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Review

Very small embryonic like(VSEL) stem cells

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Abstract

It is generally accepted that adult bone marrow(BM) contains both hematopoietic stem cells(HSCs) and mesenchymal stem cells (MSCs). Recently, a rare population of stem cells different from HSCs and MSCs were identified in murine BM and human cord blood (CB), named as very small embryonic like(VSEL) stem cells. These cells are tiny round and $CXCR4^+$ $Sca\text{-}1^+$ Lin^- $CD45^-$, expressing SSEA-1/4, Oct-4 and Nanog, which have potent of differentiation into all three germ-layer lineages, such as cardiomyocytes, neural and pancreatic cells.

Key words: very small embryonic like stem cells; embryonic stem cells

INTRODUCTION

Adult bone marrow(BM) contains both hematopoietic stem cells(HSCs) and nonhematopoietic stem cells or mesenchymal stem cells(MSCs). MSCs were previously referred to as "marrow stromal cells" or "colony-forming unit-fibroblasts"(CFU-fs), which reflect their origin and morphology in cultures. Based on their pioneering studies initiated nearly forty years ago, Friedenstein *et al*[1] were the first to propose the concept that human post natal BM contained a precursor cell for multiple mesenchymal cell lineages. Over the ensuing decades, marrow stromal cells have been characterized, based largely upon their properties *in vitro* or following transplantation in various animal model systems[2]. The term CFU-f was coined by Friedenstein to describe cells isolated from the bone marrow stroma of a variety of post natal organisms that are adherent, fibroblastic, and clonogenic in nature[3]. Under well-defined *in vitro* and *in vivo* conditions, a proportion of CFU-f can give rise to multiple mesenchymal tissues including bone, adipose tissue, cartilage, myelosupportive stroma, smooth muscle, cardiomyocytes, and tendon. Recent studies have shown that adult BM-MSCs differentiate not only into mesenchymal cells, but also into cells with the characteristics of visceral mesoderm, neuroectoderm and endoderm *in vitro* and *in vivo*[4]. When injected into an early blastocyst, single BM-MSC contributes to most, if not all, somatic cell types. Upon transplantation into a non-irradiated host, MSCs engraft and differentiate into the hematopoietic lineage, and also into the epithelium of liver, lung, gut and kidney. To date, significant controversy exists as to what is defined as a cell(or more likely a group of cells) that is referred to as an MSC. In some cases, similar or overlapping populations of primitive stem cells in the BM have probably been detected by using different experimental strategies, and hence have been assigned different names as endothelial progenitor cells[5], MSC[6], multipotent adult progenitor cells(MAPC)[4], or marrow-isolated adult multilineage inducible (MIAMI) cells[7].

Stem cell therapy holds great promise for treatment of many genetic and acquired diseases[8], and BM-MSCs are one of the most promising candidate stem cell types for this form of therapy due to their availability and the relatively simple requirements for expansion *in vitro* and

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genetic manipulation. However, despite extensive characterization *in vitro*, relatively little has been reported with respect to their biology *in vivo* and therapeutic potential[9]. It is still unclear whether MSCs reside in BM as adherent fibroblastic or non-adherent round cells; whether these cells can be served as common stem cells for both hematopoietic and stromal lineages; whether BM-MSCs are a major source for adult stem cells, and whether they migrate into various solid-organs through the circulation and differentiate into target tissue-specific cells to replace injured organ tissues and repair injured tissues.

Recently a research group had identified a population of Sca-1$^+$ Lin$^-$ CD45$^-$ stem cells in murine BM and human cord blood(CB). They named these cells "very small embryonic like(VSEL) stem cells"[10,11], which are a population of pluripotent stem cells(PSC) deposited during development and reside in BM. On one hand, after being mobilized from BM into peripheral blood, these cells may participate in the turnover of other tissue-specific stem cells that are located in peripheral niches, and may play a role in tissue organ regeneration during stress situation/organ injury[12,13]. On the other hand, if exposed to mutagens, this population of primitive stem cells may give rise to some types of cancer(e.g., pediatric sarcomas, teratomas, germinal tumors).

THE FEATURE OF VSEL STEM CELLS

VSEL stem cells which are difined as Sca-1$^+$Lin$^-$ CD45$^-$ were analyzed by multiparameter flow cytometry[10], transmission electron microscopy[10], confocal microscopy[14] and ImageStream system[14]. The morphology of VSEL stem cells are well characterized[10,14]. VSEL stem cells are small and measured as 3.63 ± 0.14 μm(2~6 μm) in diameter. Thus, VSELs are larger than peripheral blood platelets and smaller than erythrocytes. VSELs are much smaller than HSCs (6.54 ± 0.17 μm in diameter) and significantly smaller than peripheral blood granulocytes(8.08 ± 0.18 μm in diameter) or Nalm-6 lymphoblasts. VSEL stem cells can be sorted only based on Sca-1, lineage markers and CD45 expression, whatever the BM mononuclear cell (MNC) population was analyzed with or without the initial size restrictions. VSELs possess a large nucleus surrounded by a narrow rim of cytoplasm. They have significantly higher nuclear area/cytoplasmic area(N/C) ratio as compared with HSCs, granulocytes and Nalm-6 cells. At the ultrastructural level, the narrow rim of cytoplasm possesses a few mitochondria, scattered ribosomes, small profiles of endoplasmic reticulum, and a few vesicles. The nucleus is contained within a nuclear envelope with nuclear pores. Chromatin is loosely packed and consists of euchromatin. Most important DNA in the nuclei of these cells contains open-type euchromatin that is characteristic for pluripotent embryonic stem cells.

Sca-1$^+$ Lin$^-$CD45$^-$ VSEL stem cells are about 0.02% of BM MNC. The number of these cells is highest in BM from young(approximately 1-month-old) mice and decreases with age[10]. It is also significantly diminished in short living DBA/2J mice as compared to long living B6 animals[10]. VSEL stem cells also express a-chemokine G_{ai} protein-coupled seven transmembrane-spanning receptor(CXCR4), and several embryonic stem cell markers, such as stage specific embryonic antigen (SSEA)-1/4, Oct-4 and Nanog, at the protein and mRNA levels. VSEL stem cells express mRNA for Oct-4, Nanog, Rex1, Dppa3 and Rif1(mRNA for telomerase protein) at similar levels as embryonic stem cell line ES-D3 cells. Furthermore, VSEL stem cells express several markers of TCSC for neural tissue, skeletal and heart muscle, liver, pancreas, epidermis, melanocytes and intestinal epithelia at mRNA level. But they do not express MHC-Ⅰ or MHC-Ⅱ(HLA-DR) antigens, and are CD90$^-$CD105$^-$CD29$^-$[15].

Although highly purified VSEL stem cells do not proliferate and differentiate if cultured alone, they are able to differentiate into all three germ-layer lineages (cardiomyocytes, neural and pancreatic cells) when cocultured in differentiating media together with freshly isolated marrow cells[10]. When plated over a C2C12 murine myoblast cell feeder layer, murine VSEL stem cells are able to form spheres that resemble embryoid bodies and are stained positively for the fetal isoform of alkaline phosphatase. Murine VSEL-derived spheres (VSEL-DS) are immature with large nuclei containing euchromatin and, are CXCR4$^+$SSEA-1$^+$Oct-4$^+$. Cells from VSEL-DS may again(up to 5~7 passages) grow new spheres by being replated over C2C12 cells, or, expand into cells of all three germ-cell layers by being plated into cultures promoting tissue differentiation[15]. Similar spheres are also formed by VSEL cells isolated from murine fetal liver, spleen, and thymus. But it was failure to see that VSEL cells can grow into hematopoietic colonies *in vitro* or protect mice from lethally radio irradiation or form CFU-S colonies in lethally irradiated syngeneic recipients[12,13]. When co-transplanted with recipient BM MNCs to lethally irradiated mice, Sca-1$^+$Lin$^-$CD45$^-$ VSEL cells can not be contribute to long-term repopulation of the hematopoietic system as the Sca-1$^+$Lin$^-$CD45$^+$ HSCs control[10].

VSEL stem cells are mobile and respond robustly to a stromal derived factor-1(SDF-1) gradient, adhere to fibronectin and fibrinogen, and may interact with BM-derived stromal fibroblasts. Confocal microscopy and time-lapse studies have revealed that these cells attach rapidly to, undergo emperipolesis, or migrate beneath

BM-derived fibroblasts[15]. The populations of BM-adherent fibroblastic cells described in the literature(e.g.,MSC,MAPC,or MIAMI) might be "contaminated" by these tiny rare VESL stem cells "hiding" among fibroblasts. So the three germ-layer lineages differentiation experiment evidences performed on BM-derived adherent fibroblast like cells must exclude "contamination" of these VSEL stem cells.

ORIGIN OF VSEL CELLS

The researchers of VSEL stem cells envision that VSEL cells are direct descendants from the germ lineage from a developmental standpoint. As expressing markers of inner cell mass(ICM)/epiblast/ primordial germ cells(PGC), such as SSEA-1(mice), SSEA-3/4 (human), Oct-4, and Nanog, the cells identified in adult tissues are populations of PSC that are deposited in these tissues during early gastrulation/embryogenesis and are derived mostly from epiblast-derived stem cells(EPSC) and, to some extent, from some rare migrating PGC that drift from their major migratory route to the genital ridges[15]. Similar to embryonic germ(EG) cells[16,17], the VESL stem cells derived from germ lineage may erasure the somatic imprint which is paternal and maternal pattern of methylation of the H19, Igf-2, Igf-2R, and Snrpn genes , and shut down their developmental pluripotency[18] during the development, and may also regain a proper somatic imprint under certain circumstances, such as tissue/organ injury[15].

PROSPECT

In the time of ethical debates on the application of ES cells in therapy, the "pluripotent/embryonic potential" of VSEL stem cells isolated from adult tissues might be a good source for clinical application. But there is a long rugged and tortuous road between experiments and clinic. Firstly, these cells should be tested in experimental models *in vivo*, and even if VSEL stem cells are released from the BM and are able to home toward the areas of tissue/organ injury and most likely play a role in the regeneration of minor tissue injuries, those rare cells can hardly repair severe tissue damage (e.g. heart infarct or stroke) effectively. Since the number of these cells among murine BM MNCs is extremely low and decreases with age[10], how to get a sufficient number of VSEL stem cells is a pressing problem. Secondly, local degradation of SDF-1 by proteolytic enzymes released from inflammatory cells interfere with the homing of $CXCR4^+$ stem cells, VSEL stem cells may circulate in peripheral blood and return to BM or home to other organs but not to the damaged areas. Thirdly, as their somatic imprints are most probably erased, which may limit their pluripotentiality. These VSEL stem cells might be not fully functional or remain "locked" in a dormant state and need the appropriate activation signals by unidentified factors. In some cases these cells could be attracted to inflammatory areas, and if not properly incorporated into the damaged tissue they may transform and initiate tumor growth[15].

In our studies[19], we also have a hypothesis that there is a population of round and non-adherent stem cells in BM, which to some extent is similar to the VSEL stem cells theory. We demonstrated that non-adherent bone marrow cells(NA-BMCs) can be expanded in suspension and give rise to multiple mesenchymal phenotypes including fibroblastic, osteoblastic, chondrocytic and adipocytic as well as glial cell lineages *in vitro* using the "pour-off" BMC culture method. MSCs derived from NA-BMCs(NA-MSCs) from wild-type mice were transplanted into VDR gene knockout($VDR^{-/-}$) mice that had received a lethal dose of radiation. Results revealed that NA-MSC can be used to rescue lethally irradiated mice by reconstructing a hematopoietic system and repairing other damaged tissues. Adult bone marrow harbors pluripotent NA-MSCs which can migrate *in vivo* into multiple body organs. In the appropriate microenvironment, they can adhere, proliferate and differentiate into specialized cells of target tissues, and thus have a function in damaged tissue regeneration and repair.

In conclusion, VSEL stem cells are a population of small round stem cells in adult organ especially in BM, but different from MSCs and HSCs. Findings in the research on VSEL stem cells will open a new view in stem cell research and application.

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