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The developmental deposition of epiblast/germ cell-line derived cells in various organs as a hypothetical explanation of stem cell plasticity?

Magda Kucia[1], Bogusław Machalinski[2], and Mariusz Z. Ratajczak[1,2]

[1]Stem Cell Biology Program at James Graham Brown Cancer Center, University of Louisville, Louisville, KY 40202, USA; [2]Department of Physiology, Pomeranian Medical University, Szczecin, Poland

Abstract. The embryo develops from germ cell line (fertilized oocyte) and precursors of primordial germ cells (PGC) are the first population of stem cells that are specified in mice at the beginning of gastrulation in proximal primitive ectoderm (epiblast) – region adjacent to the extraembryonic ectoderm. These founder cells subsequently move through the primitive streak and give rise to several extra-embryonic mesodermal lineages and to germ cells. By day 7.25 of embryonic development, a cluster of PGC is visible at the basis of allantois. Subsequently PGC migrate through the embryo proper and colonize genital ridges, where they finally differentiate into sperm and oocytes. We hypothesize that during early development epiblast/germ line-derived cells including PGC become a founder populations of pluripotent stem cells (PSC). These cells are deposited during embryogenesis in various organs and may persist in these locations into adulthood – for example in bone marrow (BM). To support this, we recently identified in BM a population of very small embryonic-like (VSEL) stem cells that express epiblast/germ line-derived cells transcription factor Oct-4 and several other PGC markers. Similarly, cells expressing Oct-4 were also identified in several adult tissues by other investigators. Thus, pluripotent epiblast/PGC may persist beyond embryogenesis in neonatal and adult tissues. Their fate is defined by several mechanisms which regulate cell proliferation and affect status of somatic imprint on selected genes responsible for pluripotency. We hypothesize that these cells play an important role in tissue/organ regeneration and their presence in adult tissues may explain phenomenon of stem cell plasticity. In pathological situations, however they may undergo malignant transformation and give rise to tumors.

Review

Correspondence should be addressed to M.Z. Ratajczak, Email: mzrata01@louisville.edu

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INTRODUCTION

From the developmental and evolutionary point of view the main goal of the multicellular organism is to pass genes to the next generations and this process in higher organism is orchestrated by appropriate interplay between germ cell line and somatic cell lines. The germ line carries the genome from one generation to another generation and is the only cell lineage which retains true developmental totipotency. In this context somatic cell lines help germ cells to accomplish this mission effectively.

To support this concept, the most primitive totipotent stem cell that is able to form both embryo and extra-embryonal tissues (placenta) is fertilized oocyte or zygote which derives directly from the fusion of two germ cells – female oocyte and male sperm. In a zygote haploid DNA derived from oocyte is combined with haploid DNA of male germ cell sperm. However, zygote derives from the fusion of two mature germ cells, the first morphologically identifiable precursors of primordial germ cells (PGC) in mice become specified ~6.0–6.5 days *post coitum* (dpc) in proximal primitive ectoderm (epiblast) (McLaren 2003, Wylie 1999). Thus precursors of PGC are the first population of stem cells that is specified very early during development at the beginning of the gastrulation. PGC subsequently move for a short period of time first to the basis of allantois which is located in extraembryonic mesoderm and then migrate to genital ridges – where they will undergo developmental differentiation to female or male germ cells – oocytes and sperm respectively (McLaren 2003).

Therefore one individual life could be envisioned as one link in the chain of the consecutive events aimed at the transfer of the genome by germ cells from one to the next generations. In this context every life cycle begins with fusion of two germ cells (sperm and oocyte) during fertilization, specification of PGC at the beginning of gastrulation and latter on an initiation of PGC-driven oogenesis or spermatogenesis in genital ridges. The somatic cell lines play here merely a supportive role.

We will present a hypothesis that early epiblast-derived cells and/or precursors of PGC are a founder population of pluripotent stem cells (PSC) for other tissue committed stem cells in the developing embryo. Furthermore we hypothesize based on experimental evidence that in mice part of epiblast-derived stem cells and/or PGC themselves during migration through the embryo proper to the genital ridges might go astray and could be deposited in peripheral tissues. Based on this plasticity of adult stem cells could be explained by a presence of population of pluripotent germ line or epiblast-derived stem cells in different organs. It is possible that these PSC contributed to tissue/organ regeneration in some of the experiments where stem cells plasticity was demonstrated.

FROM A CONCEPT OF GERM PLASM TO PRIMORDIAL GERM CELLS

Almost 150 years ago, Weismann based on observations on some lower organisms postulated a hypothesis, that soma carries germ, which is defined by a plasm containing specific determinants which are passed from generation to generation (Weismann 1885). To support this studies in frog eggs revealed that indeed early in the development the cytoplasm in the vegetal pole becomes enriched in aggregates of mitochondria, protein and RNA and that these aggregates appear to segregate into the germ cell lineage. These aggregates were called germ plasma. This initial observation was confirmed latter on in studies performed in Xenopus and Drosophila (Machado et al. 2005, Mahowald 2001, Wall et al. 1989). In Drosophila the so called pole cells which are the very first cells formed at the posterior end of the fertilized egg are considered as precursors of germ cells (Thomson et al. 2004). These cells are highly enriched in similar type of germ plasma which is called in this species pole plasm. Similarly, in *Caenorhabditis elegans* there are described also polar granules (P granules) in the unfertilized eggs which are asymmetrically distributed to daughter cells that will give rise to germ cell lines (Wolf et al. 1983). These data confirmed that early in the development a special type of cells is segregated (germ line) which inherits from the mother's egg a special sort of cytoplasm described as germ plasma (frog), pole plasm (Drosophila) and polar granules (*Caenorhabditis elegans*) which are rich in germ-cell determinants (germ plasm). These determinants segregate in a specific population of germ cells that are specified in all the species very early in the development as a germ line. Furthermore, these determinants are probably responsible for maintaining a totipotency of germ cell lineage.

The situation is more complicated in mammals. So far, there is no clear indication for a presence of germ plasma in mammalian embryonic cells. In contrast as mentioned above in murine embryos a distinct population of PGC founder cells is specified in the proximal ectoderm (epiblast) at the beginning of the gastrulation at 6.0–6.5 dpc (Molyneaux et al. 2004, Tam et al. 1996). These cells could be identified by expression of tissue nonspecific alkaline phosphatase (TNAP) activity (MacGregor et al. 1995). They also express stem stage specific embryonic antigen-1 (SSEA-1) , mouse vasa homolog (Mvh) on the surface (Toyooka et al. 2000) and intracellular Oct-4, Stella, Fragilis, SmadI, Nobox, Hdac6 (Tres et al. 2004). PGC also express seven-transmembrane span Galphai protein coupled receptor that binds stromal derived factor-1 (SDF-1) (Ara et al. 2003a). The CXCR4-SDF-1 axis is playing a crucial role in their migration to the genital ridges (Doitsidou et al. 2002). Maturating PGC express also c-kit receptor, and kit ligand (KL) – c-kit axis is crucial for survival of these cells (Donovan 1994). PGC were also reported to contain a distinct type of round mitochondria (McLaren 2003). At this point, however, it is too speculative to claim that this could be a germ plasm equivalent in these cells.

SPECIFICATION AND DEVELOPMENTAL MIGRATION OF PGC IN MICE

As mentioned above in mice founders of PGC are specified in the proximal epiblast, pass through the primitive streak and give rise to several extraembryonic mesodermal lineages and to germ cells (Tam et al. 1996). The development of these cells is under influence of inductive signals secreted by the surrounding cells in proximal epiblast and adjacent extraembryonic ectoderm (e.g., BMP-4 and BMP-8b) (Okamura et al. 2005, Saitou et al. 2002, Ying et al. 2001). To support this notion are data showing that cells from the distal part of epiblast are also able to acquire PGC phenotype if transplanted to the proximal epiblast region.

The microscopical analysis of early embryo revealed that by 7.25 dpc a distinct cluster of approximately fifty PGC is present at the base of allantois in the extraembryonic mesoderm and subsequently expand and migrate through embryo proper towards gonadal anlagen (Ginsburg et al. 1990). As mentioned above PGC express CXCR4 and since its specific ligand SDF-1 is expressed in developing genital ridges – the SDF-1-CXCR4 axis plays a pivotal role in this migration (Molyneaux et al. 2004). At day 13.0 dpc ~30000 PGC are present in genital ridges. PGC, after they colonize genital ridges, enter a promeiotic phase and are called gonocytes (McLaren 2003).

Studies in turtles and more important in small and big mammals revealed that during migration to the genital ridges some of PGC may go astray from the main migration stream and become deposited in various tissues (Francavilla et al. 1985, Jordan 1917, Zamboni et al. 1983). On the way to the genital ridges $CXCR4^+$ PGC cells migrate through the embryo proper through so called aorta-gonad-mesonephros (AGM) region – which is a place where first definitive hematopoietic stem cells (HSC) have been identified (Medvinsky et al. 1996).

HSC from AGM region migrate to fetal liver which in mammals is during second trimester of gestation a main hematopoietic organ (Medvinsky et al. 1996). HSC similarly as PGC are also $CXCR4^+$ and their migration is regulated by SDF-1 gradient, which becomes expressed during second trimester of gestation in fetal liver. We hypothesize that in addition to HSC fetal liver may also chemoattract some migrating PGC in SDF-1 dependent manner. Furthermore, it had been even suggested that PGC may give rise in AGM to a population of definitive HSC (Rich 1995).

Latter on in the development at begin of third trimester of gestation a bone marrow (BM) becomes a major hematopoietic organ (Lensch et al. 2004). At this time a population of HSC and as we hypothesize also PGC which reside in fetal liver, move to the developing BM and perhaps other tissues. This translocation is again mediated by SDF-1 gradient which becomes expressed in developing BM tissue (Ara et al. 2003b).

To support this hypothesis we believe that population of very small embryonic like (VSEL) stem cells that our group recently isolated from the adult hematopoietic organs including BM, spleen and thymus is closely related to germ line/epiblast-derived stem cells or PGC migrating astray from the main stream (Kucia et al. 2006a). It is also likely, that $Oct\text{-}4^+$ cells that were recently identified in several adult organs and tissues could be also related to these cells (Johnson et al. 2005, Kanatsu-Shinohara et al. 2004, Kucia et al. 2006a, Mendez-Ferrer et al. 2006, Nayernia et al. 2006). This concept will be discussed later on in this paper.

PRIMORDIAL GERM CELLS AND THEIR PLURIPOTENCY

Accordingly to the definition the pluripotent stem cell (PSC) is endowed with the ability to differentiate into the stem cells giving rise to all three germ layer lineages (ecto-, meso- and endoderm) except placenta. Thus PSC should give rise in appropriate culture conditions to the functional cells being derivates from all three germ layers. PSC should also contribute to the development of all three germ layers after injection into developing blastocyst during so-called blastocyst complementation assay.

In cultures freshly isolated from embryos, PGC do not show real pluripotency, proliferate for few days, and then disappear either because they differentiate or die. However, when PGC are exposed ex vivo to three growth factors which are kit ligand (KL), leukemia inhibitory factor (LIF) and basic fibroblast growth factor (FGF-2) they continue to proliferate and form large colonies of embryonic germ cells (EG) which similarly as embryonic stem cells (ES) can be expanded indefinitely (Matsui et al. 1992, Shamblott et al. 1998). EG in contrast to PGC fully contribute to blastocyst complementation giving rise to all somatic and germ lineages (Turnpenny et al. 2003). PGC derived EG are pluripotential and had been derived from pre- and post-migratory as well from migratory PGC both in mice and human.

Furthermore, while the nuclei of migrating PGC at 8.5–9.5 dpc can be successfully used as donors for nuclear transfer, nuclei from gonadal PGC at 11.5 dpc and later are incompetent to support full-term development (Lee et al. 2002, Yamazaki et al. 2003). The pluripotency of PGC nuclei depends on status of the methylation or genomic imprinted genes (e.g., H19, Igf-2, Igf-2R, Snrpn) (Mann 2001, Szabo et al. 1995). These genes in PGC until 9.5 dpc have somatic imprint (methylation) and somatic imprint of H19, Igf-2, Igf-2R, Snrpn is crucial to maintain pluripotency of stem cells. Somatic type of imprint, however, is erased by demethylation while these cells migrate towards genital ridges at 10.5 dpc (Yamazaki et al. 2003). The erasing of the methylation (imprint) of H19, Igf-2, Igf-2R, Snrpn in early PGC could be envisioned as one of the mechanisms that shuts down PGC developmental pluripotency – and makes these cells resistant to parthenogenesis or formation of teratomas.

Proper somatic imprint is subsequently reestablished in germ cells during spermatogenesis and oogenesis, so that fertilized egg express a proper somatic imprint of these crucial genes in oocytes and sperm (Mann 2001). Since PGC-derived EG cells are pluripotent, these genes have to become again methylated during derivation of EG cell lines. The identification of all factors involved in this process is crucial in employing PGC in the future as potential source of PSC.

PRIMORDIAL GERM CELLS AS A SOURCE OF EMBRYONIC STEM CELL LINES

Totipotent zygote which gives ultimately rise to the whole organism is created by the fusion of two germ cells during a fertilization process. In contrast at the blastocyst stage level a population of cells found in inner cell mass of the blastocyst is able to give rise *in vitro* to established immortalized embryonic stem cell (ES) lines which can contribute to all germ layers in developing embryo, except placenta.

It is obvious that early PGC or EG cells are the closest equivalent of ES cells. Accordingly, EG similarly as ES cells contribute to the blastocyst development. Furthermore, both types of cells express several similar markers (e.g., Oct-4, Nanog, Dppa3, DAZl etc). These similarities in (i) gene expression profile, (ii) pluripotency of PGC derived EG cells and ES cells, (iii) an evidence that totipotent zygote is immediate product of two germ cells, and finally (iv) fact that germ line is specified so early during development – raised an interesting concept that probably all ES cells lines created so far are in fact derivates of PGC.

Accordingly, to this concept cells isolated from the inner cell mass of the blastocyste give rise during *in vitro* culture conditions to a population of early/pre-PGC which subsequently may expand as immortal ES cell pluripotent line (Zwaka et al. 2005). Thus, the way to obtain ES involves PGC specification step. Furthermore, the fact that ES cells may contribute to germ cell lines after blastocyst complementation including germ line, and living mice may be borne from these germ cells, somehow suggest that ES indeed contain and may pass to the future generations not only genes but also a hypothetical PGC-derived "germ plasm".

BONE MARROW-DERIVED VERY SMALL EMBRYONIC-LIKE (VSEL) STEM CELLS EXPRESS SEVERAL MARKERS OF PGC

Recently by employing multiparameter sorting we have identified in murine BM a homogenous population of rare (~0.01% of BMMNC) Sca-1$^+$ lin- CD45$^-$ cells that express by RQ-PCR and immunohistochemistry markers of PSC such as SSEA-1, Oct-4, Nanog and Rex-1 as well as highly express Rif-1 telomerase protein (Kucia et al. 2006a). The direct electronmicroscopical analysis revealed that these cells display several features typical for embryonic stem cells such as (i) small size (2–4 μm in diameter), (ii) large nuclei surrounded by a narrow rim of cytoplasm, and (iii) open-type chromatin (euchromatin) (Fig. 1). The status of the genomic imprint of H19 and IGF2 genes is currently under investigation in our laboratory. We also found that VSEL or cells closely related to this population may be released form BM and circulate in peripheral blood during tissue/organ injuries (e.g., heart infarct, stroke) (Kucia et al. 2004, 2006b, Wojakowski et al. 2004).

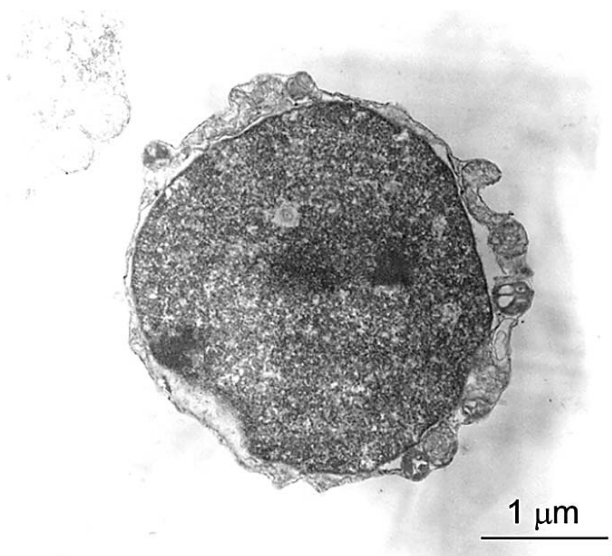

Fig. 1. TEM of Sca-1$^+$lin$^-$CD45$^-$ murine bone marrow-isolated VSEL stem cell. VSEL stem cells are small and measure 2–4 μm in diameter. They possess a relatively large nucleus surrounded by a narrow rim of cytoplasm. At the ultrastructural level the narrow rim of cytoplasm possesses a few mitochondria, scattered ribosomes, small profiles of endoplasmatic reticulum and a few vesicles. The nucleus is contained within a nuclear envelope with nuclear pores. Chromatin is loosely packed and consists of euchromatin.

Recently we noticed that ~5–10% of purified VSEL if plated over C2C12 murine sarcoma cell feeder layer are able to form spheres that closely resemble embryoid bodies which we call VSEL-derived spheres (VSEL-DS). Accordingly, cells from these VSEL-DS are composed of immature cells which large nuclei containing euchromatin, and similarly as purified VSEL are CXCR4$^+$SSEA-1$^+$Oct-4$^+$. Furthermore, VSEL-DS after replating over C2C12 cells may again (up to 5 passages) grow new spheres or if plated into cultures promoting tissue differentiation expand into cells from all three germ-cell layers (e.g., ectoderm derived neurons and oligodendrocytes, mesoderm derived myocardium and hematopoietic cells and endoderm-derived insulin producing cells). The formation of VSEL-DS was observed in a presence of C2C12 cells obtained from different sources. Furthermore, VSEL isolated from GFP$^+$ mice grew GFP$^+$ VSEL-DS which show diploid content of DNA. This suggests that VSEL-DS are in fact derived from VSEL and not from supportive C2C12 cell line as well as excludes a possibility of cell fusion to observed phenomenon. Currently, we are investigating a status of the genomic imprint of H19 and IGF2 genes in these cells.

Similar spheres were also formed by VSEL isolated from murine fetal liver, spleen and thymus. Interestingly formation of VSEL-DS was associated with young age of mice, and no VSEL-DS were observed by cells isolated from middle-aged mice (>1 years).

Since isolated by us VSEL express several markers of primordial germ cells (fetal-type alkaline phosphatase, Oct-4, SSEA-1, CXCR4, Mvh, Stella, Fragilis, Nobox, Hdac6) we hypothesize that VSEL are closely related to a population of early PGC. In this context we hypothesize, that phenomenon of VSEL-DS formation mimics a derivation of EG cells. This hypothesis, however, requires further investigations. Currently we are trying to isolate VSEL from the human tissues and our preliminary data suggests that they are present in human BM. Furthermore, as described below very recently we were successful in isolating a population of cells similar to VSEL from the human cord blood (CB). This indicates that VSEL could be also deposited during development in human tissues as well. We postulate that identified by us and purified at single cells level VSEL could become an important source of PSC for regeneration.

A POPULATION OF EMBRYONIC LIKE CELLS IS PRESENT IN HUMAN CORD BLOOD

Based on our studies on murine BM-derived VSEL we hypothesized that a similar population of cells could be also present in human hematopoietic organs and we focused on cord blood (CB). It is well known that mononuclear cells (MNC) isolated from CB contribute to organ/tissue regeneration, however, the identity of the specific cell type(s) involved remains unknown. We employed a two-step isolation procedure to purify a similar population of cells from human CB. This strategy is based on isolation of CB mononuclear cells (CB MNC) by hypotonic lysis and multiparameter FACS sorting. Accordingly, we performed (i) hypotonic lysis of CB to remove erythrocytes and to enrich for CB MNC combined with (ii) multiparameter sorting for $CXCR4^{+}AC133^{+}CD34^{+}lin^{-}CD45^{-}$ CB MNC. CB-derived VSEL (CB-VSEL) isolated this way similarly as those isolated from adult BM are very small (3–5 μm), possess large nuclei containing unorganized

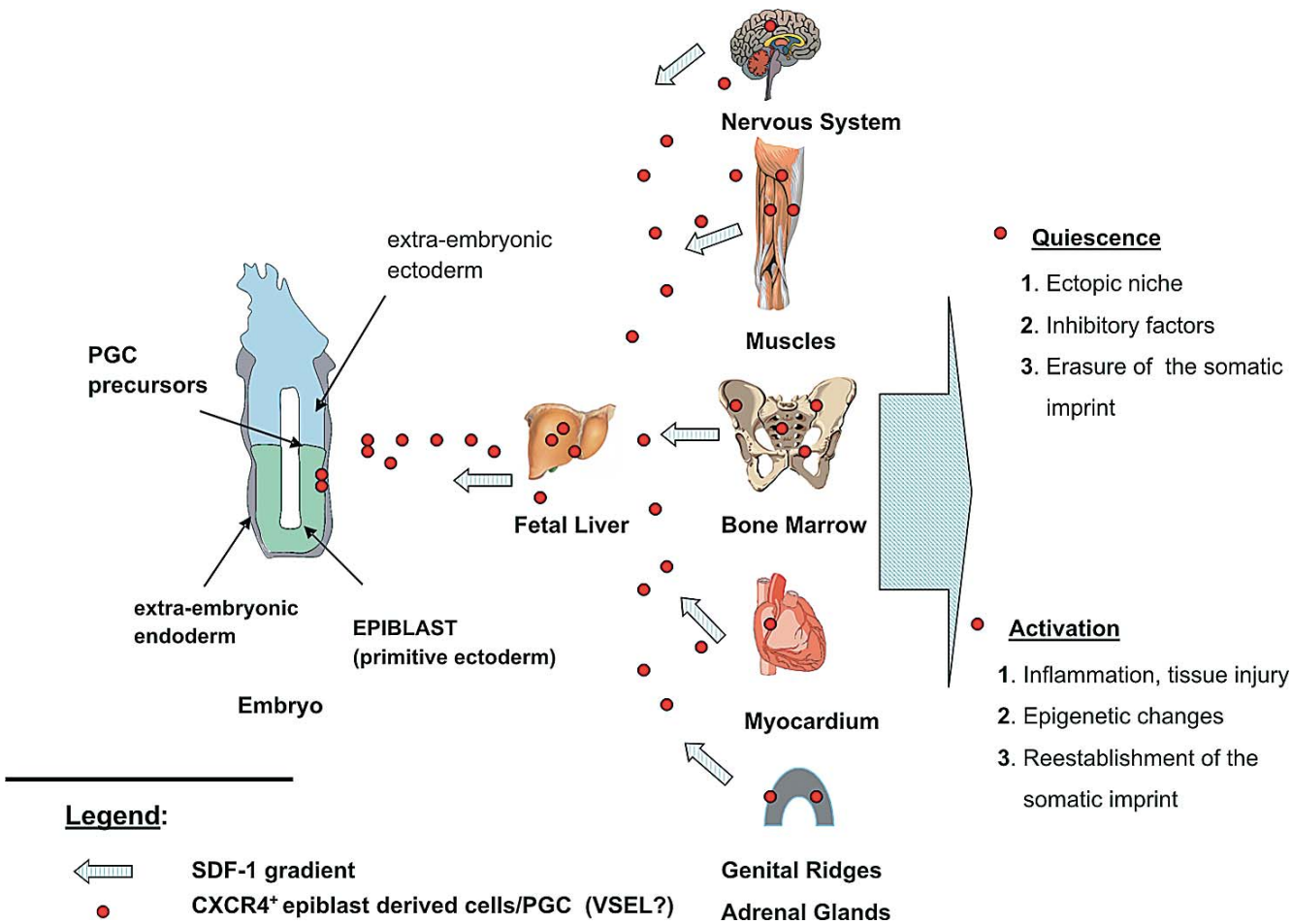

Fig. 2. Developmental deposition of epiblast/PGC cells. During early embryogenesis a population of $CXCR4^{+}$ early epiblast/primordial germ stem cells (PGC), which respond robustly to an SDF-1 gradient migrates and is deposited in various organs including fetal liver, bone marrow, genital ridges, adrenal glands, skeletal muscles, heart, skin and neural tissue. These cells may remain in these "ectopic" places quiescent because of (i) being placed into non-physiological niches, (ii) exposed to appropriate inhibitors and (iii) potential erasure of somatic imprint on selected genes that regulate pluripotency (e.g., H19, IGF-2). In contrary these cells may become again activated and began to proliferate if (i) are stimulated by the appropriate signals related to tissue/organ damage, (ii) undergo epigenetic changes and (iii) reestablish the appropriate somatic imprint. We hypothesize that purified by us from adult bone marrow VSEL are related to this population of cells. To support this hypothesis both VSEL similarly as early epiblast/PGC are $CXCR4^{+}$ $Oct\text{-}4^{+}$ $SSEA\text{-}1^{+}$ and are fetal alkaline phosphatase positive. VSEL, however, in comparison to PGC are smaller in size, which could be explained by a fact that they were isolated from the adult BM - where they reside in a "hibernated state".

euchromatin, express nuclear embryonic transcription factors Oct-4 and Nanog and surface embryonic antigen SSEA-4. *In vitro* cultures CB-VSEL are able to grow neurospheres that gave rise to neuronal lineages (beta-III tubulin$^+$, nestin$^+$, O4$^+$, MBP$^+$, GFAP$^+$) and cardiomyocytes (beta-myosin heavy chain$^+$, alpha-sarcomeric actin$^+$).

Based on this we conclude that CB contains VSEL and that the majority of these CB VSEL are lost during routine procedures employed currently for banking of CB MNC. Thus based on our observations, new more efficient methods of CB banking are needed that will enrich/preserve these cells in CB units during preparation before storage. Furthermore, we conclude that CB tissue/organ regenerating potential may be much higher than initially postulated if the proper fraction of CB MNC cells is employed and we are currently testing this hypothesis in animal models.

EVIDENCE FOR A PRESENCE OF THE PGC IN THE FETAL, NEONATAL, AND ADULT TISSUES

Based on a presence of VSEL in mice and humans we propose that epiblast-derived cells or early PGC could be deposited during development in various adult tissues (Fig. 2). To support this as mentioned above some evidence accumulated that PGC may in fact go astray during development from the main migration stream and thus could be deposited in the various tissues (Jordan 1917, Upadhyay et al. 1982, Zamboni et al. 1983). Historically the first indication that this can happen was reported almost 100 years ago on a basis of PGC migration in longhead turtles (Jordan 1917). It was reported that a certain number of germ cells migrate out of the regular route to the genital ridges and go astray being incorporated into peripheral sympathetic ganglia. Some of them are found in blood vessels of the periaortic mesenchyma and carried by the blood stream to distant regions. In this distant location, they may degenerate or persist for a certain period of time. The close association of PGC with blood vessels and the suggestion that these cells may migrate during development through the blood was proposed on the studies in equine fetuses. PGC were also found to be present in postnatal adrenal glands in mice (Zamboni et al. 1983) and PSC that are able to complete blastocyst development were also reported to be present in murine neonatal testes (Kanatsu-Shinohara et al. 2004). Furthermore, a presence of cells able to give rise to oocytes was reported in murine marrow (Johnson et al. 2005) and porcine fetal skin (Dyce et al. 2006) as well as a presence of cells able to give rise to spermatogonia was reported in postnatal murine testes (Guan et al. 2006) and BM (Nayernia et al. 2006). We believe that all these cells could be in fact PGC that are deposited during development in those various locations.

Very recently several groups of investigators reported a presence of stem cells that express embryonic/PGC marker which is Oct-4 transcription factors, being located in various anatomical locations such as bulge region of hair follicles (Yu et al. 2006), bronchial epithelium in lungs (Kim et al. 2005), renal tubular epithelium (Sagrinati et al. 2006), and heart (Mendez-Ferrer et al. 2006). Furthermore, a population of SSEA-1$^+$ stem cells was identified in retina pigment epithelium (Koso et al. 2006). The expression of these embryonic stem cell markers suggest that these cells could belong to the population of embryonic/epiblast-derived or PGC stem cells that are deposited early during development in various organs. It is likely that these cells depending on their anatomical location and niche may differ in expression of some surface markers, their size and pluripotentiality. Further studies are needed to purify these rare cells and characterize them better.

One interesting model available to track the anatomical distribution of these cells is transgenic mice which have green immunofluorescence (GFP) protein under Oct-4 promoter. We already employed these animals to sort GFP+ VSEL from the murine BM. Similar strategy could be also employed to sort these cells from other tissues and organs as well.

PSC cells deposited in the adult tissues are kept quiescent and several potential mechanisms could be involved in this process (Fig. 2). Firstly, if they are located in un-physiological ectopic niches some survival factors and self-renewing factors might be missing. Secondly, they may be under influence of some local inhibitors. Thirdly, an important regulator in their pluripotency could be a status of somatic imprint. In contrary, the potential of these cells may be unleashed if some appropriate stimulatory factors appear in the damaged tissues, or they undergo epigenetic changes – e.g. reestablishment of a proper somatic imprint.

PRACTICAL IMPLICATIONS OF PGC HYPOTHESIS

The discovery of that embryonic like stem cells (e.g. VSEL) reside in adult tissues may explain one of the most controversial issues of modern biology which is plasticity of HSC (Grove et al. 2004, Mezey et al. 2000). It is well known that the concept that HSC are plastic and might be able to trans-dedifferentiate into stem cells for other tissues created a high degree of hope and excitement. The reason for this was an assumption that HSC isolated from relatively easily accessible sources such as BM, mobilized peripheral blood (mPB) or CB could be subsequently employed as precursors for other stem cells necessary for regeneration of various solid organs (e.g., heart, brain, liver or pancreas) (Oh et al. 2004, Orlic et al. 2001, Peterson et al. 1999). This, excitement was raised in the scientific community after the first published data demonstrated a remarkable regenerative potential of HSC in animal models of heart infarct, stroke and liver damage.

However, following the first exciting and promising reports, came some disappointment, when previously published data showing a contribution of HSC to regenerate different tissues were not reproduced by other investigators, who employed in their experiments highly purified fractions of HSC (Castro et al. 2002, Orkin et al. 2002). In response to this the scientific community became polarized in their view on stem cell plasticity. These obvious discrepancies in the published results could be explained by (i) differences in tissue injury models employed, (ii) purity of stem cell populations used for regeneration, and (iii) problems in detection of tissue chimerism.

Furthermore, in addition to these "technical problems" cell fusion had been proposed to explain some of plasticity data (Terada et al. 2002, Ying et al. 2002) . Accordingly, data were presented that donor derived HSC or monocytes may fuse with differentiated cells in recipient tissues which leads to the creation of heterokaryonic daughter cells that possess a double number of chromosomes in their nuclei and express cell surface and cytoplasmic markers that are derived from both parental cells. Cell fusion, however, is extremely rare that may not fully account for the previously published positive "trans-dedifferentiation" data and fusion as a major contributor to the observed donor derived chimerism was excluded in several recently published elegant studies.

In all of these deliberations concerning stem cell plasticity the concept that BM may contain heterogeneous populations of stem cells was surprisingly not taken carefully enough into consideration. We postulate that the regeneration studies that show contribution of donor derived BM, mPB or CB cells to tissues without excluding this possibility by including the proper controls could lead to wrong interpretations. Thus, the presence of heterogeneous populations of stem cells in BM, mPB or CB should be considered first before experimental evidence is interpreted simply as trans-differentiation/plasticity of HSPC (Kucia et al. 2005, Ratajczak et al. 2003, 2004). Thus data from laboratory provide evidence that BM cells are in fact heterogeneous and that this tissue contains different types of stem cells including a rare population of pluripotent VSEL.

In fact in the past few years various populations of PSC were described in the past in the adult tissues by several investigators. The criteria to call stem cell as pluripotent require that this cell should be able to differentiate into all three germ layers and express e.g., Oct-4 transcription factor. In fact Oct-4 was reported to be expressed in some subset of so called mesenchymal stem cells (MSC) (Jiang et al. 2002a), multipotent adult progenitors (MAPC) (Jiang et al. 2002b) and marrow-isolated adult multilineage inducible (MIAMI) cells (D'Ippolito et al. 2004). The pluripotency of these cells was demonstrated in *in vitro* cultures. These cells however, were never purified and demonstrated at single cell level. We hypothesize that it is likely that the assays employed to enrich for these cells, co-isolated VSEL as well. Thus, it is possible that co-isolated VSEL were responsible for occurrence of stem cell pluripotency in employed assays. The open question remains if VSEL continuously contribute to renewal of other BM-residing cells – including population of HSC.

Finally, the identification of VSEL could also support Virchow's concept of an embryonic origin of cancer (Virchow 1855). We hypothesize that this population of CXCR4+ VSEL stem cells that is deposited early during development in BM and perhaps also in other tissues as a source of pluripotent stem cells for tissue/organ regeneration, may on the other hand if acquires mutations may give rise to some malignancies. The potential candidates for this phenomenon are not only teratomas or germ cell tumors but also several pediatric sarcomas (e.g., rhabdomyosarcoma, neurblastoma, Ewing sarcoma) and perhaps some other solid tumors in adults (e.g., seminomas, dysgerminomas, germinomas).

CONCLUSIONS

Based on presented data we hypothesize that the presence of VSEL in bone marrow, which as we hypothesize are derivates of germ line/early epiblast-derived stem cells or PGC, and not "trans-dedifferentiation" of HSC could explain some of the positive results of tissue/organ regeneration as seen by some of the investigators using BM-derived cells. To support this notion when highly purified HSC were employed for regeneration experiments, VSEL were probably excluded from the preparations of these cells, and thus the "phenomenon of trans-dedifferentiation of HSC" and their contribution to regeneration of the damaged tissues was not visible anymore. We hypothesize that VSEL colonize BM early during development, reside in BM and could be released from BM niches during tissue/organ damage and become a source of stem cells for tissue organ regeneration. Thus the developmental deposition of epiblast-derived embryonic cells or PGC in various organs could be a possible explanation of stem cell plasticity and explain why PSC were detected in adult tissues.

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