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Invited Editorial

Bone-marrow-derived stem cells – our key to longevity?

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Abstract. Bone marrow (BM) was for many years primarily regarded as the source of hematopoietic stem cells. In this review we discuss current views of the BM stem cell compartment and present data showing that BM contains not only hematopoietic but also heterogeneous non-hematopoietic stem cells. It is likely that similar or overlapping populations of primitive non-hematopoietic stem cells in BM were detected by different investigators using different experimental strategies and hence were assigned different names (e.g., mesenchymal stem cells, multipotent adult progenitor cells, or marrow-isolated adult multilineage inducible cells). However, the search still continues for true pluripotent stem cells in adult BM, which would fulfill the required criteria (e.g. complementation of blastocyst development). Recently our group has identified in BM a population of very small embryonic-like stem cells (VSELs), which express several markers characteristic for pluripotent stem cells and are found during early embryogenesis in the epiblast of the cylinder-stage embryo.

Keywords: CXCR4, embryonic stem cells, Nanog, Oct-4, SSEA, very small embryonic-like stem cells.

Introduction

Several types of non-hematopoietic stem cells (non-HSCs) have been described in adult bone marrow (BM). We postulate that the presence of these various populations of stem cells in BM is a result of the "developmental migration" of stem cells during ontogenesis and the presence of the permissive environment that attracts these cells to BM tissue. HSCs and non-HSCs are actively chemoattracted by factors secreted by BM stroma cells and osteoblasts (e.g., stromal derived factor-1, SDF-1; hepatocyte growth factor, HGF), and colonize BM by the end of the second and the beginning of the third trimester of gestation (Kucia et al. 2004; Kucia et al. 2005a; Kucia et al. 2005b; Kucia et al. 2005c; Kucia et al. 2006a; Kucia et al. 2006b; Kucia et al. 2006d; Ratajczak et al. 2003; Ratajczak et al. 2004). Accumulating evidence suggests that these non- HSCs residing in BM play some role in the homeostasis/turnover of peripheral tissues and, if needed, could be released/mobilized from BM into circulation during tissue injury and stress, facilitating the regeneration of damaged organs (Abbott et al. 2004; Gomperts et al. 2006; Kale et al. 2003; Kollet et al. 2003; Kucia et al. 2004; Kucia et al. 2006c; LaBarge et al. 2002; Long et al. 2005; Wojakowski et al. 2004).

These cells have been variously described in the literature, as: (i) endothelial progenitor cells (EPCs) (Asahara et al. 1997; Shi et al. 1998), (ii) mesenchymal stem cells (MSCs) (Peister et al. 2004; Prockop et al. 1997), (iii) multipotent adult progenitor cells (MAPCs) (Jiang et al. 2002), and (iv) marrow-isolated adult multilineage inducible (MIAMI) cells (D'Ippolito et al. 2004). It is likely that in many cases similar or overlapping populations of primitive stem cells in BM were detected by using different experimental strategies and hence were assigned different names. Unexpectedly, it has also been found that BM could be a potential source of precursors of germ cells (oocytes and spermatogonial cells) (Johnson et al.

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2005; Nayernia et al. 2006). It is still unclear whether BM contains any pluripotent stem cells (PSCs). Several attempts have been made to identify in BM such cells, which at the single-cell level in vitro could give rise to cells from all 3 germ layers (meso-, ecto- and endoderm). Recently our group has purified from BM a population of developmentally primitive stem cells, which we named very small embryonic-like stem cells (VSELs) (Kucia et al. 2006a). These cells express several markers characteristic for the embryonic stem cells that are present in the developing epiblast and differentiate into cells from all 3 germ layers.

However, the most valuable evidence for pluripotentiality of the stem cell is its contribution to the development of multiple organs and tissues in vivo after injection into the developing blastocyst, but this was so far not demonstrated in a reproducible manner for any type of stem cells isolated from the adult body.

Developmental hierarchy of the stem cell compartment – from the totipotent zygote to BM-residing stem cells

Stem cells have a potential for self-renewal and an ability to differentiate into cells that are committed to particular developmental pathways. The compartment of stem cells is organized in a hierarchical way, from the most primitive (totipotent) to already differentiated tissue-committed (monopotent) stem cells. In this context, HSCs are an example of monopotent stem cells already committed to lympho/hematopoiesis.

Figure 1 and Table 1 summarize the developmental hierarchy of the stem cell compartment. The most primitive totipotent stem cell is the zygote, which is the result of the fusion of 2 germ cells (oocyte and sperm) during fertilization. As a totipotent stem cell, the zygote is able to give rise to both the embryo and the placenta. The "artificial" counterpart of the totipotent zygote is referred to as a clonote and can be created in the laboratory with an experimental approach known as somatic nuclear transfer, involving removal of the nucleus from a somatic cell and its insertion/transfer into an enucleated oocyte (Hochedlinger et al. 2003; Rideout et al. 2001). The first blastomeres are totipotent stem cells that derive from the first divisions of the zygote or clonote. This is supported by the well-known fact that the first blastomeres, if separated from each other, can give rise to 2 or even more independent embryos (Tarkowski et al. 1959), as seen in the case of monozygotic siblings.

When the blastomeres have divided into the 32-cell stage, the embryo is known as a morula. The cells that form the morula have already lost

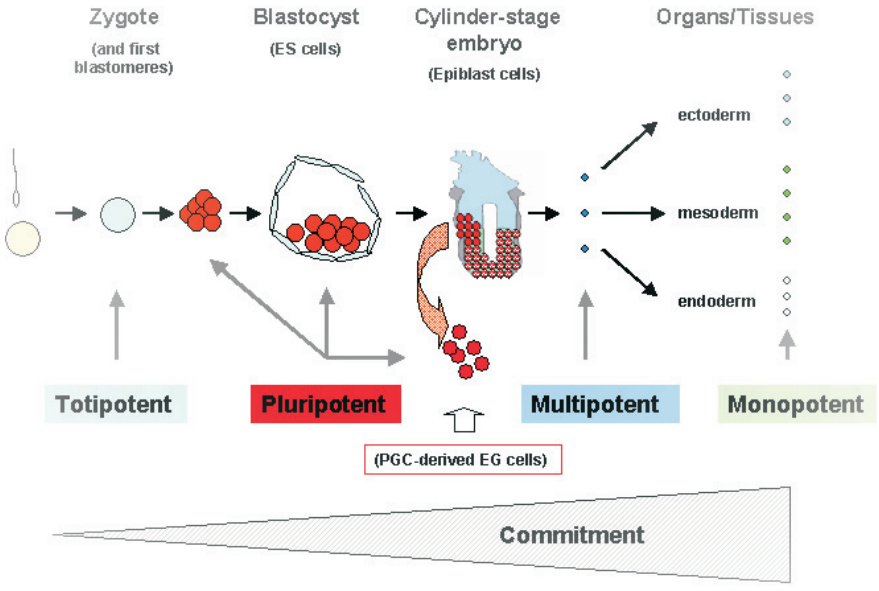

Figure 1. Scheme of developmental hierarchy in the stem cell compartment. The most primitive stem cell is a totipotent zygote or any of the first blastomeres that derive from the first divisions of the zygote. A totipotent stem cell divides into cells that will form both the embryo and placenta. Pluripotent stem cells (PSCs), which contribute only to embryo development, are cells isolated from the inner cell mass (ICM) of a blastocyst, from the epiblast of the cylinder-stage embryo (EPSCs), or could be derived in ex vivo cultures from epiblast-derived primordial germ cells (PGCs) – as a population of so-called embryonic germ (EG) cells. PSCs contribute to all 3 germ layers in the developing embryo (ecto-, meso- and endoderm). Multipotent stem cells give rise to monopotent stem cells that are committed to particular organs/tissues.

Table 1. Developmental hierarchy in the stem cell compartment

Stem cell class	Description
Totipotent	They give rise to both embryo and placenta. Physiological totipotent stem cells include zygotes and first blastomeres. Their artificial counterparts are clonotes, obtained by somatic nuclear transfer to enucleated oocytes.
Pluripotent	They give rise to all 3 germ layers of the embryo after injection to the developing blastocyst. Pluripotent stem cells are cells from the inner cell mass (ICM) of the blastocyst, epiblast (EPSCs), and stem cells obtained as immortalized cell lines: blastocyst-derived embryonic stem (ES) cells and PGC-derived embryonic germ (EG) cells.
Multipotent	They give rise to one of the germ cell layers only, either ecto- or meso- or endoderm.
Monopotent	They are tissue-committed stem cells that give rise to cells of one lineage, e.g., hematopoietic stem cells, epidermal stem cells, intestinal epithelium stem cells, neural stem cells, liver stem cells or skeletal muscle stem cells.

their totipotency and are now pluripotent. The pluripotent stem cell (PSC) is defined as being able to contribute to the development of the embryo but has lost the capacity to form the trophoblast (which gives rise to the placenta). Thus PSCs are the stem cells that contribute to all 3 germ layers (mesoderm, ectoderm and endoderm), but not to the trophoblast.

During early embryogenesis, the growing morula develops a central cavity and becomes the blastocyst (O'Farrell et al. 2004). A fully developed blastocyst contains cells that are precursors for extraembryonic tissues, and a distinct group of cells called the inner cell mass (ICM) (Evans et al. 1981; Martin et al. 1981). The cells of the ICM are also pluripotent and can give rise to all 3 germ layers of the developing embryo (Boiani et al. 2005; Bradley et al. 1984).

Another potential source of PSCs are embryonic germ (EG) cells, which are derived ex vivo from primordial germ cells (PGCs) (Matsui et al. 1992; Resnick et al. 1992; Shamblott et al. 1998; Turnpenny et al. 2003). Precursors of PGCs are the first population of stem cells that can be identified in mice at the beginning of gastrulation in the proximal primitive ectoderm (epiblast), a region adjacent to the extraembryonic ectoderm (McLaren et al. 1992; McLaren et al. 2003; McLaren et al. 2005). These founder cells subsequently move through the primitive streak and give rise to several extraembryonic mesodermal lineages and to germ cells. From a developmental point of view, PGCs are the most important population of stem cells, because – as precursors of germ cells (sperm and oocytes) – they are responsible for passing genetic information to the next generation (Donovan et al. 1998; Wylie et al. 1999). However, if isolated from the developing embryo and cultured ex vivo, these cells are mortal and undergo terminal differentiation (De Felici et al. 1983). PGCs, however, if plated over murine fetal fibroblasts in the presence of selected growth factors, e.g. leukemia inhibitory factor (LIF), basic fibroblast growth factor (bFGF), or kit ligand (Donovan et al. 1994; Resnick et al. 1998), may undergo epigenetic changes in vitro and become an immortalized population of PSCs, the embryonic germ (EG) cells (Matsui et al. 1992). EG cells in many aspects are equivalent to embryonic stem (ES) cells (Zwaka et al. 2005). Both ES and EG cells contribute to all 3 germ layers, including the germ cell lineage after injection into a blastocyst. As mentioned above, this assay (known as blastocyst complementation) is crucial for confirming in vivo functional pluripotentiality of stem cells.

During gastrulation 3 distinct germ layers of cells are established, so it is believed that the embryo must have 3 stem cell types specific for the mesoderm, ectoderm and endoderm (Figure 1). These cells are multipotent stem cells, which subsequently give rise to monopotent stem cells specific for tissues/organs that develop from a given germ layer. For example, multipotent mesodermal stem cells give rise to monopotent hematopoietic, skeletal muscle, heart, endothelial, and mesenchymal tissue-committed stem cells, while multipotent endodermal stem cells give rise to monopotent liver, pancreas, and gut epithelial cells, and multipotent ectodermal stem cells give rise to monopotent brain cells, peripheral ganglia and nerves, as well as eye, epidermal, and skin tissues.

Bone marrow non-HSCs – lessons learned from the concept postulating the phenomenon of "plasticity" of HSCs

Several investigators in the past few years demonstrated that BM-derived cells can contribute to the

regeneration of various organs and tissues (Makino et al. 1999; Lagasse et al. 2000; Jackson et al. 2001; Orlic et al. 2001; Rideout et al. 2001; Buzańska et al. 2002; Corti 2002a; Corbel et al. 2003; Hess et al. 2003; Pesce et al. 2003; Di Campli et al. 2004). These observations were mainly explained by the hypothesis that HSCs are "plastic" and thus could trans-dedifferentiate into stem cells committed to various non- hematopoietic organs and tissues (Corti et al. 2002b; Mezey et al. 2000). This is potentially possible in 2 cases: (i) a parallel switch of commitment for HSCs in the compartment of monopotent stem cells or (ii) a step back in the differentiation process of HSCs, with their dedifferentiation into multipotent (committed to one germ layer) or even pluripotent (committed to 3 germ layers) stem cells.

This hypothetical possibility that HSCs are plastic and able to trans-dedifferentiate has raised much hope that HSCs isolated from BM, mobilized peripheral blood (mPB), or cord blood (CB), could become a universal source of stem cells for tissue/organ repair. This excitement was bolstered at that time by several reports that demonstrated the remarkable regenerative potential of HSCs in animal models, for example, after heart infarct (Orlic et al. 2001), stroke (Hess et al. 2004), spinal cord injury and liver damage (Petersen et al. 1999).

However, since these first exciting and promising reports, the role of BM stem cells in the repair of damaged organs has become controversial (McKinney-Freeman et al. 2002; Orkin et al. 2002; Wagers et al. 2002). Further experiments with highly purified populations of HSCs showed them not to be effective in regenerating damaged heart (Murry et al. 2004) or brain (Castro et al. 2002).

In response to these unexpected results, the scientific community became polarized in its view of the concept of stem cell plasticity. These obvious discrepancies in published results could be explained by differences in the tissue injury models employed and/or problems in detection of tissue chimerism, and several other possibilities have been proposed to explain these discrepancies. Surprisingly, during all of these deliberations concerning stem cell plasticity and the potential contribution of BM-derived cells to organ regeneration, the concept that BM may contain heterogeneous populations of stem cells was not taken into careful consideration (Orkin et al. 2002; Ratajczak et al. 2004). We postulate that regeneration studies demonstrating a contribution of donor-derived BM, mPB or CB cells to non-hematopoietic tissues without addressing this possibility (by including the appropriate controls) has led to misleading interpretations. It is reasonable to assume that the presence of heterogeneous populations of stem cells in BM, mPB or CB should be considered before experimental evidence is interpreted as plasticity or trans-differentiation of HSCs (Kucia et al. 2005c).

Hence the presence of non-HSCs in BM, rather than "trans-dedifferentiation" of HSCs (Kucia et al. 2005a; Lagasse et al. 2000; Makino et al. 1999; Petersen et al. 1999), could explain some of the positive results of tissue/organ regeneration, as witnessed by several investigators using BM-derived cells. On the other hand, when highly purified HSCs were employed for regeneration experiments, non-HSCs were probably excluded from these cell preparations. Thus at the current state of knowledge, the phenomenon of trans-dedifferentiation of HSCs and their contribution to regeneration of damaged tissues remains questionable.

Bone marrow – the promise land for migrating stem cells

Early human embryogenesis is the most active period for the developmental migration/trafficking of stem cells. With the beginning of gastrulation and organogenesis, stem cells migrate to places where they establish rudiments of new tissues and organs. Thus at certain points of development, stem cells colonize tissue-specific niches, where they reside as a population of self-renewing cells. These cells supply new cells that effectively replace senescent ones or those undergoing apoptosis. Therefore the role of monopotent tissue-residing stem cells during adult life is restricted mainly to cell turnover, proper in a given organ or tissue.

We postulate that during later phases of embryogenesis some migrating/circulating stem cells similar to epiblast-derived cells may colonize BM. Supporting this is the fact that all monopotent tissue-committed stem cells (e.g., endothelial, neural, skeletal muscle, liver oval, renal tubular endothelium, retina pigment epithelium stem cells) express CXCR4 on their surface and follow an SDF-1 gradient (stromal derived factor –1, the chemokine binding to CXCR4) (Avecilla et al. 2004; Crane et al. 2000; Hatch et al. 2002; Ji et al. 2004; Lazarini et al. 2003; Ratajczak et al. 2003; Tachibana et al. 1998; Tögel et al. 2005; Urbich

et al. 2004; Vasyutina et al. 2005), thus potentially being able to home to BM. We suppose that the SDF-1-CXCR4 axis alone or in combination with other chemoattractants could play a crucial role in accumulation of non-HSCs in BM. These migrating stem cells find a permissive environment in BM, which allows them to survive into adulthood. It is highly possible that BM-residing non-HSCs could play a role as a reserve pool of circulating stem cells for organ/tissue regeneration during postnatal life. In this context, BM tissue becomes a "home" not only for HSCs but also for the already differentiated circulating $CXCR4^+$ tissue-committed stem/progenitor cells and more primitive $CXCR4^+$ PSCs. These cells reside in BM and could play an important role in tissue/organ repair as a mobile reserve pool of tissue-committed stem/progenitor cells.

Evidence of the heterogeneity of BM stem cells

The concept that BM may contain some non-HSCs has been postulated by several investigators and, as previously mentioned, the best evidence that BM stem cells are in fact heterogeneous was provided by experiments showing that BM-derived cells could support the regeneration of various tissues/organs (Bunting et al. 2003; Orkin et al. 2002; Ratajczak et al. 2004).

Table 2 lists different types of non-HSCs that have been postulated to reside in BM tissue. These cells will be discussed briefly below, and the similarities between these versatile populations of stem cells indicated. It is very likely that several investigators using different isolation strategies described the same populations of stem cells but gave them different names according to circumstances.

Endothelial progenitor cells (EPCs)

BM-derived cells have been shown to contain endothelial precursors in both mice and humans (Asahara et al. 1997; Asahara et al. 1999) and EPCs residing in BM could be released/mobilized into PB as a source of cells able to play a role in the vascularization of damaged organs (Kawamoto et al. 2001; Shi et al. 1998; Shintani et al. 2001; Takahashi et al. 1999). The phenotype of these cells is shown in Table 2. The level of contribution of BM-derived EPCs to organ/tissue vascularization, however, still requires further studies.

Mesenchymal stem cells (MSCs, or multipotent mesenchymal stromal cells)

MSCs are fibroblastic cells that are essential in forming the hematopoietic microenvironment in which HSCs grow and differentiate (Devine et al. 2000; Dexter et al. 1987; Pittenger et al. 1999). These cells, however, are postulated to contribute to the regeneration of mesenchymal tissues (e.g., bone, cartilage, muscle, ligament, tendon, adipose tissue and stroma) (Campagnoli et al. 2001; Pittenger et al. 1999). It is likely that MSCs may be contaminated by other populations of primitive non-HSCs. This possibility should be considered whenever a "trans-dedifferentiation" of MSCs into cells from other germ layers is demonstrated. Because various inconsistencies have come to light in the field of MSC research, the International Society for Cellular Therapy has recently recommended avoiding the name MSCs

Table 2. Non-hematopoietic stem cells found in bone marrow

Type of non-hematopoietic stem cells	Phenotype
Endothelial progenitor cells (EPCs)	Human: $CD133^+$, $CD34^+$, c-kit $(CD117)^+$, VE-cadherin$^+$, $VEGFR2^+$, $CD146^+$, vWF^+, $CD31^+$ Mouse: Sca-1^+, c-Kit $(CD117)^+$, Lin^-, $VEGFR2^+$, VE-cadherin$^+$, $Tie2^+$, $CD146^+$, vWF^+, $CD31^+$
Mesenchymal stem cells (MSCs) *	International Society for Cellular Therapy criteria: CD105+, $CD73^+$, $CD90^+$, $CD45^-$, $CD34^-$, $CD14^-$, $CD11b^-$, $CD79a^-$, $CD19^-$, $HLA\text{-}DR^-$
Multipotent adult progenitor cells (MAPCs) *	SSEA-1^+, $CD13^+$, Flk-1^{low}, Thy-1^{low}, $CD34^-$, $CD44^-$, $CD45^-$, CD117(c-kit)$^-$, MHC I^-, MHC II^-
Marrow-isolated adult multilineage inducible (MIAMI) cells *	$CD29^+$, $CD63^+$, $CD81^+$, $CD122^+$, $CD164^+$, c-met$^+$, $BMPR1B^+$, $NTRK3^+$, $CD34^-$, $CD36^-$, $CD45^-$, CD117 (c-kit)$^-$, $HLA\text{-}DR^-$
Very small embryonic-like stem cells (VSELs)	$CXCR4^+$, $AC133^+$, $CD34^+$, SSEA-1^+ (mouse) or SSEA-4^+ (human), AP^+, c-met$^+$, LIF-R^+, $CD45^-$, Lin^-, MHC I^-, $HLA\text{-}DR^-$, $CD90^-$, $CD29^-$, $CD105^-$

* (phenotype of expanded/cultured adherent cells)

Abbreviations: AP = fetal alkaline phosphatase; BMPR1B = bone morphogenetic protein receptor 1B; c-met = receptor for hepatocyte growth factor; LIF-R = receptor for leukemia inhibitory factor; NTRK3 = neurotropic tyrosine kinase receptor 3; vWF = von Willebrand factor.

and changing it into multipotent mesenchymal stromal cells instead (Horwitz et al. 2005). The phenotype of these cells is shown in Table 2.

Multipotent adult progenitor cells (MAPCs)

MAPCs are isolated from BM mononuclear cells (MNCs) as a population of $CD45^-$ $GPA\text{-}A^-$ adherent cells and they display a similar fibroblastic morphology to MSCs (Jiang et al. 2002). Interestingly, MAPCs are the only population of BM-derived stem cells that, so far as is known, contribute to all 3 germ layers after injection into a developing blastocyst, indicating their pluripotency (Jiang et al. 2002). The contribution of MAPCs to blastocyst development, however, requires confirmation by other, independent laboratories. The phenotype of these cells is shown in Table 2.

Marrow-isolated adult multilineage inducible (MIAMI) cells

This population of cells was isolated from human adult BM by culturing BM MNCs in low oxygen tension conditions on fibronectin (D'Ippolito et al. 2004). MIAMI cells were isolated from the BM of people aged from 3 to 72 years. Colonies derived from MIAMI cells expressed several markers for cells from all 3 germ layers, suggesting that, at least as determined by in vitro assays, they are endowed with pluripotency. However, these cells have not been tested so far for their ability to complete blastocyst development. The potential relationship of these cells to MSCs and MAPCs (described above) is not clear, although it is possible that these are overlapping populations of cells identified by slightly different isolation/expansion strategies. The phenotype of these cells is shown in Table 2.

Very small embryonic-like stem cells (VSELs)

Recently a homogenous population of rare $Sca\text{-}1^+$ lin^- $CD45^-$ cells (accounting for ~0.01% of BM MNCs) has been identified by our team in murine BM. They express (as determined by RQ-PCR and immunohistochemistry) markers of pluripotent stem cells, such as SSEA-1, Oct-4, Nanog and Rex-1, and Rif-1 telomerase protein (Kucia et al. 2006a). Direct electron microscopic analysis has revealed that these very small cells (2–4 μm in diameter) display several features typical for embryonic stem cells, such as (i) a large nucleus surrounded by a narrow rim of cytoplasm, and (ii) open-type chromatin (euchromatin). Interestingly, these cells despite their small size have diploid DNA and contain numerous mitochondria. They do not express MHC-1 and HLA-DR antigens and are $CD90^-$ $CD105^-$ $CD29^-$. It is important to emphasize that for the first time a sorting procedure that indicates how to purify from adult BM a distinct population of very primitive embryonic-like stem cells and, more importantly, the morphology and the surface markers of these rare cells at the single cell level, have been described.

We hypothesize that VSELs could be a population of epiblast-derived PSCs that are deposited in various tissues (e.g., BM) and survive into adulthood. We assume that these VSELs may be released from BM and circulate in PB during tissue/organ injury (e.g., heart infarct and stroke). Interestingly ~5–10% of purified VSELs, if plated over a C2C12 murine sarcoma cell feeder layer, are able to form spheres that resemble embryoid bodies. Cells from these VSEL-derived spheres (VSEL-DS) are composed of immature cells with large nuclei containing euchromatin, and like purified VSELs, they are $CXCR4^+$ $SSEA\text{-}1^+$ $Oct\text{-}4^+$.

Furthermore, VSEL-DS, after re-plating over C2C12 cells, may again (up to 5–7 passages) grow new spheres or, if plated into cultures promoting tissue differentiation, expand into cells from all 3 germ-cell layers. Since VSELs isolated from GFP^+ mice grew GFP^+ VSEL-DS, showing a diploid content of DNA, this confirms that VSEL-DS are derived from VSELs and not from the supportive C2C12 cell line; this also excludes the possibility of cell fusion. Similar spheres were also formed by VSELs isolated from murine fetal liver, spleen and thymus. Interestingly, the formation of VSEL-DS was associated with young age in mice, and no VSEL-DS were observed in cells isolated from old mice (>2 years) (Kucia et al. 2005c; Kucia et al. 2006a). This age-dependent VSEL content of BM may explain why the regeneration processes are more efficient in younger individuals. There are also differences in their content among BM MNCs between long- and short-lived mouse strains. The VSEL content of BM in long-lived (e.g., C57Bl6) is much higher than in short-lived (DBA/2J) mice (Kucia et al. 2005c). It would be interesting to identify the genes that are responsible for tissue distribution/expansion of these cells, as they could be involved in controlling the life span of mammals.

Since VSELs express several markers of primordial germ cells (fetal-type alkaline phosphatase, Oct-4, SSEA-1, CXCR4, Mvh, Stella, Fragilis, Nobox, Hdac6), they could be closely related to a population of epiblast-derived PGCs. VSELs are also highly mobile and respond robustly to an

SDF-1 gradient, adhere to fibronectin and fibrinogen, and may interact with BM-derived stromal fibroblasts. Confocal microscopy and time laps studies (Kucia et al. 2005c) revealed that these cells attach rapidly to, migrate beneath, and undergo emperipolesis in marrow-derived fibroblasts (Kucia et al. 2005c). Since fibroblasts secrete SDF-1 and other chemoattractants, they may create a homing environment for small $CXCR4^+$ VSELs. This robust interaction of VSELs with BM-derived fibroblasts has an important implication, namely that isolated BM stromal cells may be contaminated by these tiny cells from the beginning. This observation may explain the unexpected "plasticity" of marrow-derived fibroblastic cells (e.g., MSCs or MAPCs).

Recently, a very similar population of cells, which show similar morphology and markers to murine BM-derived VSELs, has been purified from human cord blood (Kucia et al. 2007). Evidence has mounted that similar cells are also present in the human BM, in particular in young patients. It is anticipated that VSELs could become an important source of pluripotent stem cells for regeneration. At this point, however, it is not clear whether VSELs contribute to blastocyst development.

Bone marrow as a source of circulating non-HSCs

A small number of BM-derived HSCs circulate in PB even in steady state conditions, as a result of self-renewal of stem cells in BM niches, but this serves as a way to keep in balance the stem cell pool in stem cell niches in various anatomical locations of the same BM. Stem cells circulating in PB can also compete for organ/tissue-specific niches. This may explain, for example, why heterogeneous populations of stem cells can also be detected in various organs (e.g., HSCs in muscle tissue). The number of HSCs released/mobilized into PB increases after injection of mobilizing cytokines (e.g., G–CSF) (Petit et al. 2002) or other agents (e.g., AMD3100) (Devine et al. 2004) as well as in several stress-related situations (e.g., tissue/organ injury, heart infarct, strenuous exercise) (Takahashi et al. 1999; Tögel et al. 2005; Wojakowski et al. 2004).

A similar phenomenon has been described for BM-residing non-HSCs, which are also able to egress from BM tissue and circulate in PB during (i) pharmacological mobilization or (ii) stress related to tissue/organ injury. It has been further reported that the number of circulating non-HSCs in PB can be increased after administration of G-CSF alone or in combination with compounds that block the CXCR4 receptor (e.g., T140 or AMD3100) (Ratajczak et al. 2004; Shyu et al. 2004). Hence the process of stem cell mobilization from BM into PB should be regarded as mobilizing not only HSCs but also BM-residing non-HSCs. Thus pharmacological mobilization could become a means to obtain non-hematopoietic stem cells, as well as HSCs from BM.

Table 3 shows several clinically relevant situations that lead to an increase in the number of BM-derived stem cells circulating in PB. Increases in their number have been reported in skeletal muscle damage (Kuznetsov et al. 2001; Long et al. 2005; Palermo et al. 2005), heart infarct (Kucia et al. 2004; Wojakowski et al. 2004), stroke (Kucia et al. 2006d), multiple bone fractures (Eghbali-Fatourechi et al. 2005), liver injury, kidney injury (Tögel et al. 2005), lung transplantation (Gomperts et al. 2006), cardiac surgery (Mieno et al. 2006), liver transplant (Lemoli et al. 2006), and limb ischemia (Jin et al. 2006). These circulating BM-derived stem cells were identified as endothelial progenitor cells (EPC), skeletal muscle satellite stem cells (Kuznetsov et al. 2001), myocardiac- (Wojakowski et al. 2004), neural-, and bone-committed stem cells (Eghbali-Fatourechi et al. 2005; Kucia et al. 2006d), and circulating MSCs, as well as so-called fibrocytes (Bucala et al. 1994; Phillips et al. 2004), which are probably a functionally changed subset of MSCs.

In summary, mounting evidence suggests that during organ/tissue damage non-HSCs are mobilized from BM (and perhaps also from other tissue-specific niches) into PB, where they circulate prior to homing to damaged tissues and participating in organ repair. It is well documented that damaged tissues upregulate the expression of several potential chemoattractants for these circulating stem cells, such as SDF-1, VEGF (vascular endothelial growth factor), HGF/SF, LIF, or FGF-2 (Hill et al. 2004; Houchen et al. 1999; Kucia et al. 2006b; Ponomaryov et al. 2000; Zhang et al. 2003). Furthermore, the hypoxia-regulated/induced transcription factor (HIF-1) plays an important role in the expression of several of these factors (Hitchon et al. 2002; Schioppa et al. 2003). In support of this notion, promoters of SDF-1, VEGF and HGF/SF contain several functional HIF-1-binding sites (Ceradini et al. 2004; Hitchon et al. 2002; Tacchini et al. 2003; Wu et al.

Table 3. Data supporting circulation of bone-marrow-derived non-hematopoietic stem cells in various clinical situations

Clinical situation	Supporting data
Skeletal muscle damage	Mouse BM-derived stem cells were reported to contribute after transplantation into irradiated muscles to skeletal muscle satellite stem cells. These cells subsequently contributed to skeletal muscle regeneration after exercise-induced damage.
Heart infarct	Human: increase in circulating $CD34^+$, $CXCR4^+$, c-Met^+ cells in peripheral blood after acute myocardial infarction (MI); increase in mRNA for Gata-4, Mef-2C and Nkx.25/Csx in circulating MNCs. Mouse: increase in mRNA for Gata-4, Mef-2C and Nkx.25/Csx in peripheral blood MNCs after experimental acute MI.
Stroke	Mouse: increase in mRNA for GFAP, Nestin and bIII-tubulin in peripheral blood (mononuclear cells) MNCs after experimentally induced stroke.
Multiple bone fractures	Human: increase in mRNA for bone-related genes (osteocalcin, bone alkaline phosphatase, collagen type I) in peripheral blood MNCs in patients with multiple bone fractures.
Liver injury	Human: increase in homing of human $CD34^+$ $CXCR4^+$ cells in NOD/SCID mouse livers damaged by exposure to CCl4.
Kidney injury	Mouse: increase in circulating $CD34^+$ $CXCR4^+$ and lin-Sca-1^+ cells in peripheral blood during acute renal failure.
Lung transplants	Human: increase in circulating $CXCR4^+$ cytokeratin-5^+ cells in peripheral blood.
Cardiac surgery	Human: increase in circulating $CD34^+$ $CXCR4^+$ cells in peripheral blood.
Liver transplant	Human: increase in circulating $CD34^+$ cells and increase in mRNA for GATA-4, cytokeratin 19 and α-fetoprotein in peripheral blood MNCs.
Limb ischemia	Mouse: BM-derived Sca-1^+ FLK-1^+ Tie-2^+ $CD34^+$ cells circulate and play a role in neovascularization.

2003). Thus hypoxic conditions created by an increasing expression of these factors may orchestrate mobilization from BM and subsequently the homing of circulating stem cells to damaged organs/tissues.

The role of BM-derived stem/progenitor cells in pathology

In parallel, evidence has also accumulated that BM-derived stem cells may play an undesirable role in the development of some pathologies (Sell et al. 2004). It is likely that if these cells are mobilized at the wrong time and home to the wrong place (e.g., into areas of chronic inflammation), they may exert unwanted effects (Reya et al. 2001; Virchow et al. 1855). For example, BM-derived stem/progenitor cells have been implicated in the pathogenesis of lung fibrosis, ocular pterygia, and diabetic neuropathy (Hasegawa et al. 2006; Hashimoto et al. 2004; Song et al. 2005).

Somewhat unexpectedly, it has been found that BM-derived stem cells may also contribute to the development of some non-hematopoietic tumors. This possibility has been recently demonstrated in a model of murine stomach cancer caused by a chronic *Helicobacter pylori* (HP) infection (Houghton et al. 2004). In this model, BM-derived cells were identified as a source of developing gastric mucosa adenocarcinomas, and the SDF-1–CXCR4 axis was implicated in the initiation of this tumor (Houghton et al. 2004). Accordingly, SDF-1 was found to be upregulated in the gastric mucosa affected by chronic inflammation due to HP infection, and postulated to be the chemoattractant responsible for attracting $CXCR4^+$ stem cells from BM. Exposed to the chronic inflammatory environment in the gastric mucosa, these $CXCR4^+$ cells (VSELs?) transformed into adenocarcinoma-initiating cells (Li et al. 2006). A similar phenomenon has recently been postulated in the pathogenesis of colon adenocarcinomas. In addition, mounting evidence also suggests that BM may be a source of EPCs and MSCs for developing cancer tissue (Dome et al. 2006; Tolar et al. 2007). Thus, BM stem cells may in various ways contribute to both initiation and expansion of the growing tumor.

Further support to the concept that BM-derived cells may initiate the growth of non-hematopoietic malignancies, was provided by a recent report that BM cells exposed in vitro to the carcinogen 3-methylcholanthrene could transform into many tumor types, including epithelial, neural, muscular, fibroblastic, blood vessel endothelial, and poorly differentiated tumors (Liu et al. 2006). Moreover, a single transformed BM cell had the

ability to self-renew, differentiate spontaneously into various types of tumor cells in vitro, express markers associated with multipotency, and form a teratoma in vivo. These data support the notion that some multipotent cancer stem cells seemed to originate from transformed BM cells (Liu et al. 2006). This provides further evidence that BM-stem cells may be the origin of several non-hematopoietic cancers, and that BM is the most likely home of non-HSCs, which are susceptible to transformation by carcinogens.

Future implications

We postulate that the "positive" data supporting the plasticity of BM-derived stem cells can be re-interpreted at least partially by the fact that BM stem cells are heterogeneous and that BM tissue contains various types of stem cells, including the rare pluripotent VSELs. The question remains, however, whether these PSCs can continuously contribute in adult life to the renewal of other stem cells, including HSCs, which are the largest population of stem cells in BM.

There are several answers to this provocative, but timely question, especially in view of the current widely performed clinical trails with BM-derived stem cells in cardiology and neurology, before these cells find potential applications in regenerative medicine. First, there is the obvious problem of isolating a sufficient number of VSELs from BM. The number of these cells among murine BM MNCs is very low (e.g., VSELs represent 1 cell per 10^4–10^5 BM MNCs) (Kucia et al. 2006a). Furthermore, our data show that these cells are more numerous in the BM of young mammals and their number decreases with age (Kucia et al. 2006a). It is also likely that VSELs released from BM, even if they are able to home to the areas of tissue/organ injury, play a role only in regeneration of minor tissue injuries. Heart infarct or stroke, on the other hand, would be considered to involve severe tissue damage, beyond the capacity of these rare cells for effective repair. Second, the allocation of these cells to the damaged areas depends on homing signals, which may be inefficient in the presence of proteolytic enzymes released from leukocytes and macrophages associated with damaged tissue. For example, matrix metalloproteinases (MMPs) released from inflammatory cells may locally degrade SDF-1, and thus perturb the homing of $CXCR4^+$ stem cells. Thus VSELs may potentially circulate as a homeless population of stem cells in peripheral blood and return to BM or home to other organs. Third, in order to reveal the full regenerative potential, these cells have to be fully functional. We cannot exclude the possibility that VSELs, while residing/being "trapped" in BM, are not fully functional but remain "locked" in a dormant state and need some appropriate activation signals by unidentified factors. As mentioned above, they have also most likely erased somatic imprint, which may limit their pluripotentiality. Finally, we have not identified so far any efficient combination of growth factors/adhesion molecules that allows efficient differentiation of VSELs without supportive feeder-layer cells (e.g., C2C12, BM stroma fibroblasts). However, on the other hand, cells isolated from VSEL-DS may differentiate into all germ layers – but as mentioned above, they already show some level of differentiation and limited self-renewal.

In conclusion, our data indicate that VSELs could potentially provide a real therapeutic alternative to the controversial use of human ES cells and therapeutic cloning. Hence, while the ethical debate on the application of ES cells in therapy continues, the potential of VSELs is ripe for exploration. Researchers must determine whether these cells could be efficiently employed in clinical treatment or whether they are merely developmental remnants found in BM and cannot be harnessed effectively for regeneration. The coming years will bring important answers to these questions.

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