

STEM CELL TYPES IN THE BONE MARROW

**Ana Maria Cristina Serban¹, Gabriela Tanasie², Daciana Nistor²,
Oana Gavriliuc²**

REZUMAT

Bolile cardiovasculare sunt afecțiuni redutabile al căror tratament nu este încă satisfăcător. Transplantul celular apare cu o importantă armă în combaterea lor. Măduva hematogenă este un rezervor promițător și bogat de celule stem pe care cercetătorii au început să le identifice și să le diferențieze. Celulele stem hematopoietice – CSH, sunt celule stem cu multe proprietăți ce prezintă markeri de suprafață CD 34 și CD45. Așa numita „side population” – SP, reprezintă majoritatea celulelor medulare cu capacitate de reînnoire a tuturor liniilor hematopoietice. Exprimă nivele crescute de angiopoetină, Tie-2 VEGF-A, PECAM, CD31. Celulele stem mezenchimale – MSC, sunt celule nonhematopoietice, ce par a fi utile și în formarea neovascularizației, exprimă c-kit și se diferențiază în multiple tipuri de țesuturi mezenchimale. MAPCs sunt o populație de celule fibroblastice ce exprimă vimentin și pot fi diferențiate în diverse tipuri celulare, incluzând hepatocitele, celulele endoteliale și neuronii. Injectate în miocard toate aceste celule refac în proporții diferite miocardul lezat fapt pentru care detaliile asupra lor lor trebuie aprofundate.

Cuvinte cheie: măduva hematogenă, celule stem, transplant celular

ABSTRACT

Cardiovascular diseases are very serious disorders and their treatment is not at all satisfactory. Stem cell therapy becomes a useful tool for healing the damaged heart. The bone marrow is a rich supply of stem cells carefully studied by the researchers. Hematopoietic stem cells (HSC) are defined by the expression of the surface markers CD34 and CD45. The “side population” cells (SP) describe a part of the bone marrow cells that have the ability of self renewal and differentiation into cells of hematopoietic lineage. They express specific markers like angiopoietin, Tie-2, VEGF-A, PECAM, CD31. Mesenchymal stem cells (MSC) are nonhematopoietic stem cells, which express c-kit, can be differentiated into mesenchymal tissue cells and can form vascular cells. MAPCs depict a population of fibroblastic cells, which express vimentin and differentiate into cells of the three germ layers including hepatocytes, endothelial cells and neurons. Injected locally in the myocardium, these cells are able to reconstruct the damaged myocardium, making them important for the research.

Key Words: bone marrow, stem cells, stem cell therapy

The already existing experimental studies and the few developed trials are conducting to the idea that the therapy through cell transplant becomes an important option and a strong hope for the patients having serious diseases (cardiovascular, degenerative neurological diseases, diabetes mellitus, cancer etc.).

The therapy through cell transplant has been developed based on the *multi-potential stem cell* that is becoming, consequently, an important scientific subject. This essential item for the cellular therapy may be defined as a primary cell, not differentiated, capable to multiply itself, to get differentiated cells and to regenerate the injured tissues.

There are many identified types of progenitor cells, obtained from different types of tissues, clinically evaluated for developing a cellular therapy, namely: embryonic stem cells, fetal cardiomyocytes, hematopoietic stem cells (HSC), mesenchymal stem cells (MSC), skeletal myoblasts, fibroblasts, primary myocardium cells, smooth muscular cells, endothelial progenitor cells.

It has already been proved that the most promising and good stem cell source is represented by the bone marrow, containing several cellular populations. Beside the differentiated cells like stromal cells, vascular cells, adipocytes, osteoblasts and osteoclasts, there are also primary cells having stem cells properties. The last types are represented by HSC and MSC that have the ability to repair the injured organs and to create other types of tissues than the original one.¹⁻⁹ This heterogeneous cell population, existing in the bone marrow, has been named BMCs (bone marrow cells) and is represented by many HSC together with a variable number of MSC and progenitor endothelial

¹Floreasca Clinical Emergency Hospital, Bucharest, ²Department of Physiology and Immunology, Victor Babes University of Medicine and Pharmacy, Timisoara

Correspondence to:
Ana Maria Cristina Serban, Floreasca Clinical Emergency Hospital, 8 Calea Floreasca, Bucharest, Tel. +40-723254887, Email: spital@urgentafloreasca.ro

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cells (EPC).¹⁰⁻¹⁴

Injecting BMCs in the infarction area is improving the heart performances, by three possible ways: (1) developing new coronary vessels, which are saving the hibernating myocardium, (2) forming new cardiomyocytes and vascular structures and (3) activating or numerical growth of the progenitor cells at the heart level by the paracrine effects of the implanted cells.

Hematopoietic stem cells (HSC). In 1961, Till and McCulloch,¹⁵⁻¹⁷ have discovered bone marrow cells with a clonogenic capacity, producing hematopoietic colonies in the spleen. They supposed that these cells are multi-potential HSC, having the property of self-renewing, multi-differentiation and colony formation. HSC isolation has become available only in the last years when their superficial markers have been identified (c-kit+, Sca-1+, Thy-1-low). The CD34 antigen is used for selecting HSC, and AC133 is another marker very useful for isolating these cells. The selection of these cells, CD34+ or AC133+ may be made also from the peripheral blood and from the blood in the umbilical cord and placenta.

Bone marrow cells are identified using their superficial markers CD34 and CD45. They are stem cells, implied in the hematopoietic process of the cell series from the blood. The precursor stem cell (pluri-potential) is the original cell. It is supposed that the hematopoietic stem cells represent about 0.01% from the cells in the bone marrow. Extensive studies on HSC have found that these cells have a great multiplying, self-reproducing and differentiation capacity all over the three cell lines - granulocytic, erythroidic and megacariocytic.

The original cells (primary or stem cells) of the hematopoietical lines develop very early. After about 18 days from the egg womb implantation, they have been identified in the Wolff and Pander islands from the vitelin bag wall. Until the 5th month of conception, they have been found also in the hematopoietic tissues of the liver and embryonic spleen, these two places being considered as the main hematopoietic sites. In adults, the original cell is found in the wide bones marrow and in children, in the long bones marrow. In the peripheral blood flowing system, they may be also found albeit in small numbers (2-10 stem cells at 10⁶ lymphocytes). They may be also isolated from the blood in the umbilical cord and placenta.

There are several features, characterizing this type of cells:

a. They are multi-potential - they give birth to all cell populations in the blood system;

b. They are multiplying and generating descendants. This capacity does not ask for an antigenic stimulus. The descendants are maturing themselves being influenced by the poietin factors, some local humoral factors, denominated after the adult cells that are resulting due to their action: erythropoietin for erythrocytes, granulopoietin for granulocytes, lymphopoietin for lymphocytes etc.

c. Morphologically, they are identical with any immunocompetent lymphocyte. It is supposed that this feature explains the impossibility of selecting a single original cell and that is why it cannot be seen. The presence of the cell colonies is considered until now as the only possibility for identifying (more precisely, their late existence recording) and for this reason they have been denominated also as colony forming unit. Experimentally, the cells from the bone marrow are spreading in shells with buckets filled with nourishing medium. At the end of the incubating time, in the bucket, each cell is generating a cell colony having different populations (erythrocytes, thrombocytes, lymphocytes etc.);

d. Have a higher plasticity. There is evidence that parts of the heart may appear from sources of the bone marrow. Namely, the myocytes derived from stem cells of the bone marrow have been detected in the peri-infarct area but at a level of only 0.02%; in exchange, the endothelial cells derived from the bone marrow were met much more frequently (3.3%). These findings raise the hypothesis of the progenitor cells plasticity, despite the fact that only very rarely they give birth to cardiac muscular cells and much more often to endothelial or small myocytes cells, in a developing stage.

Due to the plasticity of the hematopoietic stem cells from the adult bone marrow,¹⁸ successfully grafting has been realized at the level of the damaged myocardium where they differentiate into smooth muscular tissue, endothelium and cells with cardiac phenotype, thus obtaining a functional improvement of the heart.

All these reasons are justifying the bone marrow to become the main source for stem cells, aiming the clinical cardiac recovery.

e. Is very sensible at the action of the radiations but is resistant at the action of cytostatics.

The importance of HSC for therapeutic aims derives from their high potential for self-multiplication and differentiation. The possibility of regenerating the tissues using these cells represents an intense concern in the current international research activity.

The so called "side population" (SP) is characterized

by their ability to fix the Hoechst coloring matter and by their capacity of self-renewing and re-setting of all hematopoietic cell lines.^{19,20}

Although having a low appearance rate prevalence (500 at 1 million total bone marrow cells), the so called "side population" (SP) - c-Kit+, CD34- represent the majority of the bone marrow cells having the capacity of renewing and forming all the hematopoietical lines.

SP cells derived from the bone marrow do not have markers of the differentiated endothelium (factor VIII, VE-cadherin) and also no markers for the endothelial progenitor cells (the receptors of the endothelial growing factor). In exchange, they express high levels of angiopoietin-1, its receptor - Tie-2, VEGF-A, the molecule of endothelial adhesion of the plaques -1 (PECAM) and CD31.¹⁹ Most of these features represent reminiscences of the hemoangioblast but also of the endothelial cells along the embryogenesis time.

When the cells c-Kit+ from the bone marrow were injected directly in the ventricle's wall, just near the infarct area, the result was their migration into the affected area, the differentiation into cardiomyocytes and vascular cells and a partial replacement of the infarcted myocardium. The attachment and the stabilization of the transplanted cells are produced at the border between the scar and the normal myocardium and were never detected in the absence of a myocardium injury.¹⁹

The *mesenchymal stem cells* or stromal cells - (MSCs) are also found in great number in the bone marrow, where they are differentiating in multiple types of mesenchymal tissues such as bone, medullary stroma, endothelium, cartilages, tendons, ligaments, adipose tissue and striated muscle.^{19,20} Thus, MSC are multi-potential non-hematopoietic stem cells of the bone marrow.

The notion of MSCs has been introduced in 1961 by Friedenstein who has documented that medullary stroma contains osteogenetic progenitors. These cells are strongly proliferative, capable of forming colonies of different sizes and densities, made of several mesenchymal lines. Between 1980 and 1990, MSC received a special position grace to the works of Caplan who showed that MSC are fibroblastic adherent cells, isolated by centrifugation in Percoll density gradient.²¹⁻²⁷

There is not a generally accepted definition of MSC. MSC may differentiate themselves in tissues that are different of those resulted from embryonic mesoderm, giving a question mark to the denomination of mesenchymal cells. However, most of studies are using the denomination of mesenchymal stem cells

or progenitor cells for the fibroblastic adherent cells obtained from the mono-nuclear cells of the non-fractionated bone marrow.

MSC express antigens that are reacting with the monoclonal antibodies SH2 and SH3 which are recognizing CD105 and, respectively, CD73. The disadvantage of this method for isolation is the contamination with HSC and of obtaining a heterogeneous produce. MSC phenotype expresses also antigens such as CD29, CD44, CD90, CD71, CD106, CD120a and CD124. In exchange, MSC are negative for the markers of the haematopoietical lines CD34 and CD45.²⁸⁻³¹

During the antenatal period, MSC are appearing in the aorto-gonado-mesonefros region, where they are set together with HSC. Moreover, they are also found in the embryonic blood system, including the blood in the umbilical cord and amniotic liquid.

In grown-ups, the bone marrow and the peripheral blood represent the main MSC sources. Only 0.001-0.01% from the cellular population of the non-fractionated bone marrow is represented by MSC.

Generally, MSC are sticking fast on the culture plates and grow, forming colonies that become visible at a week after the insemination. Some preparations may expand until 15 cellular doublings, while others are stopping their expansion after four doublings. MSC do not differentiate themselves spontaneously but only in the presence of growing factors and cytokines.

MSC are considered as an important cellular source for the recovery therapy and also for the genetic therapy. They are easy to be transfected with ADN or with plasmides and have a great capacity of surviving and differentiation after the transplantation.

MSCs are identifiable also using the superficial markers (c-Kit) and have better potential of forming the cardiomyocytes instead of the hematopoietic cells. Some studies are stating that MSCs cells from the bone marrow and not HSC are contributing mainly at the formation of new cardiomyocytes after the infarct.

In fact, the mesenchymal cells may be oriented, in some conditions, to the myogenic line, either in the culture or together with the fetal cardiomyocytes, as a result of a direct inter-cellular contact.

When treated with 5-azacytidine they may form cardiomyocytes, expressing specific proteins such as desmin, T troponin, fosfolamban and sarcomerical proteins.^{32,33} Mesenchymal cells at the level of the bone marrow may also contribute at forming of the new vascularization.

After injection in the left ventricular cavity, they have been identified especially in the liver, in the spleen

and in the lungs but a small number have been found at the level of the myocardium. Here, the expression of the cardiac contractile proteins has appeared, of the striations that indicate the sarcomerical organization and of the specific cardiac regulation for the calcium transportation from the sarcoplasmic reticulum. It is sure that the attachment of these cells at the heart level was more pronounced when they were injected directly in the ventricular wall after an ischemic injury.

Mesenchymal cells seem to be very advantageous for the cellular therapy due both to these properties and to their great number.

Multi-potential progenitor adult cells (MAPCs) represent a sub-category of the mesenchymal stem cells. They are a homogeneous population of fibroblastoid cells which are preserving their morphology in different stages of their differentiation and express vimentin, filaments of α -SM actin³² and α -actin (specific for the smooth muscle and, respectively, for the non-muscular cells), but do not express CD31 and desmin.

MAPCs may be obtained by cultivating the BMCs in selective culture medium after removing the CD45+ and glycoprotein A+ cells. When this cell population is set at a low density on plates covered with laminin in a medium that contain EGF and PDGF, MAPCs are appearing after several months at about 20 doublings of the population.

In vitro, MAPCs are differentiating in cells of the three germinative layers, including hepatocytes, endothelial cells and neurons. This multi-differentiation appears also in vivo when the cells are injected in the mouse blastocyst. However, in mice, MAPCs are adhering only at the level of the hematopoietical tissues, lungs, liver and intestine but not at the level of the heart. It is not established yet if the MAPCs injected in the myocardium differentiate themselves to the myocytic lineage.^{12,34-37}

It is supposed that all these progenitor cell types of the bone marrow described before have the capacity of differentiate themselves into cardiomyocytes both in vitro and in vivo, improving the global cardiac function. The differentiation is stimulated among others by specific inductors such as 5-azacytidine.³³

According to some studies, although the transplanted cells express contractile proteins, the differentiation to a cardiomyocytic phenotype is not certain.³³ After differentiation, all the implanted cells do not express connexin 43 or N-cadherin, and this fact raise a question mark concerning the settlement of the inter-cellular connections.

The lower the level of cells differentiation, the higher their pluri-potentialness and their capacity

of transforming its phenotype into the host tissue. Consequently, administration of the progenitors CD34+ or c-Kit+ - Lin - regenerate the infarcted myocardium by forming endothelial cells, smooth muscular cells and cardiac cells, but also by diminishing the apoptosis at 9-48 hours from the infarct beginning.

Studies made in vitro suggest that stem cells from the bone marrow have the capacity of fusing with the host cells and to take their phenotype.^{18,33} Consequently, it is possible for the fusing phenomenon to be partially responsible for the phenotypical conversion of the grafted cells. However, the phenotypical conversion of the CD34+ cells into endothelial cells and smooth muscular cells seem to appear as predominant through trans-differentiation.

After attaching at the level of the injured myocardium, having a high migration capacity, the cells from the bone marrow are spreading all over the infarct area, part of them reaching the peripheral border of the infarct and in a close junction with the sane myocardium. They are forming small long cells having central nuclei, without generating myotubes.

Some of the progenitor cells are participating in the angiogenesis process, being identified in the luminal area of the endothelium and expressing CD31.³² In fact, it is shown that the mixed cell population from the bone marrow (MSC, hematopoietical cells, endothelial progenitors) may induce the angiogenesis by bringing angioblasts, and also by bringing angiogenetic factors such as VEGF, β FGF and Ang-1.³² The vascular density growth, obtained this way, is maintaining the viability of the grafted cells and of the residual cardiomyocytes, improving the myocardial function.³²

On another hand, it was found that the cellular therapy using the autologous not-fractioned bone marrow (containing a complex mixture of cells: CD34+, CD34, endothelial progenitors and stromal cells) does not prevent the expansion of the left ventricle and does not improve the walls contractility.³³ The transplantation of the not-fractioned bone marrow (containing 60.8% \pm 1.3% mono-nuclear cells and about 1% mesenchymal cells) in the area of the post-infarct scar does not induce any differentiation of the transplanted cells into cardiomyocytes or endothelial cells, confirming the lack of the functional benefit. The histological analysis has revealed fibrous areas without the existence of a cardiac tissue derived from the transplanted cells.

The transplantation of the not-fractioned bone marrow seems however to be benefic after the infarct onset (60 minutes), when still exists a viable peri-infarct tissue that is sending the necessary signals to

the pluri-potential cells for differentiate themselves into cardiomyocytes or endothelial cells.³³

Therefore, the transplant could be efficient by angiogenesis and saving the reversibly injured host myocardium or generating new cardiac cells. The differentiation inductors do not exist anymore in the scar stage and, taking into account the existence of the extra-cellular fibrous matrix, it is possible for the grafted cells to be transformed into fibroblasts. The inefficacy of transplanting the pure bone marrow might be the result of the small percent of pluri-potential cells, which are “diluted” in the injected heterogeneous mass.

All these data are leading to the conclusion that the bone marrow cells administrated in the myocardium may induce an important tissue regenerating level, providing a functional improvement and, consequently, the cellular transplant therapy cannot be ignored.

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