Modern Views on the Problem of Stem Cells and Potentialities of Their Use in Medicine

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Almost one hundred years ago a well-known Russian histologist A. A. Maksimov put forward a hypothesis on monophyletic origin of blood cells, thus forming the basis for the theory of ancestral (stem) cells [13]. The development of modern methods of investigation (radiation hematology, cytogenetics, molecular biology, cell cultures) directly confirmed Maksimov theory. J. E. Till, E. A. McCulloch, D. Metcalf, and other scientists known for their research in hematology [3,7,15,23] played the key role in this process. The sequence of events in the course of differentiation of a universal polypotent hemopoietic stem cell was clearly characterized (Fig. 1).

After discovery of hemopoietic stem cell similar cell populations were found in other tissues, *e.g.*, the scientists now actively investigate the properties of mesenchymal precursors.

The priority in the formation of the mesenchymal stem cell (MSC) concept belongs to the representatives of Russian biological science. The existence of MSC was first experimentally proven by A. Ya. Friedenstein *et al.* in the 1970s [10]. These scientists detected the osteogenic potential of bone marrow fibroblast-like cells in different mammals, the capacity of these cells to form discrete colonies of adherent cells *in vitro*. After heterotopic transplantation these cells formed bone, cartilage, fibrous, or fatty tissue *in vivo* [6,10].

These results promoted studies of cells superior to all other dividing elements by their proliferative potential and providing tissue renewal over the entire life span of the body. When it became clear that these cells (their common name is stem cells) can be artificially introduced into the body, attempts were made at the use of this technology (which was at that moment in fact at the primordial stage of experimental and clinical studies) with commercial purposes. However, today only bone marrow transplantation in cancer and hematological diseases and creation of umbilical and stem cell banks are officially approved.

Disagreement on the functional characteristics and potentialities of practical use of stem cells are largely caused by confusion of terms. Strictly speaking, the term "stem cell" denotes a very narrow range of totipotent cell elements. The notion "totipotent" denotes the capacity of these cells to differentiate into all, without any exclusion, cells of the body. However, in literature this term is used to denote all cells parental for at least one tissue and capable of self-maintenance (*i.e.* formation of similar cells by division). We consider that it is necessary, first, to clearly discriminate between the notions of stem cell in an adult organism and embryonic stem cell (ESC).

Theoretically, ESC possess the greatest therapeutic potential. The history of their investigation started from the moment, when it was hypothesized that teratocarcinomas originated from early totipotent embryonic cells [21]. Apart from teratocarcinoma elements, blastocyst and embryonic sex tubercle cells can serve as ESC sources [2]. It seems that use of the blastocyst is the optimal technology for ESC derivation (Fig. 2). At the first stage, proliferation of cultured blastomers is stimulated, which leads to the formation of the so-called embryonic bodies, whose elements retain totipotency and, as the majority of scientists think, unlimited self-maintenance capacity. ESC obtained directly from the embryonic bodies or subjected to directed differentiation can be used for cell therapy [2,4].

Sometimes the notion of ESC is used incorrectly. In some clinics (or stem cell banks) this term is used to denote cells isolated from fetal organs. In

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these cases the isolated cells naturally represent a heterogeneous population and the majority do not possess the properties of stem cells.

Use of ESC can be associated with numerous side effects, the most hazardous of which is the risk of tumor development, particularly teratocarcinoma. This is explained by great similarity between stem cells and tumor cells (unlimited capacity to proliferation and poor differentiation), while ESC do not undergo normal "conditioning" process in the recipient body, which is realized during normal fetal development. However, their clinical application (as a rule, commercial) is extremely tempting, and many clinics and cosmetic centers practice the use of fetal cells for the treatment of some diseases, repair of cosmetic defects, "rejuvenation" of the body, *etc.*, despite the fact that it is illegal.

On the other hand, ancestral cells can be derived not only from fetal tissues. According to modern notions, elements with high proliferative and differentiation potential are also present in adult organism. These are mainly the so-called "regional stem cells", retained in organs during the postnatal period and ensuring physiological and reparative regeneration. During alteration they migrate to the zone of injury, divide and maturate, forming the appropriate tissue cells; that is, due to these cells the tissues are restored by restitution [5,8].

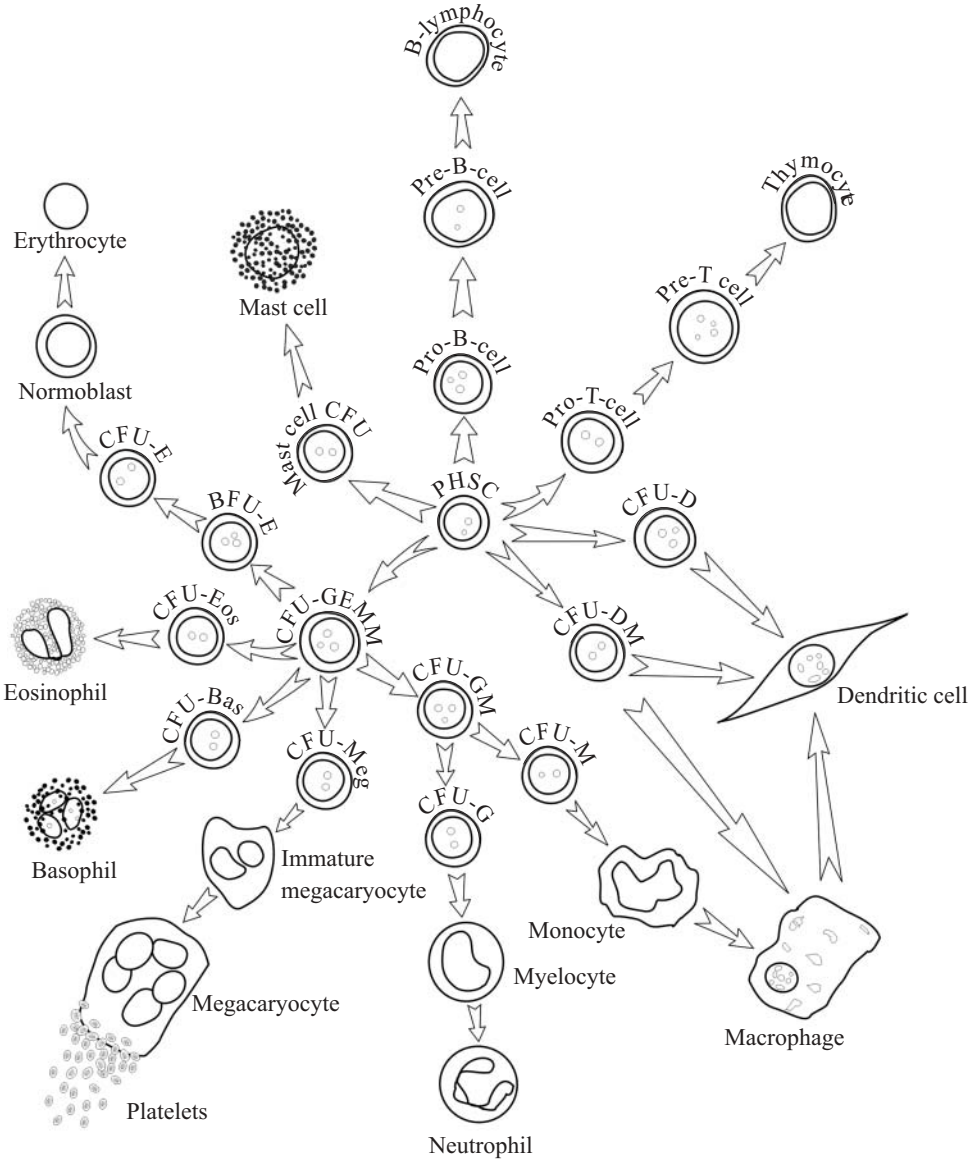

Fig. 1. Scheme of hemopoiesis. PHSC: polypotent hemopoietic stem cells; colony forming units (CFU) of: GEMM: granulocytes, erythrocytes, monocytes, and megacaryocytes; GM: granulocytes and monocytes; DM: dendritic cells and macrophages; D: dendritic cells; E: erythrocytes; G: granulocytes; M: monocytes; Eos: eosinophils; Bas: basophils; Meg: megacaryocytes; BFU-E: erythroid burst-forming units.

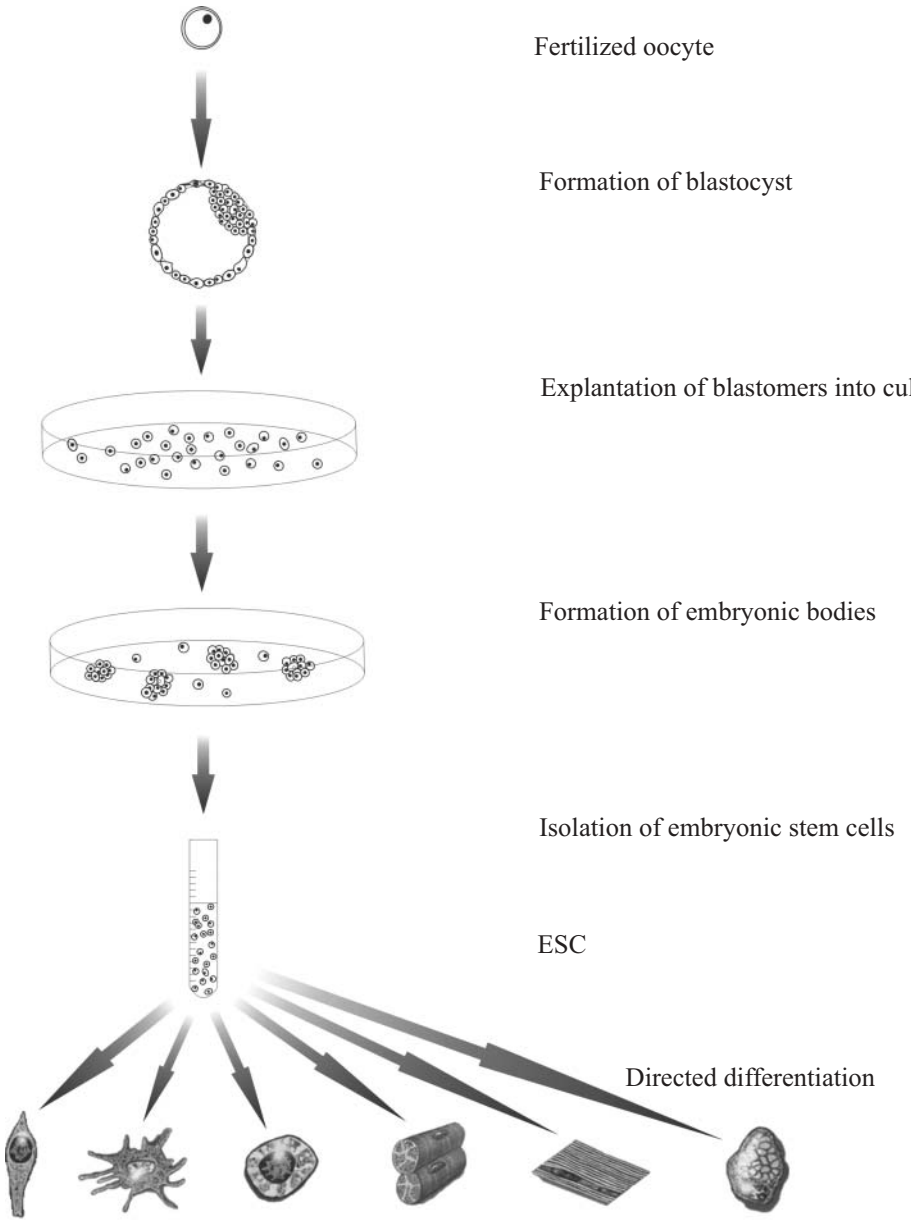

Fig. 2. Derivation of embryonic stem cells (ESC).

Though under common conditions and *in vitro* the regional precursors develop only into cells of the organ from which they were isolated, many scientists consider them multipotent. These stem cells possess a very important property: plasticity. Their differentiation spectrum in certain diseases or in recipient organs after transplantation is wider than the spectrum of a regional precursor. It was shown that bone marrow stem cells transdifferentiate into neurons in the brain and into typical myocytes in skeletal muscles. Regional stem cells of muscles, epidermis, intestinal epithelium, liver, *etc.* are also multipotent [2,5,12].

A very small number of totipotent precursors, capable of differentiating into virtually all cells, derivatives of all three embryonic leaflets, is hypothesized to exist in an adult body. According to this hypothesis, these cells are located in different organs, but they can develop not only in the direction of the tissue in which they are located [2,25]. It is unclear what is meant here: high plasticity of regional stem cells, their capacity to trans- and dedifferentiation, or it is the "relic" cells, similar to ESC, which are retained in an adult body [3,11,20].

No doubt, MSC are best studied multipotent stem cells today (Fig. 3) [17]. Due to their high plasticity, bone marrow MSC can transform, besides the connective tissue cells, into various types of muscle cells and into nerve tissue elements. Our experiments indicate that some MSC in culture under

the effect of the appropriate cytokines can give rise to hemopoietic colonies. It was also reported that under certain conditions MSC can differentiate into some epithelioid tissues. These characteristics indicate that MSC is the optimal material (among adult stem cells) for cell therapy of diseases.

MSC can be isolated from different sources: from fatty, bone, muscle tissues, according to some data even from cartilage and tendons. The bone marrow is as a rule regarded as the optimal source of MSC. Along with more mature cells, it contains uncommitted MSC characterized by a high capacity for self-renewal and great differentiation potential [5]. However, clinical use of this material is fraught with a certain risk of undesirable effects. The use of purified bone marrow cell suspension does not preclude the transfer (together with the transplantation material) of the microenvironment, essential for progenitor cell development in the undesirable direction. This was demonstrated by experimental heterotopic transplantation of the bone marrow, carried out by A. Ya. Friedenstein and M. Tavassoli

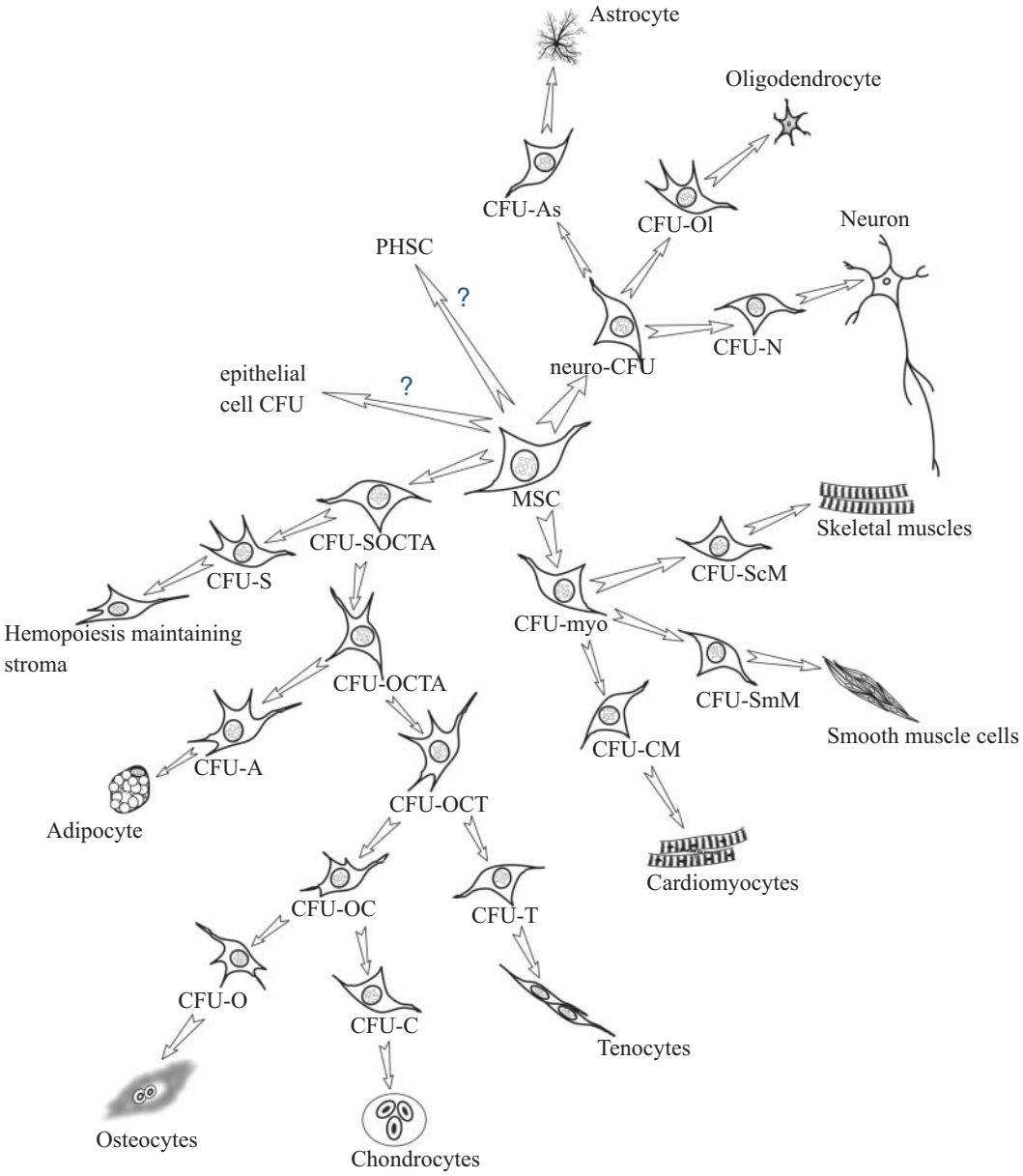

Fig. 3. Scheme of mesenchymal stem cell (MSC) differentiation. PHSC: polypotent hemopoietic stem cell; colony forming structures of: neuro: nervous tissue cells; N: neurons; As: astrocytes; Ol: oligodendrocytes; myo: muscle cells; ScM: skeletal muscles; SmM: smooth-muscle cells; CM: cardiomyocytes; SOCTA: stromal cells, osteocytes, chondrocytes, tennocytes, adipocytes; OCTA: osteocytes, chondrocytes, tennocytes, adipocytes; OCT: osteocytes, chondrocytes, tennocytes; OC: osteocytes and chondrocytes; S: stromal cells maintaining hemopoiesis; O: osteocytes; C: chondrocytes; T: tendon cells; A: adipocites.

[6,22]. Transplantation of bone marrow cells, for example, under the renal capsule led to the formation of the bone tissue at the site of injection, into which hemopoietic elements of the recipient mouse then migrated.

MSC culturing *in vitro* with the aim of inducing the irreversible differentiation in the desired direction presumably leads to reduction of their proliferative potential and hence, therapeutic efficiency.

We hypothesize that these undesired effects can be eliminated if we imitate natural functioning of stem cells in the body in manipulations with stem cells. An important feature of stem cells is their capacity to leave the tissue niche and circulate in the blood stream, which was experimentally proven for hemopoietic cells and MSC [17,19]. It is known that under certain conditions requiring triggering of regeneratory processes, the content of precursors in the blood sharply increases under the effect of host regulatory systems. For unfolding further differentiation program, circulating stem cells should get into appropriate microenvironment [17,24].

Methods for pharmacological mobilization of hemopoietic stem cells (stimulation of their release into the blood from the bone marrow) are now known. The efficiency of transplantation of hemopoietic precursors isolated from the blood is higher than that of bone marrow cells [9,14]. Experimental data indicate that MSC mobilized by means of cytokine preparations possess therapeutic activity *in situ* in some disease, *e. g.* in myocardial infarction [1,17, 18]. All these data indicate that methods of noninvasive manipulations with endogenous stem cells deserve special attention.

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