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Very small embryonic-like stem cells in cardiovascular repair

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ABSTRACT

Adult bone marrow (BM) harbors several small populations of cells which may contribute to cardiac and endothelial repair, such as endothelial progenitor cells (EPCs), mesenchymal stromal cells (MSCs) and very small embryonic-like cells (VSELs) expressing several markers of pluripotent stem cells (PSCs), such as Oct-4, Nanog and SSEA-1. Such cells were identified in mice bone marrow, peripheral blood and solid organs as well as in umbilical cord blood (UCB) and peripheral blood (PB) in humans. The adult BM-derived VSELs may undergo differentiation into cells derived for all three germ layers, including cardiomyocytes and vascular endothelial cells. VSELs can be isolated using a multiparameter live cell sorting technique with special gating strategy based on their small size, expression of stem cell markers (Sca-1 in mice, CXCR4 and CD133 in humans) and absence of hematopoietic lineage markers ($CD45^{-}$ Lin^{-}). Experiments in murine models of myocardial infarction (MI) demonstrated population of VSELs expressed also early markers of cardiac and endothelial lineages (GATA-4, Nkx2.5/Csx, VE-cadherin, von Willebrand factor) which migrated to stromal-derived factor-1 (SDF-1) and other chemoattractant gradient and underwent rapid mobilization into peripheral blood in experimental MI mice models. Recently, we demonstrated the mobilization of VSELs expressing PSC, early cardiac and endothelial markers in patients with acute MI. In addition to BM, VSELs were also identified in several murine solid organs including the heart and brain, as well as in umbilical cord blood and peripheral blood in adult humans. We hypothesized that VSELs are quiescent progeny of epiblast-derived PSCs that are deposited during organogenesis in developing organs. In experimental MI intramyocardial injection of VSELs was more efficient than that of HSCs at improving left ventricular ejection fraction and attenuation of myocardial hypertrophy. VSELs can be useful in translational studies of cardiovascular repair.

Contents

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1. Introduction

In the last decade the concept of myocardial regeneration was rapidly translated from basic science and animal experiments to application of bone marrow (BM) — derived stem and progenitor cells in multiple clinical trials including patients with acute myocardial infarction (MI) and ischemic cardiomyopathy (Abdel-Latif et al., 2007). So far, in the majority of clinical trials heterogenous population of non-selected bone marrow-derived mononuclear cells was used and currently there is no proof that any particular type of cells might be superior to others for myocardial repair in patients with MI and ischemic cardiomyopathy (Dimmeler et al., 2008).

Parallel to ongoing clinical trials with BM cells, investigating the optimal cell delivery route, dose and target population of patients, new concepts emerged which aim to enhance the capability of BM cells to induce the functional recovery of the myocardium or identifying new, more potent cell populations including cardiac stem cells (Messina et al., 2004), genetically engineered bone marrow progenitor cells (eg. Guided Bone Marrow-derived Mesenchymal Cardiopoietic Cells) (Sasaki et al., 2006), allogeneic mesenchymal stromal cells (Pittenger & Martin, 2004) and recently, various types of pluripotent stem cells (PSC), including iPS (Martinez-Fernandez et al., 2009) and very small embryonic-like stem cells (VSELs) (Kucia et al., 2006a,b,c,d). The progress of regenerative cardiovascular medicine generated the need for safe, ethically acceptable and therapeutically efficient sources of PSCs. VSELs seem to be an optimal population of cells for studies on cardiac repair as they can be efficiently isolated from adult BM, expanded in culture and differentiated. This paper reviews the recent data on VSELs and their potential application in experimental and clinical research.

2. Structural and molecular characteristics of VSELs

VSELs were initially discovered in adult mice using fluorescence activated cell sorting (FACS)-based live cell sorting technique which allowed the isolation of a rare population of Sca-1$^+$lin$^-$CD45$^-$ cells enriched for early embryonic markers (Oct-4, Nanog, SSEA-1, Rex1, Dppa3, Rif-1). Consistently with previous SDF-1 chemotaxis-based studies FACS-isolated cells expressed chemokine receptor CXCR4 (Kucia et al., 2006a,b,c,d). The morphology of the cells was investigated using transmission electron microscopy which showed their distinctive morphology and size differentiating VSELs from HSC in particular in terms of size (3–6 μm vs. 6–8 μm for HSC), chromatin structure and nucleus/cytoplasm ratio. Based on their small size, presence of PSC markers, distinct morphology (open-type chromatin, large nucleus, narrow rim of cytoplasm with multiple mitochondria) and ability to differentiate into all three germ layers, including mesoderm-derived cardiomyocytes, these cells were named very small embryonic-like stem cells (Kucia et al., 2006a,b,c,d; Wojakowski et al., 2007; Kucia et al., 2008).

Presence of characteristic markers of VSELs was confirmed using several complementary research tools. Real-time RT-PCR (RQ-PCR) showed increased levels of mRNA for PSC markers (Oct-4, Nanog, SSEA-1, Rex-1) and it was confirmed on protein level using immunofluorescent staining and ImageStream system (ISS). Also it was recently demonstrated that in VSELs the promoters of Oct4 and Nanog contain transcriptionally active chromatin excluding the possibility of amplification of pseudogenes (Shin et al., 2009).

Comparison of murine BM-derived VSELs with other populations of cells demonstrated that VSELs are smaller than hematopoietic stem cells (6.50 μm), monocytes and granulocytes and erythrocytes (4.90 μm). However, they are larger than platelets (2.12 μm) (Ratajczak et al., 2009).

In contrast to HSC, VSELs are primarily non-hematopoietic cells and their represent respective immunophenotype — absence of pan-hematopoietic marker (CD45−) and hematopoietic lineage markers (Lin−). Also freshly isolated and expanded VSELs do not from hematopoietic colonies when standard co-culture protocol using C2C12 myoblasts are used (Zuba-Surma & Ratajczak, 2010).

There are some differences of the VSEL phenotype between mice and humans (Table 1). Murine VSELs express Sca-1 antigen, whereas in human cord blood and peripheral blood of adults the population of VSELs consists of lin$^-$ CD45$^-$ positive for CXCR4, CD133 and CD34 antigens as confirmed on the mRNA level by RQ-PCR and protein level by IF and ISS. Expression of PSC markers Oct-4 and SSEA-4 was confirmed in population of small cells (~6–8 μm), therefore human VSELs are larger than murine. Presence of Oct-4 was confirmed in approximately 50–60% of VSELs isolated from human peripheral blood (Zuba-Surma & Ratajczak, 2010).

3. Strategy of isolation of VSELs

Fig. 1 illustrates the protocol for FACS-based live cell sorting from BM and PB samples. Initially BM is flushed from the femurs and nucleated cells are isolated by lysis of erythrocytes instead of using the Ficoll-based protocol as the latter might have led to the losing of very small cells. This is particularly important because VSELs represent very small population of cells, approximately 0.030 ± 0.008% of total BM cells in mice. Cell lysis is used also for isolation of VSELS from PB and CB (Zuba-Surma, E. K., Kucia, M., Abdel-Latif, A., et al., 2008; Zuba-Surma, E. K., Kucia, M., Wu, W., et al., 2008).

For sorting, cells are stained with Sca-1 (in mice) or CD133 (in humans), anti-CD45, anti-hematopoietic lineages markers (Lin) and CXCR4. Cells are sorted using a multiparameter, live sterile cell sorting systems (MoFlo, Beckman Coulter or FACSAria, Beckton Dickinson) (Kucia et al., 2006a,b,c,d). To validate the inclusion of small events (2–10 μm) in the extended lymphate synthetic beads of defined size (1–15 μm) are used (Zuba-Surma, E. K., Kucia, M., Abdel-Latif, A., et al., 2008; Zuba-Surma, E. K., Kucia, M., Wu, W., et al., 2008). Strategy of gating includes the extended lymphocyte gate which contains lymphocytes based on their size and granularity but on the left side extends to include events sized >2 μm. Such gate contains approximately 95% of VSELs (Zuba-Surma, E. K., Kucia, M., Abdel-Latif, A., et al., 2008; Zuba-Surma, E. K., Kucia, M., Wu, W., et al., 2008; Zuba-Surma & Ratajczak, 2010). ISS is particularly useful tool to confirm the presence of viable cells in sorted material, because this area of the cytogram contains a substantial number of cellular debris. Therefore decoding of the particular events on cytogram using ISS allows to confirm their typical morphology, presence of nucleous and colocalization of VSEL markers (Zuba-Surma, E. K., Kucia, M., Abdel-Latif, A., et al., 2008; Zuba-Surma, E. K., Kucia, M., Wu, W., et al., 2008; Zuba-Surma & Ratajczak, 2010).

Table 1
Major differences between isolation of murine bone marrow-derived and human umbilical cord blood-derived cells.

	Murine BM-VSELs	Human VSELs
Surface markers	Sca-1+, CXCR4+, CD133+, CD34+, SSEA-1+, AP+, c-Met+, LIF-R+, CD45−, Lin−, HLA-DR−, MHC I−, CD90−, CD29−, CD105−	CXCR4+, CD133+, CD34+, SSEA-4+, AP+, c-Met+, LIF-R+, CD45−, Lin−, HLA-DR−, MHC I−, CD90−, CD29−, CD105−
Size	4–5 μm	6–8 μm
Frequency	0.03% of nucleated cells	0.01% of nucleated cells
Tissues	Bone marrow, peripheral blond, brain, muscle, thymus, heart, liver, kidney, testes, spleen, lung, retina, pancreas, fetal liver	Bone marrow, umbilical cord blood, peripheral blood
Antibodies used for FACS-based isolation	**Anti mouse-Ly-6/E (Sca-1), anti mouse-CD45, anti mouse-lineage: CD45R/B220, Gr-1, TCRαβ, TCRγδ, CD11b, Ter119**	**Anti-human-CD133, anti-human-CD45, anti-human-lineage: CD2,CD3, CD14, CD66b, CD24, CD56, CD16, CD19, CD235a**