KAPITEL 8 / *CHAPTER 8* [8]
THEORETICAL BASIS OF APPLICATION OF STEM CELLS TRANSPLANTATION IN CLINICAL PRACTICE

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Introduction.

Over the last few years, researchers have focused on stem cell (SC) biology. It must be pointed out that progressive development of embryology, hematology, neurobiology and skeletal tissues biology is possible due to a large number of experimental studies on SC isolation and description. Besides, completion of human genome decoding fostered further studies of dependence of gene expression in SC on duality of its status: self-renewal and differentiation.

SC research offers unprecedented opportunities for the development of the new methods and approaches in cell and tissue therapy for treatment of various diseases and pathologic conditions in humans. At the same time, it is necessary to perfect methods of full-scale SC cultivation with preservation of their undifferentiated state, develop methods and discover mechanisms of guided SC differentiation *in vitro*, as well as offer effective ways of their delivery to a damaged tissue or organ and evaluation of efficacy of this procedure. However, the risk of immune rejection of allogenic SCs and probability of malignization of the transplanted cell line still remain unsolved problems in this field.

Results And Discussion

Concepts of Stem Cell, Niche and Stromal Support

So, what is "stem cell"? At present, there is no official definition of this term. It is obvious that these are non-specialized and undifferentiated cells capable of both infinite self-renewal and production of cell progeny with more limited proliferative potential and limited production of different types of differentiated cells that can originate from them. In many tissues, further subpopulations of cells with limited and, in some cases, strictly limited self-renewal capacity are called either *transit-amplifying cells* (e.g. population of skin basal keratinocytes) or *progenitor* cells (e.g. mesenchymal

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progenitor cells of bone marrow). The latter can be classified as such in between the true SCs and their differentiated derivatives. The advantage of this cell product type is limited number of division cycles involving CSs during the whole lifetime of an organism. Thus, the unlimited self-renewal capacity is not in line with SC *in vivo*. At the same time, it is practically impossible to detect differences between a stem cell and a progenitor cell.

SC destiny dependsprimarily, and perhaps exclusively, on signals of different tissues microenvironment. Degree, time, location and specificity of their conditions of stay remain unclear for most tissues. However, it is considered that distributed or contact signals emitted by the surrounding tissues and intercellular matrix and regulating survival, cell cycle as well as differentiation of SCs and their progeny are often organized in two directions: *dormant and self-renewal niche* the role of which is suppression of cell differentiation process [1] and *stromal support* extending to cells at all stages and allowing for optimization of differentiation process of the cells unaffected by the niche [2]. For example, in case with human skeletal muscles, non-random distribution of satellite cells was shown, and the majority of them were located in the close vicinity of muscle capillaries. Such privileged position is likely to favor stromal support for the process of muscle differentiation, which [stromal support] mainly consists of macrophages the large numbers of which are attracted due to regeneration process induction [3]. In reality, satellite cells guide setting of their own stromal support from the moment of their exit from the influence of a dormant niche, by attraction of circulating monocytes that, following differentiation into macrophages, interact with myocytes in order to enhance chemotaxis, stimulate monocytic growth and facilitation of angiogenesis. This kind of stromal support attracts mitogenic soluble factors for myocytes and establishes intercellular antiapoptotic contacts.

After SC division, at least one daughter cell preserves the status of this divided SC. This property is called self-renewal. Symmetric divisions increase the number of SCs, while asymmetric divisions keep SC potential in one of its daughter cells and generate further differentiated generations in another daughter cell. Divisions generating generation of two differentiated daughter cells, destroy SC potential. At

different stages of ontogenesis and depending on microenvironment, possibility of SC division, both symmetric and asymmetric, and even full differentiation, can vary [4]. Until now, it is unclear whether microenvironment recognized by SCs through intercellular contacts and cytokines, chemokines and their receptors, affects the type of SC division and their differentiation potential [5, 6].

It should also be mentioned that SC self-renewal process can be defined as balance between the existing in it signal transduction systems (STAT-activation and ERK-activation) activated by gp130 receptor dimerization. In this case, LIF family (leukemia inhibitory factor family) cytokines act as a ligand. In other words, exogenic LIF is quite enough for self-renewal and support of undifferentiated state of embryonic SCs in culture [7].

In human body, SCs are identified in the embryoblast – internal cell mass of early gestation term embryos, in some fetal tissues, in the umbilical cord and placenta, as well as in some organs of an adult body. In the latter, SCs can give rise to more than one specialized cell type inside the organ itself: for instance, neural stem cells give rise to three cell types found in the brain - neurons, glial cells and astrocytes. SCs capable of differentiation into cells types of a certain tissue where they are usually present in a dormant state demonstrate their main property –*plasticity*. When SC gives rise to various cell types associated with different organs, *multipotent stem cell* is meant.

Stem Cell Classes

Schematically, SCs can be divided into classes depending on the stage of human body development, differentiation grade and histogenesis potential:

- *totipotent cells* – first eight zygote cells resulting from the first three divisions; each of them is capable of developing into a human body, days 0-4;
- *pluripotent cells* – mainly embryoblast cells (day 5), early stages of embryo development cells and cord blood cells; capable of giving rise to more than 200 cell types of the three main germ layers;
- *multipotent cells* – cells found at late states of fetal development, adult tissues and cord blood; capable of giving rise to specific cell types, e.g. bone marrow mesenchymal stem cells;

- *unipotent cells* – can differentiate into one cell type only, e.g. oval liver cells capable of differentiation only into hepatocytes;
- *specialized cells* – fully functionally differentiated and mature cells found in adult body[3].

Embryonic Stem Cells

Embryonic stem cells (ESCs) are isolated from early stage embryos. At blastocyst stage, embryo consists of 70 trophoblast cells and around 30 cells of blastula internal cell mass – embryoblast. The latter are, in fact, pluripotent SCs giving rise to all cell types of the main germ layers – ectoderm, mesoderm and endoderm. At present, it is possible to isolate these SCs from the blastocyst with a new immunosurgical method – lysis of trophoblastic cells by certain molecules of the immune system, while embryoblast cells are maintained undifferentiated in culture as cell lines. Primordial germ cells are also isolated from the developing genital eminence [3, 51].

In order to make these cell lines useful for cell therapy, cultured embryonic stem cells and primordial germ cells require guided differentiation into a certain tissue to be transplanted to a patient. Researchers just started studying possibilities of reaching this goal.

First successful culturing of human ESCs took place in Thomson J. laboratory in 1998 [8]. Under appropriate culturing conditions, ESCs demonstrate excellent capacity for continuous self-renewal and produce a large number of similar pluripotent cells. ESC linesderived from solitary cells have high proliferation speed – 300-400 cycles of population doubling cycles. Human ESCs cultured for longer than 2 years demonstrated stable and normal chromosome set. Unfortunately, these cells generate teratomas in immunodeficient mice [9].

Culturing conditions allowing for maintenance of an undifferentiated state of human ESCs require keeping them on a feeder layer – inactivated murine embryonic fibroblasts, medium with fetal bovineserum. Upon ESC isolation from the feeder and their placement into the suspension culture, these human ESCs form the so-called embryoid bodies – dense globular cell clusters that can give rise to a large number of cell types of all three germ layers – rhythmically contracting cardiomyocytes,

pigmented and non-pigmented epithelial cells as well as nervous cells with prominent axonal and dendritic growth [10, 11, 12]. In other experiments, cells isolated from human ESCs express genes associated with liver and pancreatic functions [11]. Co-culture of human ESCs with murine bone marrow-derived stromal cells produces colonies of human hemopoietic precursor cells and, eventually, blood cells [13].

Human ESC Potential for Regenerative Medicine

Potentially, human ESCs can be regarded as inexhaustible source of cells with *in vitro* differentiation capacity for transplantation therapy, including liver, nervous system, pancreas cells etc. ESCs can also be used for more successful organ transplantation: if human ESC-derived hematopoietic SCs are successfully transplanted into the recipient's circulatory system (with obligatory immune suppression), any other implanted tissues (kidneys, pancreas) induced from the same ESCs must not, in theory, be rejected by the recipient due to the fact that immunocytes produced by hematopoietic SCs in the recipient's blood will perceive the transplanted tissues as "own". But this is a long way, and none of the known research demonstrated any ESC-induced reconstruction of organ function*in vivo* either in human or in experimental animals. Moreover, ESC tissue cultures create heterogeneous mixture of cell types, for the control and differentiation limitation of which with biochemical, cultural and molecular biological methods further research is necessary.

It is also necessary to develop methods of human ESC transplantation and tracing of whether the transplanted cells are properly developing and functioning in a post-transplantation period. In some cases, it would be important to ensure that transplanted cells or tissues are involved and positioned in an appropriate way in relation to the existing tissues. Intercellular and cell matrix interactions will also play an important role in organ functioning.

Full-scale human ESC culturing will require the abandonment of growing cells on the feeder layer [12]. For this, mechanism of ESC differentiation repression mechanisms of feeder cells must be discovered in order to find the alternative, thus minimizing the risk of transfer of animal viruses to ESCs via feeder.

Limitations and Risks of Clinical Application of Human ESCs

The two most significant risks of practical application of ESCs are as follows: tumor formation and immune rejection. It is known that human ESCs implanted to mice can cause soft tissue tumors. Presumably, such response is due to pluripotency of undifferentiated cells *in vivo*. However, in a number of works, no significant tumor formation is reported [12]. Nevertheless, thorough monitoring of integrity of the existing human embryonic cell lines is necessary for discovery and understanding of the long-term culturing effect [14].

Rejection is another serious hindrance for successful transplantation of SCs and tissues derived from them. Presumably, ESCs provoke lesser immune reaction than the whole organ transplantation. Some cell types (e.g. dendritic cells, immunocytes, endotheliocytes) express larger numbers of histocompatibility antigens than all other cells. All these types can be found in the whole organ tissues as they connect the organ to the blood stream and nervous system. However, ESC-derived tissues *in vitro*, e.g. liver tissues, should not contain the above cell types and, theoretically, the immune response is supposed to be more moderate.

It can also be assumed that ESCs could be made less reactive through the use of genetic engineering methods through elimination of their superficial antigens [12]. Reasonable compromise between the recipient and ESC-derived tissues transplanted to him/her can theoretically be reached with methods of therapeutic cloning via somatic cell nuclear transfer (SCNT) into the enucleated oocyte for the purpose of creation of histocompatible ESCs. This method is also used for reproductive cloning, but, in this case, its goal is creation of an embryo that is genetically identical to a donor of a somatic nucleus that can hypothetically be implanted into a donor uterus. However, such cells cannot be transplanted to patients with genetic diseases because they will carry the identical genetic information. In any case, understanding of how rejection of transplanted cells can be prevented is one of fundamental issues of the regenerative medicine.

It is still too early to dwell on the possibility of clinical application of human ESCs. It might be possible to add growth factors to transplants for stimulation of production of a certain cell type or several cell types. “Inductor tissues” interacting

with SCs can be transplanted together with ESCsfor reaching this goal. In other words, certain stromal support must be artificially provided for the adequate differentiation of such non-specialized cells into the final required phenotype. However, at present these opportunities are a subject of experimental research.

Trophoblast Stem Cells

Provided human trophoblast SCs (trophoblast stem cells) exist, then it is obvious that they should originate from cytotrophoblast layer of diploid trophoblast cells between syncytiotrophoblast and epiblast [15]. Human trophoblast SC lines can have a very powerful therapeutic potential, e.g. production of clinically useful hormones or cell therapy based on them for patients with placental insufficiency. Most likely, these cells can be found at later stages of placental development rather than in a blastocyst.

Postnatal Stem Cells

Apart from hematopoietic and intestinal cells regarded as SC paradigm for many years, adult body contains some other SC classes (adult stem cells):

Hematopoietic SCs: source – bone marrow; cell types produced – blood cells, endotheliocytes, liver oval cells, myocytes.

Neural SCs: source – brains; cell types produced – neurons, astrocytes, oligodendrocytes, blood cells.

Epithelial SCs: source – intestine, epidermis; cell types produced – all cells of epithelial crypt, all cells of epidermal layers.

Mesenchymal SCs: source – bone marrow; cell types produced – osteoblasts, chondrocytes, tenocytes, adipocytes, myocytes, bone marrow stromal cells, neural cells[3].

Some tissues contain specific SCs, some – multipotent SCs. For instance, skeletal muscles contain satellite cells committed to muscle phenotype at the time of differentiation *in situ*. Some epithelial cells can be regarded as stem cells, but strictly committed to epidermal differentiation. Possibly, SCs are a part of the larger reparation systems of most mammal tissue types and, most likely, of tissues of all types of vertebrate animals.

A fundamental question of cell biology regarding cell renewal in the body is

whether SCs develop from predetermined cells programmed for proliferation or from multipotent SCs with high plasticity. In spite of their large proliferative potential, SCs can remain dormant until the tissue is damaged or degraded, which is a regenerative signal for them. This has been proven both *in vitro* and *in vivo*. The cells committed to a certain line are often regarded as *committed transient* cells. These cells can be predisposed to farther expansion, like blast cells, or proliferate like multipotent cells. Thus, for each system, be it cellular or tissue, understanding of relations between expansion through proliferation and functional orientation is quite important for characterization of SC activity level. One of the key issues of modern SC biology is understanding of molecular basis of cell lines orientation – when cells become irreversibly committed to the terminal phenotype, in spite of the remaining fullness of the genome [3].

The idea that SCs remaining after early embryonic stages of development are limited by the capacity to form only that cell type, which is natural for the tissue they belong tois currently under critical scrutiny. In other words, there are data that precursors of oligodendrocytes can regain the status (dedifferentiate) of multilinear neural SCs and, thus, depending on the conditions they are in, neural SCs preserve wider range of properties [3, 16]. Besides, it has been shown that hematopoietic stem cells (HSCs) have the potential to restore the population of hepatocytes [17]. It seems that both muscle and neural tissue can be source of HSCs, and that bone marrow (BM) can, *inter alia*, contain muscle precursor cells [18, 19]. Moreover, BM stroma containing mesenchymal stem cells (MSCs) can also give rise to neurons and glia [20, 21].

Bone Marrow Stem Cells

BM of an adult contains hematopoietic (1-2%) and stromal (<0,05%) stem/progenitor cells [22]. Blood forming cells can be classified as HSCs (hematopoietic stem cells) capable of long-term continuous reconstruction of all the hematopoietic system and progenitor cells capable of short-term (1- 2 months) reconstruction [47]. HSCs give rise to different cell lineages one of which (endothelial) is capable of differentiation into cardiomyocytes. BM stromal SCs include adult MSCs

(mesenchymal stem cells) and multipotent adult progenitor cells (mesenchymal progenitor cells), and both these types are capable of multilinear differentiation [23]. In culture, MSCs stably maintain undifferentiated phenotype. These cells can be induced, for instance, for transdifferentiation into cardiomyocytes by the medium of 5-asocytidine or DNA-demethylating agents. Animal studies also demonstrated that MSCs have a potential for site-specific differentiation into cardiac muscle cells. Multipotent adult progenitor cells, also known as mesenchymal progenitor cells (MPCs) are capable of differentiation into multiple cell lines, including endothelial cells.

Bone Marrow Hematopoietic Stem Cells

This subsection does not cover issues related to reconstruction of the pool of hematopoietic CD34+ cells in hematopoietic diseases, because they are well known. However, it is necessary to point out that CD34+ population is the foundation of BM SC plasticity. However, HSCs are not limited only by CD34+ population, but the role of the rarer CD34- population in hematopoietic reconstruction and regenerative biology is not quite clear. Here, we would like to shed the light on some aspects of HSC biology, their potential and high plasticity in terms of differentiation/transdifferentiation into non-hematopoietic cell types on the example of endothelio- and cardiomyogenesis [24].

Human BM and peripheral blood contain CD34+ endothelial progenitor cells capable of proliferation and differentiation into the new vessels and adult cardiomyocytes in case of systemic administration or direct transplantation into the cardiac muscle [25]. Hypothetically, BM also contains hemangioblasts – precursor cells of hematopoietic and endothelial lines with neovascularization potential. When transplanted, lin- c-kit- cells that are not stem cells, are not capable of regeneration of cardiomyocytes, while lin- c-kit+ cells can generate cardiac cells, smooth muscle cells and endothelial cells [26, 27]. Non-hematopoietic (CD34-) subpopulation of BM cells, consisting of AC133+ cells, also has high angiogenic potential. In other words, AC133, CD34, Lin- and c-kit+ can be regarded as key markers identifying the most useful BM SC subpopulations for cardiac regeneration [28].

Thus, BM mononuclear fraction contains a large number of cells potentially capable of restoration and regeneration of the damaged myocardium with much greater effect than any other isolated cell lines. Hence, BM heterogenous mononuclear fraction is the most effective SC population for cellular cardiomyoplasty.

Cell separation based on cell exclusion of nuclear dyes Hoechst 33324 and Rhodamine 123, allows for identification of both replicating and dormant HSCs [29]. The latest data are indicative of unprecedented plasticity of these cells. For instance, when transplanted to mice, populations of presumably muscular SCs (satellite cells) contribute to hematopoiesis [30]. Purified HSC populations, on the contrary, contribute to myogenesis. The most extreme example of SC plasticity is transdifferentiation of cultured clones of neural SCs into murine hematopoietic cells [3, 30]. These cells appear to be re-educated in unknown ways by the local microenvironment, thus acquiring their new purpose.

Bone Marrow Mesenchymal Stem Cells

Here we will dwell on BM-derived substrate-dependent multipotent cells that can be multiplied *ex vivo* as homogeneous population giving rise to multiple differentiated connective tissue cell line. This is human MSC population, and this term was first use by Arnold Caplan in 1991 [31].

Flow cytometry with the use of a large number of different surface markers demonstrated that multiplied BM MSC population is more than 98% homogeneous, and under certain *in vitro* conditions, these cells easily differentiate into multiple connective tissue cell lines, including osteoblasts, chondrocytes and adipocytes [32]. As the result of orthotopic *in vivo* implantation of these cells, tissues of the above lines were obtained. Besides, MSCs can either produce or be induced to produce cytokines supporting hematopoietic cells [33]. Co-culture of MSCs with HSCs demonstrated that MSCs or human adipogenic MSCs can support HSC viability or even multiplication, while MSCs play the role of functional stroma [34].

Expression of cell surface proteins is often used for characterization of different cell types. These surface molecules are to varying degrees responsible for hetero- and homotypic interactions between cells types, and they also act as receptors for growth

factors, cytokines or intercellular matrix. Expression of these molecules by MSCs is tested via RT-PCR (reverse transcriptase – polymerase chain reaction) of mRNA, and the results are confirmed by flow cytometry. Many classes of human MSC surface molecules are known and listed below:

Specific Antigens: SH2, SH3, SH4, STRO-1, alpha-smooth muscle actin, MAB1740, Thy-;

*Cytokines and Growth Factors:*interleukins 1α, 6,7,8,11,12,14 and 15, LIF, SCF, Flt-3-ligand, GM-CSF, G-CSF, M-CSF;

Cytokine and Growth Factor Receptors :IL-1R, IL-3R, IL-4R, IL-6R, IL-7R, LIFR, SCFR, G-CSFR, IFNγR, TGFßIR, TFGßIIR, TNFIR, TNFIIR, bFGFR, PDGFR, EGFR;

*Adhesion Molecules:*ICAM-1, ICAM-2, VCAM-1, ALCAM-1, endoglin, CD44 (hyaluronan receptor), integrins αVβ3, αVβ5, integrin chains α1, α2, α3, α4, α5, αA, αV, β1, β2, β3, β4, LFA-3, L-selectin;

*Intercellular Matrix Molecules:*collagens types I, III, IV and VI, fibronectin, laminin, hyaluronan, proteoglycans [3, 51].

Absence of certain surface molecules also helps to characterize human MSCs. It is known that there is no expression of hematopoietic markersCD14, CD34 and CD45 as well as endothelial markers - von Willebrand factor and P-selectin in cultures of dispersed cells. And although no specific molecules have been found that would unambiguously identify human MSCs, the list of surface molecules gives a key to understanding of signals and interactions that can stimulate cell response and cell differentiation.

In culture, multiplication of solitary human MSC to $1x10^6$ is represented as 21 population doublings, and progeny of at least some of them give rise to colonies maintaining their multipotency. When analyzing the karyotype of 12 passages of MSCs that underwent 30 population doublings, no aberrations were found. Are MSCs capable of indefinite division? At present, there are limitations of human MSC multiplication, such as slowing of culture proliferation rate, changes in the population of multiply dispersed cells, appearance of the large spread cells that presumably don’t divide

(cellular senescence). The question whether conditions fit for indefinite multiplication of MSCs will be found remains open. However, the need for isolation of as many multipotent MSCs as possible during the current procedure is not of paramount important for research or clinical purposes. According to Pittenger et al. [32], more than 1 x 10^9 of MSCs were isolated from 25 ml of aspirated BM by the third passage with the potential for further dispersion.

Tissue Regeneration with MSCs

For the first time, the concept of BM SCs for connective tissue was presented by Owen in 1985, and it is based on the assumption that differentiated cell types in BM stroma can originate from either some common progenitor cell or SC. Later, this concept was developed by Caplan with inclusion of all the lines isolated from mesoderm, including myocytes, chondrocytes, tenocytes, osteocyte as well as dermal and stromal fibroblasts [31, 35]. More recent proofs suggest that stroma consists of differentiated and undifferentiated cells of several lines, and that in BM human MSCs co-exist with progenitor cells (MPCs) with lesser differentiation potential. Presence of multipotent MSCs in BM is in accordance with the data collected by many laboratories [36, 37, 38].

MSCs were used for demonstration of *in vivo* reparation of mesenchymal tissues on critically sized wounds orthotopically [39]. For instance, as specified by Wakatani et. al. [40], they observed reparation of joint cartilage in medial femoral condyle of a rabbit. Caplan et. al. demonstrated MSC integration into skeletal muscle of dystrophin-deficient mice, which offers opportunities of using MSCs in muscle dystrophies [3, 31].

Lately, attention is focused on the possibility of MSC transplantation into myocardium with their further differentiation. Intracoronary administration of genetically modified human MSCs into the heart of immunodeficient mice was performed. It was demonstrated that solitary MSCs had engrafted in the recipient's myocardium and were surrounded by its healthy cardiomyocytes. These cells survived for at least 2 months. After some time, the morphology of the implanted human MSCs was the same as that of cardiomyocytes surrounding them, and they started expressing

proteins specific for striated muscles, including desmin, α-myosin heavy chain, α-actinin and phospholamban at the level of the recipient's cardiomyocytes. No MyoD transcription factor was detected, which suggests absence of differentiation into skeletal myocytes, therefore the cells most likely differentiated into cardiomyocytes [41]. Such results indicate that human MSCs can acquire cardiomyocytic phenotype, which suggests that cellular cardiomyoplasty is an effective procedure for stunned or damaged heart tissue.

MSC Transplantation Possibility and Options

The main issue of MSC application in clinical practice is possibility of their transplantation, determination of progenitor cell type (*committed* or*uncommitted*) suitable for transplantation as well as optimal MSC delivery option – direct delivery (injection or implantation) or systemic infusion. The first option seems to be the one of choice for clinical strategy aiming at larger local restoration or regeneration of bone, cartilage, or tendon [3, 42, 43, 44]. On the other hand, MSC delivery with blood stream can be useful for restoration of not only local, but also systemic tissue disfunction via re-starting of their own development program. When it comes to SCs, there are opinions that MSC infusion results in their selective homing in the areas of bone marrow stoma, which, as a result, leads to better functioning of hemopoiesis-supporting stroma, thus making HSC involvement and differentiation easier [45, 46].

Whether the transplanted material is multiplied *ex vivo* in order to increase the number of progenitor cells should also be taken into consideration. This could be an important factor because there is certain divergencein stemness and functions in case of cell sub-culturing. As for hematopoietic and muscle systems, it has been demonstrated that stemness of the transplanted cells is determined by either long- or short-term repopulation of the damaged tissue. It is still unclear whether all MPCs (committed and uncommitted) are capable of supporting both long- and short-term mesengenesis.

First clinical trials demonstrated that systemic *ex vivo* infusion of dispersed autologous MPCs is possible and suitable for short-term perspective [3, 46]. On the other hand, allogenic BM transplantation (as a source of HSCs and MSCs) to children

with osteogenesis imperfecta results in significant histologic changes in trabecular bone, which is indicative of the new dense osteogenesis [3]. Accelerated growth rate and reduced frequency of bone fractures was also reported. These changes reported 3 months after transplantation are due to engraftment and functioning of the transplanted BM [47].

As an integral part of bone marrow stroma, MPC transplantation, both separately and in combination with hematopoietic progenitor cells, will favor HSC engraftment after myeloablation. It should be clarified whether MPCs have potential to substitute for stroma damaged by chemotherapy or disease, or whether they will be useful for treatment of other diseases [3].

At the same time, just like precursor cells of some mesenchymal lines, MPCs can be used as effective therapy for reduction or correction of some mesenchymal tissues damage, including osteogenesis imperfecta, osteoarthrosis, muscle dystrophy. Modern studies demonstrated the possibility of gene transfection via adeno- and retroviral vectors, therapeutically useful genes in MPCs, which will be a great contribution to extension of clinical application of such modified cells [48].

Opportunities for Clinical Use of Allogenic MSCs

BM-derived human MSCs represent a useful, readily available, and relatively well-characterized population for mesengenesis research, and it is obvious that these cells can be used for allogenic transplantation. Human MSCs express small number of I class molecules of the main histocompatibility complex and almost don’t express II class molecules and B7-costimulating molecules important for initiation of an antigen-specific immune response. *In vitro* experiments with lymphocytes from different donors indicate that human MSCs do not cause T-cell proliferation and are, in fact, capable of suppression of mixed lymphocytic reaction, which is in favor of clinical use of human allogenic MSCs [49]. Absence of an obvious immunologic response to implanted human allogenic MSCs and capacity to produce large numbers of cells from a small amount of BM aspirate make it possible to use donor cells for many recipients. Maybe, one day human BM MSCs would be used for restoration of not only mesodermal, but also endo- and ectodermal tissues.

Factors Negatively Affecting Regeneration of the Transplanted MSCs

What factors can negatively affect the restorative potential of the transplanted MSCs? There are two main ones: loss of the contact with microenvironment and/or loss of telomers. When cells are deprived of their normal stromal microenvironment, they can launch the program of differentiation at the time of migration to the affected area and thus loose their restorative potential, since they are no longer in the appropriate intercellular contact and are no longer receiving stimuli from the necessary cytokines and chemokines preserving their stemness. Apart from this, the increasing number of cell divisions (symmetric and asymmetric) can result in shortening of telomers each time when telomerase activity reduces or completely disappears [3, 50, 51].

Conclusions

Last decades of active scientific research in the field of regenerative biology brought about the hypothesis about the existence of stem cells that was represented as a modern concept. Several more decades of fundamental research will be needed for this concept to develop into clearly justified and properly organized stem cell theory. At the same time, lack of information about SC biology at this stage is not a hindrance for pursuing therapeutic usefulness of these cells, which is attractive for a wide range of clinical needs in the context of both cell and gene therapy, with both ESCs and adult SCs. However, in case with ESCs, success and progress of their further study and possibility of clinical application will largely depend on the tolerance threshold in legal and ethic aspects of work with embryonic tissues being now set forth by the public opinion of high-tech countries.