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REVIEW ARTICLE

Inducing Pluripotency in Somatic Cells: Historical Perspective and Recent Advances

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Inducing pluripotency in somatic cells is mediated by the Yamanaka factors Oct4, Sox2, Klf4, and c-Myc. The resulting induced pluripotent stem cells (iPSCs) hold great promise for regenerative medicine by virtue of their ability to differentiate into different types of functional cells. Specifically, iPSCs derived directly from patients offer a powerful platform for creating *in vitro* disease models. This facilitates elucidation of pathological mechanisms underlying human diseases and development of new therapeutic agents mitigating disease phenotypes. Furthermore, genetically and phenotypically corrected patient-derived iPSCs by gene-editing technology or the supply of specific pharmaceutical agents can be used for preclinical and clinical trials to investigate their therapeutic potential. Despite great advances in developing reprogramming methods, the efficiency of iPSC generation remains still low and varies between donor cell types, hampering the potential application of iPSC technology. This paper reviews histological timeline showing important discoveries that have led to iPSC generation and discusses recent advances in iPSC technology by highlighting donor cell types employed for iPSC generation.

Keywords: Pluripotency, Induced pluripotent stem cells, Octamer-binding transcription factor 4 (OCT4), Cellular reprogramming, Embryonic stem cells

The Birth of Embryonic Stem Cells

After fertilization, the zygote divides multiple times to form a blastocyst (1, 2). Two types of cells can be found in the blastocyst: an inner layer of cells called the epiblast and an outer layer of cells called the trophoblast (1, 2). While the trophoblast forms the extra-embryonic tissue which eventually gives rise to the placenta, the epiblast which is known as the inner cell mass (ICM) eventually develops into embryonic tissue. This ICM is considered to be a cellular source of embryonic stem cells (ESCs).

In 1981, the first ESC lines were derived from ICM of mouse blastocysts by two groups (Fig. 1) (3, 4). Specifically, Evans and Kaufman (3) derived mouse ESCs (mESCs) by plating an explanted whole blastocyst onto feeder cells and culturing in serum-containing media. Martin (4) also derived mESCs. In his case, ICM mechanically isolated from the late stage of blastocysts was plated onto feeder cells and cultured in the embryonal carcinoma cells (ECCs)-conditioned medium. The mESCs derived by these two stu-

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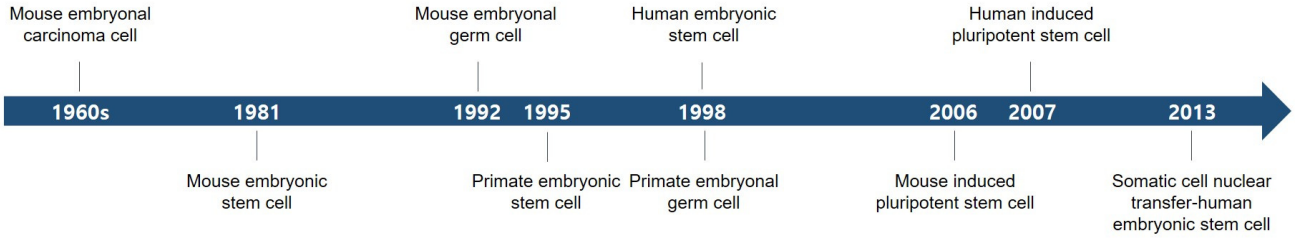

Fig. 1. Chronological timeline of important discoveries in the field of pluripotency. Mouse embryonal carcinoma cells are historically the first pluripotent stem cells which were derived from testicular tumors; mouse embryonic stem cells were first isolated and propagated in culture in 1981; mouse embryonal germ cells were derived from primordial germ cells in 1992: primate embryonic stem cells were first derived from inner cell mass of blastocysts in 1995; the first human embryonic stem cell lines were established in 1998; primate embryonal germ cells were first isolated and cultured in 1998; mouse induced pluripotent stem cells were generated in 2006; human induced pluripotent stem cells were derived by ectopic expression of OCT4, SOX2, KLF4, and c-MYC in 2007; the first human embryonic stem cells were generated by somatic cell nuclear transfer in 2013.

dies resemble ECCs in an ability to proliferate *in vitro* and to differentiate into derivatives of all three embryonic germ layers (3, 4). In contrast to ECCs, mESCs exhibit a normal diploid karyotype. Importantly, they can efficiently contribute to all adult tissues including germ cells after injection into the blastocyst, thus providing a practical way to introduce genetic modifications to the mouse. Overall, these two studies indicate that pluripotent stem cells with a normal diploid karyotype can be derived from ICM of the blastocyst and their pluripotent state can stably be maintained upon the supply of an optimal culture condition.

Soon after their discovery, many different groups have attempted to derive human ESCs (hESCs) from human embryos, but despite of their extensive efforts it took over 17 years to derive hESCs. In 1998, Thomson et al. (5) derived the first hESC lines from fresh and frozen human donated embryos (Fig. 1). Specifically, they initially obtained a total of 14 ICM which were isolated from 20 fresh and frozen human embryos. Of these 14 ICM were used, five distinct hESC lines (H1, H7, H9, H13, and H14) were successfully derived. Each hESC line display a normal karyotype, express a number of pluripotency makers including stage-specific embryonic antigen (SSEA)-3, SSEA-4, T cell receptor alpha locus (TRA)-l-60, TRA-1-81, and alkaline phosphatase, and was capable of forming teratoma in immunodeficient mice (5). Such a long-term delay of the derivation of hESCs is most likely because a culture condition which has been used for derivation and maintenance of mESCs do not really support the growth of hESCs, and thus it takes so much time to identify a right culture condition that supports the derivation and maintenance of hESCs. In 1995 and 1996, ESCs have been derived from two nonhuman primates (rhesus monkey and the common marmoset) (Fig. 1) (6, 7). Experience with derivation and culture of those primate ESCs and concomitant improvements in culture medium eventually led to the successful derivation of hESC lines. Later, it has been proved that LIF/STAT3 which is a key regulator for self-renewal of mESCs does not have same effect on hESCs (8-12). Instead, basic fibroblast growth factor and Activin A have found to be the most important factors for maintaining pluripotency of hESCs, demonstrating species-dependent signaling pathways for maintaining the pluripotent state (13-16). Furthermore, fetal bovine serum (FBS) containing growth factors, cytokines, hormones and transport proteins which is essential for the maintenance and growth of mESCs, but its inclusion to hESC culture medium results in spontaneous differentiation towards mesodermal lineage (17). Thus, FBS has been replaced by knockout serum replacement for stable maintenance of hESCs. Overall, these findings suggest that technological challenges arising from fundamental differences between mouse and hESC lines are the principal impediment.

Although the ESCs are commonly derived from ICM of blastocysts, single blastomere biopsied from 2- to 8-cell stages of pre-implantation embryos has also shown to be able to form ESC colonies (18-20). Furthermore, embryonal germ cells isolated from genital ridges of post-implantation embryos are also capable of forming ESC colonies (21, 22). Since the derivation of ESCs is possible from different cellular sources of embryos at different time points, the exact origin of ESCs and their *in vivo* counterpart remains controversial (23, 24).

Limitations of Human Embryonic Stem Cells

Derivation of hESCs has opened up exciting new opportunities to study human development and diseases (25, 26). Also, they hold tremendous promise for development of cell replacement therapies for a broad range of human

diseases. However, the use of hESCs for the cell replacement therapies is limited by ethical and political concerns, because derivation of hESCs requires the destruction of human embryos (27, 28). Furthermore, immune compatibility would be another barrier when considering transplantation of hESC-derived cells into patients (29). In fact, the genetic identity of the donor embryos from which the ESCs are derived most likely will differ from that of potential recipients. Thus, patients who will receive ESC-derived cells might have the problem of immune rejection.

Somatic cell nuclear transfer (SCNT) which involves transferring a donor cell into an enucleated oocyte could be an alternative method to generate donor-matched hESCs (Fig. 1) (18, 30). The genetic identity of SCNT-derived embryos form which the ESCs are derived will be exactly identical with that of potential recipients. Thus, patients who will receive SCNT ESC-derived cells might not encounter the problem of immune rejection. However, recipient oocytes contain over 100 times more mitochondrial DNA (mtDNA) than donor cells such that foreign mtDNA can be transmitted to the SCNT-hESCs (31). It has been reported that allogeneic mitochondria in the SCNT-ESCs may trigger an adaptive alloimmune response that impairs the survival of SCNT-ESC grafts in the host tissue (32). Moreover, the ethical concern is still remaining, since SCNT requires the perturbation of human embryos (33). Additionally, it is really challenging to obtain donor oocytes and to perform the technically intensive SCNT procedures, preventing a widespread use of this technology. Altogether, despite the enormous potential of hESCs and SCNT-hESCs for regenerative medicine, therapeutic applications of these cells are limited by ethical, technical and immunological concerns.

The Birth of Induced Pluripotent Stem Cells

It has been shown that somatic cells can be reprogrammed into pluripotent stem cells by fusion with ESCs, ECCs, or embryonic germ cells (EGCs) (34-36) or by SCNT (37). These findings provide a new idea that oocytes, ESCs, ECCs, and EGCs contain specific trans-acting factors that can confer the pluripotent state to somatic cells and evoke a new concept that ectopic expression of these trans-acting factors into somatic cells might in fact be able to induce pluripotency (38, 39). This idea has been tested by different groups with several different methods (for instance, treatment of RNA or protein extracted from ESCs, ECCs, or oocytes to somatic cells), but these efforts were unsuccessful (38, 39).

In 2006, however Takahashi and Yamanaka (40) made a breakthrough discovery that retroviral transduction of octamer-binding transcription factor 4 (OCT4), sex determining region Y-box 2 (SOX2), Krüpple-like factor 4 (KLF4), and cellular myelocytomatosis (c-MYC) can reprogram mouse embryonic and adult fibroblasts into cells closely resembling ESCs, so-called induced pluripotent stem cells (iPSCs) (Fig. 1). The resulting iPSCs are shown to be morphologically, transcriptionally, and epigenetically similar to mESCs. Subcutaneous transplantation of iPSCs into nude mice results in the formation of teratomas consisting all three germ layers, proving their *in vitro* differentiation potential (40). Furthermore, iPSCs were capable of producing chimeric embryos after injection into mouse blastocysts, proving their *in vivo* differentiation potential (40). However, these early iPSCs were failed to fully recapitulate authentic ESC properties, because they fail to express some key pluripotency genes, have incomplete promoter demethylation of Oct4, and do not yield live chimeric mice when injected into mouse blastocysts (40). Thus, this first iPSCs appeared to be rather partially reprogrammed iPSCs. Soon after this study, several groups including Yamanaka's group generated fully reprogrammed iPSCs by selecting the iPSC colonies based on the positivity of Oct4 and Nanog (41-43). Overall, these studies demonstrate that terminally differentiated somatic cells can be reprogrammed to pluripotent stem cells by ectopic expression of four reprogramming factors.

A year later after the derivation of mouse iPSCs, three groups independently reported that iPSC generation is also possible from human fibroblasts (44-46). Specifically, Takahashi et al. (45) generated iPSCs from human dermal fibroblasts by using an exact same viral system with a same set of transcription factors that have been used for mouse iPSC generation (Fig. 1). Another group headed by Yu et al. (46) generated iPSCs from human fetal fibroblasts and newborn foreskin fibroblasts by a lentiviral system with a slightly different set of transcription factors (OCT4, SOX2, NANOG, and LIN28). It seems that OCT4 and SOX2 appear to be essential for inducing pluripotency, but KLF4 and c-MYC can be functionally replaced by NANOG and LIN28. The third group headed by Park et al. (44) also generated iPSCs from human embryonic fibroblasts derived from H1 ESCs, primary dermal fibroblasts and fetal lung fibroblasts by retroviral transduction of OCT4, SOX2, KLF4, and c-MYC. The resulting human iPSCs generated by all these three groups share hESC characteristics (44-46). Specifically, they express representative pluripotent markers including OCT4, NANOG, SOX2, and TRA-I-60. They are epigenetically similar to hESCs as determined by DNA methylation status at promoter regions of OCT4 and NANOG. They are able to form embryoid bodies and teratomas where all three germ layers derivatives can be found,

fulfilling a hallmark of pluripotency. Soon after these three studies, disease-specific iPSCs have been generated from patients (47-49). Importantly, these patient-derived iPSCs provide a valuable experimental platform for creating *in vitro* disease models (38, 39, 50). Altogether, these studies demonstrate that inducing pluripotency in somatic cells is also possible in humans and fundamental features of the transcriptional network governing pluripotency remain conserved between humans and mice.

iPSCs have been generated from somatic cells of a wide variety of species, including, but not limited to, monkey, pig, rabbit, rat, and goat (51-55). iPSCs were even generated from endangered species and reanimate extinct species for aiming at helping wildlife and environmental conservation efforts (13, 56, 57). However, some species such as naked mole rat and spiny mice were found to be refractory to reprogramming (58, 59). They require either the inhibition of GSK3 and MEK1/2 signaling pathways or overexpression of additional factors to induce pluripotency (58, 59). Specific reasons why some species are refractory to reprogramming but the others are prone to reprogramming remain to be answered. Altogether, these findings demonstrate that inducing pluripotency is possible in many different species by ectopic expression of OCT4, SOX2, KLF4, and c-MYC and indicate that fundamental features of the transcriptional network governing pluripotency are conserved across species.

A Synergism of OCT4, SOX2, KLF4, and c-MYC Induces Pluripotency

The loss or gain of function studies have revealed that OCT4, SOX2, KLF4, and c-MYC are important for the establishment and maintenance of the pluripotent state in developing embryos and ESCs (60-62). Specifically, OCT4 depletion in embryos causes the loss of pluripotent characteristics in ICM (63). Furthermore, the loss of OCT4 expression in mESCs results in trophoblast differentiation (64). Therefore, OCT4 plays a pivotal role in specification and maintenance of pluripotency in embryos and ESCs. SOX2 also plays a critical role in the self-renewal and pluripotency of ESCs (65). It forms a heterodimer with OCT4 to bind to target sites and this heterodimerization has shown to be crucial for establishing and maintaining the pluripotent state in embryos and ESCs (66). Moreover, OCT4 and SOX2 act as master regulators of maintaining pluripotency in ESCs (67). KLF4 is required for both self-renewal and maintenance of pluripotency in ESCs (67-69). Furthermore, KLF4 directly interacts with OCT4 and SOX2 and regulates the expression of key transcription factors such as NANOG in ESCs (69). c-MYC is required for early embryogenesis and plays an important role in proliferation of ESCs (70, 71). Thus, these findings indicate that OCT4, SOX2, KLF4, and c-MYC are critical for establishment and maintenance of pluripotency in developing embryos and ESCs, implicating that reprogramming is achieved by a synergism of all these four factors.

Ectopic expression of OCT4, SOX2, KLF4, and c-MYC mediates a dramatic change in gene expression (72-76). They directly or indirectly bind to active somatic loci to promote inactivation of genes associated with fibroblasts at early stage of reprogramming (72, 73, 77-79). Simultaneously, they bind to regulatory regions of pluripotency genes to promote their activation. Along with global gene expression change, chromatin architectures are also reorganized by OCT4, SOX2, KLF4, and c-MYC (79, 80). The extensive loss of chromatin accessibility occurs at loci where somatic genes are located (79, 80). This loss of chromatin accessibility is closely associated with inactivation of somatic genes. The gain of chromatin accessibility also occurs around loci where pluripotency genes are located (79, 80). This gain of chromatin accessibility is largely associated with the activation of pluripotency genes. Moreover, epigenetic marks such as DNA methylation and histone modifications are also redistributed through the genome during reprogramming that is also mediated by OCT4, SOX2, KLF4, and c-MYC (74, 76, 79, 81-83). Specifically, active histone marks such as H3K27ac and H3K4me1 that are initially located on the regulatory regions of somatic genes are relocated onto regulatory regions of pluripotency genes during reprogramming (74, 76, 79). Repressive histone marks (H3K9me3, H3K27me3, etc.) that are initially located on the regulatory regions of pluripotency genes are relocated into regulatory regions of somatic genes during reprogramming (81). Furthermore, H3K79 methylation marks are normally localized on the gene body of actively transcribed genes (84). It is initially located on the gene body of somatic genes in fibroblasts, but it is redistributed to gene body of pluripotency genes during reprograming (85). Overall, these findings indicate that OCT4, SOX2, KLF4, and c-MYC induce pluripotency by mediating dramatic changes in transcriptome and epigenome.

While global changes in transcriptome and epigenome occur during reprogramming, fibroblasts undergo a morphological change; migratory mesenchymal cells are transformed to polarized epithelial cells (86). This morphological change is also mediated by OCT4, SOX2, KLF4, and c-MYC. This process called as a mesenchymal-to-epithelial transition (MET) process which is often occurred during development of organs or formation of tumors in our body,

but it also appears during reprogramming (87). In fact, this process is critical for successful iPSC generation when fibroblasts are used as a donor cell type (86). The MET is characterized by upregulation of epithelial genes (E-cadherin, Cdh1, Epcam, etc.) and down-regulation of mesenchymal genes (Snail1/2, Zeb1/2, N-cadherin, etc.) (86, 87). It has been reported that OCT4, SOX2, KLF4, and c-MYC can directly regulate expression of these epithelial and mesenchymal genes (88, 89). For instance, OCT4 and SOX2 suppress Snail1/2 and c-Myc enhances the MET process through the downregulation of transforming growth factor (TGF)-β signals by suppression of TGF-β1 and TGF-β receptor 2 expression (88, 89). Furthermore, KLF4 upregulates epithelial genes such as E-cadherin and Cdh1 during reprogramming (88, 89). Overall, these findings suggests that transformation of mesenchymal cells to epithelial cells is essential for successful iPSC generation and this process is mediated by OCT4, SOX2, KLF4, and c-MYC.

Various Donor Cell Types Used for Reprogramming

In order to use iPSCs as an efficient research tool and translate this technology into therapeutic applications, various donor cell types have been used for iPSC generation. To date, fibroblasts are the most popularly used a primary somatic cell type for iPSC generation. Various characteristics of the fibroblasts facilitate their utilization as a donor cell type (90). A fibroblast which is a cell type contributing to the formation of connective tissues can be readily obtained from different sites of our body by punch biopsy (91). Furthermore, primary fibroblasts are relatively easier to culture than other cell types (hepatocytes, oligodendrocytes, etc.). However, the punch biopsy is an invasive approach and requires local anesthesia, representing a major drawback for obtaining fibroblasts from healthy donors or patients (90, 91). Furthermore, because of the nature of its cellular properties (mesenchymal cells, early senescence, etc.), they have shown to exhibit lower reprogramming efficiency than other cell types.

Epidermal keratinocytes and renal tubular epithelial cells have been also used for iPSC generation (92, 93). The greatest advantage of using these cells as donor cell types is that they can be obtained by non-invasive methods (94, 95). Furthermore, they have shown to be much easier to reprogram than primary fibroblasts, as they are epithelial cells and thus do not require the MET process. More specifically, epidermal keratinocytes which are highly specialized epithelial cells can be obtained from plucked hair (94). This cell type seems to be a very promising donor cell type for iPSC generation, as they can reprogram into iPSCs with higher efficiency (100-fold higher than fibroblasts) (92). However, a long-term culture of primary keratinocytes has been proven to be difficult, due to their rapid differentiation and cellular senescence (92, 94). Exfoliated renal tubular epithelial cells which can be obtained from urine could be another promising donor cell type for iPSC generation, as their reprogramming efficiency is also higher than that of fibroblasts (93, 95). However, only very few cells can be obtained from urine so that it is extremely time-consuming to get a sufficient number of cells for reprogramming. Moreover, a long-term culture of primary exfoliated renal tubular epithelial cells is challenging, because they have a short lifespan in culture. The acquisition of cellular immortality therefore appears to be yet a barrier for a widespread use of these two cell types, and immortalization techniques play an instrumental role in culture to extend their cell survival that facilitates their utilization for reprogramming.

Blood cells are readily available and easily accessible donor cells in contrast to dermal fibroblasts, epidermal keratinocyte, and renal tubular epithelial cells which are required several weeks to establish a primary cell culture. Drawing blood is routinely performed in clinics for medical diagnostics and can be done without the need of anesthetics. So far, several types of cells isolated from blood were used as donor cells for reprogramming (96-102). Those include granulocyte colony stimulating factor (G-CSF) mobilized $CD34^+$ peripheral blood stem cells, T lymphocytes, peripheral blood mononuclear cells (PBMCs), and Epstein-Barr virus (EBV) immortalized B lymphocytes (lymphoblastoid cell lines, LCLs). Each cell type has pros and cons of using it as a donor cell for reprogramming.

(i) Among these four cell types, G-CSF mobilized $CD34^+$ peripheral blood stem cells have found to be the most efficient cell type for inducing pluripotency, most likely because they are immature cells that have stem cell characteristics and high plasticity compared to other cell types (99). However, isolating G-CSF mobilized $CD34^+$ peripheral blood stem cells is time-consuming, requires an antibody-based purification and involves the subcutaneous injection of G-CSF to patients that may cause some side effects.

(ii) T lymphocytes are the most abundant cells in PBMCs and can be easily isolated from them. They have shown to be readily expandable using an established protocol. Furthermore, T lymphocytes can be efficiently transduced by viral OCT4, SOX2, KLF4, c-MYC (100, 101). As such, they have shown to efficiently reprogram into iPSCs (100, 101). However, major problem of T cells as a donor cell type is that resulting iPSCs have preexisting V(D)J rearrangements

at the T-cell receptor loci which may lead to the development of T-cell lymphomas (100, 101, 103, 104).

(iii) PBMCs can be readily isolated from blood by Ficoll-Hypaque density gradient centrifugation and used for reprogramming immediately after blood drawing. Despite these beneficial effects, for some reasons the PBMCs have shown to be difficult to reprogram into iPSCs (105). It exhibits very lower reprogramming efficacies even with polycistronic expression of OCT4, SOX2, KLF4, and c-MYC (102). Recently, iPSCs have been generated from PBMCs by episomal vectors and Sendai virus (106, 107). In both cases, reprogramming efficiencies were extremely low which is 50-fold lower than fibroblasts (102, 106, 107).

(iv) LCLs which are immortalized cells can be generated by EBV infection of B lymphocytes (108). Cell repositories such as Coriell Institute for Medical Research (https://www.coriell.org/) and UK Biobank (https://www.ukbiobank.ac.uk/) store a large number of LCLs derived from a variety of patients who carry various disease states. Genotyping results and clinical histories of each patient are well documented in the cell repositories. Thus, LCLs have been popularly used for generating disease-specific iPSCs (96-98). However, the EBV is known to be associated with malignancies, Burkitt's lymphoma, B-cell lymphoproliferative syndromes, and nasopharyngeal carcinoma, Hodgkin's disease, T-cell lymphomas, and gastric carcinoma (109). Thus, LCL-derived iPSCs require an extensive safety check in order to use them for therapeutic applications.

Reprogramming Efficiency Depends on Donor Cell Types

Studies on reprogramming with a wide variety of cell types have found that different efficiencies and kinetics of reprogramming depends on donor cell types. These variations are attributed to differences in epigenetic states, differentiation status, and phenotypic characteristics of donor cell types.

It has been shown that less differentiated cells are more efficient in inducing pluripotency than terminally differentiated cells. For instance, umbilical cord blood cells and hematopoietic stem cells ($CD34^+$ peripheral blood stem cells) both of which represent immature cell types, are readily amenable for reprogramming compared to terminally differentiated cell types, such as B and T lymphocytes, with up to 28% reprogramming efficiencies (100, 101, 110). Furthermore, adipose-derived stem cells which are proliferating and multipotent stem cells formed iPSC colonies with a range of 0.4% to 7% reprogramming efficiency (111, 112). Neural stem cells (NSCs) which are resident stem cells in the central nervous system and have a differentiation capacity towards neurons, astrocytes, and oligodendrocytes can be efficiently reprogrammed into iPSCs with 3.6% efficiency which is 180-fold higher than fibroblasts (113, 114). Human dental pulp cells which are abundant dental pulp stem/progenitor cells can be efficiently reprogrammed into iPSCs with a range of 0.01% to 0.1% (115). Overall, adult stem cells or progenitor cells which have high plasticity and differentiation potential are more amenable to reprogramming than terminally differentiated cells.

As discussed above, the epithelial cells are more efficient and fast in reprogramming than mesenchymal cells (93, 94, 116-118). The high efficiency and fast kinetics are largely due to the fact that epithelial cells do not require the MET process which is necessary for successful formation of iPSC colonies (87). For instance, reprogramming of epidermal keratinocytes has shown 100-fold higher efficient and 2-fold faster compare with that of fibroblasts (92). In addition, reprogramming of renal tubular epithelial cells has shown 4-fold higher efficient compared with that of fibroblasts (93). Human nasal epithelial cells formed iPSC colonies with a range of 0.01% to 0.1% efficiency which is 50-fold higher than fibroblasts (118). Human amniotic epithelial cells reprogram faster and more efficiently than fibroblasts (94, 117). Hepatocytes which are major epithelial cells in liver and gastric epithelial cells also appear to be easier to reprogram into iPSCs than fibroblasts (116). These findings indicate that cells with epithelial properties are more amenable to reprogramming than cells with mesenchymal properties.

Epigenetic states of donor cells have shown to significantly affect iPSC generation (85, 119-123). Thus, modulating epigenetic pathways against DNA methylation, chromatic structures and histone modifications in donor cells dramatically enhances reprogramming efficiencies (85, 119-123). A diverse set of small molecules that target different epigenetic enzymes has been known to enhance reprogramming efficiency. These include valproic acid (VPA, a histone deacetylase inhibitor), sodium butyrate (NaB, a histone deacetylase inhibitor), RN-1 (a lysine-specific histone demethylase 1 [LSD1] inhibitor), RG108 (a DNA methyltransferase inhibitor), and EPZ5676 (a DOT1 like histone lysine methyltransferase inhibitor) (85, 119-123). For instance, the inhibition of the disruptor of telomeric silencing 1-like (DOT1L) by shRNA or EPZ5676 accelerated reprogramming process and significantly increased the yield of iPSC colonies (85, 121). Furthermore, the inhibition of DOT1L enables iPSC generation even without KLF4 and c-MYC and also allows iPSC generation with other OCT family members that are incompetent in reprogramming

(61, 85, 121). Moreover, NaB greatly enhances the efficiency of generating iPSCs and its treatment allows iPSC generation in the absence of KLF4 or c-MYC (122, 124). Another example would be that the inhibition of LSD1 by RN-1 or shRNA facilitated iPSC generation (119). VPA enables efficient iPSC generation even without KLF4 and c-MYC (120). Interestingly, combinations of these inhibitors exhibit additive or synergistic effects on reprogramming. For example, a combinational treatment of EPZ5676, RN-1, and NaB further increase reprogramming efficiency in comparison of their respective treatment, suggesting that there are different roadblocks in reprogramming and removing these blocks simultaneously can dramatically improve reprogramming efficiency (121). Overall, these findings delineate that specific epigenetic modifiers within donor cells act as a reprogramming barrier and the inhibition of these modifiers can elicit reprogramming in an efficient way.

Apart from the different reprogramming efficiencies and kinetics that are mediated by different types of donor cells, the number of the reprogramming factors that are required for reprogramming also depends on the donor cell types. Initially, OCT4, SOX2, KLF4, and c-MYC are discovered as a combination of transcription factors that enables iPSC generation from fibroblasts (40, 44, 45). However, with the exploitation of other cell types and the discovery of other reprogramming methods, all of four factors are not necessary for inducing pluripotency and some of them can be omitted in specific cell types or specific culture conditions. For instance, NSCs endogenously express SOX2, KLF4 and c-MYC, and thus only exogenous expression of OCT4 alone was sufficient to generate iPSCs (113, 114). In addition, inducing pluripotency in melanocytes and melanoma cells does not require exogenous expression of SOX2, since these cells express SOX2 endogenously at high levels (125). Dermal papilla cells endogenously express high levels of SOX2 and c-MYC so that these cells can be reprogrammed into iPSCs with only OCT4 and KLF4 (126). Overall, these findings indicate that the number of the reprogramming factors that are required for inducing pluripotency depends on the donor cell types.

Conclusion

Ectopic expression of OCT4, SOX2, KLF4, and c-MYC can reprogram somatic cells into iPSCs. The iPSCs closely resemble ESCs in an ability to proliferate *in vitro* and to differentiate into derivatives of all three embryonic germ layers. As such, iPSCs hold great promise for regenerative medicine. Importantly, iPSCs derived directly from patients are extremely valuable, because they can give rise to disease-relevant cells carrying disease-associated phonotypes which are normally inaccessible from body of patients. Furthermore, these disease-relevant cells greatly can be used for the development of new therapeutic agents that could alleviate disease-associated phenotypes. Genetically and phenotypically corrected patient-derived cells by Crispr-Cas9 or pharmaceutical agents can be used for preclinical and clinical trials to investigate their therapeutic potential. Our recent findings together with those of other studies clearly suggest that different efficiencies and kinetics of reprogramming depends on epigenetic states, differentiation status, and phenotypic characteristics of donor cell types. Therefore, modulating epigenomes and phenotypic characteristics of donor cells by chemical intervention and introducing additional factors might enhance reprogramming competence of donor cell types that otherwise do not exhibit reprogramming capacity.

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Potential Conflict of Interest

There is no potential conflict of interest to declare.

Authors' Contribution

Conceptualization: JP, Jueun Kim. Formal analysis: JP, Jueun Kim, BS. Funding acquisition: KPK. Investigation: JP, Jueun Kim, BS. Project administration: HRS, Johnny Kim, KPK. Supervision: HRS, Johnny Kim, KPK. Writing – original draft: JP, Jueun Kim. Writing – review and editing: Johnny Kim, KPK.

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