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Review article

Heterogeneous populations of bone marrow stem cells - are we spotting on the same cells from the different angles?

Mariusz Z. Ratajczak[1,2], Magda Kucia[1], Marcin Majka[1], Ryan Reca[2] and Janina Ratajczak[2]

[1]European Stem Cell Therapeutic Excellence Center, Medical College, Jagiellonian University, Cracow, Poland ;
[2]Stem Cell Biology Program at James Graham Brown Cancer Center and Department of Medicine, University of Louisville, Louisville, USA

Abstract: Accumulated evidence suggests that in addition to hematopoietic stem cells (HSC), bone marrow (BM) also harbors endothelial stem cells (ESC), mesenchymal stem cells (MSC), multipotential adult progenitor cells (MAPC), pluripotent stem cells (PCS) as well as tissue committed stem cells (TCSC) recently identified by us. In this review we discuss the similarities and differences between these cell populations. Furthermore, we will present the hypothesis that all of these versatile BM derived stem cells are in fact different subpopulations of TCSC. These cells accumulate in bone marrow during ontogenesis and being a mobile population of cells are released from BM into peripheral blood after tissue injury to regenerate damaged organs. Furthermore, since BM is a "hideout" for TCSC, their presence in preparations of bone marrow derived mononuclear cells should be considered before experimental evidence is interpreted simply as trans-differentiation or plasticity of HSC. Finally, our observation that the number of TCSC accumulate in the bone marrow of young animals and their numbers decrease during senescence provides a new insight into aging and may explain why the regeneration processes becomes less effective in older individuals.

Key words: CXCR4 - SDF-1 - HSC - MAPC - MSC - Stem cell plasticity

Introduction

Early during ontogenesis the stem cell compartment is organized in a hierarchical manner, beginning from the population of the most primitive pluripotent stem cells present in the inner cell mass of the blastocyst, through multipotent stem cells identified in the early gastrula, to the unipotent tissue committed stem cells (TCSC) that persist in adult tissues/organs as a pool of self-renewing cells (*e.g.* TCSC for epidermis, intestine, bone marrow, liver, heart and kidney).

The concept that bone marrow (BM) may contain heterogenous populations of stem cells was surprisingly not taken carefully enough into consideration in several recently reported experiments demonstrating so called plasticity or trans-differentiation of hematopoietic stem cells (HSC). These studies without including proper controls to exclude this possibility often may lead to wrong interpretations. Compelling evidence from others and our laboratory shows that BM harbors non-hematopoietic TCSC in addition to HSC [2, 28, 34, 37, 38, 41, 42]. We envision that several types of TCSC accumulate early in development in the BM where they find a permissive environment to survive.

Furthermore, it had been hypothesized that during development early pluripotent/multipotent embryonic-like stem cells (PSC) could be deposited in various organs, including BM. Thus, marrow could potentially contain the whole spectrum of heterogenous populations of stem cells - beginning from early PSC to TCSC. In this review we will discuss all of these possibilities, shedding more light on BM as a hiding place of versatile TCSC. We will present evidence from others and our laboratory that supports this concept and provides a perspective on a role of BM as a source of early cells for regeneration/tissue repair.

Correspondence: M.Z. Ratajczak, Stem Cell Biology Program, James Graham Brown Cancer Center, University of Louisville, 529 South Jackson Street, Louisville, KY 40202, USA;
e-mail: mzrata01@louisville.edu

Ontogenesis of bone marrow

In mammals, the fetal liver is the major hematopoietic organ during the second trimester of gestation. Bone

tissue at that time contains stem cells that generate chondrocytes, osteoblasts and fibroblasts. These cells, as widely accepted, derive from a hypothetical embryonic mesenchymal stem cell (MSC) that is a founder cell for connective tissue lineages. The MSC, however, had not yet been purified and its existence in an adult organism is based mainly on indirect evidence from cultures of BM-derived fibroblasts. These cultures if growing in selective media reveal the presence of osteoblastic, chondrocytic, and smooth muscle cells as well as adipocytes which derive from MSC present in cultures of BM-derived fibroblasts [18]. However, the possibility that BM may contain instead of MSC or in addition to them separate populations of TCSC for chondrocytes, osteoblasts and fibroblasts was not ruled out.

The rapid development/expansion of hematopoietic tissue takes place by the end of the second trimester of gestation when fibroblasts and osteoblasts in early bones began to secrete α-chemokine - stromal derived factor-1 (SDF-1) that chemoattracts HSC expressing its specific seven-transmembrane pass G-protein coupled receptor CXCR4, from the fetal liver into BM [12, 14, 26]. Studies in mice with knock-outs of SDF-1 or CXCR4 demonstrated that the SDF-1-CXCR4 axis plays a pivotal role in colonizing BM by HSC and in establishing adult marrow hematopoiesis. BM stroma in murine embryos with SDF-1 knocked out does not chemoattract HSC from the fetal liver, and on the other hand murine HSC with CXCR4 knocked out do not respond to an SDF-1 gradient and thus do not colonize BM [1]. As a result, knock-out murine embryos display defective hematopoiesis in BM. This shows that the SDF-1-CXCR4 axis is one of the key players in regulating homing/retention of HSC into bone marrow and the similarity of both knock-out phenotypes suggests that SDF-1 is the only ligand for CXCR4 and CXCR4 only binds SDF-1.

The important message from these developmental studies is that CXCR4 positive HSC from the fetal liver colonize the BM microenvironment to which they are chemoattracted by an SDF-1 gradient. At that time BM already contains developed vessels what suggests that endothelial TCSC (ESC) arrive first. Since ESC originate from a hemangioblast, a common precursor for both hematopoietic and endothelial TCSC, it is likely that hemangioblasts arrive first in BM and play a pivotal role in establishing marrow hematopoiesis before marrow is colonized by fetal liver-derived HSC. It is not clear, however, if hemangioblasts persist in BM during postnatal life. Therefore, further studies are needed to answer if adult bone marrow harbors some early embryonic pluripotent/multipotent stem cells (PSC) *e.g.*, hemangioblasts and/or MSC. Our recent data suggest that later on, in postnatal life, other TCSC (*e.g.*, for muscles, neurons, liver, heart, pancreas and kidney) in addition to HSC and ESC accumulate gradually in bone marrow tissue [23, 41]. These cells similarly as HSC express CXCR4 and are chemoattracted by SDF-1 gradient.

Bone marrow as a hiding place for CXCR4 positive cells

Compelling evidence accumulates that CXCR4 is expressed not only on HSC, but also on other types of stem cells beginning with the population of pluripotent embryonic and germ stem cells and ending with neuronal-, muscle (satellite)-, liver (oval)-, endothelial, pancreatic-, renal tubular epithelium-, retinal pigment epithelium- and lung epithelium-TCSC [5, 8, 9, 27, 29, 30, 48]. We envision that the SDF-1-CXCR4 axis plays an important role in the developmental trafficking of these cells. In support of this, mice with SDF-1/CXCR4 knockouts display several defects in the development of the heart, large vessels and central nervous system in addition to failure of colonizing bone marrow by fetal liver-derived HSC. Thus, defects of the SDF-1-CXCR4 axis seem to affect the trafficking of several types of $CXCR4^+$ TCSC [41].

Accumulating evidence supports the role of the SDF-1-CXCR4 axis in tumor metastasis [21]. Recent data suggests that tumors/leukemias develop primarily from the stem cell compartment [31, 44, 47]. Thus, the earliest neoplastic cells, founder cells for expanding/metastasizing tumors, frequently express functional CXCR4, as do stem cells from which these malignancies originate. This explains why several tumors employ the SDF-1-CXCR4 axis for metastasis, and metastasize to tissues that highly express/secrete SDF-1 (*e.g.*, bone marrow, lymph nodes or lungs). In support of this, CXCR4 was reported to play a pivotal role in metastasis of breast cancer, lung cancer and prostate cancer cells that originate as mentioned above in CXCR4 positive breast-, bronchial- and prostate-derived epithelial TCSC [7, 10, 13]. Similarly, functional CXCR4 was reported to be expressed on several pediatric sarcomas such as neuroblastoma, rhabdomyosarcoma, Ewing sarcoma, Willms tumor and retinoblastoma that originate from CXCR4 positive stem cells for neural, muscle, renal tubular epithelium, neuroectodermal and retina pigment epithelium, respectively [7, 10, 13, 16]. The data from our and other laboratories indicate that this particular group of tumors known as the so called "small round blue cell tumors" frequently metastasize to bone marrow in a SDF-1-CXCR4 dependent manner. The involvement of BM by these sarcomas may be so extensive that it mimics acute lymphoblastic leukemia.

Therefore, BM due to its extensive network of sinusoid vessels and being a source of several locally secreted growth factors and chemoattractants (*e.g.*, SDF-1) may attract $CXCR4^+$ cells circulating in the peripheral blood. These $CXCR4^+$ cells, both normal and malignant, find in the bone marrow a friendly permissive environment to survive/expand.

CXCR4 positive TCSC may circulate in peripheral blood

It is well known that HSC circulate in peripheral blood to maintain in balance hematopoiesis in the marrow tissue that is spread in distant bones. The same phenomenon was recently described for endothelial- and skeletal-TCSC [25, 40, 46]. Furthermore, reconstitution experiments of damaged muscle, kidney, lung, liver, pancreas or heart tissue strongly suggest that under some circumstances, BM-derived TCSC egress from the marrow and *via* peripheral blood migrate into damaged tissues. This migration is facilitated by several chemoattractants secreted by the damaged organs in response to injury. Evidence from our and other laboratories suggests that the SDF-1-CXCR4 axis plays a pivotal role in attracting these CXCR4$^+$ TCSC [2, 17, 23, 24, 28, 34, 37, 38, 41, 42, 48].

We postulate that circulating TCSC similarly as circulating HSC keep in balance a pool of stem cells in homologous tissues that are spread in distant areas of the body (*e.g.,* hemato/lymphopoietic tissue, muscles or neural tissue) [42]. TCSC circulate in peripheral blood probably at the highest number in young individuals during their intensive growth [23].

We envision that marrow-derived CXCR4$^+$ TCSC played a major, albeit underappreciated role in tissue/organ regeneration studies after therapeutic infusion of bone marrow-derived cells, reported in the past. Their role in tissue repair (*e.g.*, heart infarct, liver injury) was probably erroneously interpreted as trans-dedifferentiation or plasticity of HSC. The number of CXCR4$^+$ TCSC increases in peripheral blood after pharmacological mobilization (*e.g.*, G-CSF) or "organ injury" induced mobilization (*e.g.*, heart infarct, liver damage) [41]. In fact, we recently detected mRNA for liver TCSC (α-fetoprotein, CK19) and cardiac TCSC (NskX2, GATA-4) in circulating peripheral blood mononuclear cells of mice after toxic injury of the liver by CCl_4 or heart infarct [23, 41]. In both situations, mRNA for markers of endothelial TCSC (*e.g.*, VE-cadherin, von Willebrand factor) was also upregulated in mononuclear cells isolated from peripheral blood. This suggests that versatile populations of TCSC contribute to optimal regeneration of the damaged organs.

There are two important implications from the circulation of TCSC. First, TCSC accumulate in bone marrow tissue that provides them with a supportive environment. Second, since stem cell niches in various organs secrete SDF-1, circulating TCSC may compete for seeding/occupancy of these niches in peripheral tissues. This explains why in various tissue-specific niches, *e.g.* muscle or neural tissue, there are always detectable versatile circulating TCSC, *e.g.* HSC [38, 41, 42]. In support of this notion, we recently demonstrated that the SDF-1-CXCR4 axis may play a pivotal role in the "homing" of CXCR4 positive HSC into muscle tissue, as well as is responsible for the accumulation of CXCR4 positive muscle TCSC in the bone marrow [38, 42]. As a result of the circulation of TCSC, heterogenous populations of stem cells are present in various organs. The identification of these cells in cultures from non-purified tissue-derived cells could be erroneously interpreted as "lineage switch", plasticity or trans-differentation of stem cells.

The presence of heterogeneous populations of stem cells in the bone marrow

We have presented above an explanation why circulating TCSC accumulate in BM. Here we will provide more evidence that BM contains versatile populations of stem cells. The fact that these rare cells were identified by employing different strategies (*e.g.* immunoaffinity selection, *ex vivo* expansion in cultures containing marrow-fibroblasts) may explain the differences in their description and nomenclature. A brief overview on these versatile stem cells populations is presented below.

Hematopoietic stem cells (HSC)

HSC could be envisioned as TCSC for hemato/lymhopoiesis and they are the most numerous population of stem cells in BM. These cells in addition to CXCR4 express CD34, CD133, CD117 and are lineage markers negative (lin-) [11, 43]. They, however, express the hematopoietic marker CD45 [36]. HSC generate hemato/lymphopoietic cells. Our interpretation is that HSC were erroneously envisioned as a population of bone marrow-derived "plastic" cells [20, 33].

Endothelial stem/progenitor cells (ESC)

BM also contains a population of endothelial stem/progenitor cells (ESC). These cells were demonstrated to be able to (i) egress from the bone marrow, (ii) circulate in peripheral blood and (iii) contribute to regeneration of vasculature in damaged heart, pancreas, limb or lung [2]. Endothelial TCSC express CD34, VEGFR2 (KDR), TIE2 and are CD45 negative [6]. During embryonic development, both HSC and ESC derive from a common precursor called a hemangioblast. The presence of these cells in adult bone marrow is still hypothetical.

Mesenchymal stem cells (MSC)

It is accepted that MSC are present among cultured *in vitro* BM-derived fibroblasts, however, they were not yet purified as single cells from these cultures. A population of fibroblasts that contains putative MSC is CD34-, CD41-, CD133- and CD45-negative, but express Stro-1, CD-90 (Thy-1) and CD106 antigens [32]. MSC easily

differentiate in the presence of adipocytic differentiation media into adipocytes. Similarly, they may differentiate, if cultured in appropriate differentiating conditions, into osteoblasts and chondrocytes [39]. Surprisingly, it has been reported that MSC can also differentiate in the presence of neural differentiation medium into neural cells [17].

Multipotent adult progenitor cells (MAPC)

This population of cells was isolated after prolonged *in vitro* culture of adherent BMMNC depleted from $CD45^+$ and $Ter119^+$ cells plated on fibronectin covered plates in a medium supplemented with PDGF, EGF and LIF in the presence of a low serum concentration. These cells were initially described as mesodermal progenitor cells (MPC), and after it was found that they may "cross" germ layer boundaries and differentiate not only into bone-, cartilage- or smooth muscle cells (mesoderm), but also into hepatocytes (endoderm) or neural cells (ectoderm), they were renamed as multipotent adult progenitor cells (MAPC) [18, 19, 45]. MAPC which are CD34, CD44, CD45 and CD117 negative were found in human, rat and murine bone marrow, as well as in murine muscle and brain. Murine MAPC may express a low level of Sca-1 antigen.

Pluripotent stem cells (PSC)

These cells were very recently identified and isolated by FACS in a so called side-population of cells [3, 4] present in murine bone marrow and muscle tissue [15]. PSC express Sca-1 antigen but are both CD45 and c-kit negative [15]. Muscle derived PSC were able to differentiate into hematopoietic cells, myocytes, neuronal cells and adipocytes when cultured for 9-14 days *in vitro* in conditions promoting hematopoietic, myogenic, neuronal and adipocytic differentiation respectively [15]. The same group of investigators described the presence of PSC in brain, blood and intestinal epithelium [15]. However, PCS and MAPC were isolated by different approaches (FACS and prolonged *in vitro* culture from pre-purified BMMNC, respectively) and both populations of cells are CD45 and c-kit negative. They differ, however, in Sca-1 antigen expression that is high in PSC and low in MAPC [15, 39]. The most striking difference between both populations of these putative cells is the fact that MAPC in contrast to PSC do not differentiate *in vitro* along the hematopoietic lineage.

CXCR4 positive tissue committed stem cells (TCSC)

Our recent studies, in which we employed chemotactic isolation to an SDF-1 gradient (Fig. 1) combined with real time RT-PCR analysis revealed that BM contains a population of $CXCR4^+$ cells that expresses mRNA for various markers of early tissue-committed stem/progenitor cells for skeletal muscle (Myf-5, Myo-D), myocardium (Nkx2.5, GATA-4, Mef2C), neural (GFAP, nestin), endothelial (VE-cadherin, vonWillebrand), pancreas (Nkx6.1, Pdx1, Ptf1) and liver (CK19, fetoprotein) (Fig. 2). These cells are present in bone marrow and could be purified by chemotaxis to SDF-1 or enriched after staining with anti-CXCR4 antibodies by FACS. Our recent phenotyping studies revealed that these cells are $CXCR4^+$ $CD34^+$ $AC133^+$ $CD45^-$ in humans and $CXCR4^+$ $Sca\text{-}1^+$ $c\text{-}kit^+$ $CD45^-$ in mice [23, 24, 41]. We envision that TCSC is a prevalent fraction of non-hematopoietic stem cells that reside in the bone marrow and we will below provide our interpretation that these cells historically detected by different experimental strategies were described as MSC, MAPC or PSC.

Relations of $CXCR4^+$ TCSC to other bone marrow stem cells

According to these data we have postulated that the BM is not only a home for HSC but also a "hideout" for already differentiated non-hematopoietic $CXCR4^+$ TCSC [41]. Our view on relations of $CXCR4^+$ TCSC to other bone marrow stem cells is presented below.

Relation to HSC

TCSC described by us could be further purified from HSC by employing CD45 antigen as a selection marker. We found that TCSC for non-hematopoietic tissues are CD45 negative in contrast to HSC (TCSC for the hematopoietic lineage) which are CD45 positive. In support of this, murine $CXCR4^+$ $Sca\text{-}1^+$ $CD45^-$ cells, that are highly enriched in TCSC did not produce any colonies *in vitro* and did not form spleen colonies (CFU-S) after transplantation into lethally irradiated animals. In contrast, hematopoietic colonies *in vitro* as well as CFU-S were formed by murine $CXCR4^+$ $Sca\text{-}1^+$ $CD45^+$ cells. Thus, a population of murine $CXCR4^+$ $Sca\text{-}1^+$ cells contains both hematopoietic (HSC) and non-hematopoietic TCSC which could be separated by isolating $CD45^+$ and $CD45^-$ cells, respectively.

Relation to MSC and MAPC

Since in humans TCSC are enriched in a population of non-adherent $CXCR4^+$ $CD34^+$ $AC133^+$ BMMNC, these cells are different from MSC which are a population of adherent $CXCR4^-$ $CD34^-$ cells. Furthermore, since TCSC are $CXCR4^+$ $CD34^+$, they are also different from MAPC which do not express either marker. The possibility that TCSC may "contaminate" cultures of bone marrow-derived adherent MSC and MAPC will be discussed later.

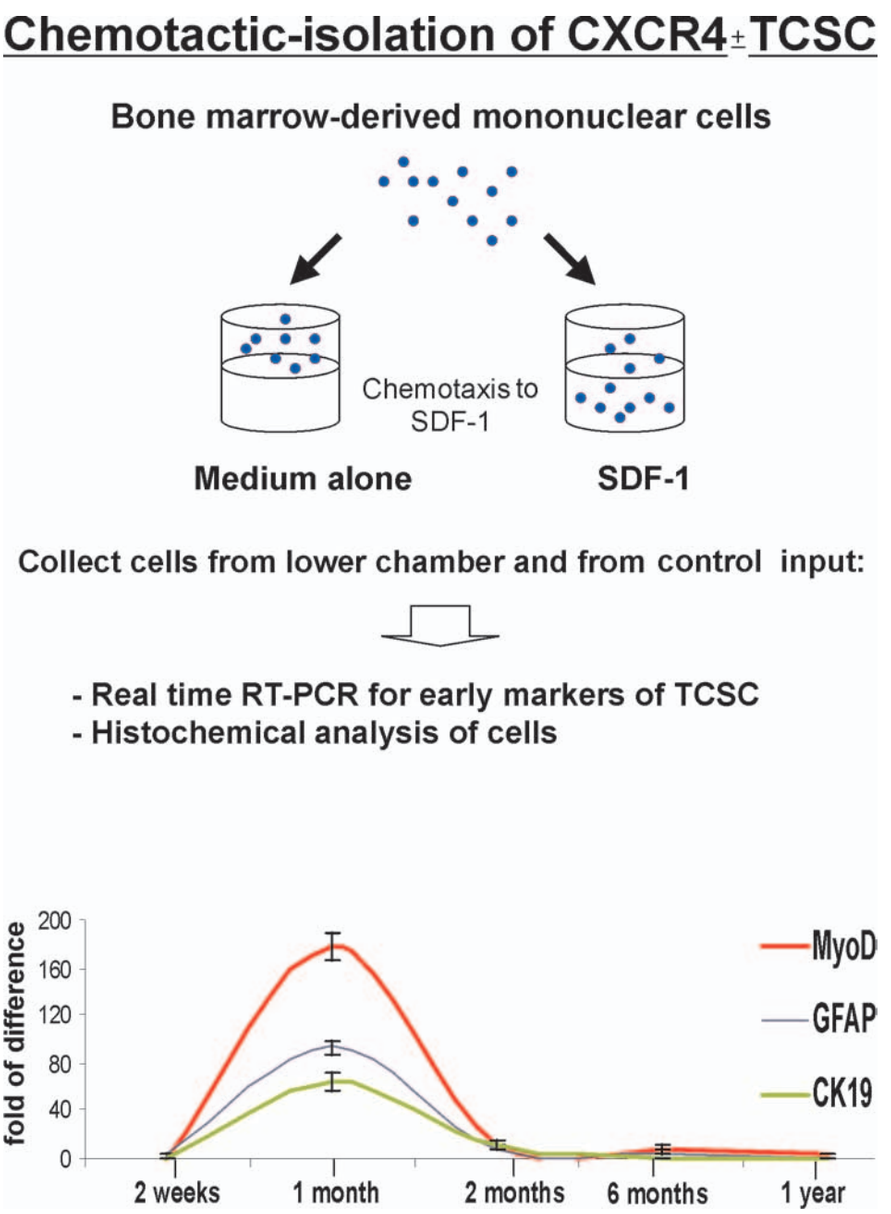

Fig. 1. Chemotactic isolation of TCSC from bone marrow. Mononuclear cells freshly isolated from bone marrow are loaded into the upper chamber of transwells and allowed to undergo chemotaxis for 5 h to the control medium or SDF-1 gradient. Subsequently cells are isolated from the control input and lower chambers and expression of mRNA for early muscle, neural and liver stem/progenitor cell markers is evaluated by real-time RT-PCR. Cells isolated by chemotacic gradient may be also stained for expression of lineage-specific proteins (*e.g.*, Myo-D, nestin or CK19).

Fig. 2. Age dependent real time RT-PCR analysis of mRNA expression for early muscle, neural and liver markers in murine BMMNC isolated by chemotaxis to SDF-1. BM cells were isolated from the lower transwell-chambers after chemotaxis to SDF-1 and expression of mRNA for early muscle (MyoD), neural (GFAP) and liver (CK19) TCSC were compared between the same number of cells from the input and lower chamber by employing real-time RT-PCR. The data are pooled together from three independent experiments (12 mice/point). Data are expressed as means ± SD.

Relation to PSC

The population of murine TCSC show a very similar phenotype (Sca-1$^+$, CD45$^-$) to murine PSC [15]. There are, however, some significant differences. For example, by employing a sensitive real time RT-PCR as well as immunostaining we noticed that several tissue-committed markers, *e.g.*, for skeletal muscle, heart muscle, liver, neural tissue and endothelium are already expressed in TCSC freshly isolated from bone marrow [41]. In contrast, PCS were reported to acquire the expression of these markers first after several days of culture *in vitro* [15]. Moreover, murine TCSC in contrast to murine PSC express c-kit [15]. These differences, however, do not preclude that among purified TCSC we may still have a rare population of PCS that are founder cells for more differentiated TCSC. In fact, we detected in purified CXCR4$^+$ TCSC the expression of mRNA for early embryonic transcription factors such as Oct-4, Rev-1 and Nanog (manuscript in preparation). However, it is also possible that expression of mRNA for these genes is not restricted for PCS and could also be expressed by more differentiated TCSC [35].

TCSC, MAPC and MSC: are we looking from different angles at the same population of stem cells?

The presence of versatile populations of stem cells that in addition to HSC and TCSC reside in BM raises an important question. Are the hypothetical MAPC and MSC in fact TCSC detected by different approaches? The rationale for this possibility is that cultures of marrow-derived adherent cells used to expand MAPC and MSC may contain some TCSC from the beginning. The hypothetical explanation of this possibility will be presented below.

First, the predominant cell type among cultured MAPC and MCS is bone marrow fibroblastic cells that secrete SDF-1. It is very likely that $CXCR4^+$ TCSC are co-isolated with these cells and subsequently, if plated into cultures, survive/expand being in close contact with fibroblasts that secrete SDF-1. It is also likely that TCSC could be embedded inside stromal fibroblasts through a mechanism known as emperipolesis. To further support our hypothesis, it was recently postulated that MAPC if plated in differentiating media may differentiate not only into cells that were identified as direct descendants of mesodermal MSC (osteoblasts, myocytes, chondroblasts and adipocytes) but also may differentiate into cardiomyocytes and even into ectoderm-derived neural cells or endoderm-derived hepatocytes. This, however, occurs very rarely - raising the possibility that MAPC cultures contain in addition to osteogenic also cardiac, neural or hepatic TCSC that are responsible for occurrence of cardiomyocytes, neural cells or hepatocytes in these cultures. We hypothesize that appearance of these cells in the cultures was erroneously interpreted as transdedifferentiation effect of MAPC.

Second, MAPC cultures are initiated from BMMNC $CD45^-$ and $GPA\text{-}A^-$ cells that are plated on fibronectin-covered plates. Since TCSC are CD45- and GPA-A-negative and as we found stick to fibronectin, they will be present in a population of cells that is plated to grow/expand MAPC. Furthermore, it is likely that the chemical composition of the culture medium and the presence of fibroblastic cells that secrete SDF-1 in these cultures may promote long-term survival of TCSC in these conditions. Thus, TCSC present in MAPC if transferred into the appropriate differentiation culture conditions may form rare colonies of *e.g.* skeletal muscle, neural cells or hepatocytes. These colonies, however, are formed by TCSC and not by "hypothetical MAPC".

Third, a similar mechanism could be responsible for plasticity of still hypothetical MSC. Since these cells are expanded in very similar conditions as MAPC, the differentiation of marrow-derived fibroblastic cells (MSC) into osteoblasts or chondrocytes could be explained by the expansion of skeletal TCSC that from the beginning "contaminate" cultures of these fibroblastic cells. This hypothesis is somehow supported by the recent observations showing that cells with properties to differentiate into osteoblasts were detected among non-adherent cells purified from bone [33]. Moreover, we recently found that $CXCR4^+$ TCSC are enriched in mRNA for early chondrocytes and adipocytes (manuscript in preparation). This supports earlier observation that bone marrow contains a mobile population of circulating skeletal TCSC [25]. These cells, if co-isolated with marrow fibroblasts, grow as small areas of osteoblastic and chondrocytic cells interpreted as functional evidence for the existence of MSC.

Thus, it is very likely that we are looking from different "keyholes" at the same population of stem cells that are hiding in a "darkroom" of the bone marrow environment. However, the question still remains if a very early pluripotent stem cell, a founder cell for all of these versatile TCSC, resides in the bone marrow. It is possible that these cells are present in BM early in the development, and such PSC and not "plastic" HSC were responsible for multilineage differentiation observed after the transplantation of single purified BM-derived "HSC" in mice [22].

TCSC and their potential role in regeneration and aging

Compelling evidence accumulated that bone marrow residing TCSC may circulate in the peripheral blood and play a role in tissue regeneration. In our recent work, we reported that the number of these cells is the highest in BM from young (1-2 month-old) mice and decreases in older animals (Fig. 2). Furthermore, our observation that the number of these cells is the highest in BM of young mice (1-2 months, what corresponds to teen age in humans) and decreases with time provides a new insight into senescence and may explain why the regeneration process becomes less effective with advancing age [23].

Our recent data show that TCSC cells also respond to HGF and LIF gradients in addition to an SDF-1 gradient. Since we demonstrated that not only SDF-1 but also HGF and LIF are upregulated in damaged tissues, we postulate that early tissue-committed $CXCR4^+$ c-Met^+ LIF-R^+ cells could be mobilized from the BM into peripheral blood from which they are subsequently chemoattracted to the damaged organs where they play a role in tissue repair/regeneration [23]. We cannot exclude, however, the involvement of other factors in regulating the trafficking of these cells in addition to SDF-1, HGF and LIF. One potential candidate is for example VEGF that was recently identified as a strong chemoattractant not only for ESC but also for neural TCSC [49].

In conclusion, evidence accumulated that BM in addition to HSC contains TCSC for different non-hematopoietic tissues (*e.g.*, muscle, neural, liver, endothelial)

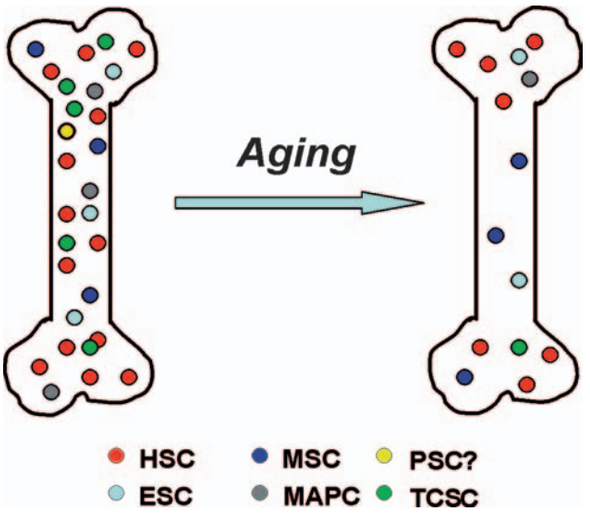

Fig. 3. Bone marrow as a source of versatile stem cells. Bones in young mammals were reported to contain a population of hematopoietic stem cells (HSC), endothelial stem cells (ESC), mesenchymal stem cells (MSC), multipotent adult progenitor cells (MAPC), pluripotent stem cells (PCS) and tissue committed stem cells (TCSC). In aging humans, bones contain HSC (mainly located in epiphyses, vertebrae and flat bones) and reduced number of ESC, MAPC and MSC. We hypothesize that all these cells could be envisioned as hematopoietic and non-hematopoietic tissue committed stem cells (TCSC). These TCSC were identified by employing different approaches (*e.g.*, cell purification, expansion in cultures containing fibroblasts) and given different names (*e.g*, MAPC or MSC). The existence of an early precursor of TCSC (hypothetical PSC) in adult marrow requires further studies.

(Fig. 3). These TCSC could "contaminate" cultures of BM-derived fibroblastic cells and in differentiating media could proliferate into various tissue specific cells (*e.g.*, osteoblasts, chondrocytes, myocytes, hepatocytes). The appearance of these cells in cultured fibroblastic cells is interpreted as functional evidence for the existence of the hypothetical MAPC or MSC. Moreover, since the BM is a "hideout" of TCSC, we suggest that their presence in BM tissue should be considered before experimental evidence is interpreted simply as transdifferentiation or plasticity of HSC. Finally, our observation that the number of these cells is the highest in the BM of young animals and decreases with age (Fig. 2) provides a new insight into aging and may explain why the regeneration process becomes less effective in older individuals. The therapeutic application of these cells for tissue repair is one of the important challenges of the regenerative medicine. The near future will show if these cells could provide an alternative therapeutic approach to the therapy based on embryonic stem cells.

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