INTRODUCTION

Stem cells represent populations of cells that can give rise to all kinds of tissue types necessary to constitute an organ. Traditional understandings on stem cells were mainly derived from hematopoietic stem cells in the model where aplastic bone marrow cells damaged by destructive radiation was repopulated using bone marrow transplantation (1). Over the last decades, studies on this cell population, namely hematopoietic stem cells have revealed much of unique properties not found in other cell types, such as self-renewal division, a mitotic division leading to a production of same stem cells, or asymmetric division, a unique division leading to un-equal production of daughter cells from same mother cells (2). Although the regulatory mechanisms controlling the self-renewing process or asymmetric division might have the key to more efficient use of stem cell in expansion culture or gene-tic modification, they still remain largely unknown awaiting further research. Another characteristic of stem cells inferred from hematopoietic stem cell is their extensive heterogeneity even after the highest purification process that currently available. The most important character of these stem cells, however, is their life-long reconstitutive activity as demonstrated by the long-term repopulating ability in animal transplantation model and specific cultures designed for in vitro assay (3). While the regulatory mechanisms for hematopoietic stem cells have been under active investigation, unexpected breakthroughs were made in other aspects of stem cell biology. One is the finding that adult hematopoietic stem cells give rise to many other tissue type in addition to blood cells, such as neuronal or muscle cells. Similar surprising findings continue to unveil the previously hidden pluripotency of adult stem. A series of these new findings in stem cell differentiation initially provoked a big chaos in the classical concept of cell development and differentiation. New concepts of retro-differentiation, plasticity in differentiation, and existence of very primitive pluripotent stem cells are emerging. Furthermore, a novel issue on stem cell identity has been addressed as to whether stem cells exist as a distinct clone in each organ and maintained throughout the development (clonal nature) or they are rather product of organ function to maintain integrity of each organ (functional nature) (4).

Another breakthrough in the stem cell area is the success in establishing human embryonic stem cells (5), which has triggered a vigorous debate between ethics and scientific merit of their use. Human embryonic stem cells can give rise to a greater numbers of tissue type from single cell nature (6-12) and continue to self-renew to the extent that adult stem cells can never achieve. Despite these attractive features in embryonic stem cells, still many hurdles ahead before clinical use
such as immune rejection by difference in histocompatibility between donor and recipients, possible tumor formation after in vivo transplantation, and problem of potential inappropriate/improper differentiation. While embryonic stem cells are at an emerging stage in the avenue of cell therapy, adult stem cells have been intensively used for hematological and cancer-related managements in clinical practice. Furthermore, recent studies are still expanding their use in many clinical situations that previously thought unrelated, such as metabolic diseases, bone diseases, or autoimmune diseases. Therefore, this review, focusing on stem cell-based cell therapy, will address discussions mostly to adult stem cells, rather than covering both types of stem cells, which should be beyond the current extent of scope.

MULTIPOTENTIALITY OF ADULT STEM CELLS

It has been a general concept that adult stem cells, in contrast to embryo-derived stem cells that have a totipotent differentiation potential, are limited in their cell types that can be derived from a given source of adult stem cells. In addition, it has been well accepted that this limitation is principally determined by their developmental origin, in such a way that the ectoderm-derived cells give rise to cells of ectodermal origin and those from the mesoderm give rise to cells of mesodermal origin. Furthermore, the developmental process has been thought to be irreversible process associated with lineage determination. However, series of new discoveries prompted the change of these classical concept awaiting emerge of new concept for cell development. In 1998, Geiger et al. (13) performed an experiment as to whether the adult cell would become like embryonic cells in a microenvironment that normal developmental process is occurring. They harvested bone marrow hematopoietic stem cells derived from transgenic mice for human beta globin gene and injected into blastocysts of developing mice. The resulting mice demonstrated a developmental chimerism, i.e., existence of donor-derived cells at various stages of development, including yolk sac, fetal liver, and adult bone marrow. The notion from this remarkable observation was that, given a certain microenvironment, the adult cells could also participate in the developmental process going backward in their developmental clock. Similarly, erythrocytes derived from adult donor did express the embryo-type hemoglobin (γ-globin and β-globin), suggesting that the gene expression program in adult genome could be reprogrammed in fetal microenvironment toward that in fetal genomic program. This intriguing observation of developmental plasticity of adult cells was rapidly extended to other models of developmental plasticity to investigate the extent of plasticity that adult stem cells can have. From early 2000, such trials brought up several remarkable observations that adult stem cells indeed have the differentiation potential beyond the developmental origin. The first evidence was obtained injecting neuronal precursor cells into blastocyst of developing mice (14). In this experiment, adult transgenic mice expressing β-galactosidase (lacZ) gene provided neural progenitor cells in the form of collection of immature cells, called neurosphere. After injection into blastocyst, the donor-derived neurosphere was tracked for their contribution to various types of cells. Surprisingly, the neurosphere, which was of ectodermal origin, was found to contribute to most of the tissues including intestine, heart, liver, mesonephron, as well as brain and notocord. This was the first demonstration that adult stem cells have a higher differentiation potential than previously thought beyond the developmental barrier, although, some criticisms were raised for possible contamination of other primitive stem cell population. However, on May 1991, Krause et al. (15) provided even stronger observations using single cell suspensions. In their experiment (schematically illustrated in Fig. 1), hematopoietic stem cells in bone marrow was purified using surface markers (CD34+ Sca-1+). The purified cells then were labeled with a lipid membrane-binding dye, PKH26, and transplanted into another mouse. Forty eight hours after transplantation, the bone marrow of primary transplanted mice were harvested and the labeled cells were isolated at a single-cell level under microscopic guidance. These single cells were inoculated into blastocysts for further development of the embryo, then tracked down for the distribution of the labeled cells.

Fig. 1. Multipotentiality of bone marrow stem cell at single cell level.
muscular dystrophy (DMD), transplantation of hematopoietic stem cells as well as muscle stem cells (SP cells, see below for description) would reconstitute the dystrophin-positive muscle cells by 10-30% when examined 12 weeks after transplantation. This observation is particularly interesting in that stem cell transplantation could be potentially used for systemic delivery of therapeutic cells to broad areas of injury in the body. Many similar observations were made for the plasticity of adult stem cells. In addition to the listed examples, many other tissues such as neuronal tissue (26, 27), renal tissue (28-30), cartilage and bone (31-33) have been shown to be derived from in vivo transplanted bone marrow cells.

Furthermore, in most of cases, the stem cell plasticity is bi-directional, i.e., bone marrow cells can differentiate into other tissues, and vice versa. For example, muscle stem cells, certain portion of hepatic tissues and neuronal tissues could differentiate into blood etc. (14, 19-21, 25, 26, 28, 33-39) (summarized in Fig. 2).

TISSUE-SPECIFIC STEM CELLS

In addition to the multipotentiality of stem cells that can give rise to various tissue types and their plasticity that can lead to different tissue types, adult stem cells provide additional potential way of tissue regeneration, i.e., through tissue-specific stem cells. It has been shown that many of adult organs have their own stem cells that retain some multipotentiality, albeit to a variable extent depending on the organ type. These cells include those from the pancreas, neuron, bone and cartilage, liver, skin, and even adipose tissues (summarized in Fig. 3).

It is, however, important to note that the limited ranges of differentiation potential does not necessarily mean their limitation for used in cell therapy. Rather, it could be a better source for stem cell therapy if it is more committed to a specific lineage of tissue when purity of cell type are to be taken.

Pancreatic stem cell

It has been known from traditional observation that the pancreatic ductal epithelium is the source of various islet-associated endocrine cell populations including alpha, beta, and delta cells in the islets of Langhans. Therefore, the pancreatic ductal epithelium has been believed to contain stem cells responsible for pancreatic endocrine cells but to easily differentiate upon in vitro culture, thereby losing the insulin-secreting ability (40, 41). In 2000, Ramiya et al. and Bonner-Weir et al. simultaneously developed series of culture method by which pancreatic ductal stem cells can proliferate maintaining their ability to differentiate into islet-progenitor cells (IPC) and accordingly ability to differentiate into insulin-secreting beta cells (42, 43). In these reports, the islet-producing cells were developed from crude ductal pancreatic epithelium and thus obtained IPCs were maintained in up to 150 serial passages (42) retaining their ability to secrete insulin and glucagons upon terminal differentiation in vitro. Subsequent injection of these islet cells into the renal capsule demonstrated that thus prepared islet cells led to neovascularization in the local environment, and secrete insulin in vivo. According to the report, the blood glucose levels of diabetic mice (non-obese diabetic: NOD) were maintained up to 5
blood expansion, ex vivo expansion and efficient tumor purging (2).

Purification of HSC: CD34 has been a gold standard marker for primitive stage HSCs and many clinical applications including tumor purging have been focused on the selective purification of CD34+ cells. Further studies revealed that still a major heterogeneity existed in the CD34+ population by CD38, AC133, and Thy-1 expression (56, 57). For example, CD34+CD38- cells are mostly enriched with most primitive stage HSCs which can be read out either by long-term in vitro culture (long-term culture initiating cells: LTC-IC) (3) or long-term in vivo NOD/SCID (non-obese diabetic/severe combined immune deficiency) repopulating cells (CRU: competitive repopulating unit) (58). In contrast, CD34+CD38+ cells are more enriched with progenitor cells restricted in their potential spectrum of lineages and in their self-renewing potential, which are often read out either by CFU-S12, CFU-14, or colony-forming assay in semi-solid medium (59). However, recent evidence revealed that additional populations that had been previously neglected (i.e., primitive CD34- cell populations) could be engrafted in NOD/SCID mice with low clonogenicity in long-term culture, suggesting that this population could be an even more primitive cell population (60).

In addition to purification of HSCs by cell surface markers, functional characteristics of HSCs using their intrinsic dye-efflux effect were also described (61). These dye-effluxing cell population, called side population (SP) cell, are characterized by dim Hoechst 33342 staining when activated by UV light due to verapamil-sensitive dye efflux function (Fig. 5). The SP cells were weak in CD34 expression, and lacked most of lineage-specific markers. Interestingly, like HSCs, multipotent stem cells from other tissues such as muscle and liver shares common phenotype, suggesting that the SP cell phenotype might be a universal stem cell marker (62).

**ONTLOGICAL DIFFERENCE IN HSCS**

HSCs have been found to exist in different forms of hematopoietic organs throughout the ontological difference, i.e., adult bone marrow, neonatal cord blood, and fetal liver. Each stage of HSCs is characterized by differential functional characteristics in terms of in vivo self-renewal capacity, in vitro proliferation potential, and optimal growth factor requirement (63-65). For example, fetal liver HSCs were characterized by the highest in vitro proliferation potential and in vivo self-renewing capacity, while adult bone marrow cells have the lowest position in both terms, and umbilical cord blood is in the intermediate position (66). The basis for these functional differences among ontologically different populations remains unknown. Previously we have performed a series of gene expression studies to investigate the distinct gene expression patterns among different stages of ontology (67). We found that series of gene expression pattern is conserved during in vivo differentiation from CD34+CD38- cells to CD34+CD38+ cells and during in vitro differentiation mediated by growth factor stimulation. Interestingly, similar difference was also conserved during ontology-related differences in gene expression in such a way that the gene expression pattern in ontologically earlier stage HSC is more close to the patterns in growth factor-stimulated cells. These findings led us to speculate that there is a certain stage of HSC activation common to in vitro stimulation and in vivo activation called “priming” and according to this hypothesis, fetal liver and umbilical cord blood HSC mimic the state already growth factor-stimulated and primed in the activation process, when compared to adult bone marrow stem cells (schematically illustrated in Fig. 6).
the stem cell plasticity, which can regenerate many tissues using different types of stem cells. The problem of this approach is that we cannot answer such questions as ‘what is the controlling mechanism?’ or ‘how does this process occur?’ To be useful for cell therapy, these phenomological descriptions of plasticity should be further dissected into the regulatory mechanisms so that the efficiency of organ regeneration by the process could reach a therapeutic level.

The third dimension of stem cell therapy would be through tissue-specific stem cells, such as pancreatic stem cells, hematopoietic stem cells for lympho-myeloid reconstitution, or liver stem cells. The advantage of the tissue-specific stem cells is that it can produce a highly homogenous population of the differentiated cells unlike pluripotent embryonic stem cells, where the possibility of improper or inappropriate differentiation of the differentiated cells unlike pluripotent embryonic stem cells is that it can produce a highly homogenous population of the differentiated cells unlike pluripotent embryonic stem cells, where the possibility of improper or inappropriate differentiation remains to be cleared. Again, however, the major obstacle of this approach is that the cell number is limited for a medically effective cell therapeutic dose.

Therefore, molecular mechanisms for the expansion of adult stem cells and differentiation of pluripotent stem cells should be elucidated before major benefit from stem cell therapy is envisioned.

REFERENCES

1. Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Doehse M, Osborne L, Wang X, Finegold M, Weissman IL, Grompe M. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med 2000; 6: 1229-34.

2. Ogawa M. Differentiation and proliferation of hematopoietic stem cells. Blood 1993; 81: 2844-53.

3. Sutherland HJ, Eaves CJ, Eaves AC, Dragowska W, Lansdorp PM. Characterization and partial purification of human marrow cells capable of initiating long-term hematopoiesis in vitro. Blood 1989; 74: 1563-70.

4. Blau HM, Brazelton TR, Weimann JM. The evolving concept of a stem cell: entity or function? Cell 2001; 105: 829-41.

5. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. Science 1998; 282: 1145-7.

6. Itskovitz-Eldor J, Schuldiner M, Karsenti D, Eden A, Yanuka O, Amit M, Soreq H, Benvenisty N. Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. Mol Med 2000; 6: 88-95.

7. Kaufman DS, Hanson ET, Lewis RL, Auerbach R, Thomson JA. Hematopoietic colony-forming cells derived from human embryonic stem cells. Proc Natl Acad Sci USA 2001; 98: 10716-21.

8. Kawasaki H, Suemori H, Mizuseki K, Watanabe K, Urano F, Ichinose H, Haruta M, Takahashi M, Yoshikawa K, Nishikawa S, Nakatsuji N, Sasai Y. Generation of dopaminergic neurons and pigmented epithelia from primated ES cells by stromal cell-derived inducing activity. Proc Natl Acad Sci USA 2002; 99: 1580-5.

9. Kehat I, Kenyagin-Karsenti D, Snir M, Segev H, Amit M, Gepstein A, Livne E, Binah O, Itskovitz-Eldor J, Gepstein L. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. J Clin Invest 2001; 108: 407-14.

10. Liu S, Qu Y, Stewart TJ, Howard MJ, Chakrabarty S, Holekamp TF, McDonald JW. Embryonic stem cells differentiate into oligodendrocytes and myelinate in culture and after spinal cord transplantation. Proc Natl Acad Sci USA 2000; 97: 6126-31.

11. Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, McKay R. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science 2001; 292: 1389-94.

12. McDonald JW, Liu XZ, Qu Y, Liu S, Mickey SK, Turesky D, Gottlieb DI, Choi DW: Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Nat Med 1999; 5: 1410-2.

13. Geiger H, Sick S, Bonifer C, Muller AM. Gliobin gene expression is reprogrammed in chimeras generated by injecting adult hematopoietic stem cells into mouse blastocysts. Cell 1998; 93: 1055-65.

14. Clarke DL, Johansson CB, Wilbertz J, Veress B, Nilsson E, Karlstrom H, Lendahl U, Friisen J. Generalized potential of adult neural stem cells. Science 2000; 288: 1660-3.

15. Krause DS, Theise ND, Collector MI, Henegar O, Hwang S, Gardner R, Neutzol S, Sharkis SJ. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 2001; 105: 369-77.

16. Grompe M, al-Dhalimy M, Finegold M, Ou CN, Burlingame T, Kennaawy NG, Soriano P. Loss of fumarylacetoacetate hydratase is responsible for the neonatal hepatic dysfunction phenotype of lethal albino mice. Genes Dev 1993; 7: 2298-307.

17. Morrison SJ, Lagasse E, Weissman IL. Demonstration that Thy (lo) subsets of mouse bone marrow that express high levels of lineage markers are not significant hematopoietic progenitors. Blood 1994; 83: 3480-90.

18. Morrison SJ, Weissman IL. The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. Immunity 1994; 1: 661-73.

19. Alison MR, Poulsom R, Jeffery R, Dhillon AP, Quaglia A, Jacob J, Novelli M, Prentice G, Williamson J, Wright NA. Hepatocytes from non-hepatic adult stem cells. Nature 2000; 406: 257.

20. Petersen BE, Bowen WC, Patene KD, Mars WM, Sullivan AK, Murase N, Boggis SS, Greenberger JS, Goff JP. Bone marrow as a potential source of hepatic oval cells. Science 1999; 284: 1168-70.

21. Orlic D, Kajstura J, Chimenti S, Jakobiuk I, Anderson SM, Li B, Pickel JG, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Bone marrow cells regenerate infarcted myocardium. Nature 2001; 410: 701-5.

22. Orlic D, Kajstura J, Chimenti S, Limana F, Jakobiuk I, Quaini F, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci USA 2001; 98: 10344-9.

23. Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, Homma S, Edwards NM, Itescu S. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat Med 2001; 7: 430-6.
getting closer to a cure? Blood 2002; 99: 768-84.

56. Gallacher L, Murdoch B, Wu DM, Karanu FN, Kerney M, Bhatia M. Isolation and characterization of human CD34(-)Lin(-) and CD34(+) Lin(-) hematopoietic stem cells using cell surface markers AC133 and CD7. Blood 2000; 95: 2813-20.

57. Mayani H, Dragowska W, Lansdorp PM. Characterization of functionally distinct subpopulations of CD34+ cord blood cells in serum-free long-term cultures supplemented with hematopoietic cytokines. Blood 1993; 82: 2664-72.

58. Bhatia M, Wang JC, Kapp U, Bonnet D, Dick JE. Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. Proc Natl Acad Sci USA 1997; 94: 5320-5.

59. Wolf NS, Priestley GV. Kinetics of early and late spleen colony development. Exp Hematol 1986; 14: 676-82.

60. Bhatia M, Bonnet D, Murdoch B, Gan OI, Dick JE. A newly discovered class of human hematopoietic cells with SCID-repopulating activity. Nat Med 1998; 4: 1038-45.

61. Goodell MA, Rosenzweig M, Kim H, Marks DF, DeMaria M, Paradis G, Grupp SA, Sieff CA, Mulligan RC, Johnson RP. Dye efflux studies suggest that hematopoietic stem cells expressing low or undetectable levels of CD34 antigen exist in multiple species. Nat Med 1997; 3: 1337-45.

62. Wulf GG, Jackson KA, Goodell MA. Somatic stem cell plasticity: current evidence and emerging concepts. Exp Hematol 2001; 29: 1361-70.

63. Holyoake TL, Nicolini FE, Eaves CJ. Functional differences between transplantable human hematopoietic stem cells from fetal liver, cord blood, and adult marrow. Exp Hematol 1999; 27: 1418-27.

64. Nicolini FE, Holyoake TL, Cashman JD, Chu PP, Lambie K, Eaves CJ. Unique differentiation programs of human fetal liver stem cells shown both in vitro and in vivo in NOD/SCID mice. Blood 1999; 94: 2686-95.

65. Wang JC, Doodens M, Dick JE. Primitive human hematopoietic cells are enriched in cord blood compared with adult bone marrow or mobilized peripheral blood as measured by the quantitative in vivo SCID-repopulating cell assay. Blood 1997; 89: 3919-24.

66. Rebel VI, Miller CL, Eaves C, Lansdorp PM. The repopulation potential of fetal liver hematopoietic stem cells in mice exceeds that of their liver adult bone marrow counterparts. Blood 1996; 87: 3500-7.

67. Oh IH, Lau A, Eaves C. During ontogeny primitive (CD34(+) CD38(-)) hematopoietic cells show altered expression of a subset of genes associated with early cytokine and differentiation responses of their adult counterparts. Blood 2000; 96: 4160-8.

68. Kim DK, Fujiki Y, Fukushima T, Ema H, Shibuya A, Nakauchi H. Comparison of hematopoietic activities of human bone marrow and umbilical cord blood CD34 positive and negative cells. Stem Cells 1999; 17: 286-94.

69. Leung W, Ramirez M, Civin CI. Quantity and quality of engrafting cells in cord blood and autologous mobilized peripheral blood. Biol Blood Marrow Transplant 1999; 5: 69-76.

70. Gluckman E. Current status of umbilical cord blood hematopoietic stem cell transplantation. Exp Hematol 2000; 28: 1197-205.

71. Rubinstein P, Carrier C, Scardavou A, Kurtzberg J, Adamson J, Migliaccio AR, Berkowitz RL, Cabbbad M, Dobrila NL, Taylor PE, Rosenfeld RE, Stevens CE. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. N Engl J Med 1998; 339: 1565-77.

72. Gluckman E, Rocha V, Boyer-Chammard A, Locatelli F, Arcese W, Pasquini R, Ortega J, Souillet G, Ferreira E, Laporte JP, Fernandez M, Chastang C. Outcome of cord-blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. N Engl J Med 1997; 337: 373-81.