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Advances in Reprogramming Somatic Cells to Induced Pluripotent Stem Cells

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Abstract

Traditionally, nuclear reprogramming of cells has been performed by transferring somatic cell nuclei into oocytes, by combining somatic and pluripotent cells together through cell fusion and through genetic integration of factors through somatic cell chromatin. All of these techniques changes gene expression which further leads to a change in cell fate. Here we discuss recent advances in generating induced pluripotent stem cells, different reprogramming methods and clinical applications of iPS cells.

Viral vectors have been used to transfer transcription factors (Oct4, Sox2, c-myc, Klf4, and nanog) to induce reprogramming of mouse fibroblasts, neural stem cells, neural progenitor cells, keratinocytes, B lymphocytes and meningeal membrane cells towards pluripotency. Human fibroblasts, neural cells, blood and keratinocytes have also been reprogrammed towards pluripotency. In this review we have discussed the use of viral vectors for reprogramming both animal and human stem cells. Currently, many studies are also involved in finding alternatives to using viral vectors carrying transcription factors for reprogramming cells. These include using plasmid transfection, piggyback transposon system and piggyback transposon system combined with a non viral vector system. Applications of these techniques have been discussed in detail including its advantages and disadvantages. Finally, current clinical applications of induced pluripotent stem cells and its limitations have also been reviewed. Thus, this review is a summary of current research advances in reprogramming cells into induced pluripotent stem cells.

Keywords

induced pluripotent stem cells; embryonic stem cells; Adult stem cell; viral vectors; transcription factor; regenerative medicine; reprogram

Introduction

In this review we will discuss recent advances in generating induced pluripotent stem (iPS) cells, different reprogramming methods and clinical applications of iPS cells. Stem cells are the special primal structures in the body that retain two distinctive properties: the ability to self-renew through mitotic cell division and thus remain in its undifferentiated state (Pera, Reubinoff, & Trounson, 2000) and the ability to differentiate into a specific cell type. Stem cells can be categorized as totipotent cells, pluripotent cells, and multipotent cells depending

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upon their differentiation potential (Odorico, Kaufman, & Thomson, 2001). Totipotent cells have total potential, which is able to differentiate into not only any cell in the organism, but also into the extraembryonic tissue associated with that organism. They can specialize into pluripotent cells that can give rise to most, but not all, of the tissues necessary for fetal development. Pluripotent cells undergo further specialization into multipotent cells that are committed to give rise to cells that have a particular function. Embryonic stem (ES) cells are pluripotent, that is, they are able to differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm, which are distinguished by their pluripotency and their capability to self-renew themselves indefinitely. Pluripotency distinguishes embryonic stem cells from adult stem cells found in adults. Embryonic stem cells can generate more than 220 cell types in the adult body, while adult stem cells are multipotent and can only produce a limited number of cell types.

Induced pluripotent stem (iPS) cells are generated by reprogramming a differentiated somatic cell into a pluripotent ES cell (Hochedlinger & Plath, 2009; Shevchenko, Medvedev, Mazurok, & Zakiian, 2009). These iPS cells are identical to human ES cells and have the ability to become every cell type in the human body. Creation of iPS cells has paved a way to reprogram a cell in its somatic state back to its embryonic state. These cells express genes and surface proteins similar to ES cells and are capable of forming teratomas which can develop into all three germ layers. These promising results, suggest that iPS cell may be used to replace certain disease damaged tissues or to study diseased cells towards developing a treatment (Amabile & Meissner, 2009). Stem cell based therapies are attractive as a clinical treatment option for a variety of diseases and disorders. Currently, there are ethical and technical limitations for adult and embryonic stem cells (Flake & Zanjani, 1997; Strong, Farrugia, & Rebutta, 2009). Some studies have also shown that due to a lack of immunological compatibility between host and donor, stem cell applications have been limited. iPS cells could generate a limitless source for tissue engineering and regenerative medicine applications. Somatic cells isolated from a patient may be reprogrammed to ES cells and then theoretically could be used to replace diseased cells in the same patient. As the reprogrammed cells have been isolated from the patient, genetic characteristics of the iPS cells would theoretically be similar to the patient cells. iPS cells can also be generated to study different disease models. Diseased mouse models could be generated from mice iPS cells and used to study and test different treatment options. Creation of specific mouse models for specific diseases could be used to study specific diseases.

A number of techniques have been developed over the years to induce pluripotency in somatic cells (Liu, 2008; Maherali & Hochedlinger, 2008; Okita, Nakagawa, Hyenjong, Ichisaka, & Yamanaka, 2008; Tweedell, 2008). These include somatic cell transfer, cell fusion, reprogramming through cell extracts and direct reprogramming. The Direct reprogramming is currently being widely investigated as it is possible to reprogram a mature nucleus by introducing a known set of genes (Gurdon & Melton, 2008). Initially retroviruses were developed to introduce these genes into the nucleus. However, more recent attention has shifted to other similar techniques to perform direct reprogramming. These techniques include the use of lentivirus, adenovirus, plasmid transfection and the piggyback transposition system (Maherali & Hochedlinger, 2008). Studies have also explored the use of non viral vector transfection of a multi-protein expression vector to reprogram both mouse and human fibroblasts (Kaji, et al., 2009a).

Several criteria need to be met to confirm the developmental potential of an iPS cell. The first is successful differentiation of reprogrammed cells. They should be able to differentiate *in vitro* and express cell specific surface makers. The second is the formation of teratomas. They should induce tumor formation which will further differentiate into all three embryonic germ layers. The next test is the formation of chimeras after injection of iPS cells into

diploid blastocytes. iPS cells should display a high percentage of tissue contribution in the host mouse towards normal development. These cells should then be able to support development of tetraploid embryos. There are limitations at each stage of generating iPS cells and maintaining them from culture to embryo. This review is an overview of recent techniques to generate iPS cells, different reprogramming methods and clinical applications of iPS cells (Table 1).

Techniques to Generate Pluripotent Stem Cells

Nuclear reprogramming denotes the morphological and molecular changes that a nucleus undergoes after transplantation into an oocytes and related changes in chromatin and gene expression. It occurs as a result of resetting the somatic cell specific epigenotype to the totipotential cell specific epigenotype by exposure to factors present in the oocytes cytoplasm. Traditionally somatic cells could be reprogrammed into pluripotent cells either by (a) somatic cell nuclear transfer into oocytes, (b) by combining factors expressed in pluripotent cells through cell fusion and (c) by genetic integration of various factors into somatic cell chromatin and (d) direct reprogramming (Do & Scholer, 2006; Fulka, First, Loi, & Moor, 1998; Hochedlinger & Jaenisch, 2006; Renard, 1998; Wade & Kikyo, 2002; Wilmut, Young, & Campbell, 1998). Germ line cells have also been reprogrammed by use of specific cell culture conditions (Barroca, et al., 2009; Surani, 2005) (Figure 1).

(a) Somatic cell nuclear transfer

Generation of reprogrammed cells through nuclear transfer has been well established and documented in mouse models (Agarwal, 2006; Beyhan, Iager, & Cibelli, 2007; Campbell, et al., 2007). Basic technique of nuclear transfer involves a somatic donor cell and an unfertilized, enucleated oocyte. The nuclear DNA from the somatic cells is transplanted into the enucleated oocyte leading to union of both components. This reconstructed cell is stimulated to initiate development of an embryo. Stimulation is achieved by either a transient increase in the intracellular-free calcium concentration induced by electrical pulse or alternatively by chemical agents. The pre-implanted embryos are then maintained in a sequential culture media to support development. Finally the developed embryo is transferred to a foster mother (Fulka, Loi, Fulka, Ptak, & Nagai, 2004). Various somatic cells, including mammary epithelial cells, cumulus cells, oviductal cells, leukocytes, hepatocytes, epithelial cells, neuronal cells, myocytes, lymphocytes and germ cells have been successfully used as donor cells for production of cloned animals (Brem & Kuhholzer, 2002; Hochedlinger & Jaenisch, 2002; Jaenisch, et al., 2004). Developed embryos contain genetic information of both the somatic cell and the new embryo. Due to differentiation, the nuclei of donor somatic cells exhibit a different pattern of markers compared to the nuclei of normal embryonic cells that have not differentiated into a specific cell type. The aim of somatic cell nuclear transfer is to generate stem and progenitor cells that are not committed to a specific lineage. Studies have shown that fully differentiated cells can be de-differentiated through nuclear transfer, thus generating reprogrammed cells. However, these cells had faulty reprogramming which lead to the death of cloned neonates after implantation or the cloned neonates were born with abnormalities (Hochedlinger & Jaenisch, 2003; Yang, et al., 2007). Examination of these failed cells revealed several nuclear defects resulting from incomplete remodeling of the donor cell nuclei and/ or from mis-regulation of gene expression of differentiated cell-specific genes. Chimeras generated from reprogrammed cancer cells exhibited a high incidence of tumor formation, suggesting that the tumorigenic potential of the donor cancer cells was not fully erased by nuclear reprogramming technique (Hochedlinger, et al., 2004). Similar cloning efficiency was noted when the nuclear transfer technique was applied to a variety of somatic cells (Tsunoda & Kato, 2000). The success rate of nuclear transfer technique to generate live births is only

1-2% (Heyman, et al., 1998; Hiiragi & Solter, 2005; Rhind, et al., 2003; Wakayama, et al., 2000)

This technique has not been demonstrated in humans as it's a technical challenge to transfer this procedure from a mouse to human model due to the inefficient number of available unfertilized oocytes. Apart from lower availability, this procedure is also dependent upon voluntary donation of these oocytes and the efficiency of this technique is also low. Out of total 304 primate oocytes, only two isogenic pluripotent stem cell lines were successfully created in a recent study (Byrne, et al., 2007). A similar low efficiency was noted in other mammalian species (Solter, 2000). More recently, somatic nuclei were transplanted into enucleated zygotes after which drug induced synchronization lead to the generation of cloned embryonic stem cells and mice. In this technique mouse zygotes were temporarily arrested in mitosis using the drug nocodazole. These enucleated zygotes were used to receive embryonic donor cell genomes. The resulting embryos developed into mice, thus supporting somatic cell reprogramming. A similar process was also performed on cultured donor cells to produce embryonic stem cell lines and the full term development of the cloned mice (Egli, Rosains, Birkhoff, & Eggan, 2007). Application of this process towards human model may be possible, however currently the application of this procedure is limited in humans.

(b) Cell Fusion

Another method involves generating reprogrammed somatic cells through cell-fusion between somatic and pluripotent embryonic stem cells. Self renewing and pluripotent embryonic stem cells derived from the inner cell mass cells of blastocytes have an intrinsic capacity for nuclear reprogramming of somatic cells after cell hybridization. The cytoplasm of pluripotent embryonic cells contains reprogramming factors which can modify the epigenetic state of the somatic nucleus back into a pluripotent state after being fused with a somatic cell (Andrews & Goodfellow, 1980; Flasz, et al., 2003; Gmur, Solter, & Knowles, 1980; Miller & Ruddle, 1976; Tada, Tada, Lefebvre, Barton, & Surani, 1997). When adult neural stem cells were introduced into the amniotic cavity of early chick embryos and the blastocoel cavity of mouse blastocytes, they retained the potential to transdifferentiate into three germ layers, including various types of somatic tissues (Rideout, Eggan, & Jaenisch, 2001). Hybrid cells generated from cell fusion have exhibited properties similar to embryonic stem cells. These reprogrammed cells also expressed reactivated pluripotent markers including Oct4, Sox2 and nanog (Cowan, Atienza, Melton, & Eggan, 2005; Do & Scholer, 2004). When these reprogrammed cells were implanted *in vivo*, they also indicated formation of three germ layers after teratoma formation. Cell fusion process has been demonstrated in both mouse (Tada, Takahama, Abe, Nakatsuji, & Tada, 2001) and human cells (Surani, 2005; Yu, Vodyanik, He, Slukvin, & Thomson, 2006). As this process involves the fusion of both somatic and embryonic stem cells, the reprogrammed cell consists of chromosomal components from both cells. Neural stem cells or bone marrow derived cells were co-cultured with embryonic stem cells suggesting that the acquisition of pluripotency by the adult neural stem cells may be mediated by spontaneous cell fusion with the inner cell mass cells following microinjection into the blastocyte. These cells were found to fuse and retain both adult markers and pluripotent potential (Kosaka, et al., 2006; Ying, Nichols, Evans, & Smith, 2002).

Currently, no technique has been devised to remove the embryonic stem cell chromosomal components which limit real time application of this technique. Moreover, due to the fusion processes inefficiency it has been difficult to even study molecules involved in reprogramming.

(c) Reprogramming through Cell Extracts

Another cell reprogramming technique is known as culture induced reprogramming. This technique is helpful to understand whether and to what extent soluble factors of the embryonic stem cell affects the nuclear reprogramming of the somatic cell. In this process cell extracts are obtained from pluripotent stem cell including embryonic stem cells, embryonic germ cells or embryonic stem cell -like cells and this crude extract is inserted into somatic cells. Specifically, chemicals are used to permeabilize somatic cell membranes which are incubated with cell extracts isolated from embryonic stem cells. These cells are then able to differentiate towards multiple cell lineages. As the isolated extract consists of pluripotent reprogramming factors, it is used to reprogram somatic cells back to its pluripotent state. One of the factors Brg1 present in the extract has been shown to play a role in nuclear reprogramming. Other cell extracts including Oct4, Utf1, Oxt2, Rex1 and Nanog are also being identified which in the future may eliminate the need for oocytes to perform nuclear reprogramming. A study by Taranger *et al.*, showed that 293T and NIH3T3 cells could be programmed by extracts of undifferentiated NCCIT cells or mouse cells. They also showed that these cells could be induced to differentiate towards neurogenic, adipogenic, osteogenic and endothelial lineage (Taranger, et al., 2005). Reprogramming through cell extracts has been applied for human cells; however they were only partially reprogrammed to pluripotency and did not demonstrate *in vivo* differentiation. Thus transdifferentiation of somatic cells has not been possible and successfully applied to *in vivo* models without the occurrence of failure.

(d) Direct Reprogramming

More recently, somatic cells have been reprogrammed by addition of selective transcription factors. These factors include Oct4, Sox2, c-myc, Klf4, Fbx15 and nanog. These factors alone or in combination were directly introduced into somatic cells through viral vectors or non viral vector system and performed direct reprogramming of somatic cells into a pluripotent cell. Successful direct reprogramming depends upon reprogramming of an adult somatic nucleus to an embryonic state. To achieve complete reprogramming, DNA methylation, histone modification and chromatin structure need to reprogram to a state which mimics embryonic development. The mechanisms of direct reprogramming are a complex process and it is unclear what role each transcription factor plays towards driving a somatic cell to pluripotency. Though, recent studies have shed light on the role played by transcription factors to promote pluripotency.

It has been shown that Oct4, Sox2 and Klf4 work together in combination to control a set of gene expression and repression programs to maintain a pluripotent cell (Loh, et al., 2006, Kim, et al., 2008). Expression of these factors including c-myc may lead to a sequence of epigenetic events which influence chromatin modifications and changes in DNA methylation which lead to a pluripotent cell. Once a somatic cell is introduced with these factors its phenotype transforms to a partially reprogrammed state (Wernig, et al., 2007, Okita, et al., 2007). Studies have shown that c-myc proteins may loosen chromatin structure of somatic cells, thus rendering them a property similar to pluripotent cells (Takahashi & Yamanaka, et al., 2006). This structure allows for Oct4 and Sox2 to bind to their target genes and the addition of Klf4 assists them to initiate a key set of ES cell genes in somatic cells (Wernig, et al., 2007). Oct4 and Sox2 then establish an autoregulatory loop which maintains this pluripotent state in somatic cells (Masui, et al., 2007).

Direct reprogramming is being widely studied because it presents a possibility of creating a reprogrammed mature nucleus just by introducing a set of known genes. However, majority of studies have used retroviruses to induce transduction which may result in random

insertion of genes within a genome. More recently, other techniques are being explored to perform direct reprogramming.

1. Retrovirus mediated ectopic expression of transcription factors

Numerous studies have used a cocktail of the transcription factors delivered through retroviruses to induce fibroblasts to be reprogrammed into a pluripotent state. One of the most commonly-used transcription factor is Oct4. It has been well established that Oct4 plays an essential role as a major regulator of pluripotency (Boiani, Eckardt, Scholer, & McLaughlin, 2002; Cheng, et al., 2007). The initial contributors to this field were Yamanaka and Takahashi. They successfully reprogrammed mouse embryonic and adult fibroblasts to pluripotent embryonic stem cell like state after introducing Oct4, Sox2, c-myc and Klf4 through retrovirus mediated transduction (Takahashi & Yamanaka, 2006). Pluripotency was confirmed through the expression of transduced Oct4 and Sox2 genes. The endogenous Oct4 and nanog genes were expressed at low levels and their promoters were methylated. Pluripotency was also confirmed by the ability of these cells to form teratomas and Fbx15 expression which is a specific expression marker for embryonic stem cells. These cells were known as 'induced pluripotent stem (iPS) cells'. Even though these iPS cells were similar to embryonic stem (ES) cells, they did not generate chimeras as they succumbed at mid gestation. Thus only part of the embryonic stem cell transcriptome was expressed in iPS cells. Following this study, many studies have started to generate pluripotent cells from reprogrammed cells using direct reprogramming.

1.1 Reprogramming Animal Somatic Cells—Methylation analysis of chromatin state of Oct4 and nanog promoter revealed an epigenetic pattern that was intermediate between both fibroblast and ES cells (Wernig, et al., 2007). Rudolf Jaenisch group first developed mouse embryonic and tail tip fibroblasts using homologous recombination in ES cells. These cells carried a neomycin resistance marker which was inserted into either the endogenous Oct4 or nanog locus. Thus the endogenous genes were silenced to prevent them from further differentiation. The cells were selected using G418. These Oct4 or nanog genes- silenced fibroblastic cells were then transfected with retroviral vectors expressing transcription factors Oct4, Sox2, c-myc and klf4. After three, six or nine days later G418 was added to the cultures and at day twenty the colonies were analyzed. They found that the induced pluripotent cells colonies were established, however, the reprogramming efficiency was just 0.05-0.1% and these nanog and Oct4 iPS cells were similar to ES cells, but the expression levels of these two genes decreased when differentiated to embryoid bodies. Oct4 expression levels are an important factor in determining the differentiation of somatic cells. If it is overexpressed then ES cells will differentiate into primitive endoderm and mesoderm. If the expression is reduced, then it induces formation of trophoderm. Thus a balance is required for the expression of Oct4 and the total amount required must be consistent during the intermediate stages of reprogramming (Hotta & Ellis, 2008; Niwa, Miyazaki, & Smith, 2000). Nanog is involved in regulating the expression of Oct4 as it is a strong activator of the Oct4 promoter (Pan, Li, Zhou, Zheng, & Pei, 2006). Some results also suggested that the induction of pluripotent state was due to virally transduced Oct4 and nanog, but maintenance of this state is due to endogenous expression of Oct4, nanog and Sox2. Sox2 expression levels were similar between iPS and wildtype ES cells. Sox2 has been shown to play an important role in epiblast and extraembryonic ectoderm formation (Avilion, et al., 2003). Sox2 also maintains pluripotent cells by regulating the levels of Oct4 expression (Masui, et al., 2007).

Mouse fibroblasts have also been reprogrammed to generate germline competent iPS cells (Okita, Ichisaka, & Yamanaka, 2007). Nanog has been associated with pluripotency and has shown to increase reprogramming efficiency after fusion with somatic cells. To evaluate

whether selection for nanog expression results in obtaining iPS cells with similarity to ES cells, Shinya Yamanaka group inserted a green fluorescent protein(GFP) –internal ribosome entry site (IRES) – Puromycin resistance gene (Puro^r) cassette into the 5' untranslated region of Nanog gene. ES cells that had stably incorporated with a nanog-GFP-IRES-pluro receptor construct were positive for GFP, but became negative when differentiation was induced. The modified ES cells with nanog were used to create transgenic mice from which mouse embryonic fibroblastic cells (MEFs) were isolated. These MEFs cells which were cultured on SNL feeder cells were introduced with Oct4, Sox2, Klf4 and c-myc and selected for GFP colonies. The colonies were comparable in morphology to ES cells. Nanog selected iPS cells were comparable to ES cells in proliferation, teratoma formation, gene expression and competency for adult chimeras. The efficiency to generate adult chimeras was low and 20% of them developed tumors due to reactivation of c-myc transgene. Development of tumors may be due to epigenetic aberration which has lead to developmental failure and abnormalities noted in nuclear transfer cloned animals (Gaudet, et al., 2003). The epigenetic status of iPS cells generated from fibroblasts has been assessed at a gene-specific, chromosome and genome wide level (Maherali, et al., 2007). The iPS cells were similar to ES cells in their epigenome, thus induced reprogramming leads to global reversion of the somatic epigenome into an ES like state. Studies have shown that reprogramming is possible without the use of c-myc but the efficiency has been low (Nakagawa, et al., 2008). Removal of other exogenous factors is also desirable as ectopic expression of Oct4 or Klf4 can induce dysplasia (Foster, et al., 2005; Hochedlinger, Yamada, Beard, & Jaenisch, 2005)

Mouse neural stem cells have been reprogrammed using both endogenous and inducible genes. In previous studies, a set of four genes were introduced through a viral vector to induce nuclear reprogramming of mouse cells. However a combination of both endogenous and exogenous genes has also been applied to induce pluripotency. Neural stem cells were reprogrammed in the presence of transcription factors Oct4, Klf4 and c-myc or MYCER and also endogenous Sox2. The results indicated reprogrammed cell differentiation into cells of each germ layer both *in vitro* and *in vivo*. Thus a combinational approach of both endogenous and exogenous genes may be applied to induce pluripotency (Duinsbergen, Eriksson, t Hoen, Frisen, & Mikkers, 2008). Neural progenitor cells have also been shown to express high levels of Sox2 and as a result maintain its neural progenitor state without differentiation (Ellis, et al., 2004). Based on these findings neural progenitor cells were only infected with Oct4, Klf4 and c-myc to induce pluripotency. Results indicated that iPS colonies did grow into stable cell lines, and formed teratomas and viable chimeras. Neural progenitor cells can be reprogrammed into iPS cells in absence of exogenous Sox2 expression. Cell types that already express one of the four reprogramming factors at appropriate levels does not require its ectopic expression. A similar study was also performed using tail-tip fibroblasts. The efficiency to generate iPS cells was lower in tail-tip fibroblasts compared to neural progenitor cells. However when neural progenitor cells were infected with all four transcription factors, its efficiency to generate iPS cells was lower than fibroblasts. Neural progenitor cells failed to reprogram in the absence of both Sox2 and c-myc. It is however possible to generate iPS cells from mouse and human fibroblast in the absence of c-myc infection (Nakagawa, et al., 2008; Wernig, Meissner, Cassady, & Jaenisch, 2008). It may be that different cell types require different levels of expression for efficient reprogramming. Thus, appropriate reprogramming factors should be selected to induce pluripotency based on cell type and cellular content.

Transcription factors Oct4, Sox2, c-myc and klf4 have been routinely used to reprogram fibroblasts. These factors are sufficient to convert fibroblasts to iPS cells which have similar characteristics to ES cells. The orphan nuclear receptor Esrrb has been used in conjunction with Oct4 and Sox2 to induce reprogramming of mouse embryonic fibroblasts to iPS cells. These cells had similar expression and epigenetic characteristics when compared to ES cells.

The Esrrb reprogrammed cells established pluripotency *in vitro* by differentiating and expression of Zfp201, Nkx2.2, Sox2, Pax5, Lbx1, Fbx1, and Dlx1. The cells also differentiated *in vivo* into three major embryonic cell lineages including mesoderm, ectoderm and endoderm through expression of lineage specific markers. Thus these results suggested that Esrrb may mediate reprogramming through upregulation of ES cell specific genes (Feng, et al., 2009).

Adult mice neural stem cells have also been successfully reprogrammed into pluripotent stem cells using only two transcription factors (Kim, et al., 2008). Fifteen different combinations of Oct4, Sox2, c-myc and Klf4 were used to reprogram neural stem cells into iPS cells using the retroviral MX vector system. Out of fifteen, six combinations were successful in inducing iPS cells from neural stem cells which was evident from GFP+ colonies and were further established into cell lines. Three factor combinations including Oct4, Klf4, c-myc; Oct4, Klf4, Sox2 and Oct4, c-myc, Sox2 were capable of generating iPS cells. Combinations without Oct4 did not induce reprogramming. Thus expression of Oct4 is capable of inducing an ES cell like expression profile (Loh, et al., 2006; Takahashi & Yamanaka, 2006). Two factor combinations also induced generations of iPS cells from neural stem cells. These include Oct4 and Klf4 and Oct4 and c-myc. Both these factors contributed to three germ layers in teratomas. Thus exogenous Oct4 either with Klf4 or c-myc is sufficient to generate iPS cells from neural stem cells. They also reported that neural stem cells express higher levels of Sox2 and c-myc than ES cells. Thus the number of reprogramming factors can be reduced when using somatic cells that endogenously express required levels of complementing factors. Another similar study reported that exogenous expression of the germline specific transcription factor Oct4 is sufficient to generate pluripotent stem cells from adult mouse neural stem cells. These induced cells were similar to ES cells both *in vitro* and *in vivo* (Kim, et al., 2009). The iPS cells could be differentiated into lineage committed populations including germ cells and germline transmission. One interesting fact of this study was that iPS cells could be generated without c-myc and Klf4, which is promising as reactivation of c-myc has caused tumor development (Okita, et al., 2007). The iPS cells induced using Oct4 gave rise to multipotential neural stem cells, cardiomyocytes with cardiac potential and chronotropic regulation and germ cells *in vitro* as well as germline transmission *in vivo*. Oct4 expression has been studied in detail both in embryo and cell lines, and it has been found to be associated with undifferentiated phenotypes. It activates transcription of those factors associated with pluripotency and represses those linked to differentiation down a specific lineage pathway (Ovitt & Scholer, 1998). Thus Oct4 alone is sufficient to directly reprogram neural stem cells to pluripotency which reduces the chance of retroviral insertional mutagenesis.

1.2 Reprogramming Human Somatic Cells—Induced pluripotent human stem cells may be effectively applied to generate patient or disease specific embryonic stem cells. These iPS cells can be used to understand disease mechanisms, to screen effective and safe drugs and ultimately treat various diseases and injuries. As human embryonic stem cells are difficult to obtain due to ethical reasons, reprogrammed somatic cells may be an effective alternative. Patient specific pluripotent stem cell lines could be generated without somatic cell nucleus. Adult human fibroblasts have been used to generate iPS cells through retroviral transduction of Oct4, Sox2, Klf4 and c-myc. The human iPS cells were similar in morphology to human ES cells. They also possessed similar proliferation, surface analysis, gene expression, epigenetic states of pluripotent cell specific genes and telomerase activity. These cells could also differentiate into cell types of endoderm, mesoderm and ectoderm both *in vitro* and in teratomas (Takahashi, et al., 2007). c-myc was used as one of the reprogramming transcription factors. However, this factor has been associated with cell death and differentiation of human ES cells (Sumi, Tsuneyoshi, Nakatsuji, & Suemori, 2007). Instead of using all four transcription factors, primary human fibroblasts have also

been reprogrammed into iPS cells with only Oct4 and Sox2. It has been shown that histone deacetylase (HDAC) can greatly improve the efficiency of reprogramming of mouse embryonic fibroblasts by genetic factors (Huangfu, Maehr, et al., 2008). Thus a small HDAC inhibitor molecule; valproic acid (VPA) was used to increase the efficiency of human primary fibroblasts in the presence of two transcription factors Oct4 and Sox2. The human iPS cells were similar to human ES cells in pluripotency, global gene expression profiles and epigenetic states. Thus these results demonstrate the possibility of reprogramming somatic cells through chemical manipulation. This could provide a safer means for therapeutic use of reprogrammed cells (Huangfu, Osafune, et al., 2008).

Human keratinocytes have also been reprogrammed to generate iPS cells. The keratinocytes were reprogrammed by retroviral transduction with Oct4, Sox2, Klf4 and c-myc (Aasen, et al., 2008) and the reprogramming efficiency of keratinocytes was compared to fibroblasts. The results showed that reprogramming of keratinocytes was a hundred fold more efficient and two fold faster compared to human fibroblasts. Those reprogrammed cells completely lost the keratinocyte specific markers such as keratin14. They were similar to human ES cells in colony formation, growth properties, expression of pluripotency associated transcription factors and surface markers including Nanog, Oct4, Sox2, Rex1, CRIPTO, Cx43, IGF1, SSEA3, SSEA4, TRA-1-60 and TRA-1-81 and global gene expression profiles and differentiation potential *in vitro* and *in vivo*. Thus this study points out keratinocytes as the most likely cell source of reprogrammed cells from plucked hair and presents an experimental model for investigating the bases of cellular reprogramming and potential advantages of using keratinocytes to generate patient-specific iPS cells.

Adipogenic differentiation of human iPS cells has also been performed and compared with ES cells (Taura, et al., 2009). Human iPS cell lines were generated by introducing Oct4, Sox2, Klf4 and c-myc transcription factors into human skin fibroblasts. Once embryoid bodies were formed, the cells were induced towards adipogenic differentiation by using an adipogenic cocktail of IBMX, dexamethasone, insulin, indomethacin and pioglitazone. After twenty two days, adipogenic transcription factors C/EBP α , CCAAT/enhancer binding protein α and peroxisome proliferator activated binding protein γ 2 were expressed. Mature adipocyte markers including leptin and adipocyte fatty acid binding protein was also expressed. All of the human iPS cell lines expressed mRNAs encoding adipogenesis-related molecules at levels that were comparable to the levels seen in human ES cell lines. In terms of lipid accumulation and transcription of adipogenesis-related molecules, human iPS derived adipocytes appear to reach at least the same level of maturity as those derived from human ES cells. Thus the adipogenic potential of iPS cells did not differ from ES cells in majority of cell lines, though adipogenic potentials were varied in each cell line. This study demonstrates that human iPS cells have an adipogenic potential comparable to human ES cells.

Majority of studies have used retroviruses to induce transduction which may result in random insertion of genes within a genome. More general disadvantages of using retroviruses include the requirement of proliferating cells for infectivity, presence of a low titer number, occurrence of insertional mutagenesis and poor *in vivo* delivery and transfection efficiency. Moreover, the storage and quality control of retroviruses is also difficult. With regards to using retroviruses for reprogramming, the major disadvantage is the occurrence of tumors. As a result, other techniques are being explored to perform direct reprogramming.

2. Lentivirus mediated ectopic expression of transcription factors

Lentivirus has been considered as a gene transfer vector since it can infect both proliferating and non-proliferating cells. It has also been shown to successfully infect hematopoietic stem

cells. Lentivirus vectors can be successfully integrated in the host chromosome without the expression of viral genes in target cells. Disadvantage of using a lentivirus vector include its limited insertion size and difficulty in storage and quality control. One of the major disadvantages of lentiviruses is that it is derived from immunodeficiency viruses which poses a safety concern. Until now, lentiviruses have only been used in animal studies and have not been used for clinical applications. With both its advantage and disadvantages, numerous studies have used lentiviruses for direct reprogramming.

A drug inducible transgenic system has been used for direct reprogramming of multiple somatic tissue/cell types (Wernig, Lengner, et al., 2008). Primary iPS cells were generated through dox inducible lentiviral introduction of Oct4, Sox2, Klf4 and c-myc into embryonic fibroblasts. iPS chimeras were created using puromycin selection. The chimeras were allowed to gestate after which a secondary set of embryonic fibroblasts were isolated, thus creating genetically homogenous secondary somatic cells that carry reprogramming factors as defined by doxycycline (dox) inducible transgenes. Cells derived from these chimeras were reprogrammed upon dox exposure and has efficiency twenty five to fifty fold greater than observed by primary transduction. Thus reprogramming occurred without the need for viral infection. The secondary cells were also used to reprogram several adult somatic cell types including brain, epidermis, intestinal epithelium, mesenchymal stem cells, tail tip fibroblast, kidney, muscle and adrenal gland through dox treatment. The results indicated that cells from many somatic tissues can be reprogrammed and different cell types require different induction levels (Wernig, Lengner, et al., 2008). The generation of genetically homogenous secondary cells increased the feasibility of chemical or biological screening and thus enhanced reprogramming efficiency. Neural progenitor cells have also been reprogrammed into iPS cells in the absence of Sox2 expression (Eminli, Utikal, Arnold, Jaenisch, & Hochedlinger, 2008). Genetically marked neural progenitor cells were isolated from neonatal mouse brains and were infected with lentiviral vectors expression Oct4, Sox2, Klf4 and c-myc. The resulting infected neural progenitor cells could generate iPS cells which expressed embryonic stem cell markers including Oct4, Sox2, Eras, Cripto and Nanog. The promoter region of Oct4 and Nanog were demethylated in iPS cells compared to heavily methylated state in neural progenitor cells.

Mouse B lymphocytes were studied for its ability to reprogram to a pluripotent state in presence of four transcription factors (Oct4, Sox2, c-myc and klf4) (Hanna, et al., 2008). Non terminally differentiated B cells were able to convert to a pluripotent state through inducible expression of four transcription factors. Mature B lymphocytes could not convert to a pluripotent state just in the presence of these four transcription factors. The mature B cells had to be altered by interrupting the active transcriptional state either by overexpressing C/EBP α transcription factor or by knockdown of Pax5. Multiple iPS cell lines were clonally derived from both non-fully and fully differentiated B lymphocytes which resulted in adult chimeras with germline contribution and injected with tetraploid blastocytes late term embryos. Thus this study successfully performed direct nuclear reprogramming of terminally differentiated adult cells to pluripotency. The cells from mouse meningeal membranes have also been reprogrammed with exogenous factors towards generating iPS cells. The meningeal membrane cells expressed elevated levels of Sox2 and thus were used to generate iPS cells. These cells were lentivirally transduced with Oct4, Sox2, Klf4 and c-myc transcription factors. The resulting selected colonies had morphological similarities to ES cells. Resulting iPS cell line expressed high levels of endogenous ES markers including Nanog, Oct4 and SSEA1. The promoter regions of Oct4 and Nanog had low methylation compared to ES cells. The colonies efficiently transformed into cells of three germ layers due to expression of GATA6 and Bmp2 (endoderm); Isl1 and T (mesoderm) and FGF5 (ectoderm) (Qin, et al., 2008). Another study has generated induced pluripotent stem cell lines from human somatic cells utilizing lentiviral vectors with

Oct4, Sox2, Nanog and Lin28 genes. The reprogrammed human somatic cells exhibited similar characteristics to ES cells. The human iPS cells had normal karyotypes, telomerase activity and surface marker expression characteristic to human ES cells. The iPS cells also had the potential to differentiate into advanced derivatives of three primary germ layers. Thus human iPS cells may prove useful to study the development and function of human tissues and for transplantation applications.

3. Other methods mediating ectopic expression of transcription factors

Apart from using the retroviral and lentiviral vectors to deliver reprogramming factors, other methods have been used to induce pluripotent stem cells. One of these methods includes generating iPS cells using adenoviral vectors that allow for transient, high-level expression of exogenous genes without integrating into the host genome (Okita, et al., 2008). Adenovirus was delivered repeatedly to maintain transgene expression for up to twelve days. The adenoviral vectors contained Oct4, Sox2, Klf4 and c-myc genes were transfected into mouse embryonic fibroblasts which resulted in the generation of iPS cells without evidence of gene integration. These cells produced teratomas when transplanted into nude mice and contributed to adult chimeras. Another study also used adenoviruses to generate iPS cells from mouse fibroblasts and liver cells (Stadtfeld, Nagaya, Utikal, Weir, & Hochedlinger, 2008). Liver cells were used because they were highly permissive for adenoviral infections. It was reported that liver cells required less viruses than fibroblasts for efficient reprogramming. The adenoviruses transiently expressed Oct4, Sox2, Klf4 and c-myc. The adenoviral iPS cells showed DNA demethylation similar to that of reprogrammed cells. They also expressed endogenous pluripotent genes, formed teratomas and contributed to multiple tissues including germline tissues in chimeric mice. Thus these studies demonstrate the feasibility of nuclear reprogramming and iPS cells generated without permanent genetic alterations.

There are many studies to find alternatives to using viral vectors for reprogramming somatic cells. These techniques include the use of plasmid transfection and the piggyback transposition system (Maherali & Hochedlinger, 2008). Studies have also explored the use of non viral vector transfection of a multi-protein expression vector to reprogram both mouse and human fibroblasts and keratinocytes, etc.

Bickenbach JR group (Grinnell, Yang, Eckert, & Bickenbach, 2007) reported that mouse epidermal interfollicular based keratinocytes transiently transfected with plasmid DNA carrying the full length mouse Oct4 transcript allows for temporal expression of Oct4 with a peak at forty eight hours and a down regulation was noted between one twenty and one sixty eight hours. Full length Oct4 induced keratinocyte expression of Oct4, Nanog, Utf1, Rex-1 and Sox2. Thus Oct4 can be expressed in mouse keratinocytes and it also activates early embryonic developmental genes. Even after Oct4 expression was down-regulated, the expression of Utf1 and Rex1 was still maintained. Both Utf1 and Rex1 are Oct4 targets and transcriptional coactivators that maintain a pluripotent state (Ben-Shushan, Thompson, Gudas, & Bergman, 1998; Okuda, et al., 1998). When Oct4 transfected keratinocytes were cultured in neuroectodermal differentiation media, cells maintained a neuronal phenotype with a triangular body and lengthy projections. Thus this suggests that in response to Oct4, keratinocytes have a greater developmental potential to maybe become a differentiated cell type including a neuron. Moreover, these neuronal like cells also expressed neuronal markers including nestin, Sox1 and NeoN. Oct4 induced differentiation of somatic cells can be reprogrammed into more developmentally potent cells through the use of a single factor.

The other non-viral methods include the piggyBac transposition system. A piggyBac system is a transposon to deliver genes in mammalian cells. The transposon includes a mobile genetic element that can be used to integrate transgenes into host cell genomes. The

piggyBac system is able to deliver large genetic elements without a significant reduction in efficiency. Unlike viral vectors, this system does not require special storage or quality control conditions. It does not need to be prepared in high titers and does not have a limited lifetime. Its transfection efficiency is increased as commercial products for gene delivery are available. Another advantage of this system over viral vectors is the non-occurrence of viral infections. This system was used to reprogram fibroblasts to iPS cells (Woltjen, et al., 2009).

An alternative non-viral vector transfection method is using a single multiprotein expression vector comprised of coding sequences of c-myc, Klf4, Oct4 and Sox2 linked with 2A peptides to reprogram both mouse and human fibroblasts (Kaji, et al., 2009b). A single plasmid was used which had a 2A peptide linked reprogramming cassette, c-myc-Klf4-Oct4-Sox2-IRES-mOrange, flanked by lox-P sites, pCAG2LMKOSMO. After the vectors were introduced in mouse fibroblasts, stable cell lines were established and exhibited reactivation of endogenous Oct4, Sox2 and c-myc genes. Once reprogramming was achieved, the transgenes were removed using transient Cre transfection. During this excision process, differentiation was inhibited by using an FGF receptor inhibitor. The Cre-excised cell lines still exhibited endogenous gene expression of Oct4, Sox2, c-myc and Klf4, thus showing robust gene expression of pluripotency markers. The reprogrammed state was confirmed by formation of adult chimeric mice. The reprogramming ability of the non viral vector system was also confirmed in human cells. The piggyback transposon was combined with the single vector reprogramming system and applied to human embryonic fibroblasts. The results indicated that reprogrammed human cell lines were successfully established from embryonic fibroblasts with robust expression of pluripotency markers. Thus this study demonstrates the complete elimination of exogenous reprogramming factors and efficiently provides iPS cells that can be applied towards regenerative medicine.

A recent study by the Eggan laboratory demonstrated that reprogramming adult cells is possible to use small chemical molecule to replace the transcription factors such as (Oct4, Sox2, c-myc, Klf4 and nanog) delivered by transgenic methods. This occurs by replacing Sox 2 transcription factor with a small molecule that inhibits transforming growth factor-beta signaling (Ichida, et al., 2009). This chemical named RepSox can replace *Sox2* in reprogramming by inhibiting Tgf- β signaling, and this inhibition promotes the completion of reprogramming through induction of the transcription factor *Nanog*. This study has shown that it is possible to replace the transgenic methods for delivering reprogramming factors with using small chemical molecules, and overcome the uncertain concerns regarding the future utility of the resulting stem cells raised with globally altering the chromatin structure by gene transduction.

Induced pluripotent human stem cells for clinical applications

iPS cells have been generated from patients with amyotrophic lateral sclerosis (ALS) and could further be differentiated into motor neurons (Dimos, et al., 2008). Primary skin cells were isolated from these patients and Klf4, Sox2, Oct4 and c-myc genes were introduced in these cells through vesicular stomatitis virus glycoprotein-pseudotyped moloney based retroviruses. The transduced fibroblasts developed colonies after two weeks with similar ES cell morphology. The iPS cell lines exhibited strong alkaline phosphate activity and also expressed ES cell associated antigens including SSEA-3, SSEA-4, TRA1-60, TRA1-81 and Nanog. The pluripotent state of these cells was confirmed due to expression of REX1, ZFP42, FOXD3, TERT, Nanog and CRIP1/TGDF1 at levels comparable to human ES cells. The cells were also capable of differentiating into three germ layers. The iPS cells were further characterized as to whether they could differentiate into a disease specific cell type. The embryoid bodies formed from iPS cells were treated with an agonist of the sonic hedgehog signaling pathway and retinoic acid. When the differentiated embryoid bodies

were allowed to adhere to a laminin coated surface, neuronal outgrowths were observed. Moreover these neuronal like cells tested positive for tubulin, β -tubulin which confirmed their neuronal phenotype. Thus this study demonstrated that patient specific iPS cells possess properties of embryonic stem cells and can be successfully directed to differentiate into motor neurons.

iPS cells have also been generated to study disease and drug development. iPS cells were successfully generated from patient isolated cells with a variety of genetic diseases including adenosine deaminase deficiency-related severe combined immunodeficiency, Shwachman-Bodian-Diamond syndrome, Gaucher disease typeII, duchenne and becker muscular dystrophy, Parkinson's disease, huntingtons disease, juvenile onset typeI diabetes mellitus, down syndrome/trisomy21 and the carrier state of lesch-nyhan syndrome (Park, et al., 2008). All iPS cell lines regardless of the diseased condition expressed pluripotency related genes including Oct4, Sox2, Nanog, Rex1, GDF3 and hTERT. All iPS cell lines differentiated *in vitro* into embryoid bodies and developed into specific lineages which was confirmed by the presence of endoderm, mesoderm and ectoderm specific markers. Thus these cell lines generated from patients can be compared with normal cell lines to understand the diseased condition at a molecular level. It also gives the opportunity to screen drugs as a treatment approach.

A more successful approach was the highly efficient neural conversion of human ES cells and iPS cells by dual inhibition of SMAD signaling (Chambers, et al., 2009). In this study an adherent cell culture condition was used to induce neural cells from human ES cells instead of relying on embryoid formation, stromal feeder coculture or selective survival conditions. They reported that by adding two inhibitors of SMAD signaling, Noggin and SB431542 they successfully induced rapid and complete neural conversion of greater than eighty percent of human ES cells. The iPS cells directly differentiated in midbrain dopamine and spinal motor neurons. Thus this study demonstrated that noggin/SB431542 based neural induction may facilitate the use of human ES and human iPS cells in regenerative medicine and disease therapy without the need of stromal feeders or embryoid body's protocols. iPS cells have also been generated from a juvenile patients skin fibroblasts with spinal muscular atrophy. The patient's mother's fibroblasts were used as a control. The fibroblasts were introduced with lentiviral constructs encoding Oct4, Sox2, Nanog and lin28. These cells were expanded in culture and maintained the disease genotype due to expression of motor neuron transcription factors. The cells generated motor neurons that showed selective deficits compared to those derived from the child's unaffected mother. This study demonstrates that human iPS cells can be used to model a specific pathology seen in a genetically inherited disease (Ebert, et al., 2009). Human blood has also been used to generate iPS cells (Loh, et al., 2009). Using the retroviral transduction method of Oct4, Sox2, klf4, c-myc and CD34⁺ mobilized human peripheral blood cells were used to generate iPS cells. These derived cells were similar to human ES cells in terms of morphology and expression of surface antigens including Tra-1-81, Nanog, Oct4, tra-1-6-, SSEA3, SSEA4 and alkaline phosphatase. The DNA methylation status of pluripotency cell specific genes and the capacity to differentiate *in vitro* and into teratomas was also comparable to human ES cells. Thus this study demonstrates that cells from human blood can be reprogrammed to generate patient-specific stem cells for diseases in which the disease causing somatic mutations are restricted to cells of hematopoietic lineage.

iPS cells have also been generated from Parkinson's disease patients without the use of viral reprogramming factors. The study showed that fibroblasts obtained from patients with Parkinson's disease were successfully reprogrammed and differentiated into dopaminergic neurons (Soldner, et al., 2009). The iPS cells were generated using doxycycline-inducible lentiviral vectors that could be excised with Cre-recombinase. Thus this represents a method

to replace viral based reprogramming. For patient therapy an alternative to viral reprogramming is required as vector expression may alter the differentiation patterns of iPS cells of induce tumor formation. The iPS cells without viral vectors maintained a pluripotent ES cell like state even after removal of the transgenes. Thus this study demonstrates that residual transgene expression in virus carrying human iPS cells can affect their molecular characteristics.

Conclusion

The breakthrough in iPS cell research was due to the initial cloning experiment of Dolly the sheep (Wilmut, Schnieke, McWhir, Kind, & Campbell, 1997). Then some studies revealed that reprogramming factors in ovular cytoplasm and ES cells may induce pluripotency in somatic cells (Kimura, Tada, Nakatsuji, & Tada, 2004). iPS cells can proliferate for months and differentiate into cells of three major germ layers. The telomerase levels of these cells are sufficiently high to promote expansion of clones at rates similar to embryonic stem cells. Colony morphology, cell surface antigens and teratoma formation has also been found to be similar to human ES cells (Yamanaka, 2008). A recent study by the Jaenisch laboratory has suggested that direct cell reprogramming is a stochastic process dependant upon an accelerated cell division rate. Utilizing Oct 4, Sox2, c-myc and Klf4 to reprogram somatic cells involves a continuous process where almost all donor cells give rise to iPS cells during continued growth. The transcription factors seem to be a key parameter in achieving pluripotency (Hanna, et al., 2009). However, many questions remain unanswered regarding these reprogrammed cells including (1) what is the precise mechanism behind reprogramming, (2) how can we direct cell differentiation towards a specific cell fate or cell type, (3) are these cell functionally similar to ES cells and (4) different cell types have different gene expression profiles and different reprogrammed cells resemble ES cells at different levels, thus do different embryonic growth factors have the same effect on different types of somatic cells (Hanna, et al., 2007). Many different stem cell sources are being studied for clinical applications studying disease models, drug development and as an autologous source of cells for tissue repair (Gonzales & Pedrazzini, 2009; Korsgren & Nilsson, 2009; Yan, et al., 2009), but it will take another decade to fully characterize these reprogrammed cells and apply them for therapeutic use in humans. Before iPS cells can be applied clinically, reduced genetic manipulation needs to be considered for gene insertion into somatic cells to prevent insertional mutagenesis. Although this process does ethically provide an alternative to the ethically problematic use of ES cells (Lopez Moratalla, 2008; Rao & Condic, 2008); to validate the use of iPS cells, human ES cells will be required. Also, human ES cells are still being characterized, so it will be difficult to functionally confirm that iPS cells are similar to human ES cells as many growth factors are still being discovered. Thus to understand the advances in iPS cell research, the advances of adult and ES cells also have to be considered (Gearhart, Pashos, & Prasad, 2007).

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Figure 1.

Diagram represents different techniques to generate pluripotent stem cells.

(a) Somatic Cell Nuclear Transfer: Nuclear DNA isolated from a somatic donor cell is introduced into an enucleated oocyte (oocyte which has its nucleus removed) to form a pre-developed embryo which is cultured until full development. The developed embryo is implanted into a foster mother ultimately resulting in cloned neonates, (b) Cell Fusion: Somatic cells and pluripotent embryonic stem cells go through cell fusion to generate reprogrammed cells. The cytoplasm of embryonic stem cells contains reprogramming factors which can alter the epigenetic state of a somatic cell into a pluripotent cell being fused with a somatic cell, (c) Reprogramming Through Cell Extracts: Cell extracts obtained from pluripotent stem cells are inserted into somatic cells which leads to nuclear reprogramming to generate a pluripotent stem cell and (d) Direct Reprogramming: A selection of transcription factors are introduced into somatic cells through viral or non viral vector sources to generate pluripotent stem cells.

Table 1

The table outlines different transcription factors used to reprogram somatic cells from different mouse or human tissue sources to pluripotent cells.

Reprogramming Transcription Factors	Cell Type	Species	Reference
Oct4, Sox2, c-myc and Klf4	Embryonic and adult fibroblasts	Mouse	Takahashi & Yamanaka, 2006
Oct4, Sox2, c-myc and Klf4	Embryonic and tail tip fibroblasts	Mouse	Wernig et al, 2007
Oct4, Sox2, c-myc and Klf4	Embryonic fibroblasts	Mouse	Okita et al, 2007
Oct4, c-myc, Klf4	Neural stem cells	Mouse	Duinsbergen et al, 2008
Oct3/4, Sox2, c-myc and Klf4	Fibroblasts	Mouse	Nakagawa et al, 2008
Oct3/4, Sox2, c-myc and Klf4	Fibroblasts	Human	Nakagawa et al, 2008
Oct4, Sox2, and Klf4	Fibroblasts	Mouse	Wernig et al, 2008
Oct4 and Klf4	Neural stem cells	Mouse	Kim et al, 2008
Oct4 and c-myc	Neural stem cells	Mouse	Kim et al, 2008
Oct4	Neural stem cells	Mouse	Kim et al, 2009
Oct4, Sox2, c-myc and Klf4	Fibroblasts	Human	Takahashi et al, 2007
Oct4 and Sox2	Fibroblasts	Human	Huangfu et al, 2008
Oct4, Sox2, c-myc and Klf4	Keratinocytes	Human	Aasen et al, 2008
Oct4, Sox2, c-myc and Klf4	Skin Fibroblasts	Human	Taura et al, 2009
Oct4, Sox2, c-myc and Klf4	Multiple somatic tissues	Mouse	Wernig et al, 2008
Oct4, c-myc and Klf4	Neural stem cells	Mouse	Eminli et al, 2008
Oct4, Sox2, c-myc and Klf4	B lymphocytes	Mouse	Hanna et al, 2008
Oct4, Sox2, c-myc and Klf4	Embryonic fibroblasts	Mouse	Okita et al, 2008
Oct4, Sox2, c-myc and Klf4	Hepatocytes and fibroblasts	Mouse	Stadtfeld et al. 2008
Oct4, Sox2 and Esrrb	Embryonic fibroblasts	Mouse	Feng et al, 2009
Oct4	Keratinocytes	Mouse	Grinnell et al, 2007
Oct4, Sox2, c-myc and Klf4+2A peptides	Fibroblasts	Mouse	Kaji et al, 2009
Oct4, Sox2, c-myc and Klf4+2A peptides	Fibroblasts	Human	Kaji et al, 2009
Oct4, Sox2, c-myc and Klf4	Keratinocytes	Human	Dimos et al, 2008
Oct4, Sox2, Nanog and lin28	Fibroblasts	Human	Ebert et al, 2009
Oct4, Sox2, c-myc and Klf4	Blood cells	Human	Loh et al, 2009