

# CHEMOTAXIS AND DIFFERENTIATION PROMOTER FACTORS OF STEM CELLS INVOLVED IN CARDIOVASCULAR PATHOLOGY

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## REZUMAT

Fixarea celulelor suşă la nivelul miocardului lezat reprezintă o suită de fenomene înlănţuite. Ele se realizează cu ajutorul mai multor tipuri de substanţe aflate în organismul uman sau administrate exogen. Există substanţe diferite ce stimulează mobilizarea (chemotactismul) celulelor stem, adeziunea la nivelul endoteliului activat, transmigrarea la acest nivel şi invazia ţesutului ţintă. Prezentarea tuturor acestor tipuri de substanţe, precum şi precizarea rolurilor lor, arată complexitatea fenomenului. Cunoaşterea citokinelor şi a factorilor de creştere ce stimulează proliferarea şi diferenţierea celulelor stem sprijină cercetarea în vederea obţinerii in vitro a cardiomicitelor şi celulelor endoteliale.

**Cuvinte cheie:** citokine, factori de creştere, factori de diferenţiere, celule stem, transplant celular

## ABSTRACT

The attaching of the stem cells to the damaged myocardium is part of a complex mechanism and it implies many different factors. Some of them stimulate the mobilization of the stem cells (chemotaxis), some of them the adhering to the activated endothelium, other factors induce the transmigration and damaged tissue invasion of the cells. In this review, we try to describe all this substances by explaining their main actions. Besides, a good knowledge of this cytokines and growth factors that promote the proliferation and differentiation of stem cells helps the researchers to obtain cardiac muscle cells and endothelial-like cells in vitro.

**Key Words:** cytokines, growth factors, differentiation factors, stem cells, stem cells therapy

Cardiac disease is the main cause of death in the developed countries. Statistics show that the cardiovascular death rate exceeds cancer death rates. It is known that life expectation for the patients suffering of cardiac failure is less than 5 years. The classic treatment is palliative and does not really improve the lost cardiac mass in the cases of cardiac failure.

Cell therapy occurred as a new possible alternative at the heart transplant operation for patients with serious cardiac failure. Stem cells from different sources, capable of differentiation into smooth muscle cells were used to replace the dead tissue.

The attaching of the stem cells to the damaged myocardium is part of a complex mechanism and it implies many different factors. Some of them stimulate

the mobilization of the stem cells (chemotaxis), some of them the adhering to the activated endothelium, other factors induce the transmigration and damaged tissue invasion of the cells. The transmigration and invasion are most important for the functional integration of the cells.

All these phenomena take place either in the case of stem cell transplantation, or in natural conditions when the injured tissue secretes chemotaxis factors, which mobilize stem cells to the target spot where the differentiation process takes place, according to the other existent factors, present at the site.

The local molecular signals that stimulate the grafting of stem cells and their ability to respond to those signals seem to be very important. Namely, many chemotaxis and adhering factors secreted by the ischemic tissue play a prominent part in this phenomenon. However, all these mechanisms are poorly understood and different stem cells have different ways of action in recovering the damaged tissue.

On the other hand, a prominent part is played by the specific factors, belonging to different tissues, which are monitoring the differentiation of the stem

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cells for focusing to a given targeted cellular line.

Many of the mechanisms and molecules involved in heart development in embryos are the same as in the adult heart in special circumstances, as an answer to the stress signals made by the dilatation and failure of the heart.

It is well known that the development of the heart begins soon after the gastrulation, when a multi-potent non-differentiated cell population turns into a cardiac phenotype, stimulated by endodermic factors.

It is not sure that the signals which provoke the differentiation of stem cells into cardiac cells in the embryogenesis process (BMPs, anti-Wnts) are the same that decide cardiac “neogenesis”.

The cytokines that act on hematopoietic stem cells (HSC) are produced *in vivo* by bone marrow non hematopoietic cells and they may be grouped, by their effects, in three classes: (Table 1)

a. Cytokines that initiate the proliferation of HSC (the cell transition from G<sub>0</sub> phase into G<sub>1</sub> phase of the cell cycle): IL<sub>6</sub>, IL<sub>11</sub>, IL<sub>12</sub>, G-CSF, LIF (leukemic inhibitor factor), thrombopoietine, Flt3 ligand;

b. Cytokines which maintain HSC proliferation: IL<sub>3</sub>, IL<sub>4</sub>, GM-CSF;

c. Cytokines with a synergic action with those from the groups mentioned before.

**Table 1.** Citokines acting on HSC.

IL <sub>1</sub>	Regulator factor of B and T lymphocyte
IL <sub>2</sub> , IL <sub>15</sub>	Growth factor of T lymphocyte
IL <sub>3</sub>	Stimulator factor of hematopoietic stem cell colonies
IL <sub>4</sub>	Stimulation of B lymphocyte proliferation and of Ab secretion
IL <sub>5</sub>	Stimulation of B lymphocyte proliferation, of Ab secretion and of eosinophil maturation and proliferation
IL <sub>6</sub>	Stimulation of B lymphocyte differentiation, of Ab secretion
IL <sub>7</sub>	Stimulation of pre-B lymphocyte production
IL <sub>9</sub>	Erythroide colony forming, proliferation of megacariocyte lineage
IL <sub>11</sub>	Stimulation of B lymphocyte, stem cells, megacariocyte
IL <sub>12</sub>	Stimulation of NK lymphocytes, of interferon synthesis and Th lymphocytes proliferation
IL <sub>13</sub>	Like IL <sub>4</sub> , inhibiting of monocytic inflammatory cytokines production, stimulation of T lymphocyte proliferation

Ab= antibody

HSC mobilization factors - G-CSF (granulocyte colony stimulating factor)<sup>2</sup> and SCF (stem cell factor) are used for *in vitro* experiments in enhancing cardiac

regeneration by mobilization of stem cells<sup>6</sup>. The effectiveness of the method is not certain and the safety is doubtful, because it was noticed the augmentation of the coronary restenosis rate and the leukocyte number increase to a leukaemia level. As leucocytosis is directly responsible for restenosis by enlarging and destabilizing of the plaque, the researchers prefer methods that increase the number of circulating progenitor cells without a great inflammation. The mobilization cytokines may be useful for selective grafting of the progenitor cells.

Some other studies reported good results by mobilizing bone marrow stem cells using SCF and G-CSF<sup>14</sup>, without major complications. These experiments underline the stem cell chemotaxis and mobilization capacity of the injured myocardium.

It has also been noticed that stem cell mobilization by hematopoietic growth factors may promote myocardial angiogenesis, but it is not able to replace the injured myocardium. Endothelial cells have been detected but not myocytes and thus, the cardiac performance was not significantly enhanced by growth factors delivery.

The cell necrosis determines the release of chromatin regulating protein, of HMGB1 (high mobility group box protein 1), acting as an extra cell “danger signal” and being able to stimulate the positioning of the stem cells at the level of the target area. Extra cell HMGB1 attracts the mesoangioblasts *in vitro* and plays *in vivo* an important part in myocardial regeneration. The chemotaxis mechanisms of HMGB 1 are not very well understood and may imply receptors which are not known yet.<sup>1</sup>

It is possible that E-selectin and P-selectin have an prominent part in the stem cell chemotaxis and adhering process at the level of the ischemic lesions.

SDF1 (stromal cell derived factor - 1) is a key factor in regulating of the progenitor cell transportation to the ischemic tissues. The release of this factor may enhance the EPC (endothelial progenitor cells) recruitment and neovascularization<sup>1</sup>.

The integrines dependent adhering of EPC is one of the possible actions of SDF-1. Especially β2-integrine is most important for cell grafting and improving EPC dependent neovascularization.<sup>1</sup>

It was also noticed that VEGF and GM-CSF had raised the level of EPC and had improved the neovascularization. EPC mobilization is also dependent on multiple proangiogenic growth factors like angiopoietin - 1, placental growth factor, eritropoietin.

There are many other ways for increasing the EPC

number, including HMG CoA reductase inhibitor (statins), estrogens and physical exercise. Many studies confirmed the efficacy of these methods for the neovascularization improvement and endothelial regeneration.

Three peptides growth factors families have been mostly studied and considered decisive in conducting the differentiation of stem cells to cardiac lineage.

- BMPs (bone morphogenetic proteins)<sup>2,3</sup>;
- TGF $\beta$  (transforming growth factor- $\beta$ )<sup>2,3</sup>;
- FGF (fibroblast growth factor)<sup>2</sup>.

It has been demonstrated the essential part of BMPs in cardiac myogenesis and later in cardiac morphogenesis. Of course, cardiogenesis is stimulated by exogenous factors like TGF- $\beta$  and BMP2 and prevented by molecules that inhibit these factors mentioned above. In vitro, the stoppage of TGF- $\beta$  / BMP2 signals by peptides or noggin action interrupts stem cell differentiation.

When cultured in a medium containing adult cardiomyocytes and cardiac promoters, the stem cells differentiate themselves into ventricular myocytes that act synchronically with the host cells, this transformation depending by the stimulation of TGF- $\beta$  or BMP2.<sup>2</sup> In fact, the differentiation of the stem cells takes place only in the presence of the TGF- $\beta$  secretitive host cells.<sup>3</sup>

In vivo, cardiac stem cell transplantation is followed by cardiac differentiation proving an efficient action of TGF- $\beta$  / BMP2<sup>3</sup> signals. Thus, in the infarcted myocardium, the delivered stem cells differentiate in functional myocytes that become an integrant part of the surrounding tissue and increase cardiac performance.

TGF- $\beta$  promotes a cardiac genetic program in the stem cells. Thus, embryonic stem cell pre-treatment with growth factors induces the formation of embryonic bodies containing large area of cardiac differentiation, normally organized. In exchange, TGF- $\beta$  / BMP2 not responding stem cells remain not differentiated and they may even turn into malign tumours.<sup>2</sup>

TGF- $\beta$  growth factors act through an enzyme - GTP-ase, sending the cardiac differentiation signals to the embryonic stem cells.<sup>4</sup>

The cardiomyocytes and the cardiac fibroblasts are the main secretive cells of this family of factors TGF- $\beta$  and BMP2. This way, the host cells can induce cardiac differentiation of the stem cells using the paracrine way of growth factors.<sup>3</sup>

Always the host medium was sufficient to induce specific differentiation of the stem cells. In the heart,

the growth factors secreted by the cardiac fibroblasts or myocytes have stimulated the cardiac differentiation after a myocardial infarction.

There are also other factors regulating and monitoring the cardiac differentiation of the stem cells, such as: leukemic inhibitor factor and retinoic acid produced by the epicardium as inductor.<sup>5</sup>

PDGF-AB (thrombocytes growth factor) increases the induction of cardiac genes and the pace-maker cells aggregation in the bone marrow stem cell culture; the number of the cardiac myocytes is also increased at the level of the myocardial scar after delivering of bone marrow stem cells. The oxytocin influences too the Sca1<sup>1</sup> cells differentiation.

An interesting revelation is thymosin  $\beta$ 4 - a protein that is connected with the embryonic heart and with the wound healing. This protein promotes the migration of the cardiac cells, the survival and restoration of the myocardium

Azacytidin (5-azase)<sup>5,7</sup> is an activating key factor for the cardiomyocytic program in the bone marrow mesenchimal cells and Sca 1 cells. 5-aza acts through regulation of different genes expression<sup>7,13,14,15</sup> and in its absence the cardiac specific markers (connexin 43) will not appear anymore.

The neureguline family (peptide growth factor) and the tyrosinkinase receptors have also been proved to be involved in the embryonic myocytes growth.<sup>3</sup>

Nowadays, more than 60 growth factors and cytokines have been identified in order to regulate either the proliferation and specific differentiation of multi-potent stem cells, or the differentiated cell action. Surely, there are also other factors, unknown yet, which intermediate the growth and the differentiation of the stem cells into new cell types.

Although the molecular signals that guide the transplanted cells attachment are not fully understood, it is important to mention that the border area of the myocardium scar expresses most of the chemotaxis signals, including the cytokines and adhering molecules.

A deeper understanding of the heart development mechanisms in embryos stages would clarify the physiopathology and molecular foundation of stem cell differentiation into adult cells.

One can conclude that the paracrine factors play a major part in the mechanism of the functional recovery after the cell transplant. Clarifying the cytokines action on stem cells will make them able to be used for the clinical therapy.

The researches on cytokines and growth factors that stimulate the proliferation and the specific differentiation of the stem cells help the fundamental

research, which can use them to induce *in vitro* the differentiation of stem cells into myocytes and endothelial cells. Just in fact, cultivating HSC and MSC (mesenchymal stem cells) in the presence of VEGF, turns them into endothelial cells.<sup>9,10,11,12,16</sup> It is also proved that PDGF-BB and TGF- $\beta$ 1 are regulating the differentiation of the same cultured stem cells into cardiomyocytes.

Although the cell therapy is in its early years and the possible disadvantages have already occurred, taking these disadvantages as improving targets, this kind of treatment may become a new and promising solution for serious diseases.

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