Very Small Embryonic-Like Stem Cells (VSELs)

An Update and Future Directions

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Results from at least 20 independent laboratories indicate that adult tissues contain rare, early-development stem cells known as very small embryonic-like stem cells (VSELs), which can differentiate into cells from >1 germ layer. Further research on these cells may provide a path forward to application of these cells in regenerative medicine that perhaps may solve several problems inherent in the use of controversial embryonic stem cells (ESCs) and somehow problematic induced pluripotent stem cells (iPSCs).

Regenerative medicine is still looking for a pluripotent/multipotent stem cell able to differentiate across germ layers. The hope to use ESCs in regenerative medicine is ethically controversial, and they have technical problems, such as the risk of teratoma formation and life-threatening arrhythmias and their potential histoincompatibility with unrelated recipients.[1–3] In response to these problems, a solution for obtaining ethically acceptable pluripotent stem cells has been proposed: generating iPSCs by genetic modification of adult cells. However, these cells have also been found to be at risk of teratoma formation and immunologic rejection and demonstrate genomic instability.[1,2] Moreover, the current results of clinical applications of iPSCs have demonstrated only paracrine effects of therapy and no contribution of these cells to damaged organs.[4] This all suggests an approaching twilight for the clinical application of ESCs and iPSCs as regenerative therapies.

More than 15 years ago, our group identified a population of small, early-development stem cells in adult tissues that express pluripotency markers and that, based on their primitive morphology and gene expression profile, were named VSELs.[5,6] The existence of these cells and their across germ layers differentiation was subsequently confirmed by at least 20 other independent groups (Online Data Supplement).

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However, although we were working on better characterizing these cells and exploring possible applications in animal models in vivo, the very existence of VSELs was questioned.[7] It is regrettable that having a problem with VSEL purification, which requires a special gating protocol, this group did not follow the detailed protocol for VSEL isolation previously published in Current Cytometry Protocols (https://currentprotocols.onlinelibrary.wiley.com/doi/abs/10.1002/0471142956.cy0929s51). This article[7] slowed progress in this area. Nevertheless, we responded to skeptics in 2 articles that pointed out why the sorting strategy they used to isolate their rare cells from hematopoietic tissues was inadequate.[5,8] Moreover, aware of the huge potential of VSELs, our group and a few other independent groups persisted in our efforts and recently developed an ex vivo strategy to expand these cells from their quiescent state without feeder layer cells or viral vectors in a chemically defined medium containing artificial serum, nicotinamide, and a cocktail of growth factors. In this Viewpoint, we will briefly summarize the current status of VSEL research.

VSEL Morphology—Seeing Is Believing

VSELs are small cells, corresponding in size to the cells in the inner cell mass of the blastocyst, and, depending on the measurement conditions (in suspension or after adhesion to slides), they measure ≈3 to 5 μm in mice and ≈5 to 7 μm in humans. Thus, they are slightly smaller than red blood cells and, therefore, require a special gating strategy during fluorescence-activated cell sorting. Transmission electron microscopy analysis revealed that they have large nuclei containing euchromatin and a thin rim of cytoplasm enriched in spherical mitochondria, which are characteristic of early-development cells.[6]

Developmental Origin of VSELs

It has been proposed that VSELs originate from cells related to the germ line, are deposited in developing organs during embryogenesis, and play a role as a backup population for monopotent tissue-committed stem cells. VSELs are quiescent but are activated during stress situations and mobilized into the circulation. The number of these cells decreases with age.[6] Overall, the presence of these early-development cells in postnatal tissues challenges the accepted hierarchy within the adult stem cell compartment in bone marrow (Figure).

VSELs and Their Link to Primordial Germ Cells

The germ line is immortal from an evolutionary point of view and transfers DNA and mitochondria to the next generation. Living organisms, including their various stem cell

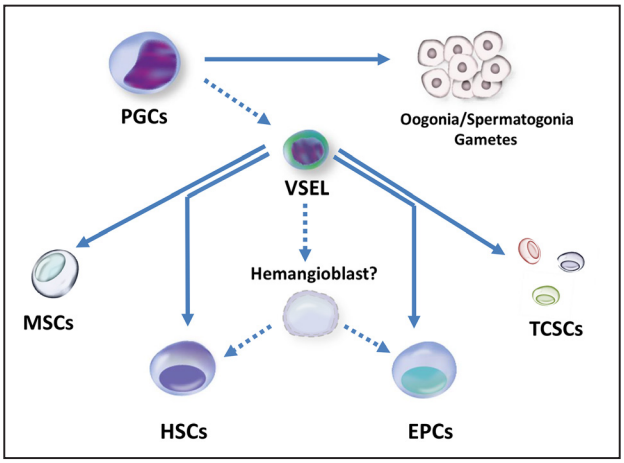

Figure. Proposed developmental interrelationship between primordial germ cells (PGCs), very small embryonic-like stem cells (VSELs), hemangioblasts, hematopoietic stem cells (HSCs), and endothelial progenitor cells (EPCs). We propose that migratory PGCs, aside from their major role in establishing gametogenesis, may be a source of certain developmentally primitive stem cells (eg, VSELs) that in bone marrow give rise to HSCs and EPCs and are a source of mesenchymal stem cells (MSCs) and in other tissues a source of tissue-committed stem cells (TCSCs). Specification of VSELs into HSCs and EPCs may involve putative hemangioblast as an intermediate precursor cell. Dotted line pathways still under investigation. Adapted from Ratajczak.[9]

compartments, develop from the fusion of gametes derived from primordial germ cells (PGCs). VSELs express several markers of PGCs, which supports the concept that the most primitive stem cells residing in adult tissues are related to PGCs (Figure).[6,9]

Gene Expression Analysis

VSELs express some ESC markers, such as stage-specific antigen (SSEA), nuclear Oct-4A (octamer-binding transcription factor 4A), Nanog, and Rex1. The true expression of these genes has been confirmed by the open structure of chromatin in its respective promoters, its association with histones promoting transcription, and by the sequencing of reverse transcription-polymerase chain reaction products. VSELs also express several markers characteristic of migrating PGCs, such as Stella and Fragilis. Our single-cell cDNA libraries revealed that the gene expression profile in murine BM-isolated VSELs, sorted as very small Sca-1^{+}lin^{-}CD45^{-} cells, varies.[10]

Quiescent State of VSELs

VSELs residing in adult tissues are highly quiescent because of the erasure of regulatory sequences for certain paternally imprinted genes (eg, at the Igf2-H19 locus) and thereby protected from insulin/insulin-like growth factor stimulation. They also express bivalent domains at genes encoding transcription factors in the homeobox family. Recent proteomic data have confirmed that genes involved in proliferation and cell signaling are expressed in VSELs at a low level and become upregulated during their expansion.

VSELs in Hematopoietic Tissues

Evidence has accumulated that VSELs are at the top of the stem cell hierarchy in normal bone marrow, giving rise to hematopoietic stem cells, mesenchymal stem cells, and endothelial progenitor cells. VSELs expand in vivo in response to stimulation by pituitary gonadotropins and gonadal sex hormones, which, from a developmental point of view, further links these cells to migrating PGCs.[6,9]

VSELs in the Gonads

It has been convincingly demonstrated that VSELs can be isolated from the ovarian surface epithelium of young and postmenopausal women, as well as from testes.[11] Recently, it has been reported that ovary-isolated VSELs differentiate into oocyte-like cells in response to sperm cells and release the zona pellucida,[12] which is the first step in the fertilization process.

VSELs in Aging

The number of VSELs correlates with longevity in certain long-living murine strains. Their number can be increased in experimental animals by caloric restriction, regular exercise, and administration of DNA modifiers, such as nicotinamide or valporic acid. By contrast, the exposure of animals to increased insulin/insulin-like growth factor signaling leads to premature aging and depletion of VSELs from the tissues.[6,13]

VSELs in Experimental Models of Tissue/Organ Injuries

Several papers have been published showing a contribution by injected purified VSELs to hematopoiesis, osteogenesis, and angiogenesis, as well as to myocardium, liver, and pulmonary alveolar epithelium in appropriate in vivo models. The well-demonstrated presence of chimerism in several organs indicates the potential of these cells to differentiate across germ layers.

Ex Vivo Expansion of VSELs

The most important breakthrough in the potential application of VSELs came with the development of more efficient ex vivo expansion strategies for these rare cells. VSELs can now be expanded ex vivo in the presence of nicotinamide or valporic acid[6] or in the presence of the small-molecule UM177[14] without transduction by DNA or RNA or by using supportive third-party feeder layer cells.

Molecular Basis Behind the Expansion of VSELs

To explain our expansion approach, both of the small molecules used in our expansion medium, nicotinamide and valproic acid, are inhibitors of the histone deacetylase Sirt-1.[6,15] This enzyme inhibits the activity of the de novo DNA methylotransferase DnmT3L, which is crucial for methylation of the regulatory regions of paternally imprinted genes. As mentioned above, these loci are demethylated (erased) during early embryogenesis in VSELs because they are in PGCs migrating to the genital ridges. These epigenetic changes explain why PGCs and VSELs are so quiescent and cannot complement blastocyst development and, what is even more important, do not grow teratomas, despite their pluripotency. The fact that Sirt-1 maintains a low intracellular level of

DnmT3L explains why it has beneficial effects on longevity by preventing premature depletion of VSELs from adult tissues. By contrast, downregulation of Sirt-1 by nicotinamide or valporic acid in culture promotes ex vivo expansion of these cells.

Future Directions and Issues to Be Solved

An open question remains whether VSEL-expanded cells will fully differentiate and integrate with other cells in the damaged tissues. It is also important to prove that they can reestablish 3-dimensional fully functional tissue structures, which will be crucial to justify their potential application in the clinic. In addition, because almost all VSEL studies to date have been performed with cells isolated from hematopoietic tissues, one can ask whether VSELs purified from other nonhematopoietic sources have the same properties and can differentiate into cells from all 3 germ layers. However, although our preliminary data show that they do not grow teratoma in immunocompromised mice, some further deep sequencing analysis is needed to evaluate the genomic stability of VSEL-derived cells after current expansion strategies using small molecular DNA-modifying agents. Furthermore, although VSELs isolated from adult tissues and expanded ex vivo could be used to regenerate damaged organs, another experimental approach would be to develop, in parallel, strategies to maintain the pool of VSELs residing in adult tissues. This goal provides a challenge for modern pharmacology: to develop drugs that protect VSELs from insulin/insulin-like growth factor signaling. Metformin, which is currently used to modulate insulin signaling and increases longevity, has, unfortunately, several side effects.

In summary, we propose that VSELs isolated from adult tissues should be studied further in solid organ injury models because they may provide a path forward that solves several problems with the use of controversial ESCs and iPSCs in regenerative medicine.

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Disclosures

University of Louisville owns IP on VSEL Technology. The authors report no conflicts.

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