Arch. Immunol. Ther. Exp. (2011) 59:369–378
DOI 10.1007/s00005-011-0139-9

REVIEW

Bone Marrow Mesenchymal Cells: How Do They Contribute to Tissue Repair and Are They Really Stem Cells?

Yasumasa Kuroda · Masaaki Kitada · Shohei Wakao · Mari Dezawa

Received: 14 January 2011 / Accepted: 7 March 2011 / Published online: 26 July 2011
© L. Hirszfeld Institute of Immunology and Experimental Therapy, Wroclaw, Poland 2011

Abstract Adult stem cells typically generate the cell types of the tissue in which they reside, and thus the range of their differentiation is considered limited. Bone marrow mesenchymal stem cells (MSCs) are different from other somatic stem cells in that they differentiate not only into the same mesodermal-lineage such as bone, cartilage, and adipocytes but also into other lineages of ectodermal and endodermal cells. Thus, MSCs are a unique type of adult stem cells. In addition, MSCs home to damaged sites, differentiate into cells specific to the tissue and contribute to tissue repair. Therefore, application of MSCs in the treatment of various diseases, including liver dysfunction, myocardial infarction, and central nervous system repair, has been initiated. Because MSCs are generally harvested as adherent cells from bone marrow aspirates, however, they comprise heterogeneous cell populations and their wide-ranging differentiation ability and repair functions are not yet clear. Recent evidence suggests that a very small subpopulation of cells that assume a repair function with the ability to differentiate into trilineage cells resides among human MSCs and effective utilization of such cells is expected to improve the repair effect of MSCs. This review summarizes recent advances in the clarification of MSC properties and discusses future perspectives.

Y. Kuroda · M. Kitada · S. Wakao · M. Dezawa (✉)
Department of Stem Cell Biology and Histology,
Tohoku University Graduate School of Medicine,
2-1, Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: mdezawa@med.tohoku.ac.jp

Y. Kuroda
e-mail: y-kuroda@med.tohoku.ac.jp

M. Kitada
e-mail: Masaaki.Kitada@gmail.com

S. Wakao
e-mail: wakao@med.tohoku.ac.jp

Keywords Mesenchymal stem cells · Adult stem cells · Transdifferenitation · Cytokines · Repair

Introduction

Bone marrow is classified by its embryologic relationship to the mesodermal cell lineage and is a very important organ for hematogenesis. Hematopoietic stem cells (HSCs), which are responsible for hematogenesis, reside in the mononucleated cell fraction of bone marrow, but other types of stem cells and precursors such as bone marrow mesenchymal stem cells (MSCs), which provide the structural and functional support for HSCs, and endothelial precursors are also contained in this fraction (Thomas 2000).

HSCs have been applied for bone marrow transplants in patients with leukemia for over 40 years, and successful results have been achieved (Thomas 2000). In addition to curing leukemia, bone marrow transplantation causes an intriguing phenomenon that cannot be explained based only on the function of the infused HSCs. As mentioned above, the bone marrow mononucleated cell fraction, the main cell population transplanted to the leukemia patients, is a mixture of several kinds of cells, including HSCs. Some patients suffering from leukemia and liver damage receiving a bone marrow transplantation partially recovered from liver damage (Thomas 2000). In the liver, transplanted cells homed to and integrated into the damaged site where they differentiated into cells expressing hepatocyte markers (Terai et al. 2002, 2003). In sex-mismatched bone marrow transplantation, donor-derived cells also integrated into various tissues: epithelial cells of the esophagus, stomach,

small intestine, and colon, endothelial cells, and keratinocytes (Thomas 2000). These phenomena suggest that cells capable of homing to damaged sites and contributing to tissue reconstruction are contained in the bone marrow. If this is the case, bone marrow transplantation would be an efficient cell therapy for treating other diseases in addition to leukemia. Moreover, bone marrow has practical advantages in that a marrow bank is already in operation, so these cells can be applied not only for auto-transplantation but also for allo-transplantation. In contrast to embryonic stem (ES) cells or fetal stem cells, the use of bone marrow is not associated with ethical problems. For these reasons, several studies have accumulated evidence for the usefulness of bone marrow for the cell therapy. Among cells, MSCs have attracted great attention in the past decade because of their high versatility. Their action is not confined to the trophic effects which is generated by the production of various kinds of cytokines and trophic factors. They are also able to home and integrate into damaged tissues and to differentiate in vivo into broad spectrum of cells that crosses the oligo-lineage boundaries between mesodermal and ectodermal or endodermal lineages, that were previously thought to be impenetrable (Prockop 1997). Thus, apart from trophic effect, MSCs have been speculated to contain a small population of cells that act as tissue repairing cells.

Friedenstein et al. (1968) developed the concept of "multipotential marrow stromal stem cells", describing cells that are adherent, clonogenic, nonphagocytic, and fibroblastic, and named them "colony-forming units-fibroblastic" (CFU-Fs). Under the appropriate conditions, CFU-Fs can give rise to a broad spectrum of differentiated mesenchymal tissues, including bone, cartilage, adipose tissue, fibrous tissue, and myelosupportive stroma.

Recent stem cell research has provided evidence that stem cells can be characterized and traced based on their expression of surface molecules. As described above, MSCs are a collective cell population harvested as adherent cells from bone marrow aspirates. They are indeed mesenchymal cells and express general mesenchymal markers. MSCs, however, are a heterogeneous cell population with regard to their expression of surface molecules (Pittenger et al. 1999). For this reason, identification of the cells responsible for differentiation and for tissue repair has been difficult.

Adult stem cells typically generate the cell types of the tissue in which they reside, and therefore the range of their differentiation capabilities is considered limited. For example, HSCs generate red blood cells and white blood cells, and neural stem cells generate neurons and glial cells (Gage 2000; Weissman and Shizuru 2008). MSCs differ from these somatic stem cells in that they differentiate not only into the same mesodermal-lineage, such as bone, cartilage, and adipocytes, but also into other lineages of ectodermal and endodermal cells (Dezawa et al. 2004, 2005; Pittenger et al. 1999; Snykers et al. 2009). In this manner, MSCs can generate cells representative of all three germ layers, and are thus thought to be pluripotent cells. On the other hand, the characteristics of MSCs differ from those of typical pluripotent stem cells such as ES cells; MSCs do not self-renew indefinitely and they are not homogeneously multipotential (Charbord 2010). In fact, the pluripotency of MSCs is unclear and from that standpoint, MSCs remain an enigma.

Mesenchymal cells with multilineage differentiation ability are mobilized into the circulating peripheral blood under many circumstances, such as serious disease, injury, or stress (Chino et al. 2008; Kuznetsov et al. 2001, 2007). These circulating mesenchymal cells are reported to integrate into the damaged site. This evidence leads us to imagine that tissue-repairing cells reside among bone marrow mesenchymal cells. Recently, new data demonstrating that pluripotent cells able to generate endodermal-, ectodermal-, and mesodermal-lineage cells from a single cell exist among mesenchymal tissues, including bone marrow and dermal fibroblasts (Kuroda et al. 2010). In this review, the features of these tissue repairing cells and their potential in regenerative medicine is discussed.

Background of MSC Transplantation

MSCs per se have been studied for many years. Till and McCulloch (1961) first revealed the clonal nature of bone marrow cells in the 1960s, and an in vitro assay for examining their clonogenic potential was later developed by Friedenstein et al. (1970). In this assay, cells that are consistently adherent, clonogenic, nonphagocytic, and fibroblastic were referred to as CFU-Fs. Subsequent studies revealed that the cells identified by Friedenstein were multipotent and able to give rise to osteocytes, chondrocytes, and adipocytes. Pittenger et al. (1999) showed that trilineal osteoblastic, chondrocytic, and adipocytic clones are present in human bone marrow and provided evidence that these cells are clearly distinct from endothelial cells or HSCs based on their surface antigen expression. They also revealed the differentiation plasticity of bone marrow cells and how their fate can be determined by environmental cues; culturing them in the presence of ascorbic acid, inorganic phosphate, and dexamethasone promotes their differentiation into osteoblasts, while administration of transforming growth factor-β induces chondrogenic markers. Since the initial description of CFU-Fs, these multipotent bone marrow cells have been given different names. Owen et al. (1987) called these cells "marrow stromal stem cells" and Caplan (1991) introduced the term "mesenchymal stem cells", which has become generally used in later reports

Together with basic studies of MSCs, these cells were determined to participate in the repair of certain tissues. For example, Petersen et al. (1999) showed that parts of the rat liver in which native hepatocytes are prevented from proliferating were repopulated by transplanted bone marrow cells. A similar phenomenon was demonstrated in humans. In patients who received a sex-mismatched bone marrow transplantation, some hepatocytes were shown to originate from the donor (Thomas 2000). In addition to these cases, MSCs are reported to repair the heart (myocardial infarction), neural tissue (stroke and spinal cord injury), airway (tracheal obstruction), skin (dermal ulcer), skeletal muscle (muscle degeneration), and intestine (ischemic intestinal diseases) (Adas et al. 2011; Chopp et al. 2000; Ferrari et al. 1998; Grove et al. 2011; Li et al. 2000; Rogers et al. 2008; Schachinger et al. 2009). Thus, MSC transplantation has already been applied in the treatment of many kinds of intractable diseases.

Transplantation of MSCs and Its Effect

There are two major effects of MSC transplantation. One is the trophic effect, which is mediated by various kinds of trophic factors and cytokines produced by MSCs (Tolar et al. 2010). The second is their tissue repairing function; the ability to recognize the damaged site, home and integrate into the site, and then finally differentiate into cells specific to the tissue (transdifferentiation) and serve as an integrated member of the functionally organizing adult tissue (Korbling and Estrov 2003). Given that MSCs normally provide trophic factors to support HSCs in the bone marrow, their trophic effect is quite reasonable. The latter effects is in some ways quite extraordinary and thus has until recently been debated as a controversial topic of MSCs. In particular, questions were raised regarding the interpretation of ''transdifferentiation'' of infused MSCs into neuronal lineage cells because some groups suggested that the transdifferentiation was rather a result of fusion between infused bone marrow cells and the host brain cells (Alvarez-Dolado et al. 2003; Terada et al. 2002). Despite the skepticism surrounding the capacity of MSCs to transdifferentiate, accumulating evidence supports this phenomenon both in vivo and in vitro, and the growing body of evidence supporting this unique property of MSCs can no longer be ignored. In the following sections, these characteristic properties of MSCs are described and summarized.

Trophic Effect of MSCs

MSCs reside in the abluminal space of marrow sinusoids to form a three-dimensional cellular network and provide structural and functional support for the generation of blood cell lineages that arise from HSCs. Because they normally produce various types of cytokines and soluble factors to support HSCs, MSCs also exert trophic effects for rescuing damaged tissues when transplanted.

Among the broad range of factors produced by MSCs, a number of them are reported to be neuroprotective. Human MSCs secrete significantly higher levels of several neurotrophic factors, such as brain derived-neurotrophic factor (BDNF), nerve growth factor (NGF), neuregulin-1, brain natriuretic peptide (BNP), interleukin-6, and fibroblast growth factor (FGF)-2 (Crigler et al. 2006; Fan et al. 2011). They also produce significant amounts of factors for dopamine neurons, such as glial-derived neurotrophic factor (GDNF) and FGF-20 (Fan et al. 2011). When infused into the cerebrospinal fluid after spinal cord injury, MSCs exert a tissue rescue effect partly via hepatocyte growth factor (HGF) (Yoshihara et al. 2007). These factors are produced by naïve MSCs, but even after neuronal differentiation, these cells produce greater quantities of granulocyte colony-stimulating factors, vascular endothelial growth factor (VEGF), and GDNF, which might have trophic effects on neural cells (Fan et al. 2011). These diffusible factors may partially account for their neurotrophic effect in alleviating deficits of neurologic diseases, such as stroke and spinal cord injury.

Angiogenesis is indispensable for tissue repair and regeneration. Human and murine MSCs also produce angiopoietin (Ang)-1, -2, Ang-like-1, -2, -3, -4, VEGF, and FGF-2, which are indispensable for tissue reconstruction (Phinney 2007).

Forced expression of a family of serine/threonine specific protein kinases (Akt) accelerates the production of HGF, VEGF, FGF-2, IGF-1, and thymosin beta 4 (TB4) in MSCs, and such genetically modified MSCs are considered efficient in cardiac repair and protection (Gnecchi et al. 2006; Markel et al. 2008; Xu et al. 2009). Even if Akt is not artificially activated, production of these factors can be detected in naïve MSCs in vitro and in microarray data. Therefore, naïve MSCs themselves are effective for cardiac protection. In particular, a significant amount of VEGF is produced by naïve MSCs, which might contribute to cardioprotection (Markel et al. 2008).

Philp et al. (2004) demonstrated that TB4 stimulates hair follicle stem cells that may lead to hair growth. Other than hair stem cells, TB4 induces stem cell migration and thus exerts a tissue repair effect. TB4 might also be involved in the MSC function after transplantation (Philp et al. 2004).

Differentiation of MSCs after Transplantation

Infusion of adult MSCs has generated unexpected phenotypes in vivo, including muscles and brains cells,

suggesting their transdifferentiation in vivo (Ferrari et al. 1998; Mezey et al. 2000). Doubts about these interpretations have been raised, however, and it has been suggested that the supposed MSC differentiation was actually a result of fusion between donor MSCs and host cells (Alvarez-Dolado et al. 2003; Terada et al. 2002). Fusion in vivo is indeed conceivable. Nevertheless, based on the frequency and ratio of MSCs integrated and differentiated into the host tissue, fusion alone cannot explain all of the phenomena observed after infusion of MSCs. Furthermore, experiments using a sophisticated Cre-lox system clearly demonstrated that MSCs can transdifferentiate into epithelial cells in vivo without fusion (Harris et al. 2004).

The state of the tissue must also be taken into consideration in the debate regarding fusion and transdifferentiation; that is, whether the tissue is intact or damaged prior to the infusion of MSCs. MSCs may behave quite differently in damaged tissue compared to intact tissues. There is a very low frequency of the integration of MSCs into uninjured intact tissues as well as their subsequent spontaneous transdifferentiation within the normal preexisting network of mature host cells (Mezey et al. 2000). In this case, even if the frequency of homing and integration is extremely low, fusion will predominate. On the other hand, MSCs are able to migrate to the damaged site, perhaps by receiving a signal from the damaged site. When the tissue is damaged, infused MSCs migrate to the injured site, integrate into the tissue via disrupted blood vessels, and then differentiate into tissue-specific cells according to the surrounding microenvironment to become a ''member'' of the tissue and contribute to the tissue repair.

Circulating MSCs

Even under normal conditions, circulating MSCs in the peripheral blood stream are extremely rare in humans (Kuznetsov et al. 2007). The role of these circulating MSCs in the normal state is unknown, but this fact suggests that MSCs originally possess the ability to be mobilized into the peripheral blood stream and to migrate to organs. Under the conditions of serious injury or stress, MSCs receive signals from the injured site and move to the bloodstream and migrate to the damaged site (Chino et al. 2008; Kuznetsov et al. 2001, 2007).

Factors and/or receptors related to these events have been suggested. Chemokines and their receptors comprise a common system to recruit immunologic cells. The CXCR4-CXCL12 and CX3CR1-CX3CL1 systems are reported to be involved in MSC migration, and among them, stromal-derived factor-1 alpha (SDF-1) and CXCR4 (the SDF-1 receptor) are strong mediators of MSCs (Ferrari et al. 2011; He et al. 2010; Liu et al. 2010; Molyneaux et al. 2003; Yu et al. 2010). SDF-1 is a CXC chemokine that is important for the trafficking of fetal and adult stem cells and for the homing of HSCs to bone marrow (Kitaori et al. 2009; Lapidot and Petit 2002).

Other than chemokine systems, urokinase receptor, necrosis factor-related apoptosis-inducing ligand receptor (TRAIL) 2 and 4, and endothelial nitric oxide synthase also act on MSCs to affect their migration (Vallabhaneni et al. 2011).

High mobility group box 1 (HMGB1) is an intracellular protein that translocates to the nucleus where it binds DNA and regulates gene expression (Meng et al. 2008). HMGB1 can be released from damaged cells in which cell membranes are permeabilized and intracellular constituents may diffuse out of the cell to bind to inflammatory cells (Meng et al. 2008). In fact, damaged neurons in a stroke model release HMGB1. Released HMGB1 efficiently recruits stem cells and is involved in subsequent tissue repair (Qiu et al. 2008). More directly, HMGB1 acts on MSCs to inhibit proliferation and to promote migration and transdifferentiation (Meng et al. 2008).

Substance P belongs to the tachykinin neuropeptide family and is a transmitter for specific sensory neurons. Recently, in a corneal injury model, substance P clearly triggered the recruitment of MSCs from the bone marrow to the injured tissue and participated in the tissue repair process (Hong et al. 2009).

To repair tissues, cells migrate to and recognize the injured site as described above. Following integration into the damaged site, they differentiate into cells that comprise the tissue to replenish the lost cells. In the next section, the differentiation ability of MSCs is discussed.

Differentiation Ability of MSCs

In the 1980s and 1990s, a number of studies extended the initial observation of Friedenstein and coworkers and clarified that MSCs are multipotent and could give rise to osteoblasts, chondrocytes, and apidocytes (Prockop 1997). Later, Pittenger et al. (1999) showed that osteoblastic, chondrocytic, and adipocytic clones are present in human bone marrow.

In addition to these mesenchymal lineages, MSCs stimulated with chemical reagents, cytokines, or gene introductions differentiate in vitro into various cell types of not only the same mesodermal-lineage cells, but also ectodermal- and endodermal-lineage cells. The MSC-inducible cell types include endothelial cells (Oswald et al. 2004), cardiac muscle cells (Makino et al. 1999), skeletal muscle cells (Dezawa et al. 2005), hepatocytes (Oyagi et al. 2006; Prindull and Zipori 2004; Snykers et al. 2009), neuronal cells (Dezawa et al. 2004; Wislet-Gendebien et al. 2005), peripheral glial cells (Dezawa et al. 2001) and insulin-producing cells (Phinney and Prockop 2007) and

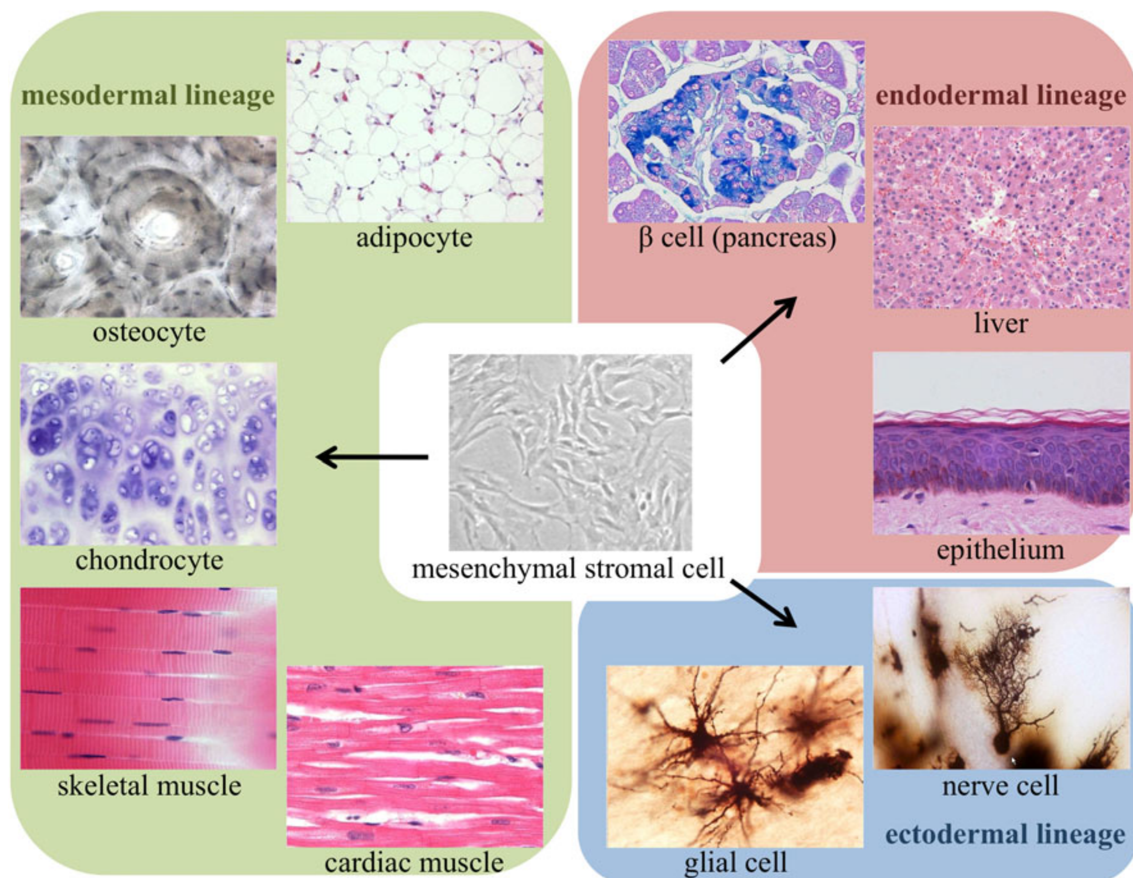

Fig. 1 Differentiation of MSCs into mesodermal, ectodermal and endodermal cells

epithelial cell (Spees et al. 2003) (Fig. 1). Because all of these verifications were performed in vitro but not in vivo, such differentiation cannot be explained by fusion. In this manner, despite the initial skepticism regarding the capacity of MSCs to differentiate into multiple lineage cells, it has recently become increasingly unreasonable to ignore the growing body of evidence supporting the wide-ranging differentiation ability of MSCs.

Even granting that MSCs are able to differentiate into multiple lineage cells, a question arises as to why they are able to differentiate into such a broad spectrum of cell types. In general, adult stem cells generate the cell types of the tissue in which they reside, and therefore the range of their differentiation capabilities is limited. From this point of view, MSCs are a unique and exceptional cell type among adult stem cells.

Heterogeneity of MSCs

Several studies aimed at unraveling the mechanisms underlying the potential of MSCs to differentiate into multiple lineage cells have encountered difficulties. MSCs are usually harvested as adherent cells from bone marrow aspirate and are thus a heterogeneous cell population (Pittenger et al. 1999). Bone marrow contains various types of adherent cells including mesenchymal stromal cells, endothelial cells, osteogenic cells, phagocytotic cells, and others (Weissman and Shizuru 2008). Therefore, any of these types of adherent cells could contaminate the MSC population, particularly in the initial step of the culture. In subcultures, however, the cell population seems to converge on general MSCs and other cell types are left out, but still there is no proof that MSCs comprise a single homogeneous cell type. Therefore, the big picture of MSCs has not been clarified, and in fact, a specific molecular marker that is exclusively expressed by MSCs has yet to be found.

Pittenger et al. (1999) was the first to analyze the surface antigens in MSCs in detail. They described that MSCs are uniformly positive for SH2, SH3, CD29, CD44, CD71, CD90, CD106, CD120a, CD124, and many other surface proteins, but are negative for markers of the hematopoietic lineage, including lipopolysaccharide receptor CD14, CD34, and the leukocyte common antigen CD45 (Pittenger et al. 1999). Cells with HSC markers are never identified among MSCs and thus they concluded that MSCs are different from the marrow fibroblastic cells formerly isolated by other groups that remain positive for the

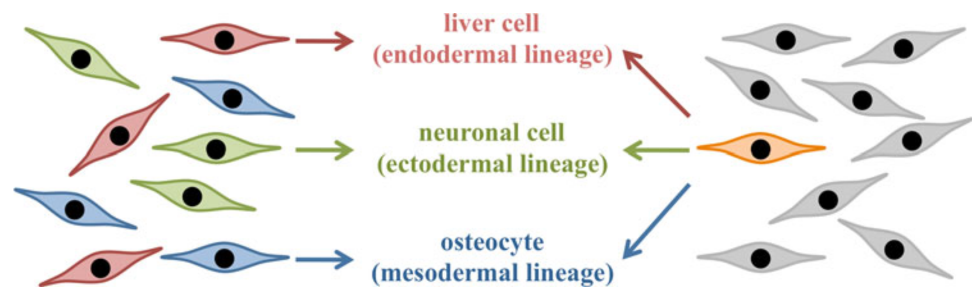

Fig. 2 Two explanations for trilineage differentiation in MSCs

hematopoietic surface antigen (Friedenstein et al. 1968). Other groups subsequently attempted to characterize the surface antigens of MSCs. Some common antigens were identified, but other antigens differed among reports. As a result, many markers have been listed, but the International Society for Cellular Therapy recently stated that MSCs are defined by their expression of CD105, CD73, and CD90, and the lack of the expression of CD45, CD34, Cd14, CD11b, CD79a, or CD19 and HLA-DR surface molecules (Horwitz et al. 2005).

MSCs are also suggested to be heterogeneous from the viewpoint of development. In early development, marrow stromal cells appear before hematopoiesis begins in a developing marrow cavity after a bony collar has formed outside of the developing rudiment. The primitive bony collar becomes eroded by osteoclasts to allow for vascular invasion and the marrow cavity is formed. Next, vascular invasion brings osteogenic cells that had previously differentiated in the periosteum into the marrow cavity. The development of sinusoids, which are characterized by cell-permeable endothelial walls, allows for penetration by blood-borne HSCs, followed by interaction of HSCs with the primitive stromal microenvironment. This interaction permits hematopoiesis to be established. In this way, MSCs belong to the mesodermal lineage (Bianco and Gehron Robey 2000).

Apart from this evidence, Takashima et al. (2007) showed that some MSCs are descendants of the neuroepithelial lineage, and they thus argued that the neural differentiation exhibited by MSCs is not truly transdifferentiation, but rather neural differentiation along the ectodermal lineage. Involvement of such cells in neural differentiation is possible, although this would not account for all the phenomena observed in MSC differentiation into lineages other than neuronal cells.

MSCs thus comprise different cell types in their origin and cell surface antigen expression.

Are MSCs Pluripotent Stem Cells?

Granted that MSCs are a heterogeneous cell population, MSCs as a whole show differentiation not only into the same mesodermal lineage cells of osteocytes, chondrocytes, and adipocytes, but also into other lineages such as into neuronal cells (ectoderm) and hepatocytes (endoderm) (Dezawa et al. 2001, 2004, 2005; Pittenger et al. 1999). For this reason, MSCs are considered pluripotent cells.

The term "pluripotency", however, requires careful verification. The definition of a "stem cell" requires that it possess two properties: self-renewal (the ability to renew itself through mitotic cell division) and potency (differentiation into a diverse range of specialized cell types). Potency specifies the differentiation potential (the potential to differentiate into different cell types) of the stem cell. For example, pluripotent stem cells can differentiate into cells derived from any of the three germ layers, ectodermal, endodermal, and mesodermal cells. Multipotent stem cells can differentiate into a number of cells, but mostly those of a related family of cells, and unipotent cells can produce only one cell type, their own, but have the self-renewal property, which distinguishes them from non-stem cells (e.g., muscle stem cells) (Dezawa et al. 2005).

MSCs are reported to behave like pluripotent stem cells. For example, Verfaillie and colleagues stated that MSCs derived from adult bone marrow, which they named multipotent adult progenitor cells (MAPC) in their report, are pluripotent stem cells, although MAPCs have been difficult to reproduce in other laboratories (Jiang et al. 2002). Marrow-isolated adult multilineage inducible cells, which were reported by Schiller and coworkers, express Oct-4 and Rex-1, known ES cell markers, and differentiate into osteocytes (mesodermal), neuronal cells (ectodermal), and pancreatic-like cells (endodermal) (D'Ippolito et al. 2004). Ratajczak and colleagues reported that a population of very small embryonic-like cells, named VSEL cells, also expressed some ES cell markers such as Oct-4, Nanog, and Rex-1, and are able to differentiate into cardiac (mesodermal), neural (ectodermal), and pancreatic (endodermal) cells (Kucia et al. 2006; Wojakowski et al. 2011). These reports suggest that MSCs are very likely to be pluripotent stem cells, but the above findings do not confirm the pluripotency of MSCs because all of the differentiations were demonstrated from a sample of heterogeneous MSC populations. In such cases, several possibilities remain: i.e., (1) there are several types of unipotent cells responsible for

each of the ectodermal, endodermal, and mesodermal-lineage cell differentiation (Fig. 2); and (2) cells with different levels of potency, including unipotent and bipotent cells are contained in MSCs. In both cases, MSCs are not pluripotent in the fullest sense, but as a whole act as if they are pluripotent. The third possibility is that a subpopulation of cells corresponding to pluripotent stem cells resides among MSCs (Fig. 2).

Conclusive evidence for pluripotency would be that MSCs are able to differentiate into cells of all three germ layers from a single cell.

Recently, adult human mesenchymal cells, such as MSCs and dermal fibroblasts were shown to contain a small number of pluripotent stem cells, named "multilineage differentiating stress enduring" (Muse) cells (Kuroda et al. 2010). These cells were initially found as stress tolerant cells; expressed pluripotency markers such as Nanog, Oct3/4, and Sox2; can be isolated from MSCs or from fibroblasts as stage-specific embryonic antigen-3-positive cells (a marker for undifferentiated human ES cells); and were able to self-renew. Most importantly, Muse cells are able to generate cells representative of all three germ layers from a single cell, thus indicating that they are pluripotent. The ratio of Muse cells in cultured MSCs is less than 1% and in fibroblasts 2–5%, but they are rarer in the fresh bone marrow mononucleated cell fraction: 1 out of 3,000 mononucleated cells (Kuroda et al. 2010).

Muse cells act as tissue repairing cells in vivo, as verified by the infusion of green fluorescent protein (GFP)-labeled naïve human Muse cells into immunodeficient mice with tissue damage either of the back skin (skin incision, transplanted cells by local injection), gastrocnemius muscle (muscle degeneration induced by cardiotoxin injection; intravenous injection), or liver (fulminant hepatitis induced by intraperitoneal injection of CCl4; intravenous injection) (Kuroda et al. 2010). Transplanted Muse cells integrate into damaged skin and differentiate into epidermal cells positive for cytokeratin 14. They were also incorporated into regenerating muscle. After 4 weeks, more than 90% of the integrated GFP^+ Muse cells had the appearance of mature myofibers with peripheral nuclei and expressed human dystrophin. Furthermore, some of the integrated Muse cells expressed satellite cell marker Pax7. In fulminant hepatitis, up to 87% of integrated GFP-labeled human Muse cells expressed human antitrypsin and human albumin after 4 weeks. In addition, human albumin is detected in mouse peripheral blood in a Western blot, suggesting that Muse cells differentiate into functional hepatocytes. Thus, Muse cells can integrate as functional cells in damaged tissue. It is possible that Muse cells undergo the phenomena previously observed in MSCs, i.e., transdifferentiation into the same mesodermal cells as well as into ectodermal and endodermal cells, and have a repair effect after transplantation into various types of damaged tissues (Kuroda et al. 2010).

Up to now, MSCs have been transplanted into patients with expectations of trophic effects. The identification of pluripotent Muse cells and their markers may lead to efficient tissue repair treatment with an advanced approach.

Tissue Repair by MSCs

As mentioned above, MSCs have highly flexible in vitro differentiation. Therefore, transplantation of MSCs that have differentiated into desired cells is a hopeful strategy. Such regulated differentiation, however, raises questions regarding their safety and quality control for the use of cytokines, reagents, and gene introduction. In fact, the use of untreated naïve MSCs is currently preferred over the use of artificially differentiated MSCs.

Bone

The initial clinical trials with MSCs were performed by Horwitz and colleagues in 1999, who demonstrated that bone marrow-derived mesenchymal cells improves the total-body bone mineral content and subsequent osteogenesis in children with osteogenesis imperfect (Horwitz et al. 1999). Since then, MSCs have been applied to patients with osteoarthritis and bone defects (Niedzwiedzki et al. 1993).

Liver

A landmark report of hepatopathy was published in 1999. Petersen et al. (1999) reported that parts of the liver were repopulated by cells that appeared to originate in the bone marrow under conditions of liver injury in which native hepatocytes were prevented from proliferating. In this model, 0.14–0.16% of hepatocytes showed evidence of a bone marrow origin. Since then, there have been many other reports of bone marrow derivation of hepatocytes in diverse models. Such phenomena have also been demonstrated in humans. Liver biopsy samples from patients with sex-mismatched (male donor, female recipient) bone marrow transplantation were examined. In these patients, 5–40% of hepatocytes contained donor-derived Y chromosomes (Petersen et al. 1999; Thomas 2000). In addition, the liver tissue with lowest injury level contained fewer bone marrow-derived hepatocytes, whereas the liver tissue with the most severe injury, such as fibrosing cholestatic recurrent hepatitis C, contained the most bone marrow-derived hepatocytes (up to 64%) (Gilchrist and Plevris 2010). Several groups have applied bone marrow cells in clinical trials for patients with liver cirrhosis by various

infusion routes, such as direct portal vein, hepatic artery, or peripheral blood (Gilchrist and Plevris 2010). In these cases, increases in hepatocyte proliferation, bilirubin, and albumin have been recognized, and complications of cirrhosis were diminished (Gordon et al. 2006; Terai et al. 2002, 2003).

Heart

In myocardial infarction, transplanted MSCs or bone marrow mononucleated cells integrate into damaged tissue and differentiate into cardiac muscle cells. Based on the evidence, several institutions in Germany have applied autologous bone marrow mononucleated cells to acute myocardial infarction patients, which has resulted in efficient cardiac function recovery for up to one year (Misao et al. 2006; Orlic et al. 2001; Schachinger et al. 2009).

Central Nervous System

Transplantation of MSCs for central nervous system (CNS) repair (rat stroke model) was first reported by Chen et al. (2003). Since their report, several studies of MSCs in stroke, spinal cord injury, and traumatic brain injury have been reported (Parr et al. 2007; Wright et al. 2011). In all of these models, MSC transplantation by either direct injection, intravenous infusion, or injection into the cerebrospinal fluid shows promising results with functional recovery. In most cases, MSCs were associated with increased vessel sprouting leading to the preservation of damaged host tissue, and provided directional guidance to regenerating axons. Different from liver and heart diseases, however, a small number of cells were positive for either glial or neuronal markers and the number was far too low to provide cellular replacement (Parr et al. 2007; Wright et al. 2011). In addition, in light of the rapidity of functional improvement, it is unlikely that cellular replacement by MSCs influenced these outcomes. Rather, transplanted MSCs may have facilitated functional recovery by releasing trophic factors, including BDNF, NGF, VEGF, HGF, BNP, and FGF-2 (Crigler et al. 2006; Fan et al. 2011). Clinical trial for CNS repair is most advanced in spinal cord injury. Park et al. (2005) reported the first trial of autologous MSCs in spinal cord injury patients, and following their report, many other groups have demonstrated an improvement in functional recovery for up to more than 1 year. Most clinical applications of bone marrow cells for the treatment of spinal cord injury have involved the use of either whole mononucleated cells or culture-expanded MSCs. The whole mononucleated cells constitute hematopoietic cells as well as endothelial cells, HSCs, and MSCs. There has been no direct verification of the clinical efficacy of mononucleated cells versus MSCs, although rat model experiments suggest there are no differences with regard to graft efficiency, glial scar reduction, or spinal cord tissue sparing (Chopp et al. 2000; Parr et al. 2007; Wright et al. 2011).

Other Tissues

MSCs are also applicable for the repair of skeletal muscle degeneration (de la Garza-Rodea et al. 2011; Ferrari et al. 1998), ischemic colonic anastomoses (Adas et al. 2011), chronic severe wounds such as skin ulcers (Rogers et al. 2008), and airway (trachea) obstruction (Grove et al. 2011).

Apart from these hopeful results using MSCs in clinical applications, a question still remains to be answered. How do homed MSCs know to differentiate into cardiac muscle cells in the heart, hepatocytes in the liver, and keratinocytes in the skin? Perhaps the microenvironment produced by the surrounding damaged tissue directs MSCs to differentiate into purposive cells, but the concrete mechanisms remain unknown. Furthermore, it is noteworthy that MSCs show less homing and integration in the CNS compared to other tissues such as liver and heart. Elucidation of these topics is important for developing a better understanding of the mechanisms of the MSC repair system.

Conclusion

The goal of regenerative medicine is to restore function by replenishing lost cells. While various types of stem cells are targeted as research subjects, a basic problem that needs to be thoughtfully considered is whether artificially established cells (i.e., ES cell and induced pluripotent stem cells) or cells in a very different developmental stage (fetal stem cells) can truly be integrated into already established adult tissues and whether those cells are able to relate to the surrounding functioning adult cells. Considering the purpose of regenerative medicine, infused cells need to become a functioning member of the adult tissue in the fullest sense. Otherwise, transplanted cells will remain unrelated and unconnected cells in adult tissues, such as adhesive plaster. In this regard, it is possible that adult-derived cells are better suited for treating adult tissues. MSCs are already applied to therapies for patients based on their efficiency in animal models, but their actual features remain poorly understood. Once the features of MSCs are clarified regarding their multilineage differentiation, homing to damaged tissues, and repair effects, MSC therapies with high efficiency may be realized. In this regard, continued basic research and preclinical studies are crucial.

Acknowledgments This work was supported by the Japan New Energy and Industrial Technology Development Organization

(NEDO) and Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO).

References

Adas G, Arikan S, Karatepe O et al (2011) Mesenchymal stem cells improve the healing of ischemic colonic anastomoses (experimental study). Langenbecks Arch Surg 396:115–126

Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM et al (2003) Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature 425:968–973

Bianco P, Gehron Robey P (2000) Marrow stromal stem cells. J Clin Invest 105:1663–1668

Caplan AI (1991) Mesenchymal stem cells. J Orthop Res 9:641–650

Charbord P (2010) Bone marrow mesenchymal stem cells: historical overview and concepts. Hum Gene Ther 21:1045–1056

Chen J, Li Y, Katakowski M et al (2003) Intravenous bone marrow stromal cell therapy reduces apoptosis and promotes endogenous cell proliferation after stroke in female rat. J Neurosci Res 73:778–786

Chino T, Tamai K, Yamazaki T et al (2008) Bone marrow cell transfer into fetal circulation can ameliorate genetic skin diseases by providing fibroblasts to the skin and inducing immune tolerance. Am J Pathol 173:803–814

Chopp M, Zhang XH, Li Y et al (2000) Spinal cord injury in rat: treatment with bone marrow stromal cell transplantation. Neuroreport 11:3001–3005

Crigler L, Robey RC, Asawachaicharn A et al (2006) Human mesenchymal stem cell subpopulations express a variety of neuro-regulatory molecules and promote neuronal cell survival and neuritogenesis. Exp Neurol 198:54–64

de la Garza-Rodea AS, van der Velde I, Boersma H et al (2011) Long-term contribution of human bone marrow mesenchymal stromal cells to skeletal muscle regeneration in mice. Cell Transplant 20:217–231

Dezawa M, Ishikawa H, Itokazu Y et al (2005) Bone marrow stromal cells generate muscle cells and repair muscle degeneration. Science 309:314–317

Dezawa M, Kanno H, Hoshino M et al (2004) Specific induction of neuronal cells from bone marrow stromal cells and application for autologous transplantation. J Clin Invest 113:1701–1710

Dezawa M, Takahashi I, Esaki M et al (2001) Sciatic nerve regeneration in rats induced by transplantation of in vitro differentiated bone-marrow stromal cells. Eur J Neurosci 14:1771–1776

D'Ippolito G, Diabira S, Howard GA et al (2004) Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential. J Cell Sci 117(Pt 14):2971–2981

Fan CG, Zhang QJ, Zhou JR (2011) Therapeutic potentials of mesenchymal stem cells derived from human umbilical cord. Stem Cell Rev 7:195–207

Ferrari D, Gulinelli S, Salvestrini V et al (2011) Purinergic stimulation of human mesenchymal stem cells potentiates their chemotactic response to CXCL12 and increases the homing capacity and production of proinflammatory cytokines. Exp Hematol 39:360–374.e1−5

Ferrari G, Cusella-De Angelis G, Coletta M et al (1998) Muscle regeneration by bone marrow-derived myogenic progenitors. Science 279:1528–1530

Friedenstein AJ, Chailakhjan RK, Lalykina KS (1970) The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet 3:393–403

Friedenstein AJ, Petrakova KV, Kurolesova AI et al (1968) Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation 6:230–247

Gage FH (2000) Mammalian neural stem cells. Science 287:1433–1438

Gilchrist ES, Plevris JN (2010) Bone marrow-derived stem cells in liver repair: 10 years down the line. Liver Transpl 16:118–129

Gnecchi M, He H, Noiseux N et al (2006) Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. FASEB J 20:661–669

Gordon MY, Levicar N, Pai M et al (2006) Characterization and clinical application of human CD34+ stem/progenitor cell populations mobilized into the blood by granulocyte colony-stimulating factor. Stem Cells 24:1822–1830

Grove DA, Xu J, Joodi R et al (2011) Attenuation of early airway obstruction by mesenchymal stem cells in a murine model of heterotopic tracheal transplantation. J Heart Lung Transplant 30:341–350

Harris RG, Herzog EL, Bruscia EM et al (2004) Lack of a fusion requirement for development of bone marrow-derived epithelia. Science 305:90–93

He X, Ma J, Jabbari E (2010) Migration of marrow stromal cells in response to sustained release of stromal-derived factor-1alpha from poly(lactide ethylene oxide fumarate) hydrogels. Int J Pharm 390:107–116

Hong HS, Lee J, Lee E et al (2009) A new role of substance P as an injury-inducible messenger for mobilization of CD29(+) stromal-like cells. Nat Med 15:425–435

Horwitz EM, Le Blanc K, Dominici M et al (2005) Clarification of the nomenclature for MSC: the International Society for Cellular Therapy position statement. Cytotherapy 7:393–395

Horwitz EM, Prockop DJ, Fitzpatrick LA et al (1999) Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med 5:309–313

Jiang Y, Jahagirdar BN, Reinhardt RL et al (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418:41–49

Kitaori T, Ito H, Schwarz EM et al (2009) Stromal cell-derived factor 1/CXCR4 signaling is critical for the recruitment of mesenchymal stem cells to the fracture site during skeletal repair in a mouse model. Arthritis Rheum 60:813–823

Korbling M, Estrov Z (2003) Adult stem cells for tissue repair—a new therapeutic concept? N Engl J Med 349:570–582

Kucia M, Reca R, Campbell FR et al (2006) A population of very small embryonic-like (VSEL) CXCR4(+)SSEA-1(+)Oct-4 + stem cells identified in adult bone marrow. Leukemia 20:857–869

Kuroda Y, Kitada M, Wakao S et al (2010) Unique multipotent cells in adult human mesenchymal cell populations. Proc Natl Acad Sci USA 107:8639–8643

Kuznetsov SA, Mankani MH, Gronthos S et al (2001) Circulating skeletal stem cells. J Cell Biol 153:1133–1140

Kuznetsov SA, Mankani MH, Leet AI et al (2007) Circulating connective tissue precursors: extreme rarity in humans and chondrogenic potential in guinea pigs. Stem Cells 25:1830–1839

Lapidot T, Petit I (2002) Current understanding of stem cell mobilization: the roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. Exp Hematol 30:973–981

Li Y, Chopp M, Chen J et al (2000) Intrastriatal transplantation of bone marrow nonhematopoietic cells improves functional recovery after stroke in adult mice. J Cereb Blood Flow Metab 20:1311–1319

Liu H, Xue W, Ge G et al (2010) Hypoxic preconditioning advances CXCR4 and CXCR7 expression by activating HIF-1alpha in MSCs. Biochem Biophys Res Commun 401:509–515

Makino S, Fukuda K, Miyoshi S et al (1999) Cardiomyocytes can be generated from marrow stromal cells in vitro. J Clin Invest 103:697–705

Markel TA, Wang Y, Herrmann JL et al (2008) VEGF is critical for stem cell-mediated cardioprotection and a crucial paracrine factor for defining the age threshold in adult and neonatal stem cell function. Am J Physiol Heart Circ Physiol 295:H2308–H2314

Meng E, Guo Z, Wang H et al (2008) High mobility group box 1 protein inhibits the proliferation of human mesenchymal stem cells and promotes their migration and differentiation along osteoblastic pathway. Stem Cells Dev 17:805–813

Mezey E, Chandross KJ, Harta G et al (2000) Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. Science 290:1779–1782

Misao Y, Takemura G, Arai M et al (2006) Bone marrow-derived myocyte-like cells and regulation of repair-related cytokines after bone marrow cell transplantation. Cardiovasc Res 69:476–490

Molyneaux KA, Zinszner H, Kunwar PS et al (2003) The chemokine SDF1/CXCL12 and its receptor CXCR4 regulate mouse germ cell migration and survival. Development 130:4279–4286

Niedzwiedzki T, Dabrowski Z, Miszta H et al (1993) Bone healing after bone marrow stromal cell transplantation to the bone defect. Biomaterials 14:115–121

Orlic D, Kajstura J, Chimenti S et al (2001) Bone marrow cells regenerate infarcted myocardium. Nature 410:701–705

Oswald J, Boxberger S, Jorgensen B et al (2004) Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem Cells 22:377–384

Owen ME, Cave J, Joyner CJ (1987) Clonal analysis in vitro of osteogenic differentiation of marrow CFU-F. J Cell Sci 87(Pt 5):731–738

Oyagi S, Hirose M, Kojima M et al (2006) Therapeutic effect of transplanting HGF-treated bone marrow mesenchymal cells into CCl4-injured rats. J Hepatol 44:742–748

Park HC, Shim YS, Ha Y et al (2005) Treatment of complete spinal cord injury patients by autologous bone marrow cell transplantation and administration of granulocyte-macrophage colony stimulating factor. Tissue Eng 11:913–922

Parr AM, Tator CH, Keating A (2007) Bone marrow-derived mesenchymal stromal cells for the repair of central nervous system injury. Bone Marrow Transplant 40:609–619

Petersen BE, Bowen WC, Patrene KD et al (1999) Bone marrow as a potential source of hepatic oval cells. Science 284:1168–1170

Philp D, Nguyen M, Scheremeta B et al (2004) Thymosin beta4 increases hair growth by activation of hair follicle stem cells. FASEB J 18:385–387

Phinney DG (2007) Biochemical heterogeneity of mesenchymal stem cell populations: clues to their therapeutic efficacy. Cell Cycle 6:2884–2889

Phinney DG, Prockop DJ (2007) Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair–current views. Stem Cells 25:2896–2902

Pittenger MF, Mackay AM, Beck SC et al (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147

Prindull G, Zipori D (2004) Environmental guidance of normal and tumor cell plasticity: epithelial mesenchymal transitions as a paradigm. Blood 103:2892–2899

Prockop DJ (1997) Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 276:71–74

Qiu J, Nishimura M, Wang Y et al (2008) Early release of HMGB-1 from neurons after the onset of brain ischemia. J Cereb Blood Flow Metab 28:927–938

Rogers LC, Bevilacqua NJ, Armstrong DG (2008) The use of marrow-derived stem cells to accelerate healing in chronic wounds. Int Wound J 5:20–25

Schachinger V, Assmus B, Erbs S et al (2009) Intracoronary infusion of bone marrow-derived mononuclear cells abrogates adverse left ventricular remodelling post-acute myocardial infarction: insights from the reinfusion of enriched progenitor cells and infarct remodelling in acute myocardial infarction (REPAIR-AMI) trial. Eur J Heart Fail 11:973–979

Snykers S, De Kock J, Rogiers V et al (2009) In vitro differentiation of embryonic and adult stem cells into hepatocytes: state of the art. Stem Cells 27:577–605

Spees JL, Olson SD, Ylostalo J et al (2003) Differentiation, cell fusion, and nuclear fusion during ex vivo repair of epithelium by human adult stem cells from bone marrow stroma. Proc Natl Acad Sci USA 100:2397–2402

Takashima Y, Era T, Nakao K et al (2007) Neuroepithelial cells supply an initial transient wave of MSC differentiation. Cell 129:1377–1388

Terada N, Hamazaki T, Oka M et al (2002) Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature 416:542–545

Terai S, Sakaida I, Yamamoto N et al (2003) An in vivo model for monitoring trans-differentiation of bone marrow cells into functional hepatocytes. J Biochem 134:551–558

Terai S, Yamamoto N, Omori K et al (2002) A new cell therapy using bone marrow cells to repair damaged liver. J Gastroenterol 37(suppl 14):162–163

Thomas ED (2000) Landmarks in the development of hematopoietic cell transplantation. World J Surg 24:815–818

Till JE, McCulloch CE (1961) A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res 14:213–222

Tolar J, Le Blanc K, Keating A et al (2010) Concise review: hitting the right spot with mesenchymal stromal cells. Stem Cells 28:1446–1455

Vallabhaneni KC, Tkachuk S, Kiyan Y et al (2011) Urokinase receptor mediates mobilization, migration, and differentiation of mesenchymal stem cells. Cardiovasc Res 90:113–121

Weissman IL, Shizuru JA (2008) The origins of the identification and isolation of hematopoietic stem cells, and their capability to induce donor-specific transplantation tolerance and treat autoimmune diseases. Blood 112:3543–3553

Wislet-Gendebien S, Hans G, Leprince P et al (2005) Plasticity of cultured mesenchymal stem cells: switch from nestin-positive to excitable neuron-like phenotype. Stem Cells 23:392–402

Wojakowski W, Kucia M, Zuba-Surma E et al (2011) Very small embryonic-like stem cells in cardiovascular repair. Pharmacol Ther 129:21–28

Wright KT, El Masri W, Osman A et al (2011) Bone marrow for the treatment of spinal cord injury: mechanisms and clinical application. Stem Cells 29:169–178

Xu YX, Chen L, Hou WK et al (2009) Mesenchymal stem cells treated with rat pancreatic extract secrete cytokines that improve the glycometabolism of diabetic rats. Transplant Proc 41:1878–1884

Yoshihara T, Ohta M, Itokazu Y et al (2007) Neuroprotective effect of bone marrow-derived mononuclear cells promoting functional recovery from spinal cord injury. J Neurotrauma 24:1026–1036

Yu J, Li M, Qu Z et al (2010) SDF-1/CXCR4-mediated migration of transplanted bone marrow stromal cells toward areas of heart myocardial infarction through activation of PI3K/Akt. J Cardiovasc Pharmacol 55:496–505