Brief Report

Reprogramming of T Cells
from Human Peripheral Blood

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Human induced pluripotent stem cells (iPSCs) derived from somatic cells of patients hold great promise for modeling human diseases. Dermal fibroblasts are frequently used for reprogramming, but require an invasive skin biopsy and a prolonged period of expansion in cell culture prior to use. Here, we report the derivation of iPSCs from multiple human blood sources including peripheral blood mononuclear cells (PBMCs) harvested by routine venipuncture. Peripheral blood-derived human iPSC lines are comparable to human embryonic stem cells (ESCs) with respect to morphology, expression of surface antigens, activation of endogenous pluripotency genes, DNA methylation, and differentiation potential. Analysis of immunoglobulin and T cell receptor gene rearrangement revealed that some of the PBMC iPSCs were derived from T cells, documenting derivation of iPSCs from terminally differentiated cell types. Importantly, peripheral blood cells can be isolated with minimal risk to the donor and can be obtained in sufficient numbers to enable reprogramming without the need for prolonged expansion in culture. Reprogramming from blood cells thus represents a fast, safe, and efficient way of generating patient-specific iPSCs.

Somatic cells can be induced to the pluripotent state by the enforced expression of several transcription factors including OCT4, SOX2, KLF4, MYC, NANOG, and LIN28 (Takahashi et al., 2007; Yu et al., 2007; Park et al., 2008a).

Human iPSCs are commonly generated from dermal fibroblasts harvested by surgical skin biopsy (Park et al., 2008b). Exposure of the dermis to ultraviolet light increases the risk for chromosomal aberrations (Ikehata et al., 2003), raising concerns for whether iPSCs will reflect the patient's constitutional genotype. For routine clinical application, it would be desirable to reprogram cell types that are safe and can be collected noninvasively in large numbers.

Blood is a cell source that can be easily obtained from patients. Mouse B and T cells are amenable to reprogramming by overexpressing Oct4, Sox2, Klf4, and Myc with the ectopic expression of Cepbα and p53 knockdown, respectively (Hanna et al., 2008; Hong et al., 2009). iPSC lines have also been generated from mouse bone marrow progenitor cells (Okabe et al., 2009). We have previously reprogrammed cytokine-mobilized human CD34+ peripheral blood cells to pluripotency, but such harvests are cumbersome, expensive, and time consuming (Loh et al., 2009). Several recent studies reported the generation of iPSCs from human bone marrow and cord blood (Ye et al., 2009; Giorgetti et al., 2009; Haase et al., 2009), but bone marrow harvesting is an invasive procedure, and cord blood is available for only a minority of individuals who have their samples banked at birth. A recent study with peripheral blood from donors with myeloproliferative disorder (MPD) isolated iPSC colonies that

contain the JAK2-V617F mutation (Ye et al., 2009), but MPD is characterized by abnormally high numbers of circulating CD34+ cells from the bone marrow. These previous studies demonstrating successful reprogramming of blood cells into iPSCs have relied on specialized blood cell sources with high proliferative potential.

CD34+ hematopoietic stem/progenitor cells mobilized into the donor's peripheral blood by pretreatment with granulocyte colony-stimulating factor (G-CSF) can be successfully reprogrammed to pluripotency (Loh et al., 2009). To test whether we can reprogram cells from routine peripheral blood (PB) sources, we obtained CD34+ purified blood samples from a healthy 49-year-old male donor who had undergone simple apheresis without cytokine priming. We also isolated mononuclear cells (PBMCs) from the peripheral blood samples collected by venipuncture of four healthy donors (28- to 49-years-old) via Ficoll density centrifugation.

To induce reprogramming of enriched CD34+ blood cells, we infected with lentiviruses expressing OCT4, SOX2, KLF4, and MYC reprogramming factors (Figure 1A). Colonies with well-defined hESC-like morphology were first observed 21 days after transduction (Figure 1B). For reprogramming of fresh peripheral blood mononuclear cells (PBMCs), we employed two rounds of lentiviral infection (day 0 and day 8) and isolated colonies
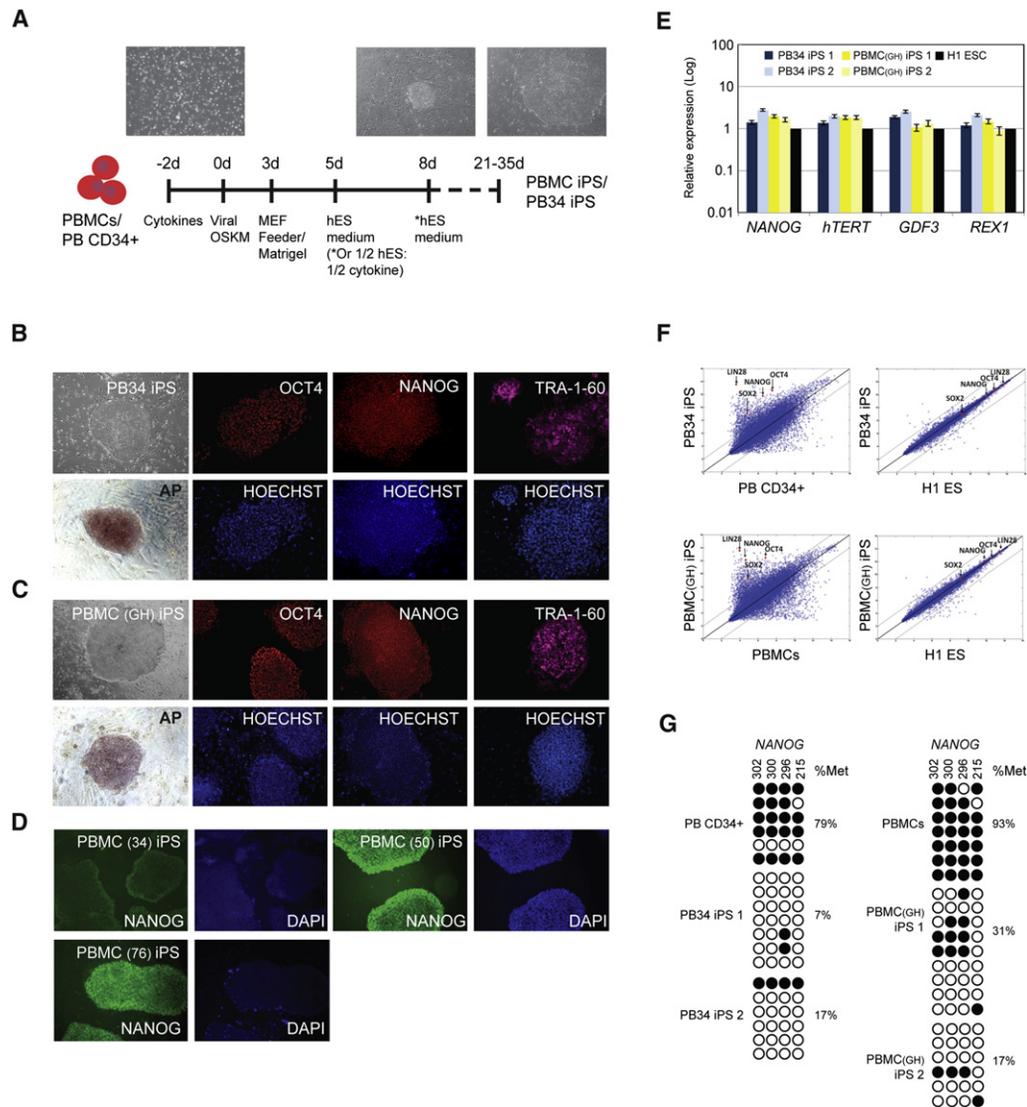
Figure 1. Reprogramming of Peripheral Blood Cells to Pluripotent iPSCs

(A) Scheme for reprogramming human peripheral blood (PB) mononuclear cells (PBMCs) and CD34^{+} cells (PB CD34^{+}). Morphology of the typical peripheral blood cells and images of hESC-like iPSC colonies are shown.

(B) Images of PB34 iPSC colonies. Bright-field images were acquired with a standard microscope (Nikon, Japan) with a 10× objective. Immunohistochemistry of PB-derived iPSC colonies expressing markers for OCT4, NANOG, Tra-1-60, and alkaline phosphatase (AP). Hoechst staining indicates the total cell content per field. Fibroblasts surrounding human iPSC colonies serve as internal negative controls for immunohistochemistry staining. Images were acquired with a standard microscope (Nikon, Japan) with a 10× objective.

(C) Images of PBMC (Donor GH) iPSC colonies. Bright-field images were acquired with a standard microscope (Nikon, Japan) with a 10× objective. Immunohistochemistry of PB-derived iPSC colonies expressing markers for OCT4, NANOG, Tra-1-60, and alkaline phosphatase (AP). Hoechst staining indicates the total cell content per field.

(D) Images of PBMC (donors 34, 50, 76) iPSC colonies. Bright-field images were acquired with a standard microscope (Nikon, Japan) with a 10× objective. Immunohistochemistry of PB-derived iPSC colonies expressing marker for NANOG. DAPI staining indicates the total cell content per field.

(E) Quantitative reverse transcription-PCR analyses for the expression of ESC-marker genes *NANOG*, *hTERT*, *GDF3*, and *REX1* in PB CD34^{+} and PBMC-derived iPSCs and human H1 ESCs (with their respective standard errors). Individual PCR reactions were normalized against β-*ACTIN* and plotted (Log$_{10}$ scale) relative to the expression level in the H1 ESCs, which was set to 1.

(F) Scatter plots comparing PB34 iPSCs and PBMC iPSCs global gene expression profiles to parental (left) and H1 human ESCs (right). The black lines indicate the linear equivalent and 2-fold changes in gene expression levels between the paired cell types. Positions of pluripotency genes *OCT4*, *SOX2*, *NANOG*, and *LIN28* in scatter plots are indicated.

(G) Bisulfite genomic sequencing of the *NANOG* promoters reveals demethylation in the iPSC lines. Each horizontal row of circles represents an individual sequencing reaction for a given amplicon. Open and filled circles represent unmethylated and methylated CpGs dinucleotides, respectively. Percentage of methylation is indicated for each cell line.

For further characterization of the peripheral blood-derived iPSC clones, see also Figure S1 and Table S1.

with distinct flat and compact morphology with clear-cut round edges reminiscent of hESCs after a slightly longer latency of around 35 days (Figure 1C). Interestingly, a previous study with a single round of lentiviral infection of PBMCs failed to observe iPSC colony formation (Haase et al., 2009). In a separate set of experiments, we tested the ability of retroviruses encoding the human reprogramming factors to generate iPSCs from human PBMCs, and despite low infection efficiency, we observed iPSC colonies after 25–35 days (Figure 1D).

With immunohistochemistry and flow cytometry, we analyzed the iPSC lines for expression of markers shared with hESCs. Consistent with their hESC-like morphology, both PB34 iPSCs and PBMC iPSCs stained positive for Tra-1-81, NANOG, OCT4, Tra-1-60, SSEA4, and alkaline phosphatase (AP) staining (Figures 1B–1D; Figures S1A–S1C available online; Chan et al., 2009). We routinely observed a reprogramming efficiency of 0.002% for PB CD34$^+$ cells (Table S1), comparable to prior experience with primary fibroblasts, mobilized PBMCs, and cord blood cell reprogramming (Takahashi et al., 2007, Park et al., 2008a, Loh et al., 2009; Haase et al., 2009). For PBMCs, we obtained hESC-like colonies at the lower efficiency of 0.0008%–0.001% (Table S1).

We further characterized the PB34 iPSC and PBMC iPSC lines for properties specific to hESCs. Efficient transgene silencing is essential for the derivation of pluripotent iPSC lines (Brambrink et al., 2008). qRT-PCR via primers specific for endogenous and total transcripts of the reprogramming factors confirmed that OCT4, SOX2, KLF4, and MYC transgenes were efficiently silenced in the blood-derived iPSCs (Figure S1D). Additional analysis via quantitative PCR revealed the activation of pluripotency markers NANOG, hTERT, REX1, and GDF3 to a level similar to the expression in H1 hESCs (Figure 1E).

We next performed global gene expression analysis of the peripheral blood-derived iPSCs comparing it to hESCs, fibroblast iPSCs, and somatic parental cells. Clustering analysis revealed a high degree of similarity among the reprogrammed iPSCs (dH1F-iPS, PBMC iPS1, PB34 iPS1, PB34 iPS2), which clustered together with the H1 and H9 ESCs and

were distant from the parental somatic cells, as determined by a Euclidean distance metric (Figure S1E). Analysis of scatter plots similarly shows a tighter correlation among reprogrammed iPSCs (PB34 iPSCs, PBMC iPSCs) and human ESCs (H1 ESCs) than between differentiated parental cells and their reprogrammed derivatives (Figure 1F). Consistent with the activation of endogenous pluripotency-associated gene expression, reprogramming of the blood cells was accompanied by the demethylation of CpG dinucleotides at the NANOG promoters (Figure 1G). Moreover, cytogenetic analysis showed normal karyotypes for the iPSC lines (Figure S1F).

Next, we evaluated the developmental potential of the iPSC lines by in vitro embryoid body differentiation, hematopoietic colony forming assays, and in vivo teratoma induction. The iPSCs readily formed embryoid bodies upon induction (Figure S2A). qRT-PCR of the differentiated cells showed strong suppression of the pluripotency genes and activation of lineage-specific genes representing the three germ layers (Figures S2B and S2C). Hematopoietic differentiation of iPSC lines resulted in erythroid, myeloid, and granulocytic colony formation (Figures 2A and 2B). Interestingly, all PB CD34$^+$-derived iPS lines we tested show greater hematopoietic colony forming activity than PBMC iPSCs (Figure 2A).

The most rigorous test for pluripotency of human ESCs is the formation of teratomas in immunodeficient mouse hosts (Lensch et al., 2007). Upon subcutaneous injection into immunodeficient $Rag2^{-/-} \gamma c^{-/-}$ mice, the iPSC lines generated well-differentiated cystic teratomas representing all three embryonic germ layers (Figures 2C and 2D). DNA fingerprinting analysis verified that these cells were indeed derived from the parental blood cells and not a result of contamination from existing hESC or iPSC lines (Table S2). The iPSC clones have been propagated for at least 20 passages as of this submission.

Because peripheral blood mononuclear cells consist of both myeloid and lymphoid elements (Figure S2D), we were interested in determining the lineage of origin of the reprogrammed cells. We tested the iPSC clones for the presence of functionally rearranged immunoglobulin and T cell receptor genes by using

probes specific for IgH, TCR-δ, and TCR-β2. Among 12 independent clones from 3 separate individuals, we failed to detect IgH recombination, indicating that none of our lines arose from B lymphocytes (Figure S2E). As reported for the mouse, reprogramming human B lymphocytes may require additional factors like CEBPα (Hanna et al., 2008). Next, we analyzed the iPSC lines for TCR-δ and TCR-β2 recombination (Figures 2E and 2F; Figure S2F). No PBMC iPSC lines demonstrated TCR-β2 recombination, whereas six of seven PBMC iPSC lines isolated from a single donor sample exhibited rearrangement of the TCR-δ locus, indicative of derivation from cells of the T lineage (Figure 2F). In contrast, PBMC iPSC lines from donors 34 and 76 lacked rearrangement of IgH, TCR-δ, and TCR-β2, indicating derivation from nonlymphoid lineages (Figure 2F; Figures S2E and S2F).

Isolation of iPSCs from T lymphocytes represents definitive proof that even terminally differentiated human cells are susceptible to reprogramming to pluripotency. Distinct protocols of cytokine stimulation and viral infection of the PBMC cells may predispose to derivation from lymphoid versus nonlymphoid hematopoietic cells from peripheral blood sources, as can preselection of lymphoid target cells prior to reprogramming (Hong et al., 2009). PBMCs from donor GH were grown in medium containing IL-3, which is known to stimulate the growth of subsets of CD4$^+$ T cells (Figure S2D; Mueller et al., 1994). In contrast, PBMCs from donors 34 and 76 were cultured in medium promoting expansion of dendritic cells and yielded iPSCs with germline IgH and TCR alleles. For applications in regenerative medicine, iPSCs containing antibody or T cell receptor gene rearrangement may be undesirable (Serwold et al., 2007).

In conclusion, we have successfully reprogrammed cells from peripheral blood sources including samples obtained through routine venipuncture. Our study provides a strategy for the reliable generation of induced pluripotent stem cells from peripheral blood mononuclear cells. Although the per-cell derivation efficiency is low, peripheral blood is an accessible source of a large number of primary cells (easily 10^5–10^6), thus enabling reliable iPSC isolation from only
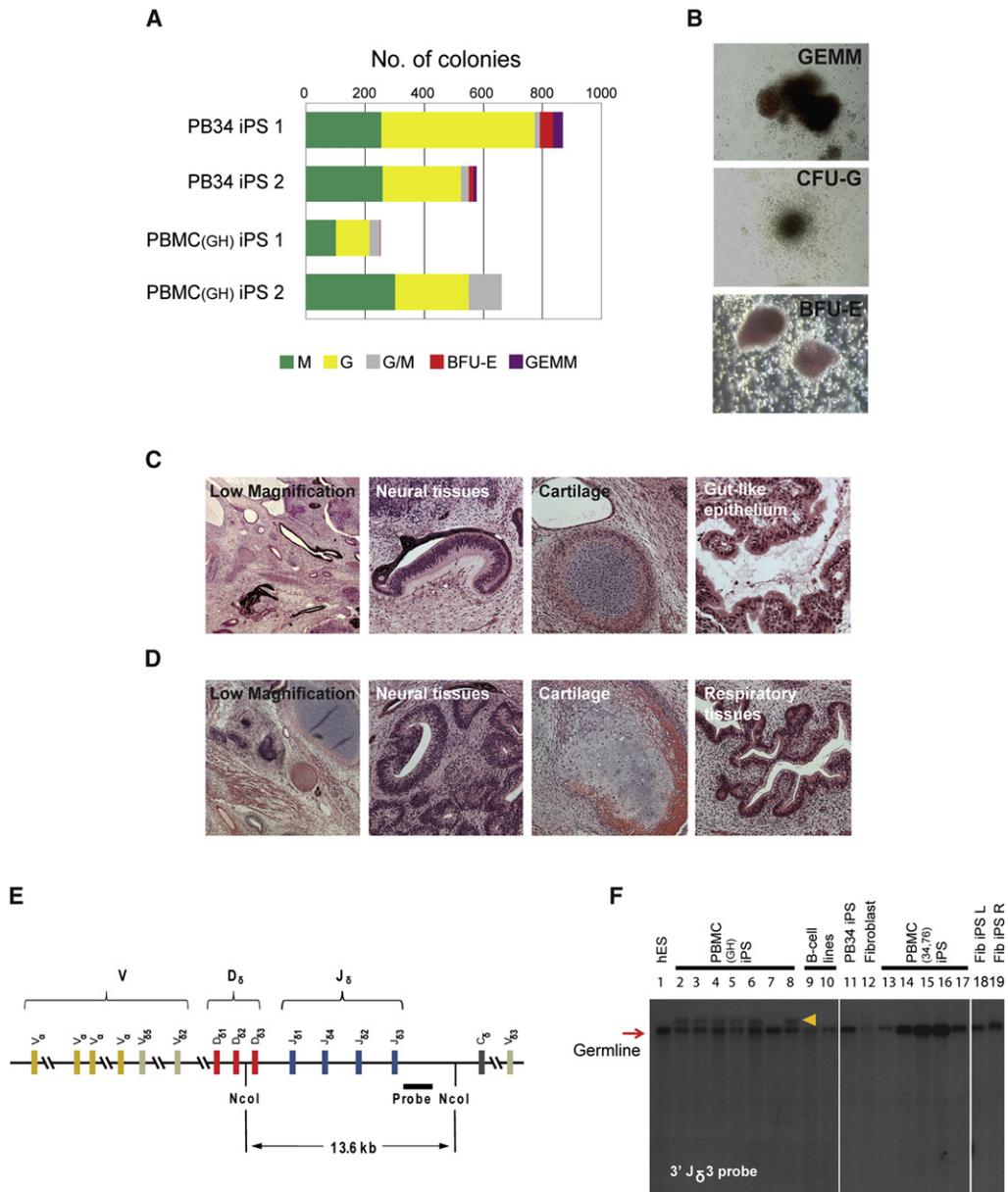
Figure 2. Pluripotency and V(D)J Rearrangement of Peripheral Blood-Derived iPSCs

(A) Embryoid bodies derived from PB34 and PBMC iPSCs yield hematopoetic colonies in semisolid methylcellulose media: burst forming unit-erythroid (BFU-E), colony forming unit-granulocyte (CFU-G), colony forming unit-macrophage (CFU-M), colony forming unit-granulocyte, macrophage (CFU-GM), and colony forming unit-granulocyte, erythroid, macrophage (CFU-GEMM). Total number of each type of colony was counted.

(B) Representative images of various types of hematopoietic colonies. Images were acquired with a standard microscope (Nikon, Japan) with a 20× objective.

(C and D) Hematoxylin and eosin staining of teratomas derived from immunodeficient mice injected with PB34 iPSCs (C) and PBMC iPSCs (D) show tissues representing all three embryonic germ layers.

(E) Genomic DNA from peripheral blood-derived iPSC lines grown was digested with NcoI and analyzed for V(D)J rearrangements at the TCR-δ (T cell receptor Delta) locus by Southern blotting with a 3'Jδ3 probe.

(F) TCR-δ V(D)J recombination of blood-derived iPSC clones. Lanes 2–8 and lanes 13–17 are PBMC iPSC lines. B cell lines on lanes 8 and 9 showed no rearrangement. TCR-δ rearrangement was observed for some PBMC-derived iPSC lines (lanes 2–6 and 8). Lanes 1, 11, 12, 18, and 19 are H1 hESCs, PB34 iPSCs, fibroblast cells, fibroblast-derived iPSCs via retrovirus and lentivirus, respectively. The red arrow indicates expected size of the germline band. Orange arrow indicates rearranged bands.

For further information on the pluripotency, V(D)J rearrangement, and fingerprint analysis performed on the peripheral blood-derived iPSC clones, see also Figure S2 and Table S2.

a few milliliters of whole blood. Future application of viral and transgene-free reprogramming or protein transduction (Kaji et al., 2009; Woltjen et al., 2009; Yu et al., 2009; Kim et al., 2009; Zhou et al., 2009) to peripheral blood reprogramming will greatly facilitate the development of efficient and safe ways of generating patient-specific pluripotent stem cells.

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Note Added in Proof

A manuscript has appeared online demonstrating isolation of iPSCs from peripheral blood, including a single line that showed evidence for both TCR-β and TCR-γ rearrangement by PCR (Kunisato, A., Wakatsuki, M., Shinba, H., Ota, T., Ishida, I., and Nagao, K. [2010]. Direct generation of induced pluripotent stem cells from human non-mobilized blood. Stem Cells Dev., in press. Published online May 24, 2010. 10.1089/scd.2010.0063).