

D. Bonnet

Biology of human bone marrow stem cells

Received: 4 July 2003 / Accepted: 18 July 2003

Abstract The bone marrow is constituted of two separate and distinct stem cells. The hematopoietic stem cells (HSC) are responsible for the production and maintenance of all the mature blood cells. The mesenchymal stem cells constituted the bone marrow stroma. In this report we review our current understanding on both stem cell populations. We also discuss the recent unexpected degree of differentiation plasticity that have been reported recently and the impacts these new discoveries may have in stem cell therapy.

Key words Hematopoietic stem cell • Mesenchymal stem cell • Xenotransplantation model • Nonobese diabetic/severe combined immunodeficient mice • Fetal sheep • Plasticity • Transdifferentiation

Introduction

The bone marrow has traditionally been seen as an organ composed of two main systems: the hematopoietic tissue proper and the associated supporting stroma. The bone marrow is also the only known organ in which two separate and distinct stem cells not only co-exist but also functionally cooperate. Originally examined because of their critical role in the formation of the hematopoietic microenvironment, marrow stem cells appeared more recently at the center stage with the recognition that mesenchymal stem cells (MSC) may be induced experimentally to undergo unorthodox differentiation like neuronal, myogenic, and liver cells. In this review we will present our current understanding of the biology of these two stem cells and we will discuss the unexpected degree of developmental or differentiation plasticity of both MSC and hematopoietic stem cells (HSC). We will examine the impacts these discoveries may have in cell-based transplantation therapies and the eventual development of clinical effective gene therapy protocols.

Hematopoietic stem cell

Definition of HSC

The hallmark properties of the HSC were defined in 1963 by Siminovitch et al. [1]. In the murine model, this group provided evidence for the existence of HSC in the bone marrow. These HSC could reconstitute the hematopoietic system and hence rescue lethally irradiated recipient animals. Using serial transplantation, they showed that HSC have self-renewal ability. Based on these experiments, HSC are defined as cells with the ability to balance self-renewal versus differentiation. They have multipotentiality (i.e., a single stem cell can produce at least 8–10 distinct lineages of mature cells); they

D. Bonnet (✉)
Hematopoietic Stem Cell Laboratory,
Cancer Research UK, London Research Institute,
44 Lincoln's Inn Fields, London WC2A3PX, UK
e-mail: dominique.bonnet@cancer.org.uk
Tel.: +44-20-7269-3282
Fax: +44-20-7269-3581

have an extensive proliferative capacity and are slowly cycling in a steady-state adult hematopoietic system.

Characterization of HSC

A conclusive way to assay stem cells is based on their capacity to repopulate the entire hematopoietic system in conditioned recipients after transplantation [2]. In mice, the phenotype and function of HSC have been characterized using competitive *in vivo* repopulation assays [3–6]. Measurement of human HSC activity has been greatly facilitated by the development of xenotransplantation assays in fetal sheep [7, 8] or immune-deficient mice [9–11].

Nonobese diabetic-severe combined immunodeficient mouse model

The engraftment of normal human hematopoietic cells in immune-deficient mice provides an assay that measures the repopulating capacity of human stem cells. Nonobese diabetic/severe combined immunodeficient mice – NOD/Lt-Sz-Scid/Scid (NOD/SCID) – have proven to be reliable for detecting human hematopoietic – repopulating cells that differentiate into multilineage mature cells and self-renew in mice. Human hematopoietic-repopulating cells identified by this assay were operationally defined as SCID-repopulating cells (SRCs). Overall, the NOD/SCID mice allowed high-level engraftment of normal and leukemic human transplants and, more importantly, enabled engraftment with lower cell doses, rendering purification strategies possible [11–13]. The only limitation of these NOD/SCID mice is their susceptibility to develop thymoma with age making long-term experiments difficult. Nevertheless, Ogawa's group have recently shown that long-term experiments can be performed using newborn mice as recipient [14]. Other mouse strains have also become available: β_2 -microglobulin knockout/NOD/SCID, Rag1 knockout/NOD, and the Nude/NOD/SCID mice [15–17]. The capabilities of each of these new strains still need to be explored.

The SRC assay as originally developed is based on intravenous injection, a complex process that requires for the candidate human HSC circulation through the blood, recognition and extravasation through bone marrow vasculature, and migration to a supportive microenvironment. This process, called “homing” has been shown to be quite inefficient even in a syngeneic murine situation [18, 19]. Cashman and Eaves [20] reported that the proportion of total injected human CB competitive units in the marrow was 7%, as determined by limiting-dilution assays in NOD/SCID mice. Thus, this assay quite possibly underestimates human hematopoietic-repopulating cell frequencies. To exclude

stem cell homing interference and focus on the intrinsic capacity of a cell to self-renew and give rise to multilineage engraftment, a few groups recently developed a highly sensitive strategy for SRC assay based on direct intra-bone marrow (IBM) injection of the candidate human stem cell [21–23]. IBM injection was found to be a more-sensitive and adequate means to measure human HSC capacity.

The fetal sheep HSC assay

This assay, based on the permissive environment of the early gestational age fetus, aims at the development of a large animal model of human hematopoiesis in sheep. The preimmune sheep fetus assay allows the long-term engraftment and multilineage expression of human HSC in the absence of irradiation or other myeloablative therapies [24–26], possibly due to the reduced number of NK cells and preimmune status in early sheep gestation [27]. An essential feature of this model is that human HSC primarily engraft host marrow and persist for long periods into post-natal life [24–26], showing multilineage expression and biological responsiveness to human cytokines [25]. The multilineage expression included T- and B-lymphoid cells [28]. Furthermore, this model is relatively specific for the human HSC pool. Indeed, while both CD34⁺ CD38⁺ and CD38⁻ subpopulations engraft the sheep, only primary recipients engrafted with CD34⁺CD38⁻ cells exhibited long-term persistence of human cells, whereas CD34⁺ CD38⁺ persist for a short period only and were unable to engraft in a secondary recipient. Although not ideal, the human/sheep xenograft model may compare with the NOD/SCID assay; in particular, the sheep model does not require myeloablation, while it allows prolonged follow-up studies after birth. However, widespread utilization of this model is hindered by its high costs.

Isolation and purification of HSC

HSCs have been enriched using a variety of techniques, including density centrifugation, activation and/or cell cycle status, and surface antigen expression; however, no unique characteristics have been found to specifically identify these elusive cells. An important point in the isolation of HSC is the one-to-one correspondence between physically purified cells and the potential ability to function as a stem cell.

Cell surface markers

Systematic functional analysis of hematopoietic cells expressing a particular cell surface antigen or other mark-

ers has led to the identification of rare populations highly enriched for stem cell (SRC and/or LTC-IC) activity. HSC do not express many of the surface antigens (“lineage markers”) that are characteristic of terminally differentiating hematopoietic cells. Thus, removal of such lineage-positive cells leaves a suspension of predominantly immature cells.

CD34⁺ human HSC

The discovery of the sialomucin CD34 as a hematopoietic cell surface antigen has transformed and accelerated studies on human hematopoietic development [29]. Cell surface expression of the CD34 antigen has rapidly become the distinguishing feature used as the basis for enumeration, isolation, and manipulation of human stem cells, because CD34 is downregulated as cells differentiate into more-abundant mature cells [30, 31]. In addition to being expressed selectively on stem cells and early progenitors during human hematopoiesis [29, 32, 33], CD34 antigen is expressed outside the hematopoietic system on vascular endothelial cells [34, 35] and some fibroblasts [36, 37]. This distribution suggests a function outside hematopoiesis. Transplant studies in several species, including baboons and mice, have shown that long-term marrow repopulation can be provided by CD34⁺ selected cells. Thus, all relevant clinical and experimental protocols are designed for CD34⁺ cells enriched by a variety of selection methods.

Other stem cell markers

CD133 represents the human homologue of prominin 5 transmembrane glycoproteins (PROML 1) [38–42]. Several studies have shown the presence of CD133 cells that coexpress CD34, *c-kit*, and other cell surface markers [42, 43]. Taken together, these studies clearly indicate that CD133 represents a significant cell surface marker for identification of human HSC, but it remains unclear whether use of this marker provides any distinct advantage over CD34 expression. Further details on the expression of AC133 in human stem cells can be found in the recent review by Bhatia [44].

Another recent marker allowing the isolation of human HSC is the vascular growth factor receptor 2 (KDR) [45]. The KDR⁺ cell fraction essentially Lin[−], are largely present in populations enriched for HSC, namely CD34⁺CD38[−], CD90⁺, and CD117^{low} cells. It has been reported that CD34⁺KDR⁺ cells are highly enriched in putative HSC (SRC and E-LTC-IC or CAFC). Conversely, hematopoietic progenitors with no self-renewal activity are restricted to and highly enriched in the CD34⁺ KDR[−] cell fraction. Several other

markers have proven useful in further dividing the population into more functionally homogeneous populations, e.g., CD90, CD117, and CD38 [46, 47].

Side population

In 1996, Goodell et al. [48] reported a new method of obtaining enriched populations of HSC from adult mouse bone marrow. This procedure exploits the ability of HSC to efflux the fluorescent dye, which, like the activity of P-glycoprotein (encoded by the MDR gene), is verapamil sensitive [49]. The Hoechst 33342 low cells thus isolated were called side population (SP) cells, and were found to have the same Lin[−]Sca1⁺CD34[−] phenotype independently identified in adult murine HSC [50]. SP cells have been since identified in adult bone marrow from several species, including human [51]. To date, a description of the functional activities of human SP cells in normal individuals has been limited to an in vitro study of cord blood [51] and more recently to an in vivo study of human fetal liver [52]. In this latter study, it was demonstrated that SP cells are present in the second-trimester human fetal liver. These cells include all transplantable HSC activity detectable in NOD/SCID mice and also other more-differentiated hematopoietic cell types. More recently, Zhou et al. [53] established the link between *Bcrp1/ABCG2* expression and the SP phenotype.

Heterogeneity of the human HSC compartment

Initially, it was assumed that in humans only cells expressing CD34 would display HSC activity, as the frequency of CD34⁺ cells is now commonly used to anticipate the adequacy of clinical hematopoietic cell transplants. Recently, however, several groups, including ours, have provided evidence of various types of human HSC that do not express detectable levels of CD34. Xenograft repopulation assays using fetal sheep and immune-deficient mice have been crucial for the identification of human CD34[−] stem cells, as little or no clonogenic cell (CFC) or long-term initiating cell (LTC-IC) activity was observed within the human Lin[−]CD34[−] cell population. Using the sheep xenograft model, Zanjani et al. [54] showed that Lin[−]CD34[−] cells contained stem cells capable of long-term repopulation and multilineage differentiation in vivo. Moreover, these cells were also able to repopulate secondary recipients, attesting to the extensive self-renewal potential of the engrafting cells. The fact that large numbers of CD34⁺ cells were found in repopulated sheep suggests that the stem cells within the Lin[−]CD34[−] cell fraction are more primitive than CD34⁺ cells. Using the NOD/SCID model, a novel human hematopoietic repopulating cell, which is devoid of lineage-

specific markers and of the CD34 antigen, has also been reported [55]. Similar to the sheep model, the development of CD34⁺ cells, as well as the more-differentiated progeny *in vivo*, suggests that CD34⁻ cells might be more primitive than the CD34⁺ stem cells. Thus, it can be concluded that the CD34⁻ SRC found within the Lin⁻CD34⁻CD38⁻ cell fraction represents a novel repopulating cell within the human hematopoietic hierarchy. The fact that the Lin⁻CD34⁻ cell fraction from mice and humans contains repopulating cells indicates an evolutionary conservation of this novel stem cell population [54–56]. The identification of CD34⁻ SRC within the Lin⁻CD34⁻ subfraction establishes that the human HSC compartment is more complex than previously recognized. However, it is not known whether CD34⁻ stem cells are important clinically. The nature of the precise relationship between CD34⁻ and CD34⁺ stem cells is still unclear [57]. Based on both progenitor capacity and repopulation characteristics, CD34⁻ stem cells appear to be functionally distinct from CD34⁺ stem cells [21, 55]. In addition, CD34⁻ cells appear to be more primitive and are capable of giving rise to CD34⁺ cells both *in vitro* and *in vivo* [21, 55, 58, 59]. A molecular difference among CD34⁻ and CD34⁺ counterparts in relation to jagged.1 response was also demonstrated [60]. Furthermore, *de novo* isolated CD34⁺ were also molecularly different cells from *in vitro* generated CD34⁺ cells arising from CD34⁻ [60]. Recently however, two independent studies indicate that, as in the mouse system, CD34 antigen expression is reversible, suggesting that CD34⁻ and CD34⁺ represent the same stem cell population at different stages of activation [61, 62].

The concept of a hierarchy in the human HSC compartment has become even more evident recently with the demonstration of a short-term versus long-term SRC population within the CD34⁺ cell fraction. Indeed, using the NOD/SCID- β_2 microglobulin-null mice, Glimm et al. [63] recently showed that these mice are sequentially engrafted by two distinct and previously unrecognized populations of transplantable human short-term repopulating hematopoietic cells (ST-SRC). One of these ST-SRC is Lin⁻CD34⁺CD38⁺ and is myeloid restricted; the other is predominantly Lin⁻CD34⁺CD38⁻ and has a broader lymphomyeloid differentiation potential. This notion of short-term versus long-term SRC has been confirmed since by Mazurier et al. [23] using intrafemoral injection of purified stem cells into NOD/SCID mice. They demonstrated that Lin⁻CD34⁺CD38^{low} have the capacity for a rapid myelo-erythroid repopulation within 2 weeks of transplantation, which disappeared at 6 weeks. Furthermore, analysis of the fate of individual SRC and their clonally derived progeny, tracked using the proviral insertion site as a clonal marker, demonstrated that some clones appeared early and were lost, whereas new clones appeared later. This elegant study by Guenechea et al. [64] provided conclusive clonal evidence that SRC differ in their repopulating potential in terms of the timing that repopulation was initiated and in the lifespan of each clone. Thus, all these

data clearly demonstrated the existence of an heterogeneity of the human HSC compartment and short-term versus long-term repopulating cells.

Engraftment of HSC

In a transplantation context, a stem cell is defined retroactively as a biological activity that can give rise to substantial measurable numbers of mature cells. Similarly, the presence of a single clonotypic marker in donor-derived cells of different lineages defines multipotentiality. Thus, following transplantation into recipient, HSC must home to and lodge in the specialized niches of the bone marrow microenvironment in order to engraft and reconstitute the bone marrow functions. At present, only partial understanding of the cellular and molecular mechanisms governing homing exists. It is believed that an intricate process involving interactions between adhesion molecules and their counter-receptors expressed on HSC and endothelial cells, directs cells. In many aspects, homing of transplanted HSC during transplantation mimics the natural movement of these cells during ontogeny. Direct involvement of particular adhesion molecules in homing has been elucidated [65, 66]. Recently, Zanjani et al. [67] demonstrated that VLA-4 played a central role in homing and engraftment of transplanted human cells to the bone marrow of fetal sheep. Another ligand receptor pairing, SDF-1-CXCR4, has also been implicated in selectively directing homing of HSC to the bone marrow. Engraftment of human cells in NOD/SCID mice was prevented by treatment with antibodies against CXCR4. Furthermore, the expression of CXCR4 on CD34⁺ human HSC has been reported and suggests a role of these chemoattractants in the homing process [68]. However, recently Rosu-Myles et al. [69] demonstrated that CXCR4 expression on human HSC was not required for effective stem cell repopulation. Redundancy between different chemoattractant molecules may be responsible for this discrepancy [70, 71]. While more is known about homing, less is understood about HSC niches [72]. Recently, the spatial organization of subpopulations of hematopoietic cells following syngeneic transplantation in mice has been investigated. The study demonstrated that the spatial distribution of transplanted cells is not a random process; candidate stem cells exhibited selective lodgement in the endosteal region of the bone [73].

Mesenchymal stem cells

Definition of bone marrow stroma and MSC

Within bone marrow, hematopoiesis occurs in association with an heterogeneous population of non-hematopoietic

cells, including mesenchymal cells, connective tissue-type cells, and their associated extracellular matrix components and growth factors, which as a collective, constitute the stroma of the bone marrow [74, 75]. Although the role played by bone marrow stromal cells in the functional support of hematopoiesis has been extensively studied [76], the precise developmental relationship between the various elements of the stroma is still lacking.

Friedenstein et al. [77] were the first to demonstrate the existence *in vitro* of stromal cell precursors using the CFU-F assay (colony forming unit resembling fibroblast). The considerable heterogeneity of the CFU-F derived colonies in terms of morphology, size, proliferation, and developmental potential led to the hypothesis for the existence of a hierarchical organization of the stroma cell compartment with a pluripotent, self-renewing stromal stem cell (also known as bone marrow stromal stem cell and/or MSC) at the top of this hierarchy [78]. Since this earlier work, a standard assay for MSC from bone marrow has been to plate the cells at low densities and count the single based generated colonies as a measure of the quality of the cells. The low incidence of clonogenic CFU-F in adult human bone marrow (1–20 per 105 mononuclear cells plated) [79] was a major limitation for their study.

Methods of MSC isolation and phenotypic characterization

Human MSC are obtained from bone marrow harvested from adult iliac crest. Controversial evidence exists concerning the existence of MSC in granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood and/or umbilical cord blood [80–83]. To isolate MSC from a bone marrow aspirate, cord blood, or peripheral blood, the samples are fractionated by density gradient centrifugation for mononuclear cell isolation, resuspended in appropriate culture medium containing selected batches of fetal bovine serum, and allowed to adhere to plastic dishes for 2–16 h; then non-adherent cells are removed and the remaining cells allowed to grow for 2–3 weeks until confluency. Confluent cells are trypsinized and allowed to expand for as many as 35–40 generations. However, cultures of human MSC are morphologically heterogeneous, even when cloned from single – cell-derived colonies [84, 85]. In pivotal studies performed by Pittenger et al. [86], human MSC were characterized as non-hematopoietic cells (lacking CD45, CD34, and CD14) that can be identified by several different monoclonal antibodies. Nevertheless, there is no agreement on the phenotypical characterization of a “pure” population of human MSC despite the panoply of surface antigens reported to be expressed on MSC [86–88]. Sekiya et al. [89] recently established conditions that maximize the yields of early progenitors and evaluate their quality. More recently,

Gronthos et al. [90] developed a novel selection protocol to purify a population of bone marrow stromal stem cells. This purification is based on the selection of STRO-1 antibody in combination with an antibody directed against vascular cell adhesion molecule 1 (VCAM-1/CD106). This STRO-1+ CD106+ cell population is non-cycling and constitutively express telomerase activity *in vivo*. These cells also demonstrate extensive proliferative potential and retain the capacity for differentiation into bone, cartilage, and adipose tissue *in vitro* [90].

Functional properties and differentiation capacities of MSC

Functionally, adult MSC are characterized by a doubling time of 33 h. They have a large expansive potential, and cell cycle studies reveal a subset (20%) of quiescent cells. MSC constitutively secrete several cytokines [91]. Among these many cytokines have a role in HSC proliferation and differentiation. MSC support human LTC-IC, providing a feeder layer for cultured HSC [92]. More recently it has been shown that the co-transplantation of expanded human MSC with HSC enhances myelopoiesis and megakaryopoiesis in NOD/SCID mice [93]. MSC can replicate as undifferentiated cells and have the potential to differentiate into several lineages of mesodermal origin, such as cartilage, bone, fat, tendon, muscle, myocardium, and marrow stroma, both *in vitro* and *in vivo*, upon culture with appropriate combination of growth factors [86]. Recently, a cell subpopulation termed multipotent adult progenitor cells (MAPCs), co-purifying with MSC from bone marrow, has been shown to differentiate, at the single cell level, not only into mesenchymal cells, but also into cells with visceral mesoderm, neuroectoderm, and endoderm characteristics *in vitro*. In fact when injected into an early blastocyst, single MAPCs contribute to most, if not all, somatic cell types [94, 95]. Under appropriate stimuli and microenvironment, the MAPCs can differentiate into cells presenting morphological, phenotypic, and functional characteristics of hepatocytes [96]. However, there is currently no direct proof of whether these MAPCs represent pluripotent embryonic stem cells that persist during adult life, and whether they are the result of de-differentiation of MSC *in vitro*.

Bone marrow stem cell plasticity

The notion of adult stem cell plasticity has been discussed in different recent review articles [97–103]. Thus, we will restrict our comments here on the recent studies involving human bone marrow stem cells, including some of our recent studies on HSC plasticity using the NOD/SCID model. The

first demonstration that this phenomenon exists in humans came from two groups [104, 105]. Two approaches were adopted. In one, the livers of female patients who had previously received a bone marrow transplant from a male donor were examined for cells of donor origin using DNA probe specific for the Y chromosome, localized using *in situ* hybridization; in the second, Y chromosome-positive cells were sought in female livers engrafted into male patients, which were later removed or biopsied for recurrent disease. In both sets of patients, Y chromosome-positive hepatocytes were readily identified. Hepatic engraftment level was highly variable, perhaps related to the severity of parenchymal damage. Recently, a biopsy study of nine liver transplant recipients found that hepatocyte chimerism was more prominent in those patients with recurrent hepatitis [106]. Another study, using biopsies from female patients who received a male bone marrow transplantation, showed evidence in the small and large intestine of male smooth muscle actin-positive myofibroblast population surrounding the crypts [107]. Using coronary artery specimens from eight subjects who received a gender-mismatched bone marrow transplantation, Caplice et al. [108] demonstrated that smooth muscle in human coronary atherosclerosis can originate from bone marrow cells. In human renal transplants, where female kidneys were grafted into male recipients, Poulosom et al. [109] showed male tubular cells expressing the epithelial marker CAM 5.2. Moreover, Grimm et al. [110] found evidence for a circulating host-derived mesenchymal cell in renal transplants that were suffering chronic rejection. Using cerebellar tissues from female patients with hematological malignancies who received male bone marrow transplant, Weimann et al. [111] showed that some bone marrow cells could contribute to Purkinje neurons. Gussoni et al. [112] reported the analysis of muscle biopsies from a muscular dystrophy patient who received bone marrow transplantation at age 1 year for X-linked severe combined immune deficiency and who was diagnosed with muscular dystrophy at age 12 years. Analysis of muscle biopsies revealed the presence of donor nuclei within a small number of muscle myofibers (0.5%–0.9%). The majority of the myofibers produce a truncated, in-frame isoform of dystrophin (not wild type). The presence of bone marrow-derived donor nuclei in the muscle of this patient documents the ability of exogenous human bone marrow cells to fuse into skeletal muscle and persist up to 13 years after transplantation [112].

Most of those studies did not distinguish whether HSC, MSC, or residual tissue-specific stem cells circulating in the bone marrow were responsible for the observed conversion. Kopen et al. [113] showed that cells that gave rise to neurons and glia were derived from cultures of adherent bone marrow stroma, suggesting that they included MSC. Using the fetal sheep xenotransplant model, Liechty et al. [114] showed that intraperitoneal injection of human MSC in fetal sheep engraft and differentiate into site-specific tissues, such as articular cartilage chondrocytes, human fat, and skeletal

muscle. After wounding the tail by clipping, even human cells of fibroblastic morphology within the dermis and dermal appendages were detected. This might indicate that circulating human MSC have the potential to assist with skin repair processes. Another study examined the myogenic potential of synovial membrane-derived human MSC (hSM-MSC) using a nude mouse model of skeletal muscle regeneration. When injected into regenerating nude muscle, hSM-MSC contributed to myofibers and to long-term persisting functional satellite cells. When administered into dystrophic muscles of immunosuppressed mdx mice, hSM-MSC restored sarcolemmal expression of dystrophin and reduced central nucleation [115]. More recently, Dr. Verfaillie's group identified mesodermal progenitor cells (MPC) that have the capacity to differentiate into osteoclasts, chondrocytes, adipocytes, skeletal myoblasts, endothelial cells, neurons, and hepatocytes [94–96, 116, 117].

We reported recently the potential of a highly purified population present in adult bone marrow and umbilical cord blood (human C1qR_p⁺ stem cells) to differentiate *in vivo* into hepatocytes [118]. These data provided the first direct demonstration that a highly purified and phenotypically defined human adult HSC population can repopulate the bone marrow and differentiate *in vivo* into functional hepatocytes using the NOD/SCID mouse model. Our results have been confirmed more recently. Using unsorted mononuclear cells from umbilical cord blood samples, Newsome et al. [119] showed indeed that after transplantation into sublethally irradiated NOD/SCID mice, some human cells present in the liver of the engrafted mice present human specific hepatocytes, biliary and endothelial markers. Using newborn NOD/SCID- β_2 -microglobulin null mice Ishikawa et al. [120] confirmed the presence of human hepatocyte-like cells in the liver of mice transplanted with human bone marrow cells. Similar results were obtained using human CD34+ cord blood cells as a source of transplanted cells [121]. In this later study, NOD/SCID mice were treated 5 weeks after transplantation with a dose of carbon tetrachloride. Human hepatocyte-like cells were detected, but in this case only after liver injury [121].

Thus, all these data indicate that human HSC, but also MSC present within the adult human marrow-possess some remarkable plasticity. In the light of the recent identification of a single mouse bone marrow-derived stem cell with multi-organ and multilineage engraftment [122], additional experiments will be required to determine the full developmental capacity of marrow-derived stem cells.

Concluding remarks

Recently important advances have been made in our understanding of the human HSC hierarchy, as well as in the characterization of the MSC population. It is evident that a num-

ber of fundamental questions still need to be resolved before these bone marrow stem cells can be used for safe and effective clinical cell and gene therapies. Despite the lack of understanding for the mechanisms responsible of the observed plasticity of certain bone marrow stem cells, the use of MSC and/or HSC for the potential treatment of human diseases, such as liver diseases and muscular dystrophy, represents an exciting new therapeutic strategy. Bone marrow stem cells represent a safe and accessible source of stem cells that can be genetically manipulated and may thus prove to be an ideal vehicle for delivering therapeutic genes to other organs. Further studies comparing in the same system the different potentials of human MSC versus HSC will also be needed. The NOD/SCID xenotransplant model might play an important role in evaluating this potential.

Acknowledgements I express my appreciation to all members of my laboratory, past and present, for their experimental and theoretical contributions to this work. This work was supported by The Association for International Cancer Research, Cancer Research UK and NIH grant R01 HL64856-01

References

1. Siminovitch L, McCulloch EA, Till JE (1963) The distribution of colony-forming cells among spleen colonies. *J Cell Comp Physiol* 62, 327–336
2. Phillips R (1991) Hematopoietic stem cells: concepts, assays, and controversies. *Semin Immunol* 3:337–347
3. Hodgson GS, Bradley TR (1979) Properties of hematopoietic stem cells surviving 5-fluorouracil treatment. *Nature* 281:381–384
4. Spangrude GJ, Heimfield S, Weissman IL (1988) Purification and characterization of murine hematopoietic stem cells. *Science* 241:58–63
5. Spangrude GJ, Johnson JR (1990) Resting and activated subsets of mouse multipotent hematopoietic stem cells. *Proc Natl Acad Sci U S A* 87:7433–7440
6. Morrison SJ, Weissman IL (1994) The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. *Immunity* 1:661–673
7. Srour EF, Zanjani ED, Cornetta K, et al (1993) Persistence of human multilineage, self-renewing lymphohematopoietic stem cells in chimeric sheep. *Blood* 82:3333–3339
8. Zanjani ED, Almeida-Porada G, Ascensao JL et al (1997) Transplantation of hematopoietic stem cells in utero. *Stem Cells* 15:79–84
9. McCune JM (1996) Development and applications of the SCID-hu mouse model. *Semin Immunol* 8:187–196
10. Torbett BE, Picchio G, Mosier DE (1991) Hu-PBL-SCID mice: a model for human immune function, AIDS, and lymphomagenesis. *Immunol Rev* 124:139–164
11. Dick JE (1996) Normal and leukemic human stem cells assayed in SCID mice. *Semin Immunol* 8:197–206
12. Shultz LD, Schweitzer PA, Christianson SW et al (1995) Multiple defects in innate and adaptive immunological function in NOD/LtSz-scid mice. *J Immunol* 154:180–191
13. Gan O, Murdoch B, Larochelle A et al (1997) Differential maintenance of primitive human SCID-repopulating cells, clonogenic progenitors, and long-term culture marrow stromal cells. *Blood* 2:641–650
14. Ishikawa F, Livingston AG, Wingard J, Nishikawa S, Ogawa M (2002) An assay for long-term engrafting human stem cells based on newborn NOD/SCID/b2-microglobulin null mice. *Exp Hematol* 30:488–494
15. Kollet O, Peled A, Byk T et al (2000) β 2 microglobulin-deficient (B2mnull) NOD/SCID mice are excellent recipients for studying human stem cell function. *Blood* 95:3102–3105
16. Shultz LD, Lang PA, Christianson SH et al (2000) NOD/LtSz-Rag1null mice: an immunodeficient and radioresistant model for engraftment of human hematolymphoid cells, HIV infection, and adoptive transfer of NOD mouse diabetogenic T cells. *J Immunol* 164:2496–2507
17. Arevalo JMG, Ertl DC, Dao MA et al (1994) A new immunodeficient mouse strain: the nude NOD/SCID for human hematopoietic cell xenotransplantation. *Blood* 94[Suppl 1]:129a
18. Spangrude GJ, Brooks DM, Tumas DB (1995) Long-term repopulation of irradiated mice with limiting numbers of purified hematopoietic stem cells: in vivo expansion of stem cell phenotype but not function. *Blood* 85:1006–1008
19. Van Der Loo JC, Ploemacher RE (1985) Marrow- and spleen seeding efficiencies of all murine hematopoietic stem cell subsets are decreased by preincubation with hematopoietic growth factors. *Blood* 85:2598–2606
20. Cashman JD, Eaves CJ (2000) High marrow seeding efficiency of human lymphomyeloid repopulating cells in irradiated NOD/SCID mice. *Blood* 96:3979–3981
21. Wang J, Kimura T, Asada R, Harada S, Yokota S, Kawamoto Y, Fujimura Y, Tsuji T, Ikehara S, Sonoda Y (2003) SCID-repopulating cell activity of human cord blood-derived CD34⁺ cells assured by intra-bone marrow injection. *Blood* 101:2924–2931
22. Yahata T, Ando K, Sato T, Miyatake H, Nakamura Y, Muguruma Y, Kato S, Hotta T (2003) A highly sensitive strategy for SCID-repopulating cell assay by direct injection of primitive human hematopoietic cells into NOD/SCID mice bone marrow. *Blood* 101:2905–2913
23. Mazurier F, Doedens M, Gan O, Dick JE (2003) Rapid myeloid repopulation after intrafemoral transplantation of NOD-SCID mice reveals a new class of human stem cells. *Nat Med* 9:959–963
24. Zanjani ED, Almeida-Porada G, Falke AW (1996) The human/sheep xenograft model: a large animal model of human hematopoiesis. *Int J Hematol* 63:179–192
25. Zanjani ED, Pallavicini MG, Ascensao JL et al (1992) Engraftment and long term expression of human fetal hematopoietic stem cells in sheep following transplantation in utero. *J Clin Invest* 89:1178–1188
26. Srour EF, Zanjani ED, Brandt JE et al (1992) Sustained human hematopoiesis in sheep transplanted in utero during early gestation with fractionated adult human bone marrow cells. *Blood* 79:1404–1412
27. Morris B, Miyasaka M (1985) Immunology of the sheep. Roche, Basel, Switzerland
28. Civin CI, Almeida-Porada G, Lee MJ et al (1996) Sustained, retransplantable, multilineage engraftment of highly purified adult human bone marrow stem cells in vivo. *Blood* 88:4102–4109

29. Civin CI, Strauss LC, Browall C et al (1985) Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody against KG-1a cells. *J Immunol* 133:157–164
30. Andrews RE, Singer JW, Bernstein ID (1989) Precursors of colony-forming cells in humans can be distinguished from colony-forming cells by expression of CD33 and CD34 antigen and light scatter. *J Exp Med* 169:1721–1731
31. Krause DS, Fackler MJ, Civin CI et al (1996) CD34: structure, biology, and clinical utility. *Blood* 87:1–13
32. Berenson RJ, Bensinger WI, Hill RS et al (1991) Engraftment after infusion of CD34+ marrow cells in patients with breast cancer or neuroblastoma. *Blood* 77:1717–1722
33. Siena S, Bregni M, Brando B et al (1991) Flow cytometry for clinical estimation of circulating hematopoietic progenitors for autologous transplantation in cancer patients. *Blood* 77:400–409
34. Fina L, Molgaard HV, Roberston D et al (1990) Expression of the CD34 gene in vascular endothelial cells. *Blood* 75:2417–2426
35. Baumhueter S, Dybdal N, Kyle C et al (1994) Global expression of murine CD34, a sialomucin-like endothelial ligand for l-selectin. *Blood* 84:2554–2565
36. Brown J, Greaves MF, Molgaard HV (1991) The gene encoding the stem cell antigen, CD34, is conserved in mouse and expressed in hematopoietic cell lines, brain, and embryonic fibroblasts. *Int Immunol* 3:75–84
37. Greaves MF, Brown J, Molgaard HV et al (1992) Molecular features of CD34: a hematopoietic progenitor cell-associated molecule. *Leukemia* 6[Suppl 1]:31–36
38. Corbeil D, Roper K, Weigmann A, Huttner WB (1998) AC133 hematopoietic stem cell antigen: human homologue of mouse kidney prominin or distinct member of a novel protein family? *Blood* 91:2625–2626
39. Miraglia S, Godfrey W, Yin AH, Atkins K, Wamke R, Holden JT, Bray RA, Walker EK, Buck DW (1997) A novel five-transmembrane hematopoietic stem cell antigen: isolation, characterization, and molecular cloning. *Blood* 90:5013–5021
40. Miraglia S, Godfrey W, Buck D (1998) A response to AC133 hematopoietic stem cell antigen: human homologue of mouse kidney prominin or distinct member of a novel protein family? *Blood* 91:4390–4391
41. Yin AH, Miraglia S, Zanjani EM et al (1997) AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood* 90:5002–5012
42. Wuchter C (2001) Impact of CD133 (AC133) and CD90 expression analysis for acute leukemia immunophenotyping. *Haematologica* 86:154–161
43. Majka M, Ratajack J, Machalinski B, Carter A, Pizzini D, Wasik MA, Gewirtz AM, Ratajack MZ (2000) Expression and function of AC133, a putative cell surface marker of primitive human hematopoietic cells. *Folia Histochem Cytobiol* 38:53–63
44. Bhatia M (2001) AC133 expression in human stem cells. *Leukemia* 15:1685–1688
45. Ziegler BL, Valtieri M, Almeida-Porada G et al (1999) KDR receptor: a key marker defining hematopoietic stem cells. *Science* 285:553–558
46. Craig W, Kay R, Cutler RB, Lansdorp PM (1993) Expression of Thy-1 on human hematopoietic progenitor cells. *J Exp Med* 177:1331–1342
47. Bhatia M, Wang JCY, Kapp U et al (1997) Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. *Proc Natl Acad Sci U S A* 94:5320–5325
48. Goodell MA, Brose K, Paradis G et al (1996) Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. *J Exp Med* 183:1797–1806
49. Yusa K, Tsuruo T (1989) Reversal mechanism of multidrug resistance by verapamil: direct binding of verapamil to P-glycoprotein on specific sites and transport of verapamil outward across the plasma membrane of K562/ADM cells. *Cancer Res* 49:5002–5006
50. Miller CL, Eaves CJ (1997) Expansion in vitro of adult murine hematopoietic stem cells with transplantable lymphomyeloid reconstitution. *Proc Natl Acad Sci U S A* 94:13648–13653
51. Goodell MA, Rosenzweig M, Kim H et al (1997) Dye efflux studies suggest that hematopoietic stem cells expressing low or undetectable levels of CD34 antigen exist in multiple species. *Nat Med* 3:1337–1345
52. Uchida N, Fujisaki T, Eaves AC, Eaves CJ (2001) Transplantable hematopoietic stem cells in human fetal liver have a CD34+ side population (SP) phenotype. *J Clin Invest* 108:1071–1077
53. Zhou S, Schuetz JD, Bunting KD, Colapietro AM, Sampath J et al (2001) The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nat Med* 7:1028–1034
54. Zanjani ED, Almeida-Porada G, Livingston AG et al (1998) Human bone marrow CD34- cells engraft in vivo and undergo multilineage expression that includes giving rise to CD34+ cells. *Exp Hematol* 26:353–360
55. Bhatia M, Bonnet D, Murdoch B et al (1998) Identification of a newly discovered class of human hematopoietic cells with SCID-repopulating activity. *Nat Med* 94:1038–1044
56. Osawa M, Hanada K, Hamada H et al (1996) Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* 273:242–245
57. Bonnet D (2001) Normal and leukemic CD34-negative human hematopoietic stem cells. *Rev Clin Exp Hematol* 5:42–61
58. Nakamura Y, Ando K, Chargui J et al (1999) Ex vivo generation of CD34+ from CD34- hematopoietic cells. *Blood* 94:4053–4058
59. Fujisaki T, Berger MG, Rose-John S, Eaves CJ (1999) Rapid differentiation of a rare subset of adult human Lin-CD34-CD38- cells stimulated by multiple growth factors. *Blood* 94:1926–1932
60. Karanu FN, Yuefei L, Gallacher L, Sakano S, Bhatia M (2003) Differential response of primitive human CD34- and CD34+ hematopoietic cells to the Notch ligand Jagged.1. *Leukemia* 17:1366–1374
61. Dao MA, Arevalo J, Nolte JA (2003) Reversibility of CD34 expression on human hematopoietic stem cells that retain the capacity for secondary reconstitution. *Blood* 101:112–118
62. Zanjani ED, Almeida-Porada G, Livingston AG, Zeng H, Ogawa M (2003) Reversible expression of CD34 by adult human bone marrow long-term engrafting hematopoietic stem cells. *Exp Hematol* 31:406–412
63. Glimm H, Eisterer W, Lee K, Cashman J, Holyoake TL, Nicolini F, Shultz LD, Von Kalle C, Eaves CJ (2001) Previous

- sly undetected human hematopoietic cell populations with short-term repopulating activity selectively engraft NOD/SCID- β 2 microglobulin-null mice. *J Clin Invest* 107:199–206
64. Guenechea G, Gan O, Dorrell C, Dick JE (2001) Distinct classes of human stem cells that differ in proliferative and self-renewal potential. *Nat Immunol* 2:75–82
 65. Papayannopoulos T, Nakamoto B (1993) Peripheralization of hematopoietic progenitors in primates treated with anti-VLA4 integrin. *Proc Natl Acad Sci U S A* 90:9374–9378
 66. Prosper F, Stroncek D, McCarthy JB, Verfaillie CM (1998) Mobilization and homing of peripheral blood is related to reversible downregulation of α 4 β 1 integrin and function. *J Clin Invest* 101:2456–2467
 67. Zanjani ED, Flake AW, Almeida-Porada G, Tran N, Papayannopoulou T (1999) Homing of human cells in the fetal sheep model: modulation by antibodies activating or inhibiting very late activation antigen 4 dependent function. *Blood* 94:2515–2522
 68. Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T, Nagler A et al (1999) Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. *Science* 283:845–848
 69. Rosu-Myles, Gallacher L, Murdoch B, Hess DA, Keeney M, Kelvin D et al (2000) The human hematopoietic stem cell compartment is heterogeneous for CXCR4 expression. *Proc Natl Acad Sci U S A* 97:14626–14631
 70. Srour EF, Jetmore A, Wolber FM, Plett PA, Abonour R, Yoder MC, Orschell-Traycoff CM (2001) Homing, cell cycle kinetics and fate of transplanted hematopoietic stem cells. *Leukemia* 15:1681–1684
 71. Whetton AD, Graham GJ (1999) Homing mobilization in the stem cell niche. *Trends Cell Biol* 9:233–238
 72. Nilsson SK, Dooner MS, Tianks CY, Weier HU, Quesenberry PJ (1997) Potential and distribution of transplanted hematopoietic stem cells in a non-ablated mouse model. *Blood* 89:4013–4020
 73. Nilsson SK, Johnston HM, Coverdale JA (2001) Spatial localization of transplanted hematopoietic stem cells: interferences for the localization of stem cell niches. *Blood* 97:2293–2299
 74. Lichtman MA (1981) The ultrastructure of hematopoietic environment of the marrow: a review. *Exp Hematol* 9:391–410
 75. Weiss L (1976) The hematopoietic microenvironment of the bone marrow: an ultrastructural study of the stroma in rats. *Anat Rec* 186:161–184
 76. Dexter TM, Allen TD, Lajtha LG (1977) Conditions controlling the proliferation of hematopoietic stem cells in vitro. *J Cell Physiol* 91:335–344
 77. Friedenstein AJ, Chailakjan 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
 78. Owen M, Fiedenstein AJ (1988) Stromal stem cells: marrow-derived osteogenic precursors. *Ciba Found Symp* 136:42–60
 79. Gronthos S, Simmons PJ (1996) The growth factor requirement of STRO-1 positive human bone marrow stromal precursors under serum-deprived conditions in vitro. *Blood* 85:929–940
 80. Fernandez M, Simon V, Herrera G, Cao C, Del Favero H, Minguell JJ (1997) Detection of stromal cells in peripheral blood peripheral cell collections from breast cancer patients. *Bone Marrow Transplant* 20:265–271
 81. Lazarus HM, Haynesworth SE, Gerson SL, Caplan AL (1997) Human bone marrow derived mesenchymal (stromal) progenitor cells (MSC) cannot be recovered from peripheral blood progenitor cell collections. *J Hematother* 6:447–455
 82. Erices A, Conget P, Minguell JJ (2000) Mesenchymal progenitor cells in human umbilical cord blood. *Br J Hematol* 109:235–242
 83. Wexler SA, Donaldson C, Denning-Kendall P, Rice C, Bradley B, Hows JM (2003) Adult bone marrow is a rich source of human mesenchymal stem cells but umbilical cord blood and mobilized adult blood are not. *Br J Hematol* 121:368–374
 84. Colter DC, Class R, DiGirolamo CM et al (2000) Rapid expansion of recycling stem cells in cultures of plastic adherent cells from human bone marrow. *Proc Natl Acad Sci U S A* 97:3213–3218
 85. Zohar R, Sodek J, McCulloch CA (1997) Characterisation of stromal progenitor cells enriched by flow cytometry. *Blood* 90:3471–3481
 86. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Dopuglas R, Mosca JD et al (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284:143–147
 87. Tocci A, Forte L (2003) Mesenchymal stem cell: use and perspectives. *Hematol J* 4:92–96
 88. Bianco P, Riminucci M, Gronthos S et al (2001) Bone marrow stromal stem cells: nature, biology and potential applications. *Stem Cells* 19:180–192
 89. Sekiya I, Larson BL, Smith JR, Pochampally R, Cui JG, Prockop DJ (2002) Expansion of human adult stem cells from bone marrow: conditions that maximize these yields of early progenitors and evaluate their quality. *Stem Cells* 20:530–541
 90. Gronthos S, Zannettino ACW, Hay SJ, Shi S, Graves SE, Kortessidi A, Simmons PJ (2003) Molecular and cellular characterisation of highly purified stromal stem cells derived from human bone marrow. *J Cell Sci* 116:1827–1835
 91. Azizi SA, Stokes D, Augelli BJ, DiGirolamo C, Prockop DJ (1998) Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats—similarities to astrocytes grafts. *Proc Natl Acad Sci U S A* 95:3908–3913
 92. Majumdar MK, Thiede MA, Haynesworth SE, Bruder SP, Gerson SL (2000) Human marrow-derived mesenchymal stem cells (MSCs) express hematopoietic cytokines and support long-term hematopoiesis when differentiated toward stromal and osteogenic lineages. *J Hematother Stem Cell Res* 9:841–848
 93. Angelopoulou M, Novelli E, Grove JE, Rinder HM, Civin C, Cheng L, Krause DS (2003) Cotransplantation of human mesenchymal stem cells enhances human myelopoiesis and megakaryocytopoiesis in NOD/SCID mice. *Exp Hematol* 31:413–420
 94. Jiang Y, Jahagirda BN, Reinhardt RL et al (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 418:41–49
 95. Reyes M, Lund T, Lenvik T, Agular D, Koodie L, Verfaillie C (2001) Purification and ex vivo expansion of postnatal human marrow mesodermal progenitor cells. *Blood* 98:2615–2625
 96. Schwartz RE, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund T, Lenvik T, Johnson S, Hu WS, Verfaillie CM (2002) Multipotent adult progenitor cells from bone marrow differ-

- entiate into functional hepatocyte-like cells. *J Clin Invest* 109:1291–1302
97. Lemischka I (1999) The power of stem cells reconsidered? *Proc Natl Acad Sci U S A* 96:14193–14195
 98. Orkin SH (2000) Stem cell alchemy. *Nat Med* 6:1212–1213
 99. Blau HM, Brazelton TR, Weimann JM (2001) The evolving concept of stem cell: entity or function? *Cell* 105:829–841
 100. Morrison SJ (2001) Stem cell potential: can anything make anything? *Curr Biol* 11:R7–R9
 101. Anderson DJ, Gage FH, Weissman IL (2001) Can stem cells cross boundaries? *Nat Med* 7:393–395
 102. Lagasse E, Shizuru JA, Uchida N, Tsukamoto A, Weissman IL (2001) Toward regenerative medicine. *Immunity* 14:425–436
 103. Wulf GG, Jackson KA, Goodell MA (2001) Somatic stem cell plasticity: current evidence and emerging concepts. *Exp Hematol* 29:1361–1370
 104. Theise ND, Nimmakayalu M, Gardner R et al (2000) Liver from marrow in humans. *Hepatocyte* 32:11–16
 105. Alison MR, Poulson R, Jeffery R et al (2000) Hepatocytes from non-hepatic adult stem cells. *Nature* 406:257
 106. Kleeberger W, Rothamel T, Glocckner S, Flemming P, Lehmann U, Kreipe H (2002) High frequency of epithelial chimerisms in liver transplants demonstrated by microdissection and STR-analysis. *Hepatology* 35:110–116
 107. Brittan M, Hunt T, Jeffery R et al (2001) Bone marrow derivation of pericyptal myofibroblasts in the mouse and human small intestine and colon. *Gut* 50:752–757
 108. Caplice NM, Bunch TJ, Stalboerger PG, Wang S, Simper D, Miller DV, Russell ST, Litzow MR, Edwards WD (2003) Smooth muscle cells in human coronary arteriosclerosis can originate from cells administered at marrow transplantation. *Proc Natl Acad Sci U S A* 100:4754–4759
 109. Poulson R, Forbes SJ, Hodivala-Dilke K et al (2001) Bone marrow contributes to renal parenchymal turnover and regeneration. *J Pathol* 195:229–235
 110. Grimm PC, Nickerson P, Jeffery J et al (2001) Neointimal and tubulointestinal infiltration by recipient mesenchymal cells in chronic renal-allograft rejection. *N Engl J Med* 345:93–97
 111. Weimann JM, Charlton CA, Brazelton TR, Hackman RC, Blau HM (2003) Contribution of transplanted bone marrow to Purkinje neurons in human adult brains. *Proc Natl Acad Sci U S A* 100:2088–2093
 112. Gussoni E, Bennett RR, Muskiewicz KR, Meyerrose T, Nolte JA, Gilgoff I, Stein J, Chan YM, Lidov HG, Bonnemann CG, Von Moers A, Morris GE, Den Dunnen JT, Chamberlain JS, Kunkel LM, Weinberg K (2002) Long-term persistence of donor nuclei in a Duchenne muscular dystrophy patient receiving bone marrow transplantation. *J Clin Invest* 110:807–814
 113. Kopen G, Prockop DJ, Phinney D (1999) Marrow stromal cells migrate through out the forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse. *Proc Natl Acad Sci U S A* 96:10711–10716
 114. Liechty K, MacKenzie T, Shaaban A et al (2000) Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep. *Nat Med* 6:1282–1286
 115. De Bari C, Dell’Accio F, Vandenabeele F, Vermeesch JR, Raymackers JM, Lyuten FP (2003) Skeletal muscle repair by adult human mesenchymal stem cells from synovial membrane. *J Cell Biol* 160:909–918
 116. Zhao LR, Duan WM, Reyes M, Verfaillie CM, Low WC (2003) Immunohistochemical identification of multipotent adult progenitor cells from human bone marrow after transplantation into the rat brain. *Brain Res Brain Res Protoc* 11:38–45
 117. Zhao LR, Duan WM, Reyes M, Keene CD, Verfaillie CM, Low WC (2002) Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats. *Exp Neurol* 174:11–20
 118. Danet G, Luongo JL, Butler G, Lu MM, Tenner AJ, Simon MC, Bonnet D (2002) C1qRp defines a new human stem cell population with hematopoietic and hepatic potential. *Proc Natl Acad Sci U S A* 99:10441–10445
 119. Newsome PN, Johannessen I, Boyle S, dalakas E, McAulay K, Samuel K, Rae F, Forrester L, Turner ML, Hayes PC, Harrison DJ, Bickmore WA, Plevris JN (2003) Human cord-blood derived cells differentiate into hepatocytes in the mouse liver with no evidenced of cellular fusion. *Gastroenterology* 124:1891–1900
 120. Ishkawa F, Drake CJ, Yang S, Fleming PA, Minamiguchi H, Visconti RP, Crosby CV, Argraves WS, Harada M, Lyndon Key L, Livingston AG, Wingard JR, Ogawa M (2003) Transplanted human cord blood cells give rises to hepatocytes in engrafted mice. *Ann N Y Acad Sci* 996:174–185
 121. Wang X, Ge S, McNamara G, Hao QL, Crooks GM, Nolte JA (2003) Albumin-expressing hepatocyte-like cells develop in the livers of immune-deficient mice that received transplants of highly purified human hematopoietic stem cells. *Blood* 101:4201–4208
 122. Krause DS, Theise ND, Collector MI et al (2001) Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* 105:369–377