Stem cells in the arrangement of bone marrow repopulation and regenerative medicine

Matične ćelije – primena u repopulaciji kostne srži i regenerativnoj medicini

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Introduction

Hematopoiesis is a permanent and complex event in which a spectrum of different mature blood cells from a small population of toti/pluri/multipotent stem cells (SCs) are produced through a variety of proliferative and differentiative processes. Hematopoietic SCs are defined as the cells with extensive self-renewal and proliferative potential, together with their ability to differentiate into all blood-cell lineages. Many studies have demonstrated that a multifactorious network of interactive cytokines and other blood-derived mediators regulate the survival, maturation, and proliferation of SCs 1–5.

Hemobiology of stem cells

Compartment of SC can be divided into embryonic and "tissue specific" or adult – i.e. bone marrow and peripheral blood derived – cells. Generally, embryonic SCs are naturally capable of differentiation into all cell types. They are the most promising, but also the most controversial type of potentially transplantable SCs 4, 6.

When a sperm fertilizes an egg, it becomes what is known as zygote. Many scientists view the zygote as the ultimate SC because it can develop into any cell – not only of the embryo, but also of the surrounding tissues, such as placenta. The zygote has the highest degree of plasticity and it is referred to as a totipotent SC. Thirty hours after fertilization, the zygote begins to divide, and by the fifth or sixth day, the cells form a kind of a bubble or blastocyst. After the first week following fertilization, the cells begin to develop the coding sequence for specific functions, which makes isolating the SCs during the blastocyst state, imperative. When removed from the blastocyst, the cells can be cultured into embryonic SCs, but these cells are not embryos. They have the capability of developing into all three types of cell lineages. Many studies have demonstrated that a multifactorious network of interactive cytokines and other blood-derived mediators regulate the survival, maturation, and proliferation of SCs 1–3.
teins (Human Leukocite Antigens – HLA) on their surface. After the 12th week, fetal SCs acquire these proteins, and they remain present on SCs from this point on, including adult SCs 4, 6.

Thus, while some advocate therapeutic use of SCs derived from cord blood, adult bone marrow or the blood stream, these sources pose the problem of possible rejection reactions. Therefore, SCs derived from these sources may have therapeutic potential only when given to the individual from whom they were derived (autologous transplantation) or from an immunologically matched donor (allogeneic transplantation) 5, 11–16.

Adult SCs are at a more advanced stage of development. These SCs are not capable of differentiating into the endoderm, ectoderm or mesoderm, because they are already at a developed stage as one of the three types of tissues and cannot be rejuvenated back to an early developmental stage. They can be found in the blood, cornea, bone marrow, dental pulp of the tooth, brain, skeletal muscle, skin, liver, pancreas and gastrointestinal tract. These cells are capable of making identical copies of themselves, and usually divide to make progenitor or precursor cells capable to develop into specific cell lines 1–4.

Adult SCs are cells that can be derived from the different parts of the body and, depending on where they are from, have different properties. They exist in several different tissues including bone marrow, blood and the brain. Some studies have suggested that adult SCs are very versatile and can develop into many different cell types. Adult SCs have already been used for more than 20 years as bone/marrow transplants to reconstitute the immune systems of patients with cancer and to treat blood cancers such as leukemia. Using the body’s own SCs means the immune system’s rejection reflex will not be aroused. However, other studies have concluded that adult SCs are only able to develop into a limited number of cell types related to the tissue that the SCs originally came from. Although a great deal of information on adult SCs has already accumulated, scientists still do not understand completely their specific properties. Research continues with the hope of one day being able to use these cells to restore or replace damaged tissues or organs. More recently, their use in treatment of cor ischemic diseases and liver disorders has also been explored. The potential for hematopoietic SCs to produce cell types other than blood cells has become the subject of intense scientific controversy, since it is still not clear whether they could be used on a clinical scale to restore tissues and organs other than blood and the immune system 1, 17–21.

Clinical application of stem cells

Thanks to the above mentioned properties (self-renewal, differentiation and proliferation), SCs are capable of providing complete and long-term reconstitution of hematopoiesis in hematological disorders, as well as altered/distorted immunity, that was the basis for the clinical use of SC transplant in allogeneic or autologous settings 12–20. In a few words, SC transplants involve the administration of high-dose chemotherapy (myeloablative or immunoablative treatment) with subsequent (re)infusion of SCs.

Historically, bone marrow was the primary source of SCs for transplant – however peripheral blood and umbilical (cord) blood are also used as SC sources 14–16, 27–30. Therefore, SCs could be collected by multiple aspirations from spon- gious bones (e.g. posterior iliac crest) or by apheresis from blood after mobilization by chemotherapy plus cytokines (rHuG-CSF) in autologous setting or by rHu–CSF alone in autologous setting 8, 11–13. The collected cells should be administered immediately after the collection through catheter placed in subclavian or some peripheral vein. If necessary, cell harvest can be subjected to different purification methods or stored in liquid or frozen state (cryopreservation) 12.

Peripheral blood transplant can be characterized by the absence of the risk of bone infections, general or epidural anesthesia, as well as by faster reconstruction of hematopoiesis. Nowadays peripheral blood SC-harvests are ever more applied in both, allogeneic and autologous transplant settings.

The intensifying of pre-transplant myeloablative therapy and the increase of the clinical use of SCs, that is CD34+ cells, as well as the introduction of the novel cell–mediated curative approaches (e.g., adoptive cell-therapy) resulted in the increased needs for both specific blood-derived cells, and practical operating procedures inducing minimized cell damages during their collection or processing and storage in liquid or frozen state 5, 11, 30–32. Therefore, a successful performance of SC transplants requires both efficient collection and (cryo) preservation procedures for obtaining an acceptable cell yield and post–thaw recovery.

As mentioned, immature adult SCs have high potential of differentiation not just into all blood cells, but into several somatic cell types (trans-differentiation or lineage-plasticity), such as osteocytes, chondrocytes, hepatocytes, myocytes, cardiomyocytes and even endothelial cells. Thanks to the above mentioned ability, toti/pluripotent SCs (having “unlimited” biological capacity), as well as mesenchymal and endothelial precursors are clinically applicable for the cell-therapy in the field of regenerative medicine – that is for the treatment of patients with myocardial, brain, vascular, liver, pancreas and some other tissue damages. In this way, pre-clinical studies showed that “implantation” of immature SCs into damaged/ischemic area induces their homing and subsequent trans-differentiation into the cell lineages of host organ, including collateral vessel formation. To be precise, angiogenic growth-factors (or genes encoding for these proteins) promote the development of collateral arteries, the process known as therapeutic angiogenesis or neovascularization 17–21.

We have previously analyzed our results of peripheral blood vs. bone marrow transplants based on the hematopoietic reconstitution efficiency. SC transplants were used for the treatment of patients with severe aplastic anemia (SAA), acute lymphoblastic leukemia (ALL), acute non-lymphoblastic leukemia (ANLL), chronic myeloid leukemia (CML), multiple myeloma (MM), Hodgkin’s and non-Hodgkin’s lymphoma, breast and ovarian cancer, extragonadal non–seminal germ cell tumor, and severe multiple sclerosis. The
mononuclear cells (MNC) yield was $10.1 \times 10^6$/kgbm in allogeneic and $7.6 \times 10^6$/kgbm in autologous setting on the average. The mean CD34+ yields for allogeneic and autologous transplants were $16.7 \times 10^6$/kgbm and $11.8 \times 10^6$/kgbm, respectively.

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The use of the selection reduces T-cell and/or tumor cell counts with $\geq 3 \log$ or more. At the same time, CD34+ cell count should be at least 70% of the total cell number present before purging $^{11,13}$. Recently, a novel antigen, CD133 (formerly named as AC133), present in a very primitive SC population has been described. The results obtained suggest that CD133+ cell selection for cell-therapy perhaps could be a better option than CD34-selection. Our results obtained by the use of positive SC selection with Isolex 300i are in accordance with data from the literature. Namely, the CD34+ recovery and purity was 62.4±4.2% and 82.4±6.3%, respectively $^3,19$. Although these methods are originally designed to purify the SCs, their appliance also removes unwanted red blood cells and residual plasma, too.

In the treatment of our 24 patients with acute or chronic cardiac infarction and myocardial failure, as well as coronary artery by-pass, cell-therapy using autologous bone marrow derived SCs was performed. Harvested cells were initially filtered, afterwards processed and resuspended in serum–free culture medium up to optimized hematocrit value as previously described $^{31}$. In myocardial cell therapy setting, the number of MNC, CD45+/CD34+ and CD34+/CD133+ are quantified $^3$. The total count of applied MNC and their viability was 8.4±6.1 $\times 10^6$ and 98.2%. The mean number of CD45+/CD34+ and CD34+/CD133+ are 10.4±7.5 $\times 10^6$ and 7.16±4.6 $\times 10^6$, respectively (Table 1). Cell suspensions were administered as an intermittent infusion into the infarcted artery across coronary catheter after previously completed primary percutaneous coronary intervention (invasive cardiological setting) or directly into myocardium (during coronary by-pass grafting). The results obtained have demonstrated that trans-differented SCs regenerates cardiac tissue in post-infarcted hearts by inducing neovascularization and myocyte-generation. Consequently, in our preclinical study, cell-therapy resulted in a considerably improved myocardial perfusion and systolic function in all patients. They tolerated the use of intensive cell-mediated treatment well, without any adverse effects.

### Table 1

<table>
<thead>
<tr>
<th>Type of transplantation</th>
<th>Blood volume [in L]</th>
<th>MNC% or TNC% harvest [in mL]</th>
<th>MNC count</th>
<th>CD34+ cell count</th>
<th>CD133+ cell count</th>
</tr>
</thead>
<tbody>
<tr>
<td>allogeneic</td>
<td>13.1±2.5</td>
<td>257.0±40.8</td>
<td>10.8±6.1 $\times 10^6$/kg</td>
<td>16.7±9.8 $\times 10^6$/kg</td>
<td>/</td>
</tr>
<tr>
<td>PBSCT</td>
<td>16.0±4.2</td>
<td>284.6±55.3</td>
<td>7.6±4.6 $\times 10^6$/kg</td>
<td>11.8±6.5 $\times 10^6$/kg</td>
<td>17.9±10.6 $\times 10^6$</td>
</tr>
<tr>
<td>total</td>
<td>15.5±4.1 (10.2–37.8)</td>
<td>279.6±53.7$^9$</td>
<td>8.2±5.9 $\times 10^6$/kg</td>
<td>12.7±7.6 $\times 10^6$/kg</td>
<td>/</td>
</tr>
<tr>
<td>CR</td>
<td>/</td>
<td>236.6±58$^\times$</td>
<td>8.4±6.1 $\times 10^6$/kg</td>
<td>10.4±7.5 $\times 10^6$/kg</td>
<td>7.16±4.6 $\times 10^6$</td>
</tr>
</tbody>
</table>

Hematopoietic reconstitution were evidently superior when peripheral blood vs. bone marrow transplants were compared, as it is presented in Figure 1 $^{13,19}$.

![Fig. 1 – Hematopoietic reconstitution after peripheral blood stem cell transplantation (PBSCT) and bone marrow transplantation (BMT). Data are expressed as mean values ± standard deviations (SD).](image-url)
Conclusion

The concept of SC lineage-plasticity has generated enormous interest in recent years. Although the mechanisms by which cells can be trans-differentiated or "reprogrammed" as a result of the action of external (extrinsic) or endogenous (intrinsin) factors are still poorly understood, the above presented clinical results and facts suggest that appropriate populations of SCs could be used as a way of recovery of reduced/lost functions of damaged tissues. Thus, the most important application of human SCs in a new millennium is the generation of cells and tissues that could be used safely in cell-therapy or regenerative medicine. Although regenerative medicine presents one really complex process and the number of potential questions is higher than the number of possible answers, considering an ever increasing use of different cell-mediated curative approaches, it should also find its rightful place in the medical practice in our country.

REFERENCES


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