

Review Article

Generation and clinical application of human T cell-derived induced pluripotent stem cells

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Pluripotent stem (iPS) cells are a very promising cell source for models of human genetic diseases and revolutionary new therapies. Successful reprogramming of human blood cells has been reported and is likely to advance the clinical application of iPS cells. In terms of a patient's own somatic cells, generating iPS cells from peripheral blood cells has advantages for clinical applications because these cells are an easily accessible cell source. Of the human peripheral blood cells, T cells can be readily cultured *in vitro* and proliferate rapidly. Furthermore, only a small amount of peripheral blood is needed to generate iPS cells from T cells, thus increasing the number of patients in whom the technique can be used. iPS cells that contain T-cell receptor (TCR) rearrangements in their genome also have the potential to be traceable markers when establishing novel transplantation therapies. The present review summarizes recent progress in the methods used to generate iPS cells and the future potential of human T cell-derived iPS cells.

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Generation and clinical application of human induced pluripotent stem cells

Induced pluripotent stem (iPS) cells were first generated from mouse fibroblasts by Yamanaka's research group at Kyoto University in 2006¹⁾. In 2007, this same group and Thomson's group at the University of Wisconsin independently succeeded in generating iPS cells from human dermal fibroblasts^{2,3)}. Yamanaka et al. identified 24 embryonic stem (ES) cell-specific transcrip-

tion factors as candidate factors responsible for cell reprogramming and introduced these factors into mouse fibroblasts using retrovirus vectors. This studies revealed that only four factors (Oct3/4, Sox2, Klf4 and c-Myc) were required to reprogram mouse fibroblasts into stem cells that were almost equivalent to ES cells¹⁾. Yamanaka et al. also discovered that human dermal fibroblasts could be reprogrammed using the same four factors. Because iPS cell can be generated from somatic cells, iPS cells

may overcome ethical issues associated with the use of human early embryos. In addition, the use of iPS cells makes autologous cell implantation possible. Therefore, iPS cells are a breakthrough cell source that may overcome the difficulties that arise with the use of ES cells.

There are two major approaches to the clinical application of iPS cells. First, many types of terminally differentiated cells that have been derived from iPS cells may be used cell transplantation therapy. There are considerable advantages to using iPS cells as a source of cells for transplantation, not least of which is the fact that the cells are derived from each patient's own somatic cells and proliferate infinitely. Second, iPS cells that have been generated from patients with particular genetic diseases may be used to create an *in vitro* disease model. Specific lineage cells with a particular disease phenotype that have come from patient-specific iPS cells may also be useful in drug screening. This could contribute to drug discovery and clarification of disease mechanisms, and may also lead to personalized medicine.

Currently, there are many methods for the generation of human iPS cells. Many combinations of reprogramming factors, many vehicles for the introduction of transgenes, and many types of somatic cells in which reprogramming factors have been introduced have been reported⁴. Although each of these methods has its own merits and drawbacks, it remains unclear which method is best. After the initial publication by Takahashi et al. of the combination of four factors (Oct3/4, Sox2, Klf4, and c-Myc) required for reprogramming¹, subsequent studies reported different combinations that reprogrammed somatic cells more efficiently⁵⁻⁷. The search for better combinations of reprogramming factors has made steady progress. Furthermore, many different types of vehicles have been used to introduce reprogramming factors into somatic cells to generate iPS cells, with research into transgene delivery systems a rapidly advancing field with a view to the clinical application of iPS cells. At first, reprogramming factors were delivered into somatic cells using moloney murine leukemia virus (MMLV)-derived retroviruses, such as pMXs^{2,8,9}; the transgenes delivered with these retrovirus vectors are usually silenced in pluripotent stem cells such as iPS and ES cells^{10,11}. In addition, lentiviral vectors have been used successfully to generate iPS cells from somatic cells^{3,12}. In iPS cells that have been generated with genome integration, transgenes have the potential to break the genes located near the insertion site. Although these transgenes are silenced through reprogramming in high-quality iPS cells, there is always the possibility that the delivered transgenes may be reactivated after cell reprogramming and that transgene reactivation may drive

oncogenesis¹³. Therefore for the clinical application of iPS cells, the insertion of transgenes into the genome is best avoided. Several methods for generating transgene-free iPS cells have been described, such as using a Cre-deletable lentivirus system¹⁴, piggyBac transposon¹⁵⁻¹⁷, adenovirus vectors¹⁸, Sendai virus vectors^{19,20}, mRNAs²¹, episomal vectors²²⁻²⁵, and recombinant proteins^{26,27}, but it remains unclear which method is best. Each of these delivery systems has its own advantages and disadvantages, but further studies are needed to determine the method most suitable for clinical application.

Generating iPS cells from hematopoietic stem cells

To generate iPS cells from patients in the clinical setting, the most appropriate type of donor cell/s needs to be taken into consideration, with the least invasive approach used to collect tissues from patients. Regardless of whether the iPS cells generated are to be used in transplantation therapy or disease research, a less invasive approach is most suitable in the clinical setting. Studies into human cell reprogramming have reported the successful reprogramming of many types of human somatic cells since the first reports of the generation of iPS cells from human dermal fibroblasts^{2,3}. In terms of clinical applications, blood cells are an attractive cell source because of the ease of sampling.

In the first report of the generation of iPS cells from human blood cells²⁸, Loh et al. transduced four factors (i.e. Oct3/4, Sox2, Klf4, and c-Myc) into CD34-positive mobilized human peripheral blood cells with retroviral vectors. iPS cells were obtained from CD34-positive cells with an efficiency of 0.01%–0.02%. In that study, mobilized peripheral blood was obtained from a donor who had received injections of granulocyte colony-stimulating factor for 3 days prior to blood sampling²⁸. However, this method has the disadvantages that it requires a relatively large amount of peripheral blood as well as the pharmacological pretreatment of patients. Shortly after that study was published, several groups reported successful reprogramming of human cord blood-derived stem cells^{29,30}. Advantages of using cord blood-derived cells include the fact that mobilization and/or biopsy is not necessary and that primary cultures do not need stochastic cell outgrowth. In addition, cord blood cells are extremely young cells, which reduces the possibility of them having accumulated genetic mutations. Another advantage is that cord blood is already banked, along with immunological information³¹. Because of this, cord blood is as an appealing cell source of iPS cells. However, sampling cord blood will not contribute to disease research in that blood will be obtained from only patients with

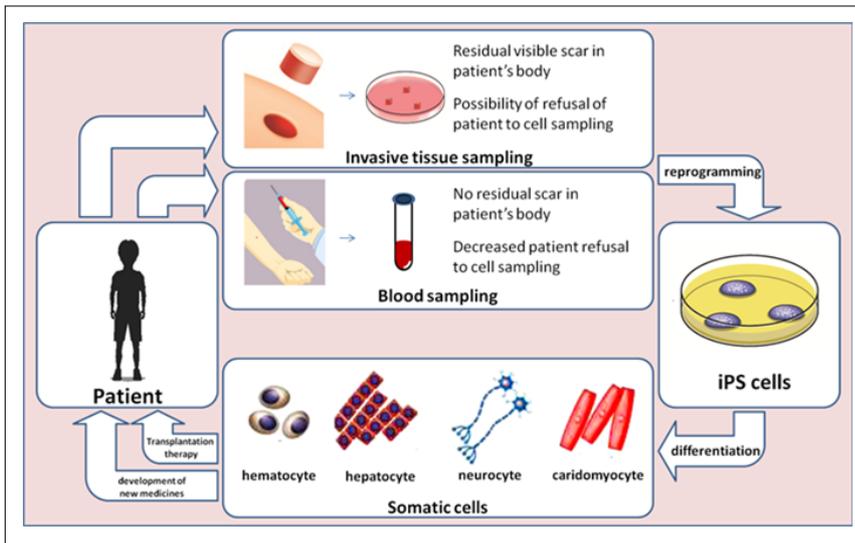


Fig.1 Generation of induced pluripotent stem (iPS) cells using a small amount of blood
 Methods of iPS cell generation that require only a relatively small amount of peripheral blood and do not require the pharmacological pretreatment of patients have considerable advantages for clinical practice. Sampling of peripheral blood is one of the least invasive procedures performed routinely in clinics and is therefore likely to increase the number of patients who can be sampled for the generation of iPS cells.

specific conditions.

Generating iPS cells from T cells

T cells are also an appealing source of cells that can be made to proliferate easily *in vitro* using a plate-bound anti-CD3 monoclonal antibody and interleukin (IL)-2³². The CD3 protein exists in the complex of T cell receptor (TCR) proteins on the cell surface of T cells and can be used as T cell-specific marker. Anti-CD3 antibody modulates the TCR-CD3 complex, which results in T cell proliferation and activation³³. IL-2 activates general T cell signaling pathways, leading eventually to the promotion of cytokine transcription, survival, cell cycle entry, and growth³⁴. Thus, a sufficient number of T cells can be obtained to generate iPS cells from a small amount of peripheral blood. Compared with previous non-invasive methods based on, for example, keratinocytes obtained from a single plucked hair³⁵ or dental tissue stem cells obtained from an extracted tooth³⁶, small amounts of peripheral blood are easy to obtain and T cell proliferation does not need stochastic cell outgrowth. Therefore, the use of T cells has several advantages in terms of the clinical application of iPS cells compared with other methods used in the past.

However, in studies in which monoclonal mice have been generated by nuclear transfer from mature lymphocytes, reprogramming a terminally differentiated T or B cell nucleus was found to be less efficient than reprogramming nuclei from other donor cell populations³⁷. Using a secondary iPS system to compare the reprogramming of different types of hematopoietic cells, Eminli et al. reported that peripheral terminally differentiated blood cells, such as mature lymphocytes, were difficult to repro-

gram compared with hematopoietic stem cells³⁸. In addition, Hong et al. reported that terminally differentiated T cells require p53 knock out for reprogramming into iPS cells³⁹: in their experiments, Hong et al. were not able to obtain iPS colonies from mouse T cells using the four transcription factors Oct4, Sox2, Klf4, and c-Myc without p53 knock out. Furthermore, Hanna et al. have reported the reprogramming of mouse terminally differentiated B cells, which are similar to T cells in that they are terminally differentiated cells that contain rearrangements in their genome⁴⁰. Hanna et al. used four transcription factors, Oct4, Sox2, Klf4, and c-Myc, to reprogram mouse B lymphocytes with doxycycline-inducible lentiviral vectors⁴⁰. Although these factors were sufficient to reprogram non-terminally differentiated B cells, reprogramming of mature B cells required additional ectopic expression of the myeloid transcription factor CCAAT/enhancer-binding-protein α (C/EBP α) or specific knockdown of the B cell transcription factor Pax5. Therefore, although T cells are a readily available cell source, as mentioned above, they are considered difficult to reprogram using only the four transcription factors Oct4, Sox2, Klf4, and c-Myc.

Recently, human peripheral blood cells, including peripheral T cells, have been successfully reprogrammed with the exogenous expression of only four transcription factors, namely Oct4, Sox2, Klf4, and c-Myc, and the technique has been reported as being a minimally invasive method of generating human iPS cells^{20, 41-44}. In these studies, mononuclear blood cells were obtained from donors and frozen samples, with the four factors introduced using either a retrovirus^{41, 44}, lentivirus^{42, 43}, or Sendai virus²⁰. In our experiments using Sendai virus, which was efficiently transduced into human activated T cells and was able to

express exogenous genes⁴⁵), the reprogramming efficiency of T cells was increased up to 0.1%, exceeding the reprogramming efficiency of a combination of fibroblasts and retrovirus. These methods have the advantages of needing relatively less peripheral blood and not requiring the pharmacological pretreatment of patients. In particular, these methods are useful for generating iPS cells from patients who have specific genetic diseases. Sampling of peripheral blood is one of the least invasive procedures performed routinely in clinics and so these methods could increase the number of patients from whom samples are taken for the generation iPS cells (Fig. 1).

T cell-derived iPS cells as a cell source for clinical therapy

There are two scenarios in which iPS cells can be used as a cell source for transplantation therapy: autotransplantation and allotransplantation. Each of these has advantages for clinical application and both are expected to develop into novel therapies. In the case of the autotransplantation of iPS cells, the iPS cells are generated from a patient's own cells, differentiated into the required cell type/s, and transplanted back into the patient. Autotransplantation of iPS cells has the advantage of avoiding immune rejection, rendering combination immunosuppressive treatment unnecessary. Allotransplantation of iPS cells involves the transplantation of iPS cells derived from an allogenic donor who has foreign human leukocyte antigen (HLA). For use in the clinical setting, this type of transplantation requires the formulation of strategies to avoid the host immune response against transplanted tissue. T cell-derived iPS cells can be generated using less invasive methods and are a suitable cell source for autotransplantation therapy, although the issue of a relatively high cost needs to be addressed before the technique can be applied in the clinical setting. T cell-derived iPS cells also has the advantage of minimal invasiveness in providing HLA-matched tissue for the target population.

The existence of TCR rearrangements has to be considered when using T cell-derived iPS cells. T cell-derived iPS cells that contain TCR rearrangements in their genome have already been demonstrated to be able to differentiate into three germ layer tissues *in vitro* and *in vivo*^{20, 41-43}. In a mouse model, a potential new immune therapy using natural killer T (NKT) cell-derived iPS cells has been reported⁴⁶. In that study, Watarai et al. successfully generated functional NKT cells *in vitro* from splenic NKT cell-derived iPS cells and demonstrated the clinical potential of iPS cell-derived NKT cells to suppress the growth of a syngeneic tumor *in vivo*. Together, these studies demonstrate

the potential of establishing new autotransplantation immune therapies using monoclonal T cells.

In addition, TCR rearrangements in iPS cells may be used as traceable markers when establishing novel transplantation therapy. Using currently available technology, there is no procedure to follow the progeny of iPS cell-derived differentiated cells after their transplantation into patients. This is important because these cells have the potential to form malignant or benign tumors. In animal models, several marker genes can be used to chart the progression and consequences of iPS cell-derived cell transplantation, such as green fluorescent protein (GFP) and luciferase. However, in the clinical setting, the insertion of exogenous marker genes into the genomes of iPS cells is undesirable. T cell-derived iPS cells already have a traceable genetic signature through rearrangements of the TCR locus. Consistent with this idea, teratomas derived from T cell-derived iPS cells have been shown to have the same signature as undifferentiated T cell-derived iPS cells^{20, 41}. Therefore, the descendants of T cell-derived iPS cells can be identified by analysis of the patterns of TCR rearrangement.

T cell-derived iPS cells as a cell source for disease research

Another possible application of iPS cells is in disease modeling. Patient-specific iPS cells that carry disease-specific genetic information would be invaluable tools for investigations into the pathogenesis of specific diseases and for drug screening. Several studies in which disease-specific iPS cells have been generated have reported the successful modeling of inherited diseases⁴⁷. The use of T cell-derived iPS cells would increase the number of patients who could be sampled for disease research because of the minimally invasive techniques involved. As noted above, obtaining samples of peripheral blood is one of the least invasive procedures performed routinely in the clinical setting and, although the detailed effects of genome rearrangements on iPS cells need to be determined, methods of generating iPS cells from T cells have the advantages of not requiring a large sample of peripheral blood and not requiring the pharmacological pretreatment of patients.

Conclusion

There are several advantages to using T cell-derived iPS cells in the clinical application of human iPS cells. In particular, the minimal invasiveness associated with the sampling of peripheral blood is appealing in the clinical setting. Despite the fact that there are some issues that require clarification, such as the

differences in reprogramming of mouse and human T cells and the effects of genome rearrangements in iPS cells, the advantages associated with the use of T cell-derived iPS cells may result in considerable advances in regenerative medicine.

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Disclosure of conflict of interests

The authors declare no competing financial interests.

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