integration-free induction method using episomal vectors. This method can induce human iPS cells efficiently and reproducibly. Regarding iPS cell induction factors, we discovered that L-Myc and the transcription factor Glis1, which is strongly expressed in the unfertilized egg, can establish iPS cells with a high efficiency and quality, replacing the oncogene c-Myc. Other reports suggest that chemicals can further enhance induction efficiency.

Each induction experiment can result in up to 100 or more independent iPS cell clones. These iPS cell clones may vary qualitatively, considering responses to in vitro directed differentiation protocols and their propensity to produce tumors. In fact, we have previously shown that the origins of mouse iPS cells have profound effects on tumorigenicity. It is therefore essential to determine the best origins, the best induction protocols, and the best methods to evaluate iPS cell clones and subclones for future clinical applications. From this point of view, the need for genetic and epigenetic analyses, such as DNA methylation, histone modification, and genomic imprinting becomes more significant. It is also important to note that iPS cell within a clone can be heterogeneous, despite their common derivation from a single progenitor cell. This is likely because the process requires multiple cell division and cannot be completed by the four exogenous factors alone. Additional endogenous factors are required to achieve full reprogramming. Better understanding of the reprogramming mechanism will facilitate more uniform and complete reprogramming during iPS cell generation.