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■ REVIEW ■

Three-Dimensional Approach to Stem Cell Therapy

Recent progress in stem cell research is opening a new hope for cell therapy in regenerative medicine. Two breakthroughs were made in the stem cell era, one, new discoveries in multipotentiality of adult stem cells beyond the traditionally appreciated extent, and the other, establishment of pluripotent stem cell from human embryo. In addition to the newly identified multipotentiality of adult stem cells, their ability to be trans-differentiated toward other tissue types (stem cell plasticity) as well as to migrate toward the site of tissue damage make adult stem cells particularly attractive choice for stem cell based therapy. Stem cell therapy for organ regeneration, therefore, could be approached from three distinct dimensions: first, direct differentiation of multi-potent stem cells toward desired tissue types; secondly, regeneration of specific tissues through in vivo stem cell plasticity, and lastly, by tissue-specific stem cells from many types of organs. While each approach in stem cell therapy poses distinctive limitations for their successful clinical applications, understanding regulatory mechanisms of stem cell self-renewal and their in vivo engraftment will mostly extend their medical efficacy of stem cell based therapy.

Key Words : *Stem Cells; Tissue Therapy; Stem Cell Therapy; Multipotentiality; Plasticity; Tissue Specific Stem Cell*

Il-Hoan Oh, Dong-Wook Kim*

Cell & Gene Therapy Institute, Catholic Research Institute of Medical Science, Catholic Hematopoietic Stem Cell Transplantation Center*, The Catholic University of Korea, Seoul, Korea

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Address for correspondence
Il-Hoan Oh, M.D.
Cell & Gene Therapy Institute, Catholic Research Institute of Medical Science, 505 Banpo-dong, Seocho-gu, Seoul 137-040, Korea
Tel : +82.2-590-2591, Fax : +82.2-591-3994
E-mail : iho@cmc.cuk.ac.kr

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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 genetic 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

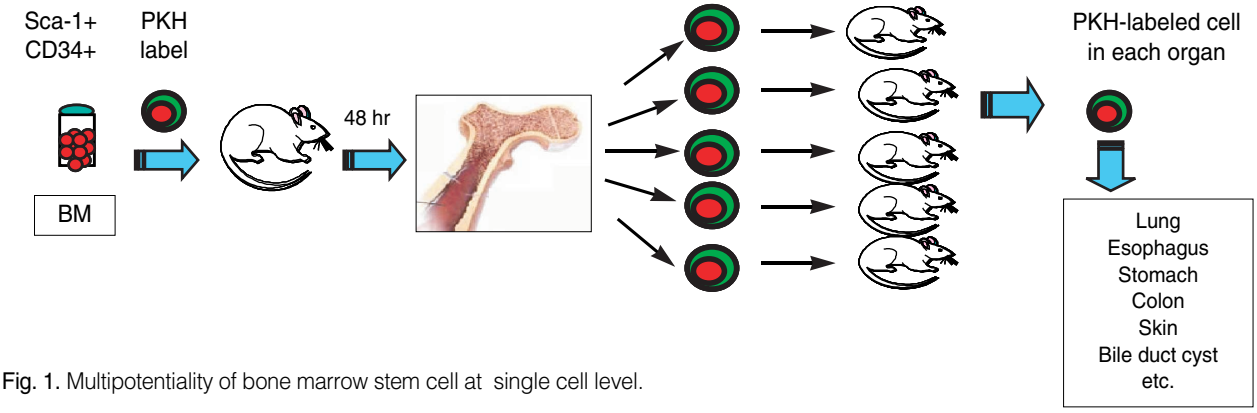

Fig. 1. Multipotentiality of bone marrow stem cell at single cell level.

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 york 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 δ-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 blastocysts, 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

throughout the whole embryos. Again, the labeled single cells of hematopoietic origin were found to give rise to almost all kinds of tissues including skin, epithelial cells of the gastrointestinal tract, bile duct cyst, liver, and lung, further establishing the notion that adult stem cells could be as pluripotent as embryonic stem cells if placed in a specific permissive microenvironment.

PLASTICITY OF STEM CELLS

In concordance with the new recognition of multipotent differentiation potential of adult stem cells, many observations were made for their differentiation toward other type tissue cells out of normal differentiation program. While the ability of a cell to give rise to a variety of different cell types is referred as "multipotentiality", ability of a particular cell to become different cell types is commonly referred to as "plasticity of differentiation". One typical experiment showing the plasticity of adult stem cells was made by Lagasse et al. (1). In their experiment, an animal model of type 1 tyrosinemia with fumaryl acetoacetate hydrolase deficiency ($FAH^{-/-}$) (16) was employed as a test model, which is characterized by hepatotoxicity due to accumulation of toxic metabolite caused by lack of FAH and hence, their dependence on 2-(2-nitro-4-trifluoro-methylbenzyol)-1,2,-cyclohexanedione (NTBC) for survival. When unpurified bone marrow cells from Rosa 26 mice (wild type for the FAH and transgenic for the β-galactosidase gene) were transplanted into lethally irradiated $FAH^{-/-}$ mice and NTBC was withdrawn, four out of nine mice remained healthy, while all of the control group mice died of hepatotoxicity. Exploration of the surviving mice after 7 months revealed hundreds of regenerating hepatic nodule in the transplanted mice, primarily consisted of hepatocytes with wild type FAH ($FAH^{+/+}$) and β-galactosidase gene, which should have been derived from transplanted bone marrow cells from the donor. Further studies showed that hematopoietic stem cells (KTLS, c-kit+ thylow Lin- Sca-1+) (17, 18) and CD45+ was the only population responsible for hepatic regeneration, with no similar phenomenon for more differentiated cells such as Lin+ or c-kit- cells. Thus it has become clear that primitive hematopoietic stem cells with all the surface markers to become blood cells, could give rise to hepatocytes in a certain in vivo condition that hepatocytes are in emergency state.

Similar observations were made (19, 20) even in a human model where a liver transplantation or bone marrow transplantation was performed in cross sexual matching. In case of bone marrow transplantation, where the donor was male and the recipient was female, Y-chromosome-positive hepatocytes were observed in the female recipient's liver, suggesting that a part of male donor's bone marrow cells contributed to the hepatogenesis in the female recipient. In contrast, in case of female-to-male liver transplantation Y chromosome-positive hepatocytes were observed in the transplanted liver, indicating that non-hepatic cells of the male recipient had contributed to the hepatogenesis.

Another interesting observation regarding stem cell plasticity was the regeneration of myocardium using bone marrow cells. In 2001, Orlic et al. (21) tried the first experiment exploring the possible conversion of hematopoietic stem cells to myocardium. In the model, coronary arteries of mice were ligated to induce myocardial infarction. Shortly after infarction, lineage-negative bone marrow cells from transgenic mice expressing enhanced green fluorescent protein (EGFP) were sorted into c-kit+ and c-kit- populations and injected into the peri-necrotic region. When the heart injected with cells were inspected 9 days after injection, 68% of infarcted region of myocardium in mice injected with c-kit+ lineage-negative cells, but not the myocardium in mice injected with c-kit- lineage-negative cells showed regeneration across the three layers of myocardium. Surprisingly again, most of the regenerated cells were EGFP+ suggesting that the bone marrow derived hematopoietic stem cells were recruited to the necrotic region and participated in the de novo regeneration of myocardium. Furthermore, with anatomical regeneration of myocardium, the functional aspect of the heart was also concomitantly improved both in systolic pressure (about 40% increase) and diastolic pressure (about 36% lower). Interestingly, in addition to regeneration of myocardium, there was simultaneous regeneration of endocardium and vessels, thus raising a hope that hematopoietic stem cell plasticity could be suitable for both repair of necrotic region and redistribution of blood flows around the coronary vessel occlusion.

An even more interesting observation made by the same group (22) was that the general increase in the circulating number of c-kit+lin- cells using granulocyte-colony stimulating factor (G-CSF) and stem cell factor (SCF) led to an increase in the availability of circulating HSC to repair the infarcted myocardium. According to the report, 70% of cytokine-mobilized mice survived 27 days post infarct, while only 17% of sham-operated control mice survived the same period. In addition, cytokine-induced cardiac repair decreased the infarct size by 40%, cavity dilation by 26%, and diastolic stress by 70%.

In concordance with the re-vascularization described by Orlic et al., Kocher et al. (23) demonstrated that intravenous injection of CD34+CD117bright cells resulted in infiltration of vascular endothelial cells around the infarct zone within 48 hr of coronary artery ligation, but such a phenomenon was not observed in unaffected myocardium or myocardium of sham-operated rats. In addition, the injection of CD34+CD117 bright cells resulted in a 3-5 fold increase in neovascularization around the infarct area associated with concomitant increase in myocardial function compared to those resulting from injection of CD34+CD117dim.

Similarly, bone marrow cells could be differentiated into skeletal muscles (24, 25). In a mice model of Duchenne's

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

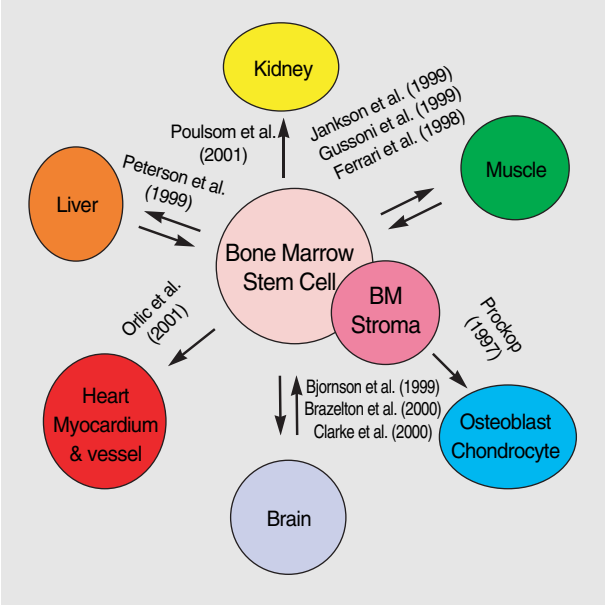

Fig. 2. Stem cell plasticity.

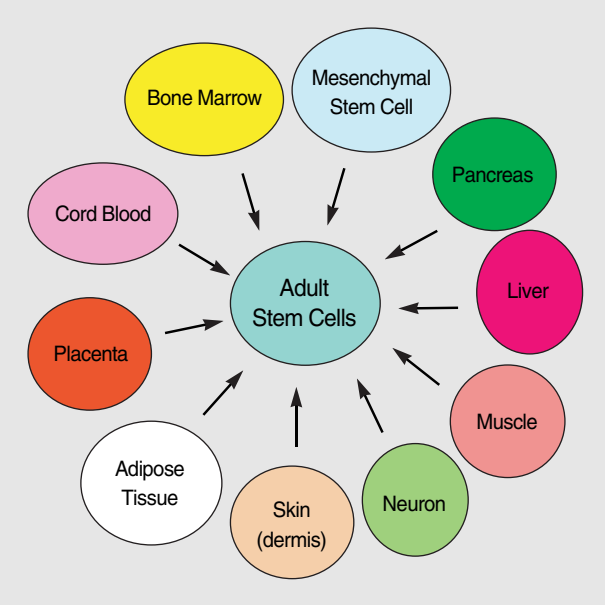

Fig. 3. Diverse source of adult stem cells.

months even in the absence of exogenous insulin administration, while the control group showed hyperglycemia (700 mg/mL) in two weeks. Interestingly, the islet cells that were used for transplantation were obtained from same strain (prediabetic NOD mice), leaving a possibility that such islet cells could again become the target of autoimmune attack as in type 1 diabetic mice. However, surprisingly, the cells protected by a polymer capsule (to protect from immune attack) and those without any polymer showed similar maintenance of transplanted cells thus suggesting possible extension of this therapeutic model to autologous transplantation settings for human diabetes.

Neuronal stem cells

Most neuronal cells are formed during the embryonic and postnatal period, but some neurons continue to proliferate in adult mammalian brain. Since 1992, Reynolds and Weis have observed that adult neuronal cells have the self-renewal capacity and that they can proliferate and differentiate into all three components of the nervous system (neurons, astrocytes, and oligodendrocytes) (44, 45). Recent progress in neuronal stem cell research found that these cells are mostly derived from ependymal cells lining the ventricle of the nervous system. These cells then proliferate in the subventricular region and migrate to olfactory bulb, where they differentiate and integrate into each neuronal structure (46). More recent report also suggests that, in adult mouse brain, the neural stem cells reside both in ependymal and subventricular zone (47). Interestingly, these neural stem cells could be identified by surface expression markers in addition to their characteristic marker "nestin". These includes their expression of notch-1, low levels of PNA (peanut agglutinin binding), and HAS (heat stable antigen) which make it possible to purify neuronal stem cells using surface marker.

Neural stem cells have well characterized advantage for cell therapeutic application, that is, they have an intrinsic ability to migrate toward the injured site as well as their capacity to renew various neuronal cells. During the brain or spinal cord injury these neural stem cells undergo extensive proliferation and migrate towards the site, either in a dorsal or lateral direction, over the 4 week period and form a scar that persists up to 1 yr. However, ependymal cell do not appear to be the only cells that have a healing effect, since in the above model of neural injury, most astrocytes have been derived from the ependymal area (Dil-postive), while oligodendrocytes and neuronal cells were Dil-negative, when ependymal cells were pre-labeled with Dil before injury (46)

In another model using neural stem cell for cell therapy, neural stem cells served as a therapeutic vehicle to deliver a therapeutic gene to the target site. In 2000, both Aboody et al. (48) and Benedetti et al. (49) demonstrated that an exogenously implanted glioblastoma, which is characterized by rapid and diffuse infiltration over the brain area and poor prognosis, was thoroughly entrapped with simultaneously administered neural stem cells. In this experiment the neural stem cells administered were cells immortalized from fetal brain by expressing c-myc. Despite the fact that they had been immortalized, the cells integrated into a neuronal structure, and stopped their proliferation, hence without causing a tumor in vivo. Interestingly, the expression of IL-4 gene (49) exerted a therapeutic effect on the glioblastoma comparable to unmodified neural stem cells, suggesting that the surrounding stem cells recruit a certain local effecter molecule which act targeting the tumor cells. More interestingly, when the immortalized neural stem cells were equipped with the gene encoding cytosine deaminase (48), the neural stem cells surrounding the tumor released this enzyme, and when 5-fluorocytosine was added systemically, the enzyme converted 5-fluorocytosine into 5-fluorouracil, and exerted a selective cytotoxic effect, resulting in up to 80% reduction in the tumor volume.

Hematopoietic stem cells

As described above, hematopoietic stem cells can give rise to all kinds of blood cells including myeloid and lymphoid cells, as well as their plasticity-related organogenesis. In addition to the organogenesis by plasticity, their tissue-specific nature itself has enabled extensive application of this stem cells in medicine (21, 50-55) (summarized in Fig. 4).

However, the more efficient use of hematopoietic stem cells (HSC) would require strategy to preserve stem cell properties, i.e., self-renewing capacity because they are highly prone to differentiation during in vitro manipulative process. Understanding of the self-renewing mechanism of HSC will enable further applications including gene therapy using HSC, cord

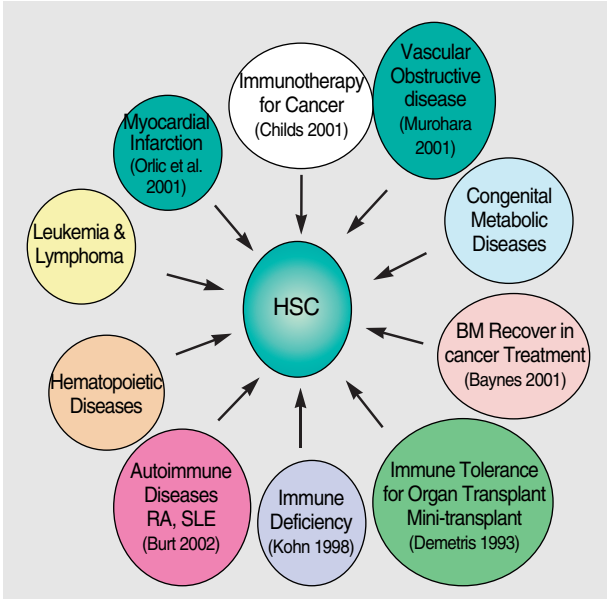

Fig. 4. Application of hematopoietic stem cells (HSCs).

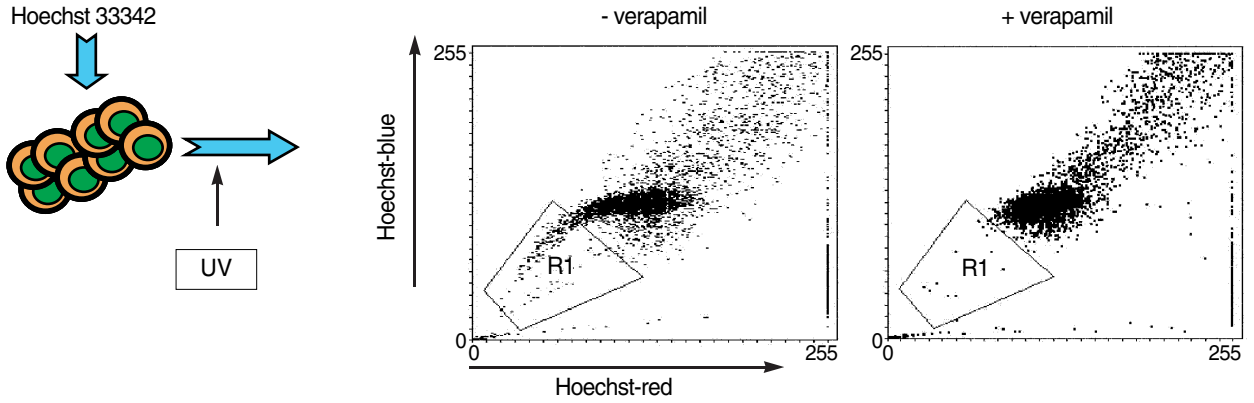

Fig. 5. Identification of SP (side population) cells as stem cell source. Cells were stained with Hoechst 33342 dye and subject to UV excitation in flowcytometer. The UV-excited Hoechst dye emits two different waves, one with >650 nm (red), and 488 nm (blue). These two lights are split into vertically arranged independent detectors. The tail part (Hoechst dim) is formed due to dye efflux mechanism of stem cells, which is dependent on the verapamil-sensitive efflux pump. The SP cells are, therefore, verified by loss of the same population by verapamil treatment.

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).

ONTOLOGICAL 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).

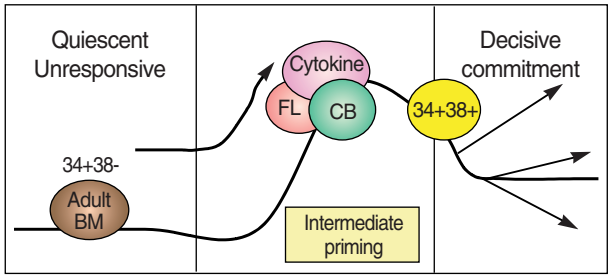

Fig. 6. Hypothetical illustration of stem cell priming and the ontological difference characterized by different priming. A series of gene expression changes were identified during ontological development from fetal liver cells to adult bone marrow cells (Oh et al., 2000). The pattern of expression changes is conserved during changes from quiescent (CD34+CD38-) population to mitotically activated (CD34+CD38+ or growth factor stimulated) populations. Therefore, we set a hypothesis that there is a certain stage of gene expression status in stem cell i.e., "intermediate priming" that a suppressed quiescent stem cell needs to pass through in order to become mitotically active and sensitive to extra cellular signal and, and after that, more decisive commitment to lineage-restricted cells is occurring. According to this hypothesis, fetal liver cells and cord blood cells are already in a state that is primed in the intermediate stage compared to adult bone marrow cells, and hence show more immediate and higher proliferation potential.

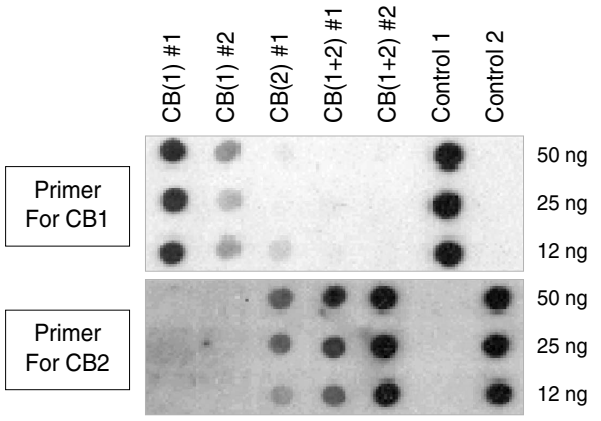

Fig. 7. Differential contribution of mixed cord blood (CB) transplantation. Shown above is the representative data obtained from full six-loci matched (HLA-A,B,DR) double cord blood mixing transplantation into the NOD/SCID mice. The polymorphism in HLA-DP locus was used to discriminate the cells derived from each donor (CB (1) and CB (2)). The intensity of each dot is related to the amount of engraftment from specific donor cells as hybridized by donor specific probe corresponding to the distinctive sequence in HLA-DP region. # represents number of transplanted NOD/SCID mice in each group and control 1 and 2 are the lanes containing purified DNA as a positive control.

THERAPEUTIC APPLICATION OF UMBILICAL CORD BLOOD

Previous bone marrow transplantations were mostly performed using adult bone marrow stem cells, often hard to find the donor in allogeneic transplantations, or often contaminated with tumor cells in autologous transplants. In addition to their advantage in finding a donor and their safety from infection or tumor cell contamination, the umbilical cord blood has many functional advantages over adult bone marrow stem cells (63, 68, 69). Accordingly, cord blood stem cell transplantation has become a world-wide trend as a new alternative way of bone marrow reconstitution in many disease conditions including genetic diseases and malignant tumors, evidenced by the simultaneous clinical trials both in American blood bank and 'Eurocord' (70-72).

However, despite many advantages of cord blood in transplantation, the total cell number available has been the major limitation for a broader ranges of recipients in addition to the current applications mainly limited to children. Recently we have been trying to overcome this limit in the total cell number by co-transplantation of double unit cord blood to determine if any additive effect could be seen. When using several sets of cord blood pair varying in the HLA matching (from full 6-loci match to full mismatch), there was a consistent deviation toward a one single unit (>9:1 ratio) out of two independent donors (illustrated in Fig. 7). Similar deviations were also observed in a human model, where mixed double cord transplantation was done in one leukemic patient, with engraftment pattern predominantly contributed by a single donor (manuscript in preparation). Therefore additional strategies to overcome these limitations need to be developed for wider application of umbilical cord blood.

CONCLUSION

As discussed so far, stem cell therapy is a powerful tool for organ regeneration and de novo production of cells to replace damaged tissues. The organ regeneration based upon stem cell therapy could be approached in a three-dimensional way. The first dimension is using multi-potential and/or pluripotent stem cells such as embryonic stem cells or multipotent adult stem cells. However, the use of adult stem cells is limited by the extremely low frequency and the amount available in a given organ, although it is advantageous in that it seldom forms a tumor and that it shows organ-specific differentiation. In contrast, embryonic stem cells should overcome the hurdles ahead. They should be driven down to specific differentiated cells before transplantation in order to prevent tumor formation in vivo, and that therapeutic cloning is almost inevitable in order to overcome the immune mediated rejections, which is a very inefficient process with low success rate of normal development. Overcoming these hurdles in embryonic stem cells, like the strategy to expand adult stem cells, will broaden the potential choice of the source in cell therapy.

The second dimension of cell therapy would be through

the stem cell plasticity, which can regenerate many tissues using different types of stem cells. The problem of this approach is that we can not answer such questions as 'what is the controlling mechanisms?' or 'how does this process occur?' To be useful for cell therapy, these phenomenological 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 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.

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