Patient-Specific Pluripotent Stem Cells Become Even More Accessible

Shinya Yamanaka[1,2,*]
[1]Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto 606-8507, Japan
[2]Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA
*Correspondence: yamanaka@cira.kyoto-u.ac.jp
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In this issue of *Cell Stem Cell*, Staerk et al. (2010), Seki et al. (2010), and Loh et al. (2010) each describe the derivation of human iPSCs from peripheral blood. Although seemingly incremental, this advance brings the stem cell field an important step closer to eventual clinical use.

In this issue of *Cell Stem Cell*, three groups report the generation of human induced pluripotent stem cells (iPSCs) from peripheral blood cells obtained from individuals who had received no pretreatment (Staerk et al., 2010; Seki et al., 2010; and Loh et al., 2010). Human iPSCs were originally generated by introducing just a few defined factors into skin fibroblasts (Takahashi et al., 2007; Yu et al., 2007) and have since been derived from various lineages and sources of somatic cells, including cytokine-mobilized peripheral blood cells (Loh et al., 2009). Therefore, from a scientific point of view, the achievements described in these three new papers may seem to represent a relatively small step forward. However, practically and technically speaking, their findings represent a huge and important progression in the field.

Patient-specific iPSCs provide unprecedented opportunities not only in regenerative medicine but also in other medical sciences, drug discovery, and toxicology. Stem cell therapies using patient-specific iPSCs would be free from immune rejection and ethical issues regarding the usage of human embryos (Yamanaka, 2009). Patient-specific iPSCs, especially those derived from individuals suffering from monogenic diseases, are extremely useful in constructing in vitro models, which facilitate understanding of pathological mechanisms and screening for more effective and safer drugs. Many laboratories and hospitals all over the world are now generating iPSCs from patients with a wide range of diseases.

In most cases to date, skin fibroblasts are the cell type from which patient iPSCs are generated. Acquiring a sample of this sort generally involves performing a skin biopsy, which requires patients to undergo procedures such as local anesthesia, an incision, and suturing. None of these interventions are free from potential complications, particularly risk of infection. In our experience, it is relatively easy to obtain consent for this process from patients suffering from rare and monogenic diseases. It has been more difficult, however, to obtain permission from patients suffering from multifactorial and common diseases and also from healthy individuals who are needed to donate tissue samples for use in deriving control iPSC lines. In addition, even relatively noninvasive procedures such as a skin biopsy may worsen the symptoms of some diseases, such as fibrodysplasia ossificans progressiva (FOP). Another concern about using skin as a source for iPSC line derivation is the risk that the starting cells harbor chromosomal aberrations caused by UV irradiation. After biopsy, it takes at least a month to expand fibroblasts for iPSC induction. Undesired mutations may occur during this period. These limitations prevent many scientists from utilizing the iPSC technology.

Sampling of peripheral blood is one of the most commonly performed and least invasive clinical procedures. In 2009, granulocyte colony stimulating factor (G-SCF) mobilized $CD34^+$ blood cells were used as a source to derive hiPSCs (Loh et al., 2009). However, such pretreatment is expensive and time-consuming and may have some detrimental effects on donors. For these reasons, this procedure has not been widely conducted for the purposes of cell harvesting for biopsies or iPSC derivation. Thus, the generation of iPSCs from a small amount of peripheral blood collected from non-pretreated donors is an important step in facilitating the usage of iPSC in the various applications described above. Instead of requiring patients to undergo an invasive skin biopsy, all we need may be a small amount (as little as 1 ml) of extra blood sample. Importantly, additional procedures are not necessary, given that blood sampling is routinely conducted on patients and also on healthy people at routine medical checkups. Furthermore, blood sampling is significantly less expensive than performing a skin biopsy. Finally, according to the methods described by the Jaenisch, Fukuda, and Daley groups, iPSCs can be induced within several days after blood sampling, thus the risk of undesired mutations can be minimized. It is reasonable to predict that the field may see a dramatic shift from using skin fibroblasts to peripheral blood as a source of iPSCs in the very near future.

The three groups all used the original four factors (Oct4, Sox2, Klf4, and c-Myc) (Takahashi and Yamanaka, 2006) to derive iPSCs. In two of the three reports (Staerk et al., 2010 and Loh et al., 2010), the reported efficiency of iPSC generation from blood cells is lower than that typically determined with fibroblasts (Maherali and Hochedlinger, 2008), and thus relatively large volume blood samples (30 to 350 ml) were used by these groups. Lentiviruses or retroviruses were used to introduce the four factors to the target blood cells. In these two studies, the authors showed that iPSC clones were derived from terminally differentiated T cells as well as nonlymphoid lineage (possibly myeloid cells).

In the third paper by Seki et al. (2010), the induction efficiency is much higher

and only 1 ml of blood sample was used. The four factors were introduced with Sendai viruses. In this study, all the iPSC clones examined were proven to derive from terminally differentiated T lymphocytes. This efficiency and specificity of iPSC induction is probably attributable to efficient gene delivery and expression of transgenes in human T cells by Sendai viruses. The report by Seki et al. is also attractive because Sendai viruses can provide integration-free iPSC clones.

Several issues must still be resolved prior to the wide adoption and use of iPSCs derived from peripheral blood cells. First, the differentiation ability of these iPSCs should be examined further. PB-derived iPSCs may retain epigenetic memories of having been blood cells and therefore may exhibit better differentiation into hematopoietic lineages, relative to other cell types. Overcoming any safety issues is another important challenge. We have shown previously that the cell of origin has a huge impact on the safety of resulting iPSCs, in that mouse iPSC clones from adult tail tip fibroblasts contained a significantly larger proportion of cells refractory to differentiation cues than did those lines derived from embryonic fibroblasts (Miura et al., 2009). Thus, the safeness of iPSC clones from adult terminally differentiated blood cells should be carefully evaluated. Another issue is how long and to what extent iPSC clones from terminally differentiated cells can be expanded. Finally, the impact of the presence of preexisting TCR rearrangements on the properties of iPSC needs to be determined (Serwold et al., 2007). If these issues are resolved, the use of skin biopsies for iPSC generation may become a thing of the past.

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Note Added in Proof

A paper related to these three studies recently appeared online ahead of editing. The authors generated hiPSCs from nonmobilized blood at low efficiency using retroviral transduction of OCT3/4, SOX2, KLF4, and c-MYC (Kunisato, A., Wakatsuki, M., Shinba, H., Ota, T., Ishida, I., and Nagao, K. [2010]. Direct generation of induced pluripotent stem cells from human nonmobilized blood. Stem Cells Dev., in press. Published online May 24, 2010. 10.1089/scd.2010.0063).

Tumor Oncogenotypes and Lung Cancer Stem Cell Identity

James P. Sullivan[1] and John D. Minna[1,*]
[1]The Hamon Center for Therapeutic Oncology Research, Simmons Cancer Center, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA
*Correspondence: john.minna@utsw.edu
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In this issue of *Cell Stem Cell*, Curtis et. al. (2010) reveal that the identities of lung cancer stem cell populations differ depending on the specific tumor oncogenotype in three murine lung adenocarcinoma models. These findings highlight the importance of determining the cancer stem cell oncogenotype for genotypically diverse malignancies.

According to the cancer stem cell model, only self-renewing, stem-like tumor cells possess the capacity to initiate tumor formation and distant metastases. In this regard a stem cell hierarchy, possibly inherited from a transformed adult stem cell, is thought to exist in many cancers, and that long-term clinical remission can only be achieved in these cancers by eliminating or incapacitating the cancer stem cell population. Indeed, the prospect for stem cells serving as the driving force for tumor initiation and progression has lead to a concerted effort to identify and characterize stem cells and cancer stem cells in many tissue types. Although cancer stem cells have been identified and described in numerous human malignancies, the biology of lung cancer stem cells remains less well studied. This difference is due in part to the lack of validated