REVIEWS

Mesenchymal Stem Cells

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The formation of the concept of a mesenchymal stem cell (MSC) is a priority of Russian biological science. A. Ya. Fridenshtein and his colleagues were the first who experimentally proved the existence of MSC. Osteogenic potential of fibroblast-like bone marrow cells of different mammalian species was demonstrated [25,26]. Fibroblast-like bone marrow cells often formed discrete adhesive colonies *in vitro* [27,28,47]. After hetero- and orthotopic transplantation *in vivo* cloned cells from these colonies formed bone, cartilaginous, fibrous, and adipose tissues [48]. Intensive self-renewal and multipotency of fibroblast-like colony-forming cells from the bone marrow allowed Fridenshtein and Owen to formulate a concept of multipotent mesenchymal precursor cells (MPC) [62].

An ordered chain of finely regulated cell proliferation, migration, differentiation, and maturation processes underlies the formation of the majority of cell lineages in adult organisms. The earliest cell elements in this chain are stem cells (SC). Along with extensive self-renewal capacity, SC possess a great differentiation potential. Apart from well studied hemopoietic and intestinal SC, other SC classes were recently discovered in adult organism.

Until recently it was considered that SC in adults can give rise to cell lines specific to tissues where these cells are located; however, new facts necessitated revision of this concept. Hemopoietic SC capable of differentiating into all cell elements of the blood, can also be a source of hepatic oval cells [65]; neural SC, precursors of neurons and glia [2,3], serve as the source of early and committed hemopoietic precursors [10]. MSC, a source of bone, cartilaginous, and adipose tissue cells, can differentiate into neural cells [46]. Tissue growth and reparation are associated with migration of uncommitted precursor cells from other tissues. During muscle tissue reparation mesenchymal SC migrate from the bone marrow into skeletal muscles [24].

Hence, in addition to capacity to unlimited division and reproduction of a wide spectrum of descendants of a certain differentiation line, adult SC are characterized by high plasticity. The existence of a rare type of somatic pluripotent SC, common precursors of all SC in an adult organism, is hypothesized [79]. Another important characteristic of SC is their migration from the tissue niche into circulation, which was experimentally proven for hemopoietic and MSC [69,73]. For activation of the differentiation program, circulating SC should get into an appropriate microenvironment [75,78].

A potent stimulus for investigation of SC is the possibility of their clinical use in cell and gene therapy. The bone marrow contains multipotent MSC, which can be easily isolated and cultured *in vitro*. It is therefore interesting to analyze some fundamental aspects of MSC biology and the possibilities of their clinical use.

MSC descendants are involved in the formation of bones, cartilages, tendons, adipose and muscle tissues, and stroma maintaining the hemopoiesis [12,19,51]. The term MPC is used to denote MSC and their committed descendants capable of differentiating into at least two types of mature cells, which are present in the bone marrow and some mesenchymal tissues [16,19,57,82].

Bone Marrow MPC

The bone marrow is the most frequent source of MPC. Mononuclear cells transferred into a culture

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medium(at low density) form colonies of adhesive cells, which serve as the source of MPC. Morphologically these colonies consist of fibroblast-like cells [13,26]. Only about 10% MPC actively proliferate [17]. The content of MPC in adult bone marrow is 2-5 cells per 10^6 mononuclears [45]. This parameter decreases with age. Despite their high proliferative potential, MPC retain normal karyotype and telomerase activity after short-term culturing *ex vivo* [66]; long-term culturing leads to MPC aging and apoptosis [17,20]. The immunophenotype of MPC is not unique: these cells carry markers characteristic of mesenchymal, endothelial, epithelial, and muscle cells. MPC do not express CD45, CD34, and CD14 antigens of hemopoietic cells [66].

MPC produce some hemopoietic and nonhemopoietic growth factors, interleukins, and chemokins. Some cytokines are produced constitutively, others only after stimulation [33]. MPC express receptors of some cytokines and growth factors. These data suggest that bone marrow MPC contribute to the formation and functioning of stromal microenvironment, which produces inductive and regulatory signals for not only MPC, but also hemopoietic precursors and other nonmesenchymal SC in the bone marrow [14,42,70]. MPC produce matrix components, such as fibronectin, laminin, collagen, and proteoglycans [15,66,67]. They express some counter-receptors involved in cell-cell and cell-matrix interactions. For example, MPC actively express CD44 receptor for hyaluronan and osteopontin, which play the key role in the organization of extracellular matrix in the bone marrow and bone tissue, respectively [55,56,81].

Study of the differentiation potential of MPC *in vitro* and *in vivo* showed that bone marrow MPC undergo terminal differentiation and can form bone, cartilage, muscle, nerve, and adipose tissues, tendons, and hemopoiesis-maintaining stroma [6,19, 24,46,47,64].

Uncommitted MPC in MPC Cultures

In stationary bone marrow cultures MPC are presented by a minor population of small agranular cells (RS-1 cells) with low colony-formation capacity not expressing Ki-67 proliferating cell antigen. By their antigenic profile, resting RS-1 cells differ from rapidly growing committed precursors [16]. High proliferative potential of committed precursors manifests in the presence of RS-1 cells. RS-1 cells proliferate under the effect of factors secreted by the most mature MPC, *i. e.* are a subclass of uncommitted MSC capable of recycling. In bone marrow cultures, 5-fluorouracyl-resistant cells are characterized by a low RNA content and intensive expression of ornithine decarboxylase gene (marker of nonproliferating cells) [37,38]. Resting cells and MPC not treated with 5-fluorouracyl are almost identical by their antigenic profile: resting cells express adult SC marker CD117 [54,80]. Resting cells represent a population of uncommitted MPC, because they do not express Cbfa-1 and PRAP-γ-2 markers typical of cells committed to osteo- and adipogenesis, respectively [66]. After long-term incubation with embryonic bovine serum slowly proliferating resting cells give rise to rapidly growing terminally differentiating committed precursors. Clones of cells with SC characteristics were obtained, whose growth is maintained by fibroblast growth factor-2; they do not differentiate into mesenchymal cells in the absence of conditions for commitment [39].

Committed Precursors in MPC Cultures

Clone analysis of bone marrow-derived MPC showed that 30% clones can differentiate into osteo-, chondro- and adipocytes, while others are differentiated into osteo- and chondrocytes or possess only osteogenic potential [57]. A clone of MPC (BMC-9) was isolated, which under appropriate conditions can differentiate into cells with phenotypical and functional characteristics of osteoblasts, chondrocytes, adipocytes, and hemopoiesis-maintaining stromal cells [19].

Bone marrow represents a depot for uncommitted MSC. These cells possessing self-renewal and differentiation potentials supply the organism with mesenchymal cells throughout life [12].

Tissue Sources of MPC

Primary cell culture isolated from human skeletal muscles contains stellate cells and multinuclear myotubules. In culture suplemented with equine serum stellate cells grow without signs of differentiation, but in the presence of dexamethasone they differentiate and acquire phenotype of skeletal and smooth muscles, bone, cartilaginous, and adipose tissue [31]. Hence, both committed and uncommitted MPC are present in skeletal muscles. MPC were also found in the myocardium of newborn rats. In the presence of dexamethasone these adhesive stellate cells differentiate into adipocytes, osteoblasts, chondrocytes, smooth muscle cells, skeletal muscle myotubules, and cardiomyocytes [76]. It was shown that the population of precursor cells in the skeletal muscles originates from uncommitted bone marrow MPC and differs from myogenic satellite cells [24].

Several subclasses of MPC were identified in primary human bone tissue culture. Cells expressing stromal precursor cell marker STRO-1 and not carrying osteoblast marker alkaline phosphatase have the preosteoblast phenotype, because they are characterized by low capacity to the formation of mineralized bone matrix and do not express osteopontin and parathyroid hormone receptor. Other subclasses are represented by intermediate and terminally differentiated osteoblasts and originate from STRO-1-positive cells not expressing alkaline phosphatase [32]. Nonimmortalized cloned cells isolated from human trabecular bones can differentiate into mature osteo- and adipocytes. The type of differentiation is determined by the presence of inducing factors, such as long-stranded fatty acids, interleukin-1β, tumor necrosis factor-α, and transforming growth factor-β [61]. Cell clone RCJ 3.1 isolated from rat fetus clavicle is differentiated into mesenchymal cells of different phenotypes. In the presence of ascorbic acid, β-glycerophosphate, and dexamethasone multinuclear myocytes appear. They are followed by adipocytes, chondrocytes, and mineralized nodules of bone tissue. The population of granular cells from the rat fetal periosteum exhibits properties of uncommitted MPC. These cells divide slowly, do not express differentiation markers, and *in vitro* give rise to chondro-, osteo-, adipocytes, and smooth muscle cells [29].

The cartilaginous tissue is characterized by limited reparative potential, presumably due to low content of MPC or differentiation and growth factors. It was assumed that MPC committed to chondro- and osteogenesis migrate to cartilaginous tissues from other tissue sources [11].

Achilles tendon cells from young rabbits retain expression of tenocyte differentiation markers, type I collagen and decorin, in primary cultures and after the first passage but change their phenotype during further culturing [9]. The tissue source and commitment conditions for tendon MPC are unknown [40].

Adipose tissue SC include adipocyte precursors at different stages of maturing. Stromal vascular cells are the least differentiated adipocyte precursors in adipose tissue. Along with bone marrow-derived MPC, these cells can differentiate into adipocytes in the presence of glucocorticoids, insulin-like growth factor-1, and insulin [68]. Stromal vascular cells can differentiate into both adipo- and chondrocytes [50]. Bone marrow adipose tissue contains cells differentiating into adipocytes or osteoblasts [63].

Blood vessels are usually formed from endothelial tubes coated with smooth muscle cells/pericytes originating from undifferentiated perivascular MPC [34]. These cells express smooth muscle α-actin and receptor for growth factor of a platelet origin. They can differentiate at least into smooth muscle cells.

Development of MPC. Mobilization and Microenvironment

It is important to know whether MPC detected in various tissues are constantly present there or their pool is replenished due to migration from the bone marrow. It was recently shown that MPC are present not only in adult bone marrow and other mesenchymal tissues, but also in umbilical blood. Similarly to adult bone marrow MPC these cells adhere to plastic; they do not differ from adult MPC by morphology, expression of cell surface and cytoplasm antigens, and differentiation potential. Umbilical blood MPC cultures contain 5-10% resting cells - uncommitted MPC. Their content in the umbilical blood is inversely proportional to fetal age, *i. e.* MPC migrate into different tissues during early ontogeny [23]. Apart from distant migration along blood vessels, MPC migrate locally in tissues during cartilage reparation, muscle regeneration, and other reparation processes [8,11].

The maintenance of SC compartment depends on externally modulated cell autonomic regulators, in particular, factors controlling asymmetrical cell division, expression of genes determining stages of commitment and number of cell divisions [56]. The fate of SC is determined by external signals from the microenvironment or SC niche [4,5]. Information exchange between uncommitted and committed precursors and between precursors and neighboring cells is realized within this niche. It was hypothesized that along with mesenchymal and nonmesenchymal cells and their products (growth factors and extracellular matrix molecules), selective modulators (differentiation inductors) participate in the formation of spatial and time relationships in the MPC microenvironment. MPC niches formed after local injuries to mesenchymal tissue qualitatively differ from those in intact tissues characterized by regular physiological regeneration of MPC pools. This difference is extremely important for specialization of cell phenotype in normal and damage-induced microenvironments [75]. More detailed study of mesenchymal microenvironment is essential for basic support of programs of clinical cell transplantology using MPC.

MPC in Ontogeny

The main events of embryogenesis (differentiation, growth, and morphogenesis) are realized with participation of SC of different differentiation lineages.

The need in SC is maximum at this stage of ontogeny. Later, their role is determined by physiological demands of the organism in cell renewal and compensation of tissue damage. Starting from postnatal ontogeny, the number of SC gradually decreases. By the age of 40 years the osteogenic potential of human MSC decreases to a minimum [21].

MSC in Immunity Modulation

Human MSC express or can be induced to expression of molecules mediating antigen-specific interactions with T lymphocytes [18,66], in particular adhesion molecules (VCAM-1, ICAM-1, L-selectin, CD72, and LFA-3) and classes I and II HLA molecules. MSC are characterized by low expression of ICAM-1 and HLA class II molecules and higher expression of HLA class I molecules. Proinflammatory cytokines interferon-γ, tumor necrosis factor-β, and interleukin-1β stimulate expression of these molecules. MSC do not express costimulatory M7.1 and B7.2 molecules. Resting allogenic T cells cultured with human MSC do not proliferate and do not express T cell activation molecules CD25 and CD134 [43]. The absence of proliferative response can not be explained by alloantigen deficiency, because MSC were treated with interferon-γ before culturing with allogenic T cells. Retrovirus transduction of B7.1 or B7.2 in MSC does not restore proliferative response. MSC pulse-labeled with tetanus antitoxin induce hyporeactivity of T cells of the corresponding specificity [71]. However MSC are not tolerogenic for resting alloreactive T cells. If T cells were cultured with allogenic MSC or irradiated mononuclear cells of MSC donor for 7 days and after 3 days each culture was restimulated with irradiated mononuclears, the kinetics of secondary immune response in these cultures was similar [44]. Hence, MSC can prime resting T cells without inducing their proliferation.

Indirect presentation of MSC alloantigens can occur *in vivo*, therefore it is important to evaluate the capacity of activated T cells to respond to MSC. Activated T cells do not proliferate in response to allogenic MSC even if MSC are treated with interferon-γ and transduced with costimulatory B7 molecules. Activated T cells cultured with MSC for 7 days and 3 days without stimulatory cells, did not proliferate in response to irradiated peripheral blood mononuclear cells, but produced interleukins-2, -4, and –10, growth-transforming factor-β, tumor necrosis factor-α, and interferon-γ [44]. Removal of $CD8^+$ cells canceled areactivity. Hence, MSC induce $CD8^+$ suppressors in the population of activated T cells [52]. T cell activation via indirect presentation of MSC alloantigens followed by confrontation with MSC does not lead to further T cell expansion and includes a suppressor component preventing MSC rejection.

MSC inhibit proliferation of alloreactive T cells in a mixed lymphocyte culture. This was observed after addition of MSC at the start and at the intermediate stage of 7-day culturing [43]. The effect of MSC is not genetically restricted.

Immunosuppressor nature of MSC allows their use not only for tissue reparation and replacement, but also for preventing undesirable immune reactions. For example, addition of MSC to bone marrow cells intended for transplantation can stimulate graft take and attenuate graft-versus-host disease [60]. MSC integrated into organs subjected to autoimmune attack or into donor organs intended for transplantation can provide local immunosuppressor or tolerogenic environment in the organism without changing systemic immune reactions.

In a canine model MSC retained viability in allogenic recipients during at least 6 weeks without infiltration with host cells or inflammatory response in the implantation site. In baboons, single intravenous injection of MSC prolonged the life span of allogenic skin transplants by 50% [7].

Clinical Use of MSC and MPC

The prospects of clinical use of MSC and other MPC are determined by their capacity to repair defects in mesenchymal tissues. MPC seem to be promising for replacement cell therapy, because they can be easily isolated from bone marrow aspirates, cultured, and transfected with exogenous genes. MPC differentiate into different cell types (osteo-, chondro-, tendo-, adipocytes, *etc*.) depending on microenvironment. The strategy is determined on the type of the defect. Local implantation of MPC is more preferable in local tissue defects, while their injection into the circulation is effective only in systemic dysfunction of organs and/or tissues.

In clinical practice MPC were first used for the treatment of a patient with degenerative arthritis. MSC isolated from the patient bone marrow were cultured and injected directly into the joint [12]. Locally injected MSC promoted repair of cartilaginous tissue of rabbit knee joint after surgical damage. In rodents MSC incorporated into ceramic carcass promoted healing of bone defects [30].

MPC injected into systemic circulation repopulate not only the bone marrow, but also other tissues. MPC from normal mice intravenously injected in high doses to young mice with mutation in type I collagen gene replaced of up to 30% cells in the

recipient bone and cartilaginous tissue [64]. These results prompted clinical use of MPC for the treatment of bone defects in children with severe forms of incomplete osteogenesis caused by mutations of type I collagen gene. After depletion of bone marrow cells the recipient children received the bone marrow from HLA-compatible healthy donors. The authors believed that nonfractionated bone marrow contained sufficient amount of MSC cells for compensation of severe bone defect [49]. The same strategy (transplantation of allogenic bone marrow) was used in the treatment of children with incomplete osteogenesis. Transplantation led to positive histological changes in trabecular bones, accelerated growth, and decreased incidence of bone fractures [58]. Positive clinical result was attained in the treatment of a girl with hypophosphatasia, who received allogenic bone marrow and osteoblasts from her father [22].

Development of experimental approaches to the use of genetically modified MSC is in progress. After transfection of factor IX gene in human MSC and subsequent transfer of transfectant cells to immunodeficient SCID mice, secretion of this factor was observed for 8 weeks after transplantation [41]. In transfectant cells factor IX was posttranslationally modified with γ-glutamyl carboxylase. The therapeutic level of factor IX persisted in the plasma for 12 days after injection of MSC transduced with a retrovirus factor encoding human factor IX to a dog with hemophilia B [36]. Mouse MSC secreting human interleukin-3 effectively maintained hemopoiesis in immunodeficient mice after simultaneous injection with human hemopoietic SC. These transfectant cells effectively maintained hemopoiesis determined by donor SC for more than 9 months [59].

Human MSC can be used in clinical transplantology for promoting expansion of hemopoietic SC and their early precommitted descendants. After high-dose drug therapy, patients with breast cancer were injected with autologous hemopoietic stem cells and MSC. The counts of peripheral blood neutrophils and platelets were restored after 8 and 9 days, respectively [72].

Conclusion

Recently, considerably progress was attained in studies of MSC and MPC biology and development of strategy of clinical use of MSC. Methods for isolation, culturing, and *ex vivo* growth of these cells, and evaluation of their differentiation potential were developed. Numerous molecular markers of these cells were characterized, but unique marker of MSC was not identified.

Therapeutic use of MSC and their early committed descendants appears to be a promising trend. Donor MSC can be used for restoration of bone marrow stroma after radiation and chemotherapy and in combination with bone marrow cells for restoration of hemopoiesis. The use of MSC in incomplete osteogenesis seems to be promising. One more perspective trend is replacement MSC-therapy aimed at repair of locomotor defects caused by traumas or diseases. The development of this trend is stimulated by progress in bioengineering, specifically in construction of matrix biomaterials or biomimetics forming carcasses populated MSC descendants [48].

Correction of genetic defects of mesenchymal tissues with genetically modified donor MSC is undoubtedly perspective [35]. Tolerogenicity of MSC allows their use for allotransplantation and opens prospects for MSC therapy of autoimmune diseases.

Of the recent publications devoted to clinical use of MSC therapy we should like to draw attention to the use of umbilical blood cells as a source of MSC [53]. High content of MSC in biopreparations obtained at early stages of ontogeny determined the good prospects of their utilization. Isolation of MSC from fetal bone marrow and liver seems to be effective. Theoretically, a possible limitation for the use of this biomaterial is inadequate microenvironment of SC in fetus and adult organism (different SC niches). However, speaking about stimulation of a regenerative process, which is in fact a phenomenon of embryogenesis recapitulation, we can expect that adult microenvironment is adequate for fetal MSC. The capacity of fetal SC to integrate into vital systems of adult recipients was demonstrated for SC of other differentiation lines [1].

Hence, pioneer investigations of A. Ya. Fridenshtein and his colleagues gave an impetus for studies of MSC characteristics. By the present time we passed a long way from fundamental research to clinical application of MSC. Conditions for optimization of methods for therapy of many heretofore incurable diseases are now created.

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