Published in final edited form as:

Stem Cell Rev. 2010 June ; 6(2): 307–316. doi:10.1007/s12015-010-9143-4.

Epiblast/Germ Line Hypothesis of Cancer Development Revisited: Lesson from the Presence of Oct-4⁺ Cells in Adult Tissues

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Abstract

The morphology of several tumors mimics developmentally early tissues; tumors often express early developmental markers characteristic for the germ line lineage. Recently, our group identified a population of very small stem cells (SCs) in murine bone marrow (BM) and other adult organs that express several markers characteristic for epiblast/germ line-derived SCs. We named these rare cells “Very Small Embryonic/Epiblast-like Stem Cells (VSELs).” We hypothesized that these cells that express both epiblast and germ line markers are deposited during early gastrulation in developing tissues and organs and play an important role in the turnover of tissue-committed (TC) SCs. To support this, we envision that the germ line is not only the origin of SCs, but also remains as a scaffold or back-up for the SC compartment in adult life. Furthermore, we noticed that VSELs are protected from uncontrolled proliferation and teratoma formation by a unique DNA methylation pattern in some developmentally crucial imprinted genes, which show hypomethylation or erasure of imprints in paternally methylated genes and hypermethylation of imprints in the maternally methylated. In pathological situations, however, we hypothesize that VSELs could be involved in the development of several malignancies. Therefore, potential involvement of VSELs in cancerogenesis could support century-old concepts of embryonic rest- or germ line-origin hypotheses of cancer development. However, we are aware that this working hypothesis requires further direct experimental confirmation.

Keywords

VSELs; Oct-4; Cancer testis antigens; Germ line

Introduction

Recent augmenting evidence suggests that malignancy arises from accumulation of mutations in normal stem/progenitor cells rather than by the de-differentiation of already differentiated cells [1–4]. Thus, self-renewing SCs residing in organs and tissues, but not mature differentiated somatic cells: a) acquire/accumulate mutations; and b) maintain them in the SC

compartment by passing them during the lifetime to daughter SCs. To support this SC theory of cancer development, recent research from several laboratories provides direct evidence that several malignancies (e.g., brain tumors, melanomas, and prostate, colon, and lung cancers) may in fact originate in the SC compartment [3,5–7]).

Our team recently identified a developmentally primitive population of so-called “VSELs” in adult tissues. As we hypothesize, these cells are deposited in early developing tissues during organogenesis and express several epiblast/germ lineage markers. VSELs remain quiescent in adult tissues due to the epigenetic modulation of some crucial somatic imprinted genes. Perturbation of these mechanisms may lead to their neoplastic transformation.

However, the concept that developmentally primitive cells capable of transforming into tumor cells exist in adult tissues is not so novel. In the 19th and 20th centuries, several investigators (e.g., Rudolf Virchow, Julius Cohnheim, and John Beard) proposed the so-called “embryonic rest hypothesis of cancer development.” They suggested that adult tissues may contain embryonic remnants that normally lie dormant, but that can be activated to become cancerous. Based on this theory, cancer may develop in populations of cells that are left in a dormant state in developing organs during embryogenesis. To support this concept, Wright (1910) proposed a germinal cell origin of a pediatric sarcoma, i.e., Willm's tumor (nephroblastoma), and John Beard (1911) postulated that tumors may arise from displaced and activated trophoblasts or even displaced germ cells (GCs).

In this review, we present a concept that was described by our team whereby VSELs may reconcile past theories of the embryonic rest hypothesis of cancer's origin as well as current theories maintaining cancer as a SC disorder. However, the hypothesis presented in this review of the epiblast/germ line origin of cancer and potential involvement of VSELs in this process needs further experimental support.

SCs and Embryonic Development

An adult organism develops from the most primitive totipotent SC called a zygote, which is an oocyte fertilized by a sperm cell. This totipotent zygote, the “mother of all stem cells” in the developing body, first gives rise to pluripotent (P)SCs that form the morula. Subsequently, the morula's PSCs are the origin of the SCs committed to trophoblasts that will give rise to the placenta and the PSC population that forms the inner cell mass (ICM) of the blastocyst. These cells from the ICM of the blastocyst give rise to the epiblast, a part of the developing embryo that is the origin of SCs committed to all three germ layers, i.e., meso-, ecto-, and endoderm [8,9].

Thus, the epiblast could be considered the origin for SCs committed for all organs and tissues in developing the embryo proper. PSCs in the epiblast undergo a sequel of specification events, first into multipotent SCs and then into versatile TCSCs, which play a role in the formation and rejuvenation of various organs [10]. An important question to emerge is whether some of these primitive epiblast-forming PSCs can escape specification into more differentiated populations of SCs and retain their pluripotential character, thus surviving among differentiated daughter TCSCs.

Recently, our group obtained several pieces of evidence that may lend some support for this aforementioned possibility. Accordingly, we identified a population of very primitive SCs in adult tissues that express numerous markers characteristic for epiblast/germ line SCs [11], which we termed “VSELs.” We hypothesized that these VSELs are deposited during early gastrulation in developing tissues and organs, survive into adulthood, and play an important role as a back-up scaffold population of PSCs in the turnover of TCSCs [12,13].

VSELs were initially isolated from murine BM by employing fluorescence-activated cell sorting (FACS) as a population of Sca-1⁺Lin⁻CD45⁻ cells (Fig. 1). When the initial experiments obtained in our laboratory indicated the unique and very small size of these rare cells, we focused on cellular events smaller than 5–6 µm in diameter. Standard FACS procedures exclude all events smaller than 5–6 µm in diameter as cellular debris, erythrocytes, and platelets, which may explain why VSELs were not purified earlier. Direct electron microscopical analysis revealed that these cells display several features typical for embryonic (E)SCs such as: i) small size (~3.6 µm in diameter); ii) a large nucleus surrounded by a narrow rim of cytoplasm; and iii) open-type chromatin (euchromatin). We also employed ImageStream system (ISS) analysis to further evaluate these rare cells. ISS technology was developed as a novel method for multiparameter cell analysis and as a supportive tool for flow cytometry (FC). It integrates the features of FC and fluorescent microscopy by collecting images of acquired cells for offline digital image analysis [14]. Analysis employing ISS confirmed the very small size of VSELs as well as other features related to their primitive status, such as a high nuclear to cytoplasmic ratio. Recently, VSELs were also isolated as a population of rare Sca-1⁺Lin⁻CD45⁻ small cells (smaller than erythrocytes) from several other adult tissues including brain, liver, pancreas, kidney, muscles, heart, testes, and thymus [14].

As determined by real-time reverse transcription polymerase chain reaction (RT-PCR) using sequence-specific primers and by immunohistochemistry, these cells express several markers of PSCs such as Oct-4, stage-specific embryonic antigen-1 (SSEA-1), Nanog, and Rex-1 as well as Rif-1 telomerase protein [11,14]. In addition, VSELs express several other markers of epiblast/germ line cells, i.e., fetal-type alkaline phosphatase, Mvh, Stella, Fragilis, Nobox, and Hdac6. VSELs also highly express telomerase activity and, similarly to ESCs, do not express major histocompatibility class I (MHC-I) or human leukocyte antigen D-related (HLA-DR) antigens and are CD90⁻ CD105⁻ CD29⁻. Interestingly, VSELs possess diploid DNA and contain numerous mitochondria despite their small size.

With some recent reports casting doubts on whether Oct-4 could be truly expressed in cells isolated from adult tissues, we were prompted to reappraise the expression of Oct-4 in VSELs. To rule out the argument that Oct-4 expression is the result of misinterpreted RT-PCR (e.g., amplification of Oct-4 pseudogenes) and/or immunostaining results, we: a) employed Oct-4-specific primers that do not amplify pseudogenes; b) removed contaminating genomic DNA during RNA isolation by deoxyribonuclease I (DNase I) treatment; c) confirmed the PCR products by DNA sequencing; and d) showed the intranuclear localization of the Oct-4 protein [15]. More importantly, we performed an epigenetic analysis (DNA methylation and histone modifications) of the Oct-4 promoter. Our data provided strong molecular evidence at the chromatin level that the Oct-4 gene is truly transcribed in VSELs isolated from adult BM [15]. Utilizing a similar approach, we also provided evidence for the open status of the Nanog promoter [15].

We postulate that the presence of pluripotent VSELs in adult tissues may reconcile all previously published data stating that adult tissues may contain a population of PSCs [16–19]. The existence of pluripotent cells in adult tissues had been proposed by several investigators [20–22]. However, such cells that reside in adult tissues were never purified or identified at the single cell level.

Germ Line as Origin and “Scaffold” of the SC System in the Adult Tissues

From a developmental point of view, cells that are “immortal” in mammals are those that belong to the germ line (Fig. 2). The biological mission of the germ line is to pass genomic and mitochondrial DNA to the next generations. Therefore, the germ line creates “mortal soma”, which will help germ line-specified gamete cells to fulfill this reproductive mission [23,24].

Germ line potential is first established in zygote, which is a result of the fusion of two GCs, i.e., the oocyte and sperm, during the process of fertilization and is subsequently maintained in blastomers of the morula and in the ICM of the blastocyst. At the developmental level of the blastula, a part of the cells that surrounds the ICM of the blastocyst “buds out” from the germ line lineage and differentiates toward the trophoblast, which will give rise to the placenta. Subsequently, immediately after implantation of the blastocyst in the uterus, a germ line potential is maintained in the cells of epiblast [8], a part of the embryo from which all three germ layers (meso-, ecto- and endoderm) develop.

In the epiblast in mice 7.25 days post-conception (dpc), a portion of the proximal epiblast PSCs is specified into a population of primordial (P)GCs that will migrate first to the extra-embryonic ectoderm and then return through the primitive streak to the embryo proper and migrate to the genital ridges. Once in the genital ridges, PGCs differentiate into gametes (oocytes or sperm) [25,26]. We postulate that around the time of PGC specification, the remaining epiblast PSCs, which we envision to be related to the germ line lineage, become specified to TCSCs for developing tissues and organs [27]. These primitive epiblast/germ line-derived PCSs are probably not completely eliminated from the developing organism by the differentiation process and some of them may survive into adulthood as VSELs. We envision that VSELs residing in adult organs could play a role as a potential backup for TCSCs [12,15], not only as a source of primitive precursor cells involved in organ and tissue homeostasis, but also as the source of a mobile pool of SCs actively involved in tissue and organ regeneration in emergency situations.

In 1880, August Weissman introduced an important corollary to the biogenic law, “that cells living today can trace their ancestry back to ancient times”, which could imply the existence of a common ancestral cell. The population of PGCs as mentioned above is the most important from a biological point of view because, as precursors of GCs, they are directly responsible for passing genetic information to the next generation. As such, the germ line may correspond to Weissmann's concept. However and somewhat surprisingly, as demonstrated when these developmentally early cells are isolated from the developing embryo during the time they migrate to the genital ridges and are cultured *ex vivo*, they will undergo rapid terminal differentiation or apoptosis [28]. More importantly, they also lack the currently approved criteria of pluripotentiality, such as complementation of blastocyst development and teratoma formation after inoculation into experimental animals [29–31]. Moreover, it is well known that nuclei isolated from PGCs are not able to provide fully functional nuclei during nuclear transfer in the process of clonote formation. This indicates that PGCs must be protected in some way from uncontrolled expansion by certain important regulatory mechanisms.

One explanation for this obvious lack of pluripotentiality is that migrating PGCs (~8.5 to 11.5 dpc) undergo epigenetic modification and erasure of the somatic imprint on differently methylated regions (DMRs) of somatic imprinted genes [31–33]. Somatic imprinted genes show a different methylation pattern in some of the genes located either on maternal or paternal chromosomes, such as H19-insulin-like growth factor 2 (Igf2), Igf2 receptor (Igf2R), Ras protein-specific guanine nucleotide-releasing factor 1 (Rasgrf1), and Snrpn. As a result of the imprint in all somatic cells, for example, Igf2 and Rasgrf1 are expressed from the paternal chromosome while H19 is expressed from the maternal chromosome [34–36]. In contrast, the imprint is erased or modified in PGCs. The process of erasure of the somatic imprint occurs in PGCs very early during gastrulation when these cells begin to migrate to the genital ridges [29]. It is postulated that erasure of imprinting is one of the basic mechanisms that prevents: i) uncontrolled proliferation; ii) parthenogenesis; and iii) potential teratoma formation by these cells. However, this process is reversible and the proper imprint is established during gametogenesis on developing oocytes and sperm cells after the first meiotic division, when the chromosomes are already reduced to haploid numbers.

However, as demonstrated in the past, if PGCs (8.5~11.5 dpc) are plated over murine fetal fibroblasts in the presence of selected growth factors, i.e., leukemia inhibitory factor (LIF), basic fibroblast growth factor (bFGF), and kit ligand (KL), they may undergo epigenetic changes forced by in vitro culture conditions that partially regain the somatic imprint. This epigenetic change endows PGCs with “immortality” [37–39]. The immortalized population of PGCs known as embryonic (E)GCs is the equivalent to ESCs in many aspects [40]. For example, similar to ESCs, EGCs that regained imprinting: a) contribute to all three germ layers including the GC lineage after injection into a blastocyst (blastocyst complementation assay); b) form teratomas after injection into living mice [41–43]; and c) provide functional nuclei for the clone after nuclear transfer. Thus, it is evident that a proper somatic imprint is vital for cells from the germline to retain full pluripotentiality.

Based on this, we postulated and recently confirmed that erasure of the somatic imprint of some crucial developmental genes (e.g., *Igf2-H19* and *Rasgrf1*) is involved in controlling the quiescent status of VSELs [15]. The molecular basis of VSELs quiescence is discussed in more detail below.

Somatic Imprint-related Molecular Mechanisms that Govern Quiescence of VSELs

As mentioned above, we recently confirmed our hypothesis that VSELs show some unique genomic imprinting patterns that regulate their quiescent status (Fig 3a). Accordingly, we noticed that paternally DMRs (*Igf2-H19* and *Rasgrf1*) in VSELs (Table 1 and Fig. 3b) are hypomethylated similarly as in PGCs, which is in contrast to maternally DMRs (*Kcnq1*, *Igf2R*, and *Peg1*) that remain hypermethylated in VSELs [15].

It is well known that imprinted genes play crucial roles in fetal growth, development, pluripotency of SCs, and tumorigenesis. As a result of the unique reprogramming of genomic imprinting, VSELs show upregulation of growth-repressive imprinted genes (*H19*, *p57^{KIP2}*, *Igf2R*) and down-regulation of growth-promoting genes (*Igf2*, *Rasgrf1*; Table 1 and Fig. 3c). Because *Igf2* has been described as an important autocrine growth-factor that promotes expansion of several cell types [44,45] and, conversely, *H19* regulatory mRNA was found to inhibit cell proliferation [46], the changes in expression of both these genes are probably responsible for the quiescent status of VSELs. Importantly, the significance of a proper mono-allelic imprint of the *Igf2-H19* gene was demonstrated as being crucial in generating viable parthenogenetic mice using two haploid sets of maternal genomes [41]. In addition, because the non-signaling receptor *Igf2R* functions as a decoy receptor for *Igf2* that prevents its availability for *Igf1R* [47], upregulation of *Igf2R* in VSELs may additionally protect VSELs from autocrine and paracrine stimulation by *Igf2* [15]. Another important gene down-regulated by changes in DMR methylation is *Rasgrf1*, a protein involved in *Igf1R* signaling transduction [48]. Thus, our data suggest that VSELs show some changes in the expression of genes that are related to insulin/Igf-signaling machinery. However, the elucidation of a potential role of these factors in quiescence and activation of VSELs requires further and more complex studies. Furthermore, we noticed that VSELs highly express the *p57^{KIP2}* transcript as a result of hypermethylation of DMR in the *Kcnq1* locus [15].

Therefore, we postulate that potential modulation of mechanisms controlling genomic imprinting in VSELs would be crucial for developing more powerful strategies to unleash the regenerative potential of these cells for efficient employment in the clinical setting (Fig. 3a).

Data Supporting “Epiblast/Germ Line Hypothesis of Cancer Development”: A Potential VSEL Link?

We envision that the “embryonic rest hypothesis of cancer development” proposed in the 19th and 20th centuries could be explained by the presence of VSELs in adult tissues. Furthermore, VSELs could somehow reconcile past theories of the embryonic rest hypothesis of cancer's origin as well as current theories maintaining cancer as a SC disorder. To support this, there are several direct and indirect indications that cancer could originate in the cells closely related to the germ line.

First, it has been postulated that the “classical” germ line tumors such as ovarian-, yolk sac-, mediastinal GC-, and brain GC tumors as well as seminomas, teratomas, and teratocarcinomas could be attributed to a population of migrating PGCs. These cells may have gone astray during developmental migration en route to the genital ridges and been deposited in adult tissues [49–51].

Second, it is well known that various tumor cell types (e.g., gastric-, lung-, liver-, renal-, and bladder carcinomas as well as melanomas, medulloblastomas, pediatric sarcomas, and germinal tumors [52,53]) frequently express so-called Cancer Testis (C/T) antigens (~40 identified so far) that are normally expressed in the germ line only. This indicates that cancer may originate in germ line cells. However, the expression of these genes in cancer cells could be alternatively explained as a re-expression of the primitive germ line potential in mutated or epigenetically changed cancer cells. Of note, we reported that VSELs express several C/T antigens that are encoded both on the X chromosome (MageB3, Ssbx2) and on the non-X chromosome (BORIS) [54]. Based on this, VSELs could be a population of SCs that gives rise to C/T antigen-expressing tumors.

The third indication is the well-known expression of either the beta subunit of human chorionic gonadotropin (hCG) [55] or its fragments and/or carcinoembryonic antigen (CEA) by different tumor types. Expression of these germ line markers in cancer cells could support the germ line origin of tumors. Alternatively, as in the case of C/T antigens, such marker expression could indicate a re-expression of the primitive germ line potential in mutated cells.

Finally, several tumors (e.g., gastric-, lung-, bladder-, and oral mucosa carcinomas and germinal tumors) may express Oct-4 transcription factor, which is a marker of embryonic, epiblast, and germ line PSCs [56]. However, the true expression of Oct-4 in cancer cells must be considered first after excluding the possible expression of Oct-4 pseudogenes. We reported that our VSELs highly express Oct-4 at the mRNA and protein levels; this observation was subsequently confirmed by demonstrating hypomethylated Oct-4 promoter and its association with acetylated open-type chromatin-associated histones [15].

VSELs as a Missing Link to Reconcile the Embryonic Rest/Germ Line Origin of Cancer Hypothesis and Contemporary SCs Theory of Cancer Development

In Table 2, we propose potential roles of Oct-4⁺ epiblast/germ line-derived cells (e.g., VSELs) in the development of various malignancies. First, if the genomic imprinting in VSELs is not erased, these rare cells may retain post-developmental in vivo pluripotency and could grow teratomas and teratocarcinomas. Second, if developmentally early PGCs, which are closely related to VSELs, deviate from their major migratory route on the way the genital ridges and ultimately settle down in various organs, they may give rise to germinal tumors (ovarian-, yolk sac-, mediastinal GC-, and brain GC tumors as well as seminomas). Such tumors are found not only in the gonads and retroperitoneal spaces, but in also in the thorax mediastinum and brain. Third, if VSELs undergo some critical epigenetic changes (e.g., acquire hypermethylation on

the DMR for the Igf2-H19 locus or Rasgrf1), they may develop into several types of pediatric sarcomas (e.g., neuroblastoma, rhabdomyosarcoma, Ewing-sarcoma, or Willm's tumor). In support of this, some visible correlation exists between the number of these Oct-4⁺ cells that persist in postnatal tissues and the coincidence of these types of tumors seen in pediatric patients and young adults [57]. Furthermore, patients with Beckwith–Wiedemann syndrome, who frequently develop sarcomas (e.g., neuroblastoma or rhabdomyosarcoma), display perturbation in the expression of imprinted genes. Accordingly, such patients are diagnosed with a loss of imprint on the H19-Igf2 locus, which leads to overexpression of pro-proliferative Igf2 and downregulation of growth inhibitory H19. This syndrome is also often connected with the downregulation of cyclin kinase inhibitor p57^{KIP2}, another imprinted gene that, as we demonstrated, controls quiescence of VSELs and is over-expressed in these primitive cells. These potential methods of how VSELs could hypothetically contribute to cancer initiation and expansion through acquiring mutations and/or modulating the status of genomic imprints are shown as the 1st scenario in Fig. 4.

Furthermore, Oct-4⁺ VSELs mobilized into peripheral blood or residing locally in tissues may be chemoattracted into damaged tissues and fuse with somatic cells (Table 2 and Fig. 2). Thus, it is possible that if VSELs are mobilized at the wrong time into peripheral blood and deposited in areas of chronic inflammation instead of playing a role in regeneration, they may contribute to the development of other malignancies (e.g., stomach cancer or lung cancer) by a fusion mechanism. Such fusion leads to the formation of heterokaryons and aneuploid cells that display chromosomal instability [58,59]. Our data indicate that VSELs are in fact highly fusogenic cells and we hypothesize that they could be potential fusion partners for somatic cells. After fusion, VSELs would provide transcripts that are characteristic for early developmental cells (e.g., Oct-4, Nanog, Klf-4) to the somatic fusion partner while somatic cells would supply chromosomes to VSELs that show proper genomic imprinting. The formation of such heterokaryons could be the first step in the selection of aneuploid “immortal” malignant cell clones, shown as the 2nd scenario in Fig. 4.

Finally, as shown in the 3rd scenario in Fig. 4, mobile VSELs could be chemoattracted by the expanding hypoxic microenvironment of tumor tissues [e.g., in a stroma-derived factor-1 (SDF-1)-, hepatocyte growth factor/scatter factor (HGF/SF)-, or vascular endothelial growth factor (VEGF)-dependent manner] and potentially be involved in tumor expansion and growth by providing vessels and stroma. In this case, VSELs could wrongly recognize the expanding hypoxic environment in the tumor as regenerating tissue.

Conclusions

We hypothesize that a unique population of VSELs recently identified by our team that express several epiblast/germ line markers could be the origin of several tumors. To support this notion, several markers that are expressed in VSELs (e.g., Oct-4, SSEA-1, or C/T antigens) have been described in malignancies present in both pediatric and adult patients. Overall, we envision that VSELs can initiate cancer or contribute to its growth by acquiring mutations, maintaining genomic imprinting, and fusing with other somatic cells and by providing stroma and endothelial precursors, respectively. Therefore, potential involvement of VSELs in cancerogenesis could support the century-old concepts of embryonic rest- or germ line-origin hypotheses of cancer development. However, we are aware that this working hypothesis requires further direct experimental confirmation.

Acknowledgments

This work is supported by NIH grants R01 CA106281-01 and R01 DK074720 and the Stella and Henry Hoenig Endowment to MZR NIH grant P20RR018733 from the National Center for Research Resources to MK and European Union structural funds, Innovative Economy Operational Program POIG. 01.01.02-00-109/09-00.

Abbreviations

BM	Bone marrow
BMMNC	Bone marrow mononuclear cell
C/T	Cancer testis
DM	Differently methylated region
dpc	Days post-conception
EGC	Embryonic germ cell
ESC	Embryonic stem cell
FACS	Fluorescence-activated cell sorting
FC	Flow cytometry
GC	Germ cell
HSC	Hematopoietic stem cell
ICM	Inner cell mass
Igf1R	Insulin-like growth factor 1 receptor
Igf2	Insulin-like growth factor 2
Igf2R	Igf2 receptor
ISS	ImageStream system
PGC	Primordial germ cell
PSC	Pluripotent stem cell
Rasgrf1	Ras protein-specific guanine nucleotide-releasing factor 1
SC	Stem cell
SSEA-1	Stage-specific embryonic antigen-1
TCSC	Tissue-committed stem cell
VSEL	Very small embryonic/epiblast-like stem cell

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Fig. 1.

BM preparation and gating strategy for sorting of murine BM-derived VSELs by FACS. Panel **a** Gating strategy for VSEL isolation by FACS: Agranular, small events ranging from 2 μ m to 10 μ m are included in gate R1 after comparison with six differently sized bead particles with standard diameters of 1, 2, 4, 6, 10, and 15 μ m; cells are visualized by dot plot showing forward scatter (FSC) vs. side scatter (SSC) signals, which are related to the size and granularity/complexity of the cell, respectively. Panel **b** Cells from region R1 are further analyzed for Sca-1 and Lin expression and only Sca-1⁺/Lin⁻ events are included into region R2. Panel **c** Population from region R2 is subsequently distinguished based on CD45 marker expression into CD45⁻ and CD45⁺ subpopulations visualized on histogram. CD45⁻/Lin⁻/Sca-1⁺ cells (VSELs) are sorted as objects enclosed in a logical gate including regions R1, R2, and R3, while CD45⁺/Lin⁻/Sca-1⁺ cells (HSCs) are from a gate including regions R1, R2, and R4. Percentages show the average content of each cellular subpopulation in total BM nucleated cells

Fig. 2.

The overall hypothesis presented in this review of the developmental deposition of Oct-4⁺ epiblast/germ line-derived VSELs in adult tissues. It is known that the germ line carries the genome (nuclear and mitochondrial DNA) from one generation to the next. Somatic SCs “bud out” from the germ line during ontogenesis and form soma, which help germ line-derived gametes fulfill this mission. The germ potential is established in the fertilized oocyte (zygote) during fertilization and subsequently retained in the morula, ICM of the blastocyst, epiblast, PGCs, and mature GCs (gonocytes–oocytes and sperm). The first cells that bud out from the germ lineage are trophoectodermal cells that give rise to the placenta. Subsequently, we envision that during gastrulation, the epiblast is a source of PSCs for all three germ layers (meso-, ecto-, and endoderm) as well as PGCs. We hypothesize that at this stage, some epiblast-derived SCs could be deposited as Oct-4⁺ PSCs in peripheral tissues and organs (red circles). In addition, some migrating PGCs could deviate from their major migratory route to the genital ridges and be deposited as well. Furthermore, we hypothesize that epiblast/germ line-derived SCs (VSELs) deposited in the developing tissues undergo erasure of their somatic imprint similarly to PGCs. This “erasure of methylation of somatic imprinted genes” protects the developing organism from the unwanted possibility of teratoma formation, but at the same time restrains their pluripotentiality.

Fig. 3.

Reprogramming of somatic imprints control the pluripotentiality and quiescence of VSELs. Panel **a** During development, PGCs and VSELs undergo similar epigenetic reprogramming of somatic imprinted genes; however, they also retain expression of some pluripotent genes (e.g., Oct-4). In particular, the erasure of somatic imprints in these cells is responsible for preventing them from aberrant teratoma formation, but at the same time restrains their pluripotentiality. In contrast, differentiated somatic cells lose their pluripotency by turning off the transcription of pluripotent genes through stable DNA methylation of their promoters (e.g., Oct-4). However, they retain the somatic pattern of the genomic imprint. Thus, somatic cells may be dedifferentiated to PSCs by expression of pluripotent genes (*blue box*—iPSC protocol). In contrast, PGCs that express pluripotential genes, but erase the somatic imprint, may become pluripotent EGCs by proper remethylation of some of erased imprinted genes (*green box*—EGC protocol). We hypothesize that similar modulation of parent-of-origin-specific reprogramming of somatic imprints in VSELs that enforces their quiescent status in tissues may “unleash” their pluripotentiality and reverse them to fully pluripotent status (*dark blue box*—VSEL protocol). Panel **b** Bisulfite sequencing results of DNA methylation of indicated imprinted loci in VSELs and HSCs. Mean values for the percentage of methylated CpG sites are shown as the mean±S.D. from at least three independent experiments. Dashed red line indicates the somatic imprinting pattern (50%). Panel **c** Relative quantification of the expression level of maternally expressed/proliferation-repressing imprinted genes (H19, p57^{KIP2}, and Igf2R, *blue box*) and paternally expressed/proliferation-promoting genes (Igf2 and Rasgrf1, *red box*) in VSELs and HSCs. The relative expression level is represented as the fold difference to the value of BM mononuclear cells (BMMNCs) and shown as the mean from at least four independent experiments. VSELs, HSCs, and BMMNCs were separated by FACS from 4 week-old mice

Fig. 4.

Three scenarios postulated in this review for involvement of VSELs in cancerogenesis. *1st scenario:* We envision that this particular scenario plays a significant role in the pathogenesis of pediatric tumors. Accordingly, VSELs may give rise to cancer (stem) cells (*dark irregular cell*) if the genomic imprinting is not erased or the VSEL acquires mutations and, as a result of this, potentially gives rise to teratomas/teratocarcinomas or sarcomas (e.g., rhabdomyosarcoma, neuroblastoma, nephroblastoma), respectively. *2nd scenario:* VSELs may fuse with somatic cells and give rise to heterokaryons (*blue cells with nucleus*). Tetraploid heterokaryons undergo subsequent selection and give rise to aneuploid cancer (stem) cells. We envision that this scenario in particular plays a more important role in the pathogenesis of tumors developing on the basis of chronic inflammation/tissue injury in adult patients. *3rd scenario:* VSELs may indirectly contribute to tumorigenesis by providing vasculature and stroma cells for the growing tumor tissue

Table 1

Methylation status of crucial imprinted genes in murine VSELs

	VSEL	Hematopoietic (H)SC	Mesenchymal (M)SC	ESC
Igf2-H19	↓	N	N	↑
Rasgrf1	↓	N	N	N
Igf2R	↑	N	N	↑↑
KCNQ1	↑	N	N	↓
Peg1/Mest	↑	N	N	N
SNRPN	N	N	N	N

hypermethylation,

hypomethylation, *N* normal status

Table 2Potential role of Oct-4⁺ epiblast/germ line-derived cells (e.g., VSELs) in cancerogenesis

Tumor types observed	Potential VSEL-related mechanisms that may lead to cancerogenesis
Teratomas, Teratocarcinomas	Persistent somatic imprinting in Oct-4 ⁺ epiblast/germ line-derived cells (e.g., VSELs) that are deposited during development in adult tissues, additional mutations.
Germinal tumors (e.g., Germinomas, Seminomas, Teratomas, Dermoid cyst, Hydatidiform mole)	Cells left along PGC migratory route, persistent genomic imprinting, additional mutations.
Pediatric Sarcomas or “small round blue cell tumors” (e.g., rhabdomyosarcoma, neuroblastoma, Ewing sarcoma, -nephroblastoma, Wilms tumor)	Mutated epiblast-derived Oct-4 ⁺ cells in various peripheral tissues. For example, loss of imprint on H19-Igf2 locus in Beckwith-Wiedemann syndrome leads to overexpression of Igf2 and downregulation of H19.
Other solid malignancies originating due to chronic tissue injury, chronic inflammations	Involvement of circulating Oct-4 ⁺ cells that home into damaged tissues, additional mutations? Cell fusion leading to formation of heterokaryons/aneuploid cells and chromosomal instability?