ratios were detected in 253G1-derived samples around day 20 of differentiation (Fig. 6A and B), whereas no such cells were detected in 201B7-derived samples (data not shown). Interestingly, hemophagocytosis-like scenes, where the small cells were phagocytized by macrophages, were often observed (Fig. 6C). Because the morphologies of the small cells resembled to hematopoietic stem/progenitor cells, we checked their colony-forming activities (Fig. 6D). Colony assays performed around day 20 indicated that 253G1-derived cells had comparable CFU-G ($3.3 \pm 2.3/10^4$ cells; $n=3$), CFU-GM ($3.3 \pm 1.2/10^4$ cells; $n=3$), CFU-M ($15.0 \pm 1.0/10^4$ cells; $n=3$) to those of hESCs. On the other hand, few hematopoietic colonies were observed in the case of 201B7 at any time points (data not shown).

Thus, hiPSCs can generate reproducible HPCs with equivalent colony-forming activities to hESC-derived HPCs, although some lines of hiPSCs suffer from defective hematopoietic differentiation.

Discussion

In this article, we have provided the counterexamples to a previously reported finding that hiPSC-derived hemangioblast, the common progenitor of hematopoietic and endothelial cells, suffered from early senescence. In that report, hiPSC-derived HPCs was shown to have substantially decreased colony-forming activities and the majority of hiPSC-derived endothelial cells senesced after one passage (Feng et al., 2010). However, our data have clearly shown that the issue of early senescence can be overcome by selecting appropriate lines of hiPSCs and applying proper differentiation methods to them. Moreover, our results proved that retroviral insertion of reprogramming transgenes was not the cause of early senescence contrary to the discussion by the authors (Feng et al., 2010). We have also shown that, after sequential passages, hiPSC-derived VECs enter senescence as in the cases of hESC-derived VECs and primary human VECs, guaranteeing that hiPSC-derived VECs bear very low tumorigeneity, if any.

The key to our success in producing hiPSC-derived VECs that bear as high growth potentials as hESC-derived counterparts may reside, at least in part, in our usage of multiple hematopoietic cytokines in addition to VEGF. As we have shown previously, the six cytokines, SCF, IL6, IL3, BMP4, Flt3-L, and VEGF, as a whole work for the stable and high-purity production of subculturable VECs (Saeki et al, 2008). Interestingly, we are also observing that, under serum-free conditions, the presence of hematopoietic cytokine cocktail is crucial for the formation of spheres and their subsequent growth on gelatin-coated plates (M.N., unpublished finding). Thus, the usage of hematopoietic cytokine cocktail is advantageous not only for an achievement of high-efficiency differentiation but also survival and proliferation of the differentiated cells. Alternatively, the differentiation process per se, which is often followed by apoptosis, might include antiapoptotic processes as far as the differentiated cells keep surviving. In any event, stressful conditions should be avoided as much as possible from the differentiation procedures of hESCs/hiPSCs as in the case of their maintenance culture, where chromosomal aberrations are reportedly induced via stressful handling of the cells (Draper et al., 2004, Mitalipova et al., 2005).

As we mentioned, two lines of hiPSCs, 253G4 and 201B2, failed in directed differentiation into VECs. The 253G4- and 201B2-derived cells showed poor cord-forming activities and lacked VEC marker expressions, although they possessed Ac-LDL-uptaking capacities and were subculturable over 10 passages (data not shown). Their disadvantageous natures concerning VEC differentiation may be resulted from the possible line dependency in differentiation propensity among hiPSCs as reported in the case of hESCs (Osafune et al., 2008). Indeed, 253G4 and 201B2 showed very poor or no hemaotopoietic differentiation (data not shown). The finding that hiPSC lines with poor VEC-differentiating potentials bear little hemaotocyte-producing capacities seems very reasonable, because hematopoietic cells are derived from a specific population of vascular endothelial cells (Eilken et al., 2009).

Our findings together indicate that, although hiPSCs may be imposed line-dependent limitations in their differentiation capacities, they are not put inevitable fates of differentiation-dependent early senescence.

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Author Disclosure Statement

The authors declare that no conflicting financial interests exist.

References

Draper, J.S., Smith, K., Gokhale, P., et al. (2004). Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. Nat. Biotechnol. 22, 53–54.

Eilken, H.M., Nishikawa, S., and Schroeder, T. (2009). Continuous single-cell imaging of blood generation from haemogenic endothelium. Nature 457, 896–900.

Feng, Q., Lu, S.J., Klimanskaya, I., et al. (2010). Hemangioblastic derivatives from human induced pluripotent stem cells exhibit limited expansion and early senescence. Stem Cells 28, 704–712.

Fusaki, N., Ban, H., Nishiyama, A., et al. (2009). Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. 85, 348–362.

Kaji, K., Norrby, K., Paca, A., et al. (2009). Virus-free induction of pluripotency and subsequent excision of reprogramming factors. Nature 458, 771–775.

Kim, D., Kim, C.H., Moon, J.I., et al. (2009). Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell 4, 472–476.

Mitalipova, M.M., Rao, R.R., Hoyer, D.M., et al. (2005). Preserving the genetic integrity of human embryonic stem cells. Nat. Biotechnol. 23, 19–20.

Miura, K., Okada, Y., Aoi, T., et al. (2009). Variation in the safety of induced pluripotent stem cell lines. Nat. Biotechnol. 27, 743–745.
Nakagawa, M., Koyanagi, M., Tanabe, K., et al. (2008). Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nat. Biotechnol. 26, 101–106.
Nakagawa, M., Takizawa, N., Narita, M., et al. (2010). Promotion of direct reprogramming by transformation-deficient Myc. Proc. Natl. Acad. Sci. USA 107, 14152–14157.
Nakahara, M., Nakamura, N., Matsuyama, S., et al. (2009). High-efficiency production of subculturable vascular endothelial cells from feeder-free human embryonic stem cells without cell-sorting technique. Cloning Stem Cells 11, 509–522.
Nelson, T.J., Martinez-Fernandez, A., Yamada, S., et al. (2009). Repair of acute myocardial infarction by human stemness factors induced pluripotent stem cells. Circulation 120, 408–416.
Okita, K., Ichisaka, T., and Yamanaka, S. (2007) Generation of germline-competent induced pluripotent stem cells. Nature 448, 313–317.
Okita, K., Nakagawa, M., Hyenjong, H., et al. (2008). Generation of mouse induced pluripotent stem cells without viral vectors. Science 322, 949–953.
Osafune, K., Caron, L., Borowiak, M., et al. (2008). Marked differences in differentiation propensity among human embryonic stem cell lines. Nat. Biotechnol. 26, 313–315.
Saeki, K., Yogiashi, Y., Nakahara, M., et al. (2008). Highly efficient and feeder-free production of subculturable vascular endothelial cells from primate embryonic stem cells. J. Cell Physiol. 217, 261–280.
Saeki, K., Saeki, K., Nakahara, M., et al. (2009). A feeder-free and efficient production of functional neutrophils from human embryonic stem cells. Stem Cells 27, 59–67.
Seki, T., Yuasa, S., Oda, M., et al. (2010). Generation of induced pluripotent stem cells from human terminally differentiated circulating T cells. Cell Stem Cell, 7, 11–14.
Suemori, H., Yasuchika, K., Hasegawa, K., et al. (2006). Efficient establishment of human embryonic stem cell lines and long-term maintenance with stable karyotype by enzymatic bulk passage. Biochem. Biophys. Res. Commun. 345, 926–932.
Takahashi, K., Tanabe, K., Ohnuki, M., et al. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861–872.
Tesar, P.J., Chenoweth, J.G., Brook, F.A., et al. (2007). New cell lines from mouse epiblast share defining features with human embryonic stem cells. Nature 448, 196–199.
Vallier, L., Touboul, T., Chng, Z., et al. (2009). Early cell fate decisions of human embryonic stem cells and mouse epiblast stem cells are controlled by the same signalling pathways. PLoS One 4, e6082.
Warren, L., Manos, P.D., Ahfeldt, T., et al. (2010). Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell 7, 618–630.
Woltjen, K., Michael, I.P., Mohseni, P., et al. (2009). piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 458, 766–770.
Yu, J., Hu, K., Smuga-Otto, K., et al. (2009). Human induced pluripotent stem cells free of vector and transgene sequences. Science 324, 797–801.
Zhou, H., Wu, S., Joo, J.Y., et al. (2009). Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 4, 381–384.

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