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Research Article

Stem cells and mesenchymal stem cell markers

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Abstract:
Stem cells are undifferentiated cell type characterized by colonogenic ability, self-renewal and multi-lineage differentiation. They are classified into the following categories: embryonic stem cells [ESC], somatic stem cells [or adult stem cells] and induced pluripotent stem cells [iPSC]. Stem cells represent area of interest for wide range of scientists, as they are promising tool for regenerative therapy. Their differentiation ability is significantly affected by various factors of the local environment. Additional research will provide more information about the optimal cell culture conditions when stem cells are cultivated for clinical purpose, to avoid side effects like uncontrolled cell proliferation and premature differentiation.

Keywords: stem cells, mesenchymal stem cells, stem cell markers.

Stem Cells:

Stem cells are undifferentiated cell type characterized by colonogenic ability, self-renewal and multi-lineage differentiation [1,2]. Depending on the specific environment and culture conditions, these cells can keep their stem cell properties or can differentiate into various cell types [2]. The process of differentiation is complex and modulated by various external factors and intracellular signaling pathways.

Undifferentiated cells in combination with active molecules [i. e. local and systemic growth factors] and extracellular matrix [ECM] are the basis of tissue regeneration. It has been found that these undifferentiated cells are capable of initiating new specialized cell lines and promoting protein synthesis [3,4]. Detailed research of the stem cell would lead to significant progress in regenerative therapy. The introduction of these cells into clinical practice can contribute to a faster recovery of damaged organs and their function due to different pathologies.

In 1964, Till and McCulloch investigate murine bone marrow cells that are able to retain their ability of self-renewal and form colonies after radiation exposure [5]. Later, Friedenstein [6] demonstrate the presence of a small population of precursor cells in bone marrow different than the hematopoietic cells. these cells were able to initiate ectopic bone formation. They are small fibroblast-like cells that adhere to plastic cell culture dishes. Caplan [7] name them Mesenchymal Stem Cells [MSC], based on their origin. In addition, hematopoietic stem cells and endothelial cell stem cells are also known [8].

First of all, bone marrow MSC have been broadly investigated, as they still represent cell of strong scientific interest [9,10]. For this purpose, researchers usually compare the properties of various isolated MSCs to those of bone marrow derived MSC. MSC are isolated from humans and various animal species: i.e. mice [11], pigs [12], monkeys [13], etc.

Stem cells are classified into the following categories: embryonic stem cells [ESC], somatic stem cells [or adult stem cells] and induced pluripotent stem cells [iPSC]. ESC are pluripotent and are derived from the internal cell mass or blastocyst [14]. They can be transformed into cells of all three germ layers [ecto-, endo- and mesoderm] and can proliferate for a long time without undergoing differentiation stage. The term "embryonic stem cells" has been proposed to differentiate embryonic pluripotent stem cells from those derived from teratocarcinomas [11]. In 1981, Martin for the first time managed to isolate the ESC from the inner side of blastocyst [11]. As a result of ethical considerations, experimental work with this cell type nowadays is limited.

Another type of stem cell is so-called iPSC. They are obtained by genetic transformation and cell reprogramming of somatic cells [15]. Like the ESC, they are able to differentiate into cells from all three germ layers [16]. The genetic modifications of the iPSC place them at a potential risk of undesirable modifications in their properties, such as uncontrolled proliferation and limited potential for tissue regeneration [17].

The application of ECS and iPSC in therapeutics poses high risk of tumorogenesis and additional side effects [18]. However, the benefit of conducting in vitro and pre-clinical studies with both cell types should not be underestimated. This can give answers to many questions arising, related to the effects of various active molecules [including growthfactors] and antitumor agents.

Adult postnatal or somatic stem cells [SSC] could be found all over the body, presented as colonies of undifferentiated quiescent cells. They have more limited differentiation potential when compared to embryonic ones and serve to

maintain and restore organs when damaged, i.e. provide the tissue homeostasis [19]. Typically, CCS are expected to undergo specific differentiation towards the cell type, identical to those of their tissue of origin [16]. Wide range of studies has recently revealed their ability to differentiate into cells of different tissue origin [20,21]. Their differentiation ability is significantly affected by the local environment. Additional research will provide more information about the optimal cell culture conditions when stem cells are cultivated for clinical purpose.

Within the past 20 years, the so-called Cancer stem cells [CSC] have been found [22]. They possess properties quite similar to the normal stem cells, including the ability of self-renewal and differentiation into various tumor cell subtypes. CSC are capable of initiating tumor growth and metastasis. There are new therapeutic methods proposed for neoplasm management, aiming to suppress the function of the CSC [22,23].

Mesenchymal Stem Cells [Msc]:

The ability of MSK of plastic adherence allows their detailed in vitro investigation [20]. The adhering properties provides their separation from non-adherent hematopoietic cells. Some hematopoietic cells also initially adhere to the bottom of cell culture dishes [24]. However, subsequently they are detached and washed away, as only the fibroblast-like cells remain in the dishes. The isolated MSC cultures are heterogeneous, as endothelial cells, smooth muscle cells, macrophages, osteoblasts, and all other cells having the ability to adhere, could be presented in the culture.

Somatic MSC have more limited differentiation potential when compared to ESC. However, MSC could be isolated withouth ethical concerns and no data about spontaneous tumorigenesis in vivo after their application has been reported. Many scientists are still searching for the optimal and the most appropriate site of the human body that could serve as a promising sourse of MSC. Appart from bone marrow [25], these cells are isolated from placenta [26], blood [27], fat [28], etc. In 1999, Pittenger [29] isolates and cultivates human bone marrow MSC. Their ability to differentiate into osteogenic, adipogenic and chondrogenic types have been demonstrated. Subsequently, more studies have been carried out to prove their stem cell properties and multilineage differentiation.

The in vitro cell culture conditions are of primary importance for the fate of the isolated and cultivated stem cells. Usually cells are cultivated in autocrine media supplemented with basic active substances for cell growth. Fetal calf serum [FCS] is frequently added as it is a powerful stimulator of cell proliferation and growth. It is a product of animal origin and the long-term cell cultivation in FCS-containing medium is associated with high risk of immune reactions and infections when isolated for medical purposes [30]. Use of FCS should be avoided when MSC are isolated and prepared for clinical use, as the risk of adverse reactions and complications should be eliminated. In broad spectrum of studies FCS substitutes, such as insulin-trasferin-selenium-ITS or platelet aggregates [TK] [31] are applied. All the researches have concluded that stem cells can be successfully cultivated in serum-free media.

MSC can affect the immune response. It has been shown that stem cells are able to inhibit the production of proinflammatory cytokines such as TNF-α [tumor necrosis factor-α] and IFN- γ [interferon-γ], and on the other hand, they increase the synthesis of anti-inflammatory cytokines [IL-4, IL -10 [interleukin]] [32]. MSC are suggested to be applied in the management of host-versus-graft reaction in transplanted patients and to stimulate the process of graft incorporation into the recipient site. Clinical experiments have already been carried out using MSC for the treatment of host-versus-graft reaction [32].

MSC are very susceptible to gene modification. They may act as carriers of specific genes and can form depots of anti-tumor agents in the tumors themselves [33]. However, this field of research is still not well investigated.

All the features that MSC possessed make them ideal candidates for the development of cellular regenerative therapy. This would allow the development of new, biologic and less invasive approaches for the management of various congenital, degenerative diseases, traumas and tumors. Results from clinical trials with MSC are currently insufficient and have to be supported by broad spectrum of experiments including large number of patients [1]. There is insufficient data about the factors and mechanisms responsible for the phenotypic expression of MSC properties, as well as about the cell response to different signals and molecules [2]. Of primary importance to the regenerative stem cell therapy is the creation of optimal in vitro culture conditions for MSC prior to their clinical application. The environment should eliminate the risk of uncontrolled cell proliferation or irregular cell differentiation after implantation of stem cells. Many studies investigate the effect of various active molecules on the proliferation, differentiation, and protein synthesis in MSC [24,34,35]. However, many issues still remain unclear.

Mesenchymal Stem Cell Markers:

The routine application of MSC in different clinical fields requires detailed information about their characteristics. An accurate knowledge of all regulation stages, from the gene expression to protein production and markers expression, is required. Isolated mesenchymal cells represent a heterogeneous cell culture where they differ in their morphology, proliferation rate, differentiation potential and expression of surface antigens. A number of surface and intracellular markers are described in the literature and could be routinely used for cell characterization and investigation of their stem cell phenotype [24]. Many experiments are conducted to detect a single surface marker expressed equally by all MSC. However, there is no unique marker found in all stem cells, as their phenotype significantly varies between different cells and species.

The International Society for Cellular Therapy [ISCT] defines the minimum criteria that MSC must possess [36]. These are: 1. ability of adhering / adhering to surfaces of cell culture dishes under appropriate conditions; 2. multipotency, - the ability to differentiate into various cell types - osteoblasts,

adipocytes, chondrocytes, etc. in vitro; 3. expressing markers typical for MSC such as CD73, CD90, CD105 and lack of CD11b, CD14, CD19, CD34, CD45, CD79α makers expression.

Characterization based on these cell surface proteins, called clusters of differentiation [CD] is a very important step in stem cells research. Human MSC do not express hematopoietic and endothelial markers [CD133, CD11] [9]. A very common surface marker for characterization of MSC, especially those of dental origin, is STRO-1. It interacts with cell surface antigens and non-hematopoietic bone marrow cells [37]. Antibodies against CD73, [membrane-bound ecto-5'-nucleotidase], CD90 [Thy-1], CD105 [endoglin], CD166, CD271, CD146 also interact with undifferentiated mesenchymal cells [2,9]. CD146 is one of the major markers for perivascular and multipotent progenitor cells in human connective tissue. It is also typical for endothelial cells, smooth muscle cells, schwann cells, and some neoplastic cells [38]. The exact function of this protein is not fully understood but is known to be relevant for cell morphology, adhesion, cytoskeleton reorganization, migration, transmembrane signaling, etc. A number of studies have shown that genuinely CD146-positive cell populations have all the characteristics of MSC [39,40]. Another typical marker for MSC is CD271 [low affinity nerve growth factor]. An interesting fact is that its expression in some cells decreases after prolonged cultivation in vitro [41]. Many of the surface markers applied for MSC characterization, are also used to identify CSC. Some of the commonly used for this purpose are CD44, CD90, CD133, etc. Various stem cell surface and intracellular markers can be detected in MSC of different tissue origin. On the other hand, each MSC population differs in the degree of particular marker expression. This may be due to the properties of the particular stem cell source, the differentiation stage of the cells, the isolation and cultivation method, or the individual features of the species. For these reasons, the characterization and separation of cell cultures should be based on the labeling of at least two different markers. Otherwise, it can not be claimed that the obtained cell population is homogeneous. Separation of cells based on the expression of surface markers affects the “purity” of the cell culture. There are various methods of selecting certain cells that express target surface antigens; such are Fluorescent Activated Cell Sorting [FACS] and Magnetic Activated Cell Sorting [MACS] [42].

Conclusion:

Currently MSC is area of interest in various scientific fields. There is a lot of information and studies revealing their properties. However, the data about the management of their properties followed by their potential clinical application is still insufficient. Additional research about the effects of various agents on the stem cell properties may help revealing new therapeutic approaches in the stem cell therapy and regenerative medicine.

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