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STEM CELLS - BIOLOGICAL UPDATE AND CELL THERAPY PROGRESS

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Abstract

In recent years, the advances in stem cell research have suggested that the human body may have a higher plasticity than it was originally expected.

Until now, four categories of stem cells were isolated and cultured in vivo: embryonic stem cells, fetal stem cells, adult stem cells and induced pluripotent stem cells (hiPSCs).

Although multiple studies were published, several issues concerning the stem cells are still debated, such as: the molecular mechanisms of differentiation, the methods to prevent teratoma formation or the ethical and religious issues regarding especially the embryonic stem cell research.

The direct differentiation of stem cells into specialized cells: cardiac myocytes, neural cells, pancreatic islets cells, may represent an option in treating incurable diseases such as: neurodegenerative diseases, type I diabetes, hematologic or cardiac diseases.

Nevertheless, stem cell-based therapies, based on stem cell transplantation, remain mainly at the experimental stages and their major limitation is the development of teratoma and cancer after transplantation. The induced pluripotent stem cells (hiPSCs) represent a prime candidate for future cell therapy research because of their significant self-renewal and differentiation potential and the lack of ethical issues.

This article presents an overview of the biological advances in the study of stem cells and the current progress made in the field of regenerative medicine.

Keywords: stem cells, cell therapy

Introduction

In the 1960s the Canadian scientists Ernest A. McCulloch and James E. Till laid down the first bricks of the stem cell research foundation by proving the presence of colony-forming cells (now known as hematopoietic stem cells) in the mouse bone marrow [1,2]. Until now, several varieties of stem cells have been isolated and cultured *in vitro*. Concerning their origin they have been divided in three wide categories: embryonic, fetal, and adult stem cells [3,4,5]. In addition to these, a fourth category was discovered by Shinya Yamanaka in 2007, which made a breakthrough by reprogramming human skin fibroblasts back into pluripotent cells. We call these cells human induced pluripotent stem cells or shortly: hiPSCs. These can differentiate into all three germ layers similarly to the embryonic stem cells, but, unlike the latter, hiPSCs come with significantly less ethical and religious issues, thus greatly simplifying the research work [6]. Consequently, significant advances have been made in the disease

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modeling field for the simple fact that these hiPSCs offer the possibility to study the physiology of the diseases on models that are the closest to the human reality, also carrying the disease-specific genetic background [7,8]. Along with this, drug testing is getting closer to creating better, more specific, and safer drugs, eventually reaching the ultimate purpose: personalized treatments [9,10].

Since 1960s, numerous articles focusing on stem cells have been published, bringing us closer to a better understanding of their origin, function, morphology, molecular biology and, eventually, therapeutic applications. Regarding the latter, one of the oldest stem cell therapies is transplantation of bone marrow from donor to leukemic patients. Transplantation of umbilical cord cells to patients that suffer from hematological diseases, a more recent method and an alternative to the first, shows long term results similar to the previous, as reported in some studies [11]. Still, nowadays, big hopes are laid in the direct differentiation of stem cells towards specialized cell types such as pancreatic islets cells, neurons and cardiac myocytes [12,13,14]. Reaching this point and successfully transplanting them in the affected organs or tissues might consist in a new approach for treating incurable diseases such as neurodegenerative diseases, type I diabetes, and cardiac diseases.

A better mastering of the isolation, differentiation mechanisms, and preventing the stem cells to evolve into teratomas and/or terato-carcinomas are some of the obstacles that have to be overcome in order to use stem cells for therapeutic purposes. In this review we try to bring up to date the biological advances in the study of stem cells as well as the progress made towards using them in the field of cell therapy.

Stem cells are unspecialized cells that present two very important properties: self-renewal and plasticity

Stem cells are unspecialized cells capable to proliferate and differentiate. Consequently to the differentiation they acquire a more specialized phenotype by adopting a specific genetic expression profile. In the end they become specialized cells that present characteristic properties [12,13,14]. A good example is the embryonic stem cell which is capable to differentiate not only into all three embryonic germ layers (endoderm, mesoderm, and ectoderm) but also in the embryonic annexes (e.g. placenta, amniotic membranes etc), eventually leading to the possibility to obtain any of the more than 220 cell types found in the human organism. Therefore, we say that the embryonic stem cell at morula stage is totipotent [4]. Following the process of ontogenesis, stem cells can be found at any stage of development. Indeed, studies show that fetal stem cells can be isolated and show promising applications in perinatal and regenerative medicine [15,16,17]. Moreover, nowadays, several types of adult stem cells can be obtained not only from bone marrow but also from adipose tissue, skeletal muscle, myocardium and pancreas [5]. The typical example of adult stem cells are the hematopoietic stem cells that can differentiate into cells that carry specific functions such as antigen synthesis, transport of gases and other [18]. In addition, these are also frequently used for therapeutic purposes in patients suffering from leukemia [19].

For a cell to be categorized as a stem cell two very important conditions must be fulfilled. First, the cell must have the property of self-renewal [20]. This means that it has the ability to preserve its undifferentiated status even after numerous cycles of cell division. For this to be possible stem cells undergo a special type of division called asymmetric division. As it follows, one of the resulted cells will be identical to the mother cell, maintaining the pool of stem cells, whereas the second will undergo differentiation becoming, eventually, a specialized cell. Transcription factors that play a key role in the molecular mechanisms of this process are: SOX2, NOTCH, WNT, PTEN, p53, Myc, as well as the ones from the HOX group (4, 7, 9, and 10), and Musashi-1 [21,22].

The other important property that defines the stem cell phenotype is plasticity. This makes reference to the capacity of cells to differentiate by adopting a genetic expression profile that will eventually lead towards a specific cell phenotype. Generally, the more undifferentiated the stem cell, the broader the specter of differentiation. Consequently, according to their ability to differentiate into wider panels of specialized cells, stem cells are sorted into 4 classes, as shown bellow [23].

Totipotent stem cells are present in the zygote until it achieves the conformation of 8 cells. They can differentiate into cells that result from all three germ layers including placental cells. Unspecialized cells, they can undergo symmetric and asymmetric division and exhibit phenotypic and molecular specific markers such as Oct 3/4, Nanog, SEEA1, SEEA 3 and 4. They present a great proliferative capacity producing embryoid bodies, but also teratomas and/or teratocarcinomas.

Pluripotent stem cells can be isolated from the internal mass of the blastocyst. Undergoing differentiation they can form cells from all three germ layers (endoderm, mesoderm, and ectoderm) but lack the ability to form placental or extra-embryonic cells.

Multipotent stem cells are found in fetal organisms in the process of evolution but also in the adult organisms. They can differentiate into a limited number of cells restricted to a certain germinal layer.

Unipotent stem cells differentiate resulting in a single type of cell and are found in the adult organisms. [24]

Sources of stem cells and their applications

As already mentioned, several varieties of stem cells have already been successfully isolated and cultured *in vitro*. Nowadays, they are grouped in four broad categories: embryonic stem cells, fetal stem cells, adult stem cells, and induced pluripotent stem cells. In the following we will discuss each one of these categories bringing up to date their effective applications and future prospects.

1. Embryonic stem cells

1.1. Properties

Human embryonic stem cells (hESCs) were first isolated from inner cell mass of the blastocyst by Thomson et al. in 1998 [3]. In addition, more recent studies show that morulae and single blastomeres may constitute alternative sources of hESCs [25,26]. Classically, the characteristic cell surface markers expressed by hESCs are stage-specific embryonic antigen-3 (SSEA-3), SSEA-4, tumor rejection antigen-1-60 (TRA-1-60), TRA-1-81, and alkaline phosphatase [3]. Also, there are studies that propose a range of cell surface proteins as novel cell markers for a better delimitation and isolation of hESCs. Regarding these, CD9, CD30, CD50, CD90, and CD200s in combination with SSEA-5 showed promising results in sorting undifferentiated hESCs from a pool of cells that were committed towards a neural-directed differentiation. The results showed a remarkable reduction in teratoma formation [27]. Besides the specific cell surface markers, a series of genes are also used to detect uncommitted hESCs, being more expressed at that stage. From these, NANOG, OCT4 and SOX2 are the three typically transcription factors used to demonstrate the pluripotency of the cells. Additionally, a range of genes consisting in Rex-1, Dnmt3b, Lin-28, Tdgf1, FoxD3, Tert, Utf-1, Gal, Cx43, Gdf3, Gtcm1, Terf1, Terf2, Lefty A, and Lefty B are also commonly used for the same purpose [28,29].

The capacity of differentiation towards all three germ layers brings a series of advantages such as applications in regenerative medicine. We will thoroughly discuss this in the following section. A major drawback for using the hESCs though, is the great deal of ethical and religious issues they come along with, the embryo being destroyed upon their isolation.

1.2. Applications of hESCs

Attempts in obtaining myogenic progenitors date back from 2006 when a group successfully obtained myogenic cells from mouse skeletal muscle. Using a technique initially utilized for obtaining neurogenic progenitors, they reported the presence of non-adherent microspheres capable to maintain their abilities to proliferate and differentiate for at least several months. Notably, they were also capable to participate in muscle regeneration. Interestingly, after initializing differentiation, instead of obtaining multinucleated cells they observed the presence of thin mononucleated and elongated myosin- and myogenin-positive cells that presented spontaneous contractility and cell surface adherence, suggesting a very primitive phenotype [30]. A more recent study, this time using hESCs in a sphere-based culture, reported positive results in the propagation of human myogenic progenitors. Even so, the inability to maintain their proliferative ability for longer than 12 weeks and exhibiting a slight heterogeneity with the presence of neural progenitors shows that this method requires further optimization [31]. This may bring valuable insights on the mechanisms in muscle tissue development, growth factors and drug discovery, toxicology, and regenerative medicine.

In 2009, the Geron Company received the FDA authorization to use and differentiate embryonic stem cells into oligodendrocytes. The latter were then used in phase 1 trials for treating acute spinal cord injuries. Even though the results were promising the study was discontinued in 2012 due to financial reasons.

In 2010 two other trials were approved for the company called Advanced Cell Technology. Their purpose was to improve the sight of patients suffering from Stargardt macular dystrophy and dry age-related macular degeneration. For this, they used retinal pigmented cells obtained from hESC. The preliminary results show no recordings of tumors or ectopic tissue. Also, no reject phenomena were registered. Nowadays there are 6 clinical trials all of them sharing the purpose of treating Stargardt macular dystrophy and dry age-related macular degeneration.

1.3. Future prospects of hESCs

Regarding the therapeutic use of hESCs, theoretically they can be differentiated into any type of cell that forms the organism. At the moment, the lack of knowledge concerning their biology is a limiting factor that decreases the potential of their use. Another drawback that presents a high risk for using them in the clinical practice is the generation of teratomas and teratocarcinomas. Due to their high plasticity it is necessary to establish standardized protocols of differentiation, more accurate and reproducible.

2. Fetal stem cells

2.1. Properties

As hESCs come with a great deal of ethical and religious issues another valuable source of stem cells is represented by the fetal tissues samples [32,33] or, after birth, by the fetal adnexa such as fetal membranes and placenta [34].

Antenatal sources of stem cells (SCs) include fetal blood from umbilical cord and amniotic fluid. From the first, it is possible to isolate fetal hematopoietic stem cells (fHSCs) as well as fetal mesenchymal (fMSCs), the latter in a much lower quantity, about 0.4% in the first trimester presenting a decreasing trend with the gestation advance [4]. Fetal bone marrow, liver, kidney, lungs and also umbilical cord constitute other sources of fMSCs [33,35,36]. fHSCs express CD34 and can differentiate towards all hematopoietic lineages, whereas fMSCs express

intracelular markers such as fibronectin, laminin, vimentin and mesenchymal markers, like CD105, CD73, CD45, CD34, CD14 and can differentiate in cells from adipose, cartilage and bone tissues but also towards cardiac muscle and neuron cells [37]. From the amniotic fluid it is possible to isolate stem cells that come from connective tissue and dermal fibroblasts. They share markers and properties with the MSCs and therefore are called amniotic-fluid-derived mesenchymal stem cells (AF-MSCs) [4].

Postnatal sources of stem cells can be found in the structures that form the placenta. Even though their number decreases along the gestation they still remain more numerous than the adult stem cells after isolation. As it follows, four distinct types of cells regarding their site of origin have been obtained from the structures that form the placenta: human amniotic epithelial cells (hAECs), human amniotic mesenchymal stromal cells (hAMSCs), human chorionic mesenchymal stromal cells (hCMSCs), and human chorionic trofoblastic cells (hCTCs) [38].

HAECs express low amount of human leukocyte antigen (HLA) type A,B,C a very important aspect in the possibility of their transplantation between different individuals. Moreover, they have been shown to exhibit other cell surface markers like ABCG2/BCRP, CD9, CD24, E-cadherin, integrins alfa6 and beta1, c-met, SSEA 3 and 4, TRA-1-60 and TRA-1-81. However, the surface markers that are missing or are expressed in low quantities are SSEA-1, CD34, CD133, CD117 (c-kit) and CCR4 (CC chemokine receptor) [39]. As molecular markers they express Oct 3/4, Sox-2, and Nanog. Studies show that hAECs can differentiate towards all three germ layers, thus they are pluripotent [40].

Cells isolated from the mesenchymal compartment (hAMSCs and hCMSCs) exhibit markers similar to those found in the stem cells isolated from the bone marrow, except for the hematopoietic markers CD34 and CD45 and the monocytic marker CD14. The presence of SSEA-3 and SSEA-4 has been shown through mARN analysis. Very interesting, they express low levels of HLA-ABC and no HLA-DR [41].

2.2. Applications

Concerning the fHSCs, the most used source, by far, resides in the blood from the ombilical cord. It is also the oldest source of stem cells used in the clinical practice, the first transplant being successfully carried out in a patient with Fanconi anemia 27 years ago. This kind of treatment is used in patients who suffer from malignant hemopathies or metabolic storage disease that can benefit of allogenic transplant of hematopoietic stem cells [42,43]. At the moment, there are 600.000 units stored and more than 30.000 performed transplants. Since 2007 to date there have been a number of 11 ongoing trials, the majority of them in the field of neuropathology (amiotrophic lateral sclerosis, cerebral palsy, cerebral atrophy, Huntington's disease and Parkinson's disease) [44].

2.3. Future prospects

Stem cells obtained from the umbilical cord will be used in treating different hematological pathologies in the future. Moreover, the ongoing clinical trials will bring new data regarding the cellular therapy of neurologic diseases. Similarly to hESCs, the hFSCs come with a still insufficiently exploited potential. Their low HLA level as well as the particular immunosuppressant properties they exhibit makes them the ideal candidate for cellular therapy. Unlike the hESCs, they come with little ethical issues.

3. Adult stem cells

3.1. Properties

Just as fetal stem cells, adult stem cells come with little ethical or religious issues since they are harvested from adult organisms. The typical adult stem cell is the hematopoietic stem cell (HSC) first isolated from mouse bone marrow in 1961 by Ernest A. McCulloch and James E. Till, under the name of spleen-colony forming units (CFU-S) [1,2]. Later, in 1982, Friedenshtein isolated and described another group of cells, also located in the bone marrow, that presented stem cell characteristics, but exhibiting a wider panel of differentiation than HSCs. They were called colony forming units-fibroblasts (CFU-F), now known as mesenchymal stem cells (MSCs) [5].

Bone marrow and peripheral blood are viable sources of HSCs [45,46]. The typical cell surface markers expressed in HSCs are CD34, CD 90 and CD133, while they lack expression of CD38 [47,48,49]. Also, studies show that kinase insert domain receptor (KDR) and cub domain protein 1 (CDP1) positive cells in the CD34 + fraction show enrichment of the primitive HSCs [50,51]. In addition to these, other markers such as CD49f and aldehyde dehydrogenase (ALDH) are debated and proposed as new HSCs specific markers in a review from 2013 [27]. HSCs possess the capacity to differentiate towards all cells that form the hematopoietic system.

MSCs are adult multipotent SCs that can be isolated not only from bone marrow stroma [52], but also from other tissues such as adipose tissue [53], neural tissue [54], olfactory mucosa [55.], heart tissue [56], skin [57], gingiva [58], and many others. Classically, MSCs exhibit CD105, CD73 and CD90 as specific cell surface markers and lack expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface molecules. Also, MSCs have the ability to differentiate into adipocytes, osteoblasts, and chondroblasts [59]. Moreover, studies show that they can also differentiate into non-mesenchymal cells such as pancreatic islets [57], neuron-like cells [55], and hepatocytes [60].

3.2. Applications

The hematopoietic stem cells are the only adult stem cells currently used for bone marrow transplants in patients who suffer from different types of malignant hemopathies. Also, there are two trials approved by Food and Drugs Administration (FDA) regarding the use of neural stem cells

in patients that suffer from Parkinson's disease and spinal cord injury [61,62]. Likewise, the use of stem cells obtained from the marrow is in an advanced phase in treating the pulmonary pathology related to premature births.

3.3. Future prospects

The understanding of the molecular mechanisms that control the phenotype of the stem cells has revolutionized the field of biology. However, our knowledge is still extremely limited, which leads to a low level of their use in the clinical practice. Finding new markers that may sort the different populations of stem cells into classes as well as the identification of new mechanisms involved in cellular differentiation (e.g. microRNA, lncRNA) will broaden the specter of opportunities in the medicine of the future.

4. Human iPS cells

4.1. Properties

In 2007 Yamanaka was the first to succeed in reprogramming human dermal fibroblasts (HDF) back into pluripotent stem cells through viral transduction of human Oct3/4, Sox2, Klf4, and c-Myc (OSKM cocktail) genes. Moreover, he also managed to reprogram human fibroblast-like synoviocytes (HFLS) obtaining similar results [6]. Using Oct3/4, Sox2, Nanog, and Lin28, Thomson managed to induce pluripotent cells from somatic cells, thus proving true the concept Yamanaka had stated [63]. Both of them being invasive, studies have shown that it is possible to obtain hiPSCs even from urine cells, avoiding scar and bleeding, allowing sampling cells even from patients that suffer from hemophilia [64]. Theoretically, any type of somatic cells can be reprogrammed, eventually generating induced pluripotent stem cells [65].

HiPSCs express cell surface markers specific for hESCs such as SSEA-3, SSEA-4, TRA-1-60, TRA-1-8, alkaline phosphatase, and Nanog protein. Moreover, they express OCT3/4, SOX2, NANOG, growth and differentiation factor 3 (GDF3), reduced expression 1 (REX1), fibroblast growth factor 4 (FGF4), embryonic cell-specific gene 1 (ESG1), developmental pluripotency-associated 2 (DPPA2), DPPA4, and telomerase reverse transcriptase (hTERT) at levels similar to those found in hESCs [6].

HiPSCs are pluripotent stem cells retaining the capacity to differentiate into cells that originate from all three germ layers. As it follows, studies show that cardiac myocytes [66], neural cells [67], hepatocytes [64], pancreatic islets [68], and many others have been obtained through direct differentiation of hiPSCs.

4.2. Applications

One of the purposes of regenerative medicine is the generation of specialized cells for every individual patient. The achievement of this type of cells brings new opportunities in the fields of disease modeling and drug testing. Some examples that are currently under evaluation are familial dysautonomia [69], prolonged QT interval [70,71], and dyskeratosis congenita. At the moment, their use in the clinical practice still remains a desire. However, Jaenisch et al managed to prove the utility of these cells on two murine models, one with sickle cells disease [72], and the other, with Parkinson disease [73], respectively.

4.3. Future prospects

These cells hide an immense potential for future use. Lacking any ethical issues, researchers can generate personalized lines of cells from which it will be possible to obtain differentiated cells. The latter could be used in treating diseases that are now considered incurable. The neurodegenerative diseases are one of the most studied at the present time and as examples stand Huntington's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, Fronto-Temporal Dementia, and Alzheimer's disease [74]. Also, the cardiac and metabolic conditions are worth mentioning [75]. The use of these cells in the future clinical practice will truly open the path towards personalized medicine.

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