Induced Pluripotent Stem Cells (iPS): Reverse the Course of Life

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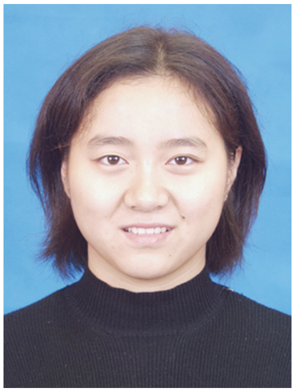

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Abstract

Mouse embryonic stem cells (ESCs) were derived nearly 25 years ago, from the inner cell mass of blastocyst stage embryos and were being defined as pluripotent. It's thought that ESCs have enormous potential for regenerative medicine. But there are still some key questions should be answered before the pluripotent cells can be used in clinics. Nuclear transfer solves the problem of immune rejection, but the technical and ethical problems still exclude ESCs from using on regenerative medicine. Recent studies have successfully generated induced pluripotent stem (iPS) cells and proved pluripotency. Now people try to improve induction methods, in order to obtain iPS cells more safely and with higher efficiency. They also use it as a model to study reprograming mechanisms during iPS cells induction. The generation of iPS cells from patients might provide us the model of human diseases, which will be valuable in searching novel drug target and cell replacement therapies.

Mouse embryonic stem cells (ESCs) were derived nearly 25 years ago, from the inner cell mass of blastocyst stage embryos [1] [2]. Mouse ESCs are capable of indefinite self-renewal in vitro, and are able to differentiate into derivative of the 3 germ layers, thus being defined as pluripotent [3]. Ten years ago, embryonic stem cells were also produced from human blastocyst [4]. ESCs have enormous potential for regenerative medicine. In the field of cell therapies, it might be used on replacing wounded organs or curing diseases, such as diabetes, neuron-degenerative disorders. In the field of tissue engineering, it might be used on tissue repair or regeneration. But there are still some key questions remaining before the pluripotent cells can be used in clinics [5]. First of all, there is one major challenge need to be solved before ESCs can be used in clinics -- the immune rejection of the ESCs by patients' own immune system. The induced pluripotent stem cells from adult somatic cells is considered to be an effectual measure to avoid this problem. Currently, there are several available methods to generate pluripotent stem cells from adult somatic cells. Nuclear transfer can reprogram somatic cells and create cloned animals or produce embryonic stem cells [6] [7]. But the technical and ethical problems of nuclear transfer stem cells preclude it from the use on regenerative medicine.

Derivation of Induced Pluripotent Stem (iPS) Cell Lines

Yamanaka first sifted four key factors from 24 genes which can induce pluripotency in somatic cells. He introduced 4 genes into mouse embryonic fibroblasts by retroviral transduction. They improved that molecular reprogramming of mouse somatic cells into iPS cells only need 4 factors: Oct4, Sox2, Klf4, and c-Myc[8]. They deduced that c-Myc induces the activation of chromatin structure of somatic cells properties, also induces apoptosis and senescence, which are suppressed by KLF4. Oct-3/4 turn the developmental direction of the cells from tumor cells to ES-like cells, and Sox2 establishes the pluripotent stage[9].

The year after Yamanaka's study, Jaenisch repeated the work and he also studied DNA methylation, gene expression and chromatin state of iPS cells. They found that iPS cells can form viable chimaeras, contribute to germ line, they even got late-term embryos by tetraploid complementation, but all iPS embryos could not survive after d14.5 [10] [11].

But, does iPS have true pluripotency? cell lines tetraploid complementation is a gold standard to determine the pluripotency of ES cells. Two years later, in 2009, Qi Zhou reported that their iPS cells are capable of generating viable, fertile live-born progeny by tetraploid complementation. These iPS cells maintain a pluripotent potential similar to those of ES cells [12] [13]. Nowadays, most of scientists believe that biological functions of induced pluripotent stem cells are indistinguishable from those of ES cells.

In November 2007, Yamanaka and Thomson simultaneously used iPS technique on human somatic cells and derived human iPS cells [14] [15]. Now, the technique has been used to generate induce pluripotent cells from other species, including primates [16], pig [17] [18] [19] and rat [20].

Improvement of Induction Methods

Even proved the pluripotency of iPS cells, there are still many other factors preclude it from the application on clinic. First of all, the exogenous gene integration is a major obstacle, which might increase the risk of tumorigenicity. In 2008, Hochedlinger first generated mouse iPS cells from fibroblasts and liver cells by using nonintegrating adenoviruses[21]. One month later, Yamanaka also reported the generation of mouse iPS cells without viral vectors [22]. These results provided strong evidence that transient expression of key factors can induce reprogramming successfully. Yamanaka pointed out that virus-free iPS cells address a critical safety concern for potential use of iPS cells in regenerative medicine. But compared with the retrovirus iPS (0.1%), virus-free iPS cells is in extremely low level of efficiency (0.005‰)[21]. In 2009, Thomson derived human iPS cells with nonintegrating episomal vectors. After removal of the episome, they could obtain iPS cells completely free of exogenous genes. These proliferative and developmental potential iPS cells are similar with human ES cells [23].

Since the iPS technique was reported by Yamanaka, we can found that reprogramming by viral infection is a slow and inefficient process. In 2008, Melton reported that valproic acid (VPA), an HDAC inhibitor, can greatly improve reprogramming efficiency by more than 100-fold, to 9.6%-14% [24]. Deng first reported other two factors can improve the reprogramming efficiency for human iPS cells. They found that p53 siRNA and UTF1 significantly elevate the efficiency of iPSC generation up to 100-fold [25]. Hochedlinger reported a new iPS technique system in 2008 which was defined as "doxycycline (DOX)-inducible transgenes" by infecting with DOX-inducible lentiviruses. The infected fibroblasts were cultured in the medium with DOX. This "secondary" system can also enhance the efficiency of reprogramming [26]. Recently, Blelloch reported that microRNAs (miRNAs) enhance the production of mouse iPS cells [27].

In addition, many scientists indicated that genetic transformation with exogenous genes, especially oncogenes c-Myc might increases tumorigenicity in the chimeras and progeny mice, and handicap iPS to human therapeutic applications. In 2008, Yamanaka generated mouse and human iPS cell lines without c-Myc, which proved that the omission of the c-Myc retrovirus significantly reduced the risk of tumorigenicity in chimeras [28].

Reprogramming Mechanisms

Scientists also use iPS cells as an excellent model to study reprogramming mechanism. Austin Smith reported that brain-derived neural stem (NS) cells can acquire undifferentiated morphology rapidly but critical attribute of true pluripotency. They combined dual inhibition (2i) of mitogen-activated protein kinase signalling and glycogen synthase kinase-3 (GSK3) with self-renewal cytokine leukaemia inhibitory factor (LIF), which induced stable up-regulation of Oct4 and Nanog, and generated iPS cell line with competence of somatic and gremlin chimaeras. Using 2i /LIF, they found that Sox2 and c-Myc are unnecessary for the reprogramming, and Oct4 and Klf4 are sufficient to convert NS cells into chimaera-forming iPS cells[29]. In the same month, Melton reported that, with valproic acid (VPA), they can reprogram human fibroblasts with only two factors, Oct4 and Sox2, without the need for the oncogenes c-Myc or Klf4[30]. The two factor–induced human iPS cells were similar to human ES cells in global gene expression and epigenetic states.

Application of iPS Cells on Cell Therapies and Disease Model

The generation of pluripotent stem cells from patient might contribute to study that patient's disease as a disease modeling, to discover novel drug candidates and cell replacement therapies. Dimos generated human iPS cells from a woman diagnosed with a familial form of amyotrophic lateral sclerosis (ALS). They successfully differentiated iPS cells into motor neurons, which are destroyed in ALS[32]. Thomson generated iPS cells from skin fibroblast derived from a patient with spinal muscular atrophy (SMA) and differentiated these iPS cells into motor neurons. They also studied how iPS-SMA cells respond to drug treatment and hoped it could be useful for novel drug selection in future studies[33]. Another group generated iPS cells from Fanconi anaemia patients by correcting these Fanconi-anaemia-specific iPS cells and differentiating into phenotypically normal haematopoietic progenitors [34]. Jaenisch healed the mouse with humanized sickle cell anemia disease by repairing gene defects of iPS cell line derived from itself, and in the same year, he improved symptoms of rats with Parkinson's disease with the same technique[36]. These studies indicate that iPS cell technology can be used on curing disease by gene modification, and promote the application on cell therapy.

For the purpose of regenerative medicine, some other scientists focus on inducing adult cells into other cell types for tissue repairing and regeneration. Melton transfected three factors: Ngn3, Pdx1 and Mafa, into pancreatic exocrine cells, and induced them into cells that closely resemble beta-cells. The induced beta-cells are similar with endogenous islet beta-cells[37]. This study presented that, for the cell therapy purpose, we can induce one kind of cell type into the other with specific gene expressions, without reprogramming it to a pluripotent stem cell state.

Since Yamanaka first reported iPS technique, research achievements of iPS change with each passing day. All of the results provide evidence that, in the future, people may apply induced reprogramming to regenerative medicine. However, iPS cells safety and efficiency must be improved first.

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