Review Article

Stem cell plasticity in hematopoietic system

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Bone marrow (BM) contains hematopoietic stem cells (HSC) which differentiate into all mature blood cells and marrow stromal cells that provide the microenvironment for hematopoietic stem/progenitor cells along with the capability to differentiate into mature cells of multiple mesenchymal tissues including fat, bone and cartilage. Recent studies indicate that adult BM also contains cells which can differentiate into nonhematopoietic cells of ectodermal, mesodermal and endodermal tissues other than hematopoietic tissues, including liver, pancreas, kidney, lung, skin, GI tract, heart, skeletal muscles and neural tissues. Studies describing this multipotentiality of BM cells have become a focus of interest because clinical applications in the treatment of damaged or degenerative diseases would be at hand using easily obtainable cells. However, presently, definitive evidence explaining the mechanism of this multipotentiality of bone marrow stem cells is lacking. In this review, we summarize recent progresses and controversies in the multipotentiality of adult bone marrow-derived stem cells to non-hematopoietic tissues.

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Introduction

A stem cell has the unique capacity to self-renew and to give rise to specialized cells of certain tissues. Traditionally, stem cells have been divided into two major groups: 1) embryonic stem (ES) cells and 2) somatic stem cells.

ES cells are pluripotent stem cell lines derived from the inner cell mass of fertilized ova without use of immortalizing or transformating agents. They have the capacity to give rise to differentiated progeny representative of all three embryonic germ layers. They also can be propagated as homogenous stem cells in culture and expanded without apparent limit. More remarkably, ES cells retain the character of embryonic founder cells, even after prolonged culture. Nowadays, human ES cells could be established and extensive studies have been done based from the viewpoints of further academic analysis and its clinical application. Although human ES cells have the potential to generate new tissues in regenerative medicine, the generation of human ES cells requires the ethically problematic destruction of a human embryo that otherwise would have had the potential to develop to term. In addition, the mechanism and manipulation method into specialized tissue cells remains to be resolved more extensively, along with the problem of the occurrence of teratoma formation after transplantation.

On the other hand, adult tissues characterized by a high cell turnover rate such as hematopoietic system have been demonstrated to contain somatic stem cell populations. These cells maintain the life-long production of the functional daughter cells of the tissue in addition to sustaining their own numbers through regulated differentiation and self-renewal divisions[1]. Recently, it has become clear that other adult tissues containing functional cells with much longer life spans such as brain, muscle and liver also contain cells with stem cell properties[2-5]. Moreover, interestingly, a potential new paradigm in somatic stem cells has emerged in the last decade: the concept that somatic stem cells may have far broader differentiation capacity than originally thought. Studies describing this plasticity of somatic stem cells have become a focus of interest because clinical applications in the treatment of damaged or degenerated tissues would

be at hand. The first report of plasticity of somatic stem cells was done by Bjornson et al. with provocative headings like " turning brain into blood "[6], although attempts to duplicate some of these spectacular findings failed[7]. Subsequently, a number of studies on somatic stem cell plasticity have been reported *in vitro* and *in vivo*. Although numerous studies have been done, many of the findings in this new field are controversial due, at least in part, to the fact that 1) reliability of the techniques used to assess *in vitro/vivo* plasticity, 2) lack of paradigm to explain post-natal switching of cell fate and 3) ambiguity for therapeutic use from the viewpoint of controlled manipulation or safety. This has led a stream of headlines such as " Cell fusion leads to confusion "[8], " Biologists question adult-stem-cell versatility "[9], " Plasticity: time for a reappraisal? "[10], " Is transdifferentiation in trouble? "[11] and " Are somatic stem cells pluripotent or lineage-restricted? "[12]. In this review, we will consider these issues, being focused on hematopoietic system[13-17].

Bone marrow subpopulations

The bone marrow can be viewed as a tissue organized into two subpopulations: the hematopoietic compartment, ultimately providing the organism with mature blood cells of all lineages, and the stromal cell compartment, providing the microenvironment for self-renewal, proliferation and differentiation of hematopoietic stem/progenitor cells. In addition, the stromal cell compartment harbors vascular progenitor cells[18], and mesenchymal stem cells capable of differentiation into cells of various connective tissues[19,20].

1) Hematopoietic stem cells (HSCs)

The absolutely reliable assay for HSCs is their ability to reconstitute the hematopoietic system in a myeloablated host. This is because both extensive self-renewal of the transplantated HSCs and their differentiation into all mature blood cell types are requisite to fulfill the definition of HSCs and the reconstitution of bone marrow (BM) can satisfy these two requirements. To isolate HSCs, various surface markers on hematopoietic cells are frequently useful. In mice, at first, a lineage depletion step, in which all cells bearing lineage-specific markers (e.g. CD11b for macrophages and granulocytes, CD3 for T cells, B-220 for B cells, and Ter-119 for red blood cells) are removed, is used for starting step. The resultant population, referred to as lin^-, contains 10 to 100 times enrichment of HSCs when comparing with the starting material. Further purification of HSCs can be performed in several ways. Lin^- cells that exclude Hoechst and Rhodamine dyes ($Hoechst^{low}Rhodamine^{low}$)[21] are considerably enriched for HSCs, as well as lin^-CD34^+ or $lin^-Sca\text{-}1^+c\text{-}kit^+Thy1^{low}$ population[22,23]. $CD34^-lin^-$ population also reconstitute hematopoiesis. In humans, the $CD34^+CD38^-$ population is enriched for HSCs. Side Population (SP) cells are also enriched for HSCs[24]. They are called SP cells because they have a unique ability to extrude Hoechst dye. When examined by FACS analysis they fall into a separate population that is to the side of the rest of the cells, referred to as Main Population (MP), on a dotplot of emission data in the blue vs. red spectrum.

Several recent studies indicate that the same BM populations that are enriched for HSC are also enriched for BM derived stem cells (BMSC) with multipotentiality. As one of representative experiments, Krause et al. reported that the clonal origin of HSCs capable of engrafting in non-hematopoietic tissues was demonstrated conclusively[25]. In this study, a cell fraction enriched for HSCs was labeled with the membrane-bound dye PKH26 and injected into lethally irradiated recipients. After 48 hours, mitotically quiescent, PKH26-positive cells homing to the BM were isolated and single cells were injected into lethally irradiated, sex-mismatched recipients. At 11 months after transplantation, the engraftment of non-hematopoietic, ontogenetically distinct tissues as well as the reconstitution of the hematopoietic system was confirmed. As no injury other than irradiation prior to transplantation was given upon the recipients, the mechanism of transdifferentiation in respect of homing, proliferation, and differentiation remains unclear. Although the issue of cell fusion and functional activity of these transdifferentiated cells remains to be resolved, this study definitely revealed that the progeny of a single BMSC population with HSC characteristic can produce cells of non-hematopoietic tissues.

On the contrary, Wagers et al. demonstrated the extremely low frequency for developmental plasticity of adult HSCs[26]. They examined rigorously the cell fate potential of prospectively isolated, long-term reconstituting HSCs using chimeric animals generated by transplantation of a single $GFP^+c\text{-}kit^+Thy1.1^{low}Lin^-Sca\text{-}1^+$ (KTLS) BM HSC. Although single KTLS HSCs contributed substantially to the generation of mature hematopoietic cells, most tissues showed no evidence of GFP^+ nonhematopoietic cells.

At present, the correlation between HSC and BMSC is ambiguous. The well-desinged experiments remains to be planned to elucidate whether BMSC populations are enriched for a prehematopoietic cells that maintain greater pluripotentiality than HSC, or whether HSCs can transdifferentiate.

2) Mesenchymal stem cell (MSCs)

The microenvironment of the hematopoietic cells is comprised of stromal cells, a diverse population consisting of fibroblasts, smooth muscle cells, endothelial cells and others. These cells not only provide a scaffold to the developing stem and progenitor cells, but also produce extracellular matrix components and soluble proteins. Decades ago, *in vitro* propagation of adult mesenchymal stem cells within the stromal cell population, defined as colony-forming units-fibroblastic (CFU-F), was reported[27,28].

Recent cell separation techniques allowed for further isolation and characterization of CFU-F, which proved capable of differentiation into adipogenic chondrogenic and osteogenic lineages. Very recently, a population of highly plastic adult marrow-derived cells was characterized by the Verfaillie group[29-31]. These cells, termed multipotent adult progenitor cells (MAPCs), resemble embryonic stem cells in that they could be expanded for at least 80-120 population doublings without apparent exhaustion or telomerase shortening, and in that they contributed to tissue formation derived from mesoderm, endoderm, or ectoderm. Moreover, quantitative repopulation of hematopoietic and other tissues was confirmed in the absence of any injury-causing conditioning regimens without transforming events. In these studies, fusion formation, which has been suspected to be responsible for at least some of the transdifferentiation findings, was excluded by serial cytogenetic analysis[32,33]. Further confirmatory studies need to be done to evaluate MAPC characteristics.

From bone marrow to skeletal muscle

Several studies have demonstrated that marrow derived cells can differentiate into skeletal muscle cells. First, Ferrari et al. used direct inject of BM-derived cells into damaged muscle to induce differentiation of BM derived cells into skeletal muscle myocytes[34]. They used whole BM cells from transgenic animals that express β-galactosidase under the myosin light chain 3F promoter, which is expressed only in skeletal muscle myocytes. BM-derived cells developed into β-gal expressing myocytes 2-5 weeks after injection. After whole BM transplanttion followed by skeletal muscle injury, donor derived cells were found to contribute to the newly formed healing muscle. Other elegant study using transplantation of GFP^+ marrow analyzed the engraftment kinetics of BM derived myocytes after transplantation of whole BM[35]. Confocal microscopy confirmed that early engraftment of small numbers of donor derived myocytes increased up to 3.5% of the muscle fibers in response to muscle damage with exercise. In addition, this study described the progression from donor-derived uninucleate cells to multinucleate muscle cells, implying the normal muscle development. However, in these experiments, it is not clear which subpopulation(s) within BM contributed the differentiation into myocytes. Ferrari et al. evaluated the differentiation potency after separating into the adherent and nonadherent subpopulation, resulting that both subpopulations were capable of generating skeletal muscle myocytes.

To clarify the subpopulation within BM to differentiate into myocytes, we purified GFP labeled-purified HSCs, $lineage^-$ $CD45^+Sca\text{-}1^+c\text{-}kit^+$ cells, followed by transplantation into irradiated mice, and thereafter examined the contribution of transplanted cells for muscle regeneration sequentially. We demon-stareted two different roles of HSC population for muscle regeneration process, one leading to direct regeneration of damaged muscles in the early phase and the other to conversion into satellite cells in the late phase[36]. Of course, this does not exclude the possibility of non-hematopoietic stem cells. On the other hand, Shi D, et al. reported that marrow-derived stromal cells mainly contribute to myogenesis through fusion, than hematopoietic cells[37].

The potential clinical utility of this finding was demonstrated in a mouse model of muscular dystrophy. In the report by Gussoni et al., SP cells, isolated from BM of congenic male wild type mice, were used in an attempt to revert the phenotype of female mice with a spontaneous mutation in the dystrophin gene (Dmd^{mdx})[38]. This mouse serves as a model for Duchenne's muscular dystrophy. After transplantation of 2000-5000 male wild type SP cells into female Dmd^{mdx} mice, up to 4% of myofibers were stained positive for dystrophin at 12 weeks after transplantation. Ten to thirty % of these contained fused donor nuclei. However, functionality of SP cell-derived myocytes was not clear because they do not have a clear clinical phenotype.

While numerous reports indicate that adult BM-derived cells can contribute to skeletal muscle differentiation, *in vivo* in adult mouse, the generally low frequency of these events has made it difficult to study the molecular and cellular pathways involved. However, Brazelton et al. reported that in panniculus carnosus, one of specific muscles, up to 5% of myoblast incorporated BM-derived cells, showing the difference of transdifferentiation efficiency among skeletal muscles[39]. They suggested that the difference of molecular basis for muscle regeneration is different among muscles.

A clinical case report of a boy was diagnosed with relatively

mild Duchenne's muscular dystrophy (DMD) at age 12, who had undergone an allogeneic BM transplantation for complicated X-linked SCID at 1 year of age. Immunohistochemical analysis of skeletal muscles proposed the possibility that healthy muscle fibers forming from the donor marrow have decreased the severity of DMD[40]. However, at thirteen years after allogeneic BM transplantation, when this case was 14 years old, there were rare donor derived nuclei that expressed normal dystrophin (0.5%-0.9%) in the skeletal muscle fibers. Patients with DMD have a wide range of disease severity, therefore it is not clear in this case to evaluate whether donor derived myocytes improved the muscle function in this patient with relatively mild DMD phenotype.

From bone marrow to cardiac muscle

Cardiovascular disease is a major health problem in developed countries, therefore studies describing regeneration of the infarcted heart by MB derived stem cells raised enormous interests. Therapeutic benefit was demonstrated in mice with experimentally induced myocardial infarction which received intracardiac injection of BM derived cells during the initial post-infarct period. Whole BM cells or enriched murine HSC ($lin^-c\text{-}kit^+$) population, injected in the periventricular zone of the left ventricle, contributed up to 54% of newly formed myocardium, including cardiac muscle and endothelium[41]. This outcome was thought to be derived from the following three pathways: 1) increased vascularity due to BM cells differentiating into endothelial cells, 2) myogenic repair due to differentiation of BM cells into cardiac myocytes, and 3) production of cytokines or other factors that promote myogenic repair and prevent fibrosis. Similar observations were made with the use of SP cell from BM or $CD34^+$ cells, although contributing only marginally to newly formed cardiac muscle and/or vasculature[18,42]. On the other hand, several research teams reported that *in vitro* treatment of murine BM MSCs resulted in the formation of myotubule-like structures, where cardiac myocyte-like ultrastructures were observed in electron microscopy[43]. Following 2-3 weeks of culture, spontaneous and synchronised contraction was observed. These experiments imply that BM cell phenotype and purity will affect the experimental outcome. A related study demonstrated that Orlic et al. used a cytokine stimulation protocol with granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF), followed by coronary artery ligation, to evaluate the outcome[44]. The cytokine stimulation regime led to a 250-fold increase in circulating hematopoietic progenitor cell numbers. As compared to control groups, mice receiving the cytokine treatment showed 68% mortality reduction, 40% reduction in infarct size, and 26% reduction in ventricular dilatation. While it might be that these growth factors exert ameliorative effects on the infarcted heart unrelated to stem cell engraftment, this result is interesting and deserves further investigation. On the contrary, other investigators have reported that this apparent transformation is a result of cell fusion[45-47].

In humans, after transplantation of female hearts into males, up to 15% of cardiac myocytes can be recipient derived[48]. These observations show a high level of cardiac chimerism caused by the migration of primitive cells from the recipient to the grafted hearts. Two recent phase I studies using autolougous BM cells into the human heart post-infarct were reported. In patients who had autologous BM cells injected directly into their damaged myocardium, some improvement in cardiac function was documented based on medication usage, quality of life, and MRI-based studies of function at the site of injection[49]. In another report, after autologous $AC133^+$ BM cells were injected into infarct borders following coronary artery bypass grafting, improved perfusion and cardiac functin were observed[50]. In interpreting the results of these Phase I studies, cautions must be paid because no control subjects were compared and small numbers of patients were assessed. In addition, it is not clear to evaluate whether this improvement occurred due to generation of BM derived myocytes because these were autologous transplants.

From bone marrow to liver

BM cell engraftment as hepatocytes using male to female BM transplants in mice, rats, and humans was demonstrated in response to liver damage, which might promote BM cell to hepatocyte transition[51-55]. In rats, a combination of hepatotoxin, which induces widespread liver damage, and 2-acetylaminofluorine, which prevents endogenous liver repair, was used. In these rats, the combination of Y chromosome FISH and transgene expression demonstrated that BM cells were the source of the resultant hepatocytes. In mice, myeloablation prior to BM transplantation by the irradiation and/or chemotherapy caused liver damage, and donor derived hepatocytes were identified by Y chromosome FISH. In humans, the effect of other forms of liver damage could be assessed in liver samples of men who received liver transplantation from female donors. In these patients, the degree of subsequent damage to the transplanted liver

correlated with the extent of host-derived hepatocyte engraftment.

As one of the most excting demonstrations of BM cell plasticity into liver, Lagasse et al. showed that as few as 50 c-kit^{high} Thyl^{low}lin^{-}Sca-1^{+} (KTLS) HSCs rescued the phenotype of mice bearing a fumarylacetoacerare hydrolase (FAH) mutation causing fatal hereditary tyrosinemia type I without the treatment with NTBC[56]. These experiment demonstrated and extended the notion that cells purified as HSC contained liver-repopulating activity[51]. A major strength of this study was that the hepatocytes dedrived from BM cells were shown to be functional. Follow up experiments revealed that liver repopulation and functional rescue were almost exclusively due to cellular fusion[57]. Moreover, cellular fusion was independent of liver injury and appeared to be stochastically determined.

On the contrary, there is a report that this fusion observed may be a result of the genetic alterations in the FAH-deficient mouse, which has chromosomal abnormalities including aberrant karyokinesis or cytokinesis and multinucleation[58]. Jang Y-Y, et al. reported that a heterogenous bone marrow population might have more potential for fusion and a highly enriched population of HSC become liver cells when cocultured with injuried liver separated by a abarrier, which implies the denial of fusion during liver cell differentiation from HSC[59].

Several studies have examined human liver after sex mismatched liver or BM transplantation[53,60]. Male recipients of female livers and female recipients of male BM had hepatocytes containing Y chromosome, which can only be marrow derived unless fusion had occurred. Although these reports did not describe the functionality of generated liver cells, these data are exciting that it might become possible to provide *in vivo* replacement of diseased liver without the need for whole liver transplantation.

From bone marrow to nervous system

The brain tissue consists of neurons and glia cells, the latter of which can be subdivided into macroglia (astroglia and oligodendroglia) and microglia. Although microglia are considered to be derived from hematopoietic cells, the generation of macroglia and neurons from BM derived cells would be speculated stem cell plasticity. Two different experiments demonstrated that BM derived cells could serve as progenitors of non-hematopoietic cells in the murine central nervous system (CNS). In one experiment, lethally irradiated adult mice which received whole BM intravenously produced dono-derived brain cells with neural antigens NeuN and class-III β-tubulin[61]. Another experiment showed that in a strain of mice incapable of developing cells of the myeloid and lymphoid lineages, intraperitoneally transplanted adult BM cells migrated into the brain and differentiated into cells that expressed neuron-specific antigens[62]. These studies demonstrated the plasticity of BM cells in both adults and developng animals. Functional roles for these neuronal cells, which could be suspected to be immature due to the lack of axons, has yet to be shown.

MSC also might be useful in curing CNS diseases. MSC could be induced to differentiate into neuron-like cell *in vitro*[63]. These neuronal cells expressed neuron-specific antigens, but functional evaluation has not yet been demonstrated. In addition their ability to differentiate into neuron-like cells, MSC could also differentiate into oligodendocytes *in vivo*. When MSC from GFP expressing mice were microinjcted into a demyelinated spinal cord or fresh BM mononuclear cells were injected intravenously, remyelination occurred due to the transplanated cells[64,65]. The origin of cells and their characteristics were evaluated by expression of GFP and their appearance under electron microscopy with the staining of myelin basic protein. Interestingly in this case, function was inferred from the viewpoint of improved conduction velocity of axons. Sanchez-Romos et al. and Woodbury et al. independently reported the transdifferentiation of human BM MSCs into neural cells in culture[63,66]. In these experiments, chemical inducing reagents and growth factors such as basic FGF were used either alone or in combination to induce BM MSCs. The evidence of the differentiation to neuronal cells was based on morphology, antigenic markers and protein expressions. The neural specific markers expressed in these culture cells included neural specific enolase, neurofilament-M, tau, nestin, and glial fibrillary acidic protein. However, functional ability of these differentiated neuronal cells to produce an action potential has not been demonstrated, provoking doubts about the concept to transdifferentiation of BM MSCs into neuronal cells[67].

From bone marrow to pancreas

In mouse diabetic model, Hess et al. showed that transplantation of adult BM derived cells expressing c-kit reduces hyperglycemia in mice with STZ-induced pancreatic damage[68]. Although quantitative analysis of the pancreas revealed a low frequency of donor insulin-positive cells, these cells were not present at the onset of blood glucose reduction. Instead, the majority of transplanted cells were localized to ductal and islet

structures, and their presence was accompanied by a proliferation of recipient pancreatic cells that resulted in insulin production. The capacity of transplanted BM derived stem cells to initiate endogenous pancreatic tissue regeneration represents a previously unrecognized means by which these cells can contribute to the restoration of organ function.

In other experiment, 4 to 6 weeks following transplantation of male GFP^+ BM to female recipients, GFP^+ cells were isolated from the pancreatic islets of the recepient mice after digestion into single cells and FACS sorting[69]. Immunohistochemistry for insulin and FISH for Y-chromosome on the isolated cells confirmed that GFP^+ pancreatic cells were donor derived β cells. Furthermore, RT-PCR analysis confirmed expression of many islet cell markers including insulin I, insulin II, GLUT-2, IPF-I, HNF1α , HNF1b, PAX6 while being uniformly negative for CD45. Overall, 1.7-3% of islet cells in the recipients were donor derived. When grown *in vitro* under conditions standard for islet cells, BM derived cells had normal morphology and secreted insulin in response to glcose and/or exendin.

From bone marrow to gastrointestinal tract

In an elegant experiment, Krause et al. demonstrated that injection of a single BM derived stem cell with long term repopulating ability in mice leads to low numbers of donor derived esophageal and bowel epithelial cells[25]. On the other hand, Jiang, et al. demonstrated MAPC administered intravenously could engraft as gastrointestinal crypt cells, the functional stem cells of the gastrointestinal epithelium[29]. In human, engraftment as epithelial cells in gastrointestinal tract was reported after allogeneic BM transplantation[60]. In women who received BM transplantation with male BM, Y^+ epithelia could be detected in the esophagus and stomach as well as in the small and large bowel. Areas with chronic inflammation including gastric ulcers and graft versus host disease had a higher percentage of Y^+, cytokeratin$^+$, $CD45^-$ cells[70].

From bone marrow to lung

In the lung, two types of stem cells are identified: clara cells, which is the stem and progenitor cells for airway epithelia cells, and type II pneumocytes, that is the stem cells of alveoli. They can both self-renew to produce type II pneumocytes and differentiate into type I pneumocytes. Krause et al. reported that unfractioned BM cells or $CD34^+lin^-$ cells could differentiate into bronchiolar epithelia and type II pneumocytes after transplantation onto lethally irradiated female mice[25].

Lethal irradiation caused histologic evidence of pneumonitis including alveolar breakdown and hemorrhage beginning at day 3. The kinetics of engraftment implied that the high degree of BM cell engraftment as type II pneumocytes was derived from BM cells to repair extensive irradiation-induced damage[71]. Within the first 2 weeks after transplantation, the number of donor derived pneumocytes increased gradually and after 2 months, 1-20% of type II pneumocytes wad donor derived.

From bone marrow to kidney

A functional benefit for BM cell differentiation into renal tubular cells could be demonstrated in a model of ischemic renal disease. After wild type mice were transplantaed with whole BM cells from ROSA-26 mice after sublethal irradiation, rare β-galactosidase$^+$ renal tubule cells developed in the recipients' kidneys[72]. The observation of the increase in circulating $lin^-Sca\text{-}1^+$ cells following ischemic injury of the kidney prompted the investigators to evaluate these mobilized cells in order to repair the damaged kidney. Renal ischemia was induced in wild type mice by surgical clamping of the renal artery followed by reperfusion, which had received BM transplantation using $lin^-Sca\text{-}1^+$ ROSA-26 BM cells. The rise in BUN induced by renal ischemia 48 hours after lethal irradiation was significantly reduced in mice that were transplanted with $lin^-Sca\text{-}1^+$ BM cells and β-galactosidase$^+$ renal tubule epithelial cells were detected as early as 48 hours after ischemic injury.

In human, two studies demonstrated that after the transplantation of female kidneys into male recipients, Y chromosome positive epithelial cells could develop in the transplanted kidneys[73,74]. Additional studies showed the engraftment of BM cells into nonepithelial mesangial cells and interstitial cells within kidney[75-77].

From bone marrow to skin

In both mice and humans, Y^+cytokeratin$^+$ cells could be detected in the skin of female recipients, followed by BM transplantation from a male donor[25,60,78]. In human studies, donor derived keratinocytes were cytokeratin$^+$ and $CD45^-$[60]. However, even though 4 -14% of keratinocytes in human skin were Y^+, keratinocytes grown *in vitro* from the same skin biopsies failed to demonstrate any Y^+ donor cells. These findings could be explained in at least following two ways: either the donor derived keratinocytes required different culture conditions than those used, or the donor derived stem cells became keratinocytes without passing through an intervening tissue-specific stem

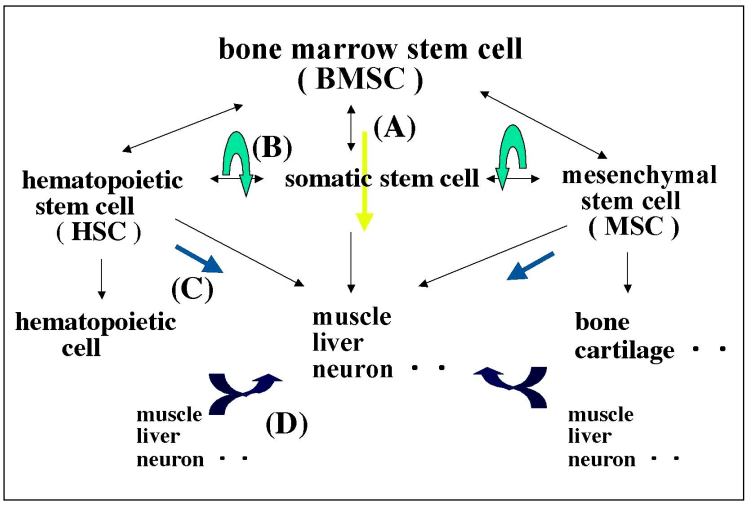

Fig. 1 Proposed mechanisms of plasticity
Four different colored arrows represent mechanisms of differentiation from BM derived cel ls into nonhematopoietic phenotypes. (A): This model predicts the presence of a highly pluripotent cell that has not yet committed to the hematopoietic lineage and maintains the capability to differentiate into multiple diverse cells. (B,C): HSC changes its gene expression pattern to that of an alternate cell type via dedifferentiation/redifferentiation pathway directly or indirectly. (D): If fusion is the mechanism of plasticity, a BM-derived cell fuses with a nonhematopoietic cell and the nucleus of a BM-derived cell takes on the gene expression pattern of the nonhematopoietic cell type.

cell state[78].

Mechanisms of plasticity

Generally, almost all the studies documenting plasticity have been reported using models of tissue injury to induce homing and differentiation of BM-derived stem cells. Tissue damage results from apoptosis/necrosis, being suspected to change microenvironment favorable for the crossing of lineage barriers. In spite of the enormous results demonstrating transdifferentiation capability of BM-derived cells, several recent studies have cast doubts or cautions in this field of stem cell biology.

Transdifferentiation or plasticity refers to the ability of one committed cell type to change its characteristics to that of a completely different cell type. **Fig.1** shows four possibilities explaining plasticity. Possible mechanism for this change in potency requires dedifferentiation at first, followed by maturation along an alternative pathway directly or indirectly. Simultaneously, another possibility could be proposed. This mechanism is that BM cells that differentiate into these diverse cell types represent a population of highly pluripotent stem cells located in the BM, which have not yet committed to blood. This possibility could be evaluated definitively by single cell transplantation experiments. In the study reported by Krause et al., single BM first fractionated (Fr25) via elutriation, and then lineage depleted (lin^-) cells from male mouse donors were infused into irradiated female recipients[25]. The progenies of donor stem cells were found in the epithelium of lung, liver, kidney, intestine and skin with engraftment frequency of 0.2-20% at 11 months after transplantation as well as in the recipient's BM. In the report of Jiang et al., a single BM MAPC was found to differentiate into visceral mesodermal, neurodermal, and endodermal cells in culture[29]. When injected into early blastcysts, these single MAPC were also shown to contribute to various somatic cell types. When these cells were transplanted into adult animals, they were found to differentiate into epithelium of liver, lung and gut along with hematopoietic cells.

Recently, an alternative mechanism for platicity was proposed: that is fusion. The fusion of a BM-derived cell with a nonhematopoietic cell to form a heterokaryon could convert the gene expression pattern of the original BM cell type to that of the fusion partner. Two groups have independently demonstrated that co-culture of postnatal cells with embryonic stem (ES) cells led to transformation of hybrid cells. In one study, primary neural stem cells co-cultured with ES cells fused with ES cells and resembles some of the phenotypic properties of ES cells[33]. A similar study showed that BM cells grown with ES cells in the presence of LIF and IL-3 could develop into ES-like cells after fusion[32] In either case, the progeny was tetra- and hexaploid. Although the fusion rate was estimated to be 1 in 10000 to 1 million cells, they open the possibility that cells fuse without specific fusionic stimulation. Therefore, further studies in this field need to be tested whether fusion might be responsible for change in the gene expression patterns of BM-derived stem cells. When cell-cell fusion is responsible for reprogramming the gene expression pattern of an adult cell, this still represents plasticity, but the cells involved need not to be stem cells. Ingenious experiments were designed to assess whether BM derived cells fuse with recipient cells. BM derived stem cells from male stop-lox-GFP mice, in which the cells express eGFP only after recombination by cre recombinase, were transplanted into female recipient animals that expressed

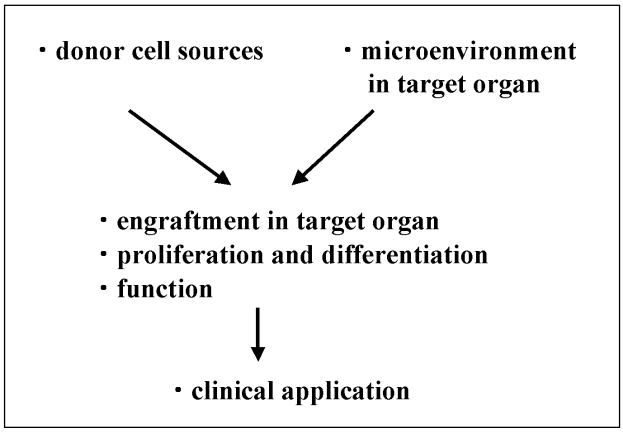

Fig. 2 Required items for plasticity research
At present, only the identification of donor BM-derived cells in various organs have been attempted. From now, the mechanisms underlying these phenomena should be studied precisely. The microenvironment supporting the differentiation of transplanted BM-derived cells, caused by tissue injury, may pave the way for efficient transdifferentiation without tissue injury. The functional evaluation is indispensable for applying to clinical use.

cre recombinase in all otheir cells. If fusion were to occur, the cre recombinase from the donor cell would induce recombination and subsequent GFP expression from the donor cell nuclei. Y chromosome positive pancreatic β cells were found as expected. However, GFP expression could not be detected suggesting that fusion had not occurred[69]. In spite of these data suggesting that fusion is not the underlying cause of BM derived stem cell differentiation into mature nonhematopoietic cells, the opposite papers were reported in the case of severely injuried liver[57,79]. In both cases, donor derived BM stem cells were transplanted into $FAH^{-/-}$ mice, and engraftment into hepatocytes occurred after the $FAH^{-/-}$ mice were weaned off the drug NTBC, which allows them to survive in the absence of the FAH enzyme. In the transplanted mice that survived NTBC withdrawal, the majority of the hepatocytes that were FAH^{+} (donor derived) also had markers of the recipient cells suggesting that fusion had occurred. Subsequently, Alvarez-Dlado et al. and Weimann et al. showed the evidence for cell fusion of BM derived cells with neurons and cardiomyocytes[80,81].

It is not yet known whether fusion is responsible for much of the plasticity results. Even if it were, this should deserve to be examined. If the fused cells are functional and healthy, these cells could be of great physiologic significance. The concern would be that the resulting cells carry high potential for malignant transformation. Such avenues of research will require extensive investigation to evaluate whether the fusion data represent an even more profound challenge to our existing paradigms of cell differentiation and development[82].

Moreover, for future clinical application, it is indispensable to evaluate the multipotentiality and its functionality of transdifferentiated human BM cells *in vivo*. Recently, we have developed new recipient mouse mice, NOD/SCID/γc^{null} mice, which permit the reconstitution of BM after transplantation of human $CD34^{+}$ cells of umbilical cord[83]. These NOD/SCID/γc^{null} mice, double homozygous for the severe combined immunodeficiency (SCID) mutation and interleukin-2Rγ (IL-2Rγ) allelic mutation (γc^{null}), were generated by 8 backcross matings of C57BL/6J- mice and NOD/Shi-*scid* mice. It is suggested that multiple immunological dysfunctions, including cytokine production capability, in addition to functional incompetence of T, B, and NK cells, may lead to the high engraftment levels of xenograft in NOD/SCID/γc^{null} mice. Interestingly, the reconstitution of a human immune system could be confirmed in these laboratory animals, supporting the notion that the *in vivo* multipotentiality and subsequent functional evaluation of transplanted human cells could be examined in these mice[84].

Ultimately, we want to apply the phenomenon of plasticity to treating disease or tissue injury in patients. In order to better understand the mechanisms responsible for the differentiation of BM cells into mature functional nonhematopoietic cell types, further progress will need to be made in many steps (**Fig.2**). At first, we need to clearly indicate the donor cell sources and specify the cell subpopulation. Different injuries and diseases will likely select for different cell types, therefore these conditions should be optimized, resulting into the identification of the essential molecular mechanism required for plasticity. Also, we must improve detection methods so that the cell source, cell phenotype, and cell function can be evaluated clearly. Finally it is important to understand that the development of clinical applications can only occur concurrently with studies to elucidate the underlying cellular and molecular mechanisms.

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