Abstract:

The concept of stem cells was born in the early 19th century by the observation that some cells can produce other cells. Since then until today, but especially in recent decades, the knowledge on the stem cells biology was increased so rapidly that today efforts are being made to extend their therapeutic use in a wide range of diseases in the frames of the new field of regenerative medicine.

In this article, the main characteristics of stem cells are reviewed and there is a quick reference on important discoveries which were milestones in the evolution of the stem cells study.

Key words: embryonic stem cell (ES), hematopoietic stem cell (HSC), nuclear transfer (SCNT), induced pluripotent stem cell (iPS), cloning.
1. Introduction.

Stem cells, in the broadest sense, are a cell population which is characterized by two properties: the self-renewal and differentiating capacity. The fact that they have the ability to control proliferation, differentiation and apoptosis, distinguishes them from the tumor cells.

Self-renewal is defined as their ability to undergo endless cycles of symmetric mitotic divisions whence two daughter cells arise, identical to the progenitor. Differentiating capability is defined as their ability to differentiate into specialized cell types when they receive or lack specific environmental signals. Under these conditions, a stem cell undergoes an asymmetric mitotic division whence a stem cell identical to the progenitor and a stem cell committed to differentiate phenotypically maturing along a limited and exclusive lineage arise.

The first totipotent stem cell is the zygote that is the fusion product of an ovum and a spermatozoon. By the zygote proliferation, the morula and then the blastocyst emerge, which in human corresponds to a 4 to 5 days fetus and consists of 50 to 150 cells. From the inner cell mass of the blastocyst the pluripotent embryonic stem cells (ES) arise, which can differentiate into any mature cell type of all three germ layers. Furthermore, by the ES, the pluripotent stem cells will also arise, also referred as adult stem cells, which are committed to mature into a variety of offsprings, are situated in the adult body and they constitute the replacing pool of dysfunctional or senescent cells, in the frame of maintaining an organism’s homeostasis.

Stem cells differentiating ability and the possibility of their genetic manipulation in vitro are the reasons of being investigated as a therapeutic approach to diseases, where dysfunctional cells and / or loss of healthy cells are during the course of the disease.

Despite the fact that, at least in theory, the concept of stem cell has been known for
over 100 years, their isolation and biology study are issues relating to the recent decades.

2. Embryonic stem cells (ESCs).

ESCs are derived either from the morula or the inner cell mass of the blastocyst and are characterized by unlimited self-renewal capacity as well as differentiation capacity into cells of all three germ layers [1], under certain incitement conditions for a specific cell lineage.

In order for ESCs to maintain their pluripotency in vitro, optimum cultivation conditions or genetic manipulation are required [2]. Researches have shown that three transcriptional factors, in particular Nanog, Oct4 and Sox2, which suppress differentiation genes, ensure ESCs pluripotency [3]. Ian Chambers, who isolated the Nanog gene, characterized it as the main gene that makes ESCs immortal [4]. Oct4 factor is the main regulator of pluripotency since its exact level regulates ESCs fate. A possible Oct4 deficiency leads cells to differentiation into trophectoderm while an increase leads to primitive endoderm and mesoderm. The Sox2 gene is also involved in regulating embryonic development and in determining the cell fate. Many studies have focused on the necessary signaling pathways maintaining ESCs in a pluripotent state as well as the signals manipulating differentiation, because their injection, in the absence of differentiation control, results in teratomas [5].

Studies with human ESCs in relation with the ability to differentiate into various cell types, have demonstrated that they can differentiate into neural cells [6,7], keratinocytes [8], melanocytes [9] (ectoderm derivatives), connective tissue cells, blood cells subpopulations and cardiovascular tissue [10,11,12,13] (mesodermal derivatives), pancreatic, hepatocellular and lung tissue [14,15,16] (endoderm derivatives). Thus, ESCs are an attractive source of regenerative tissue. But it should be noted that the morphological and immuno-histological characterization of a cell do not coincide nor ensure its functional status and therefore the
magnitude of its normal function potential.

The characterization of an embryonic stem cell includes many surface antigens and is the subject of continuous research [17]. The most commonly used surface antigens of human ESCs are the glycolipids SSEA3 and SSEA4, the keratan sulfate antigens tra-1-60 and tra-1-81, etc. It is important that human ESCs express the class I major histocompatibility complex which, given that it is up-regulated during differentiation [18], increases the immunological rejection risk in case of transplantation. Apart from this risk, ethical concerns and conflicts due to the necessity of an embryo’s destruction in order to obtain ESCs [19], led the research towards the manufacture and production of embryonic or embryonic-like stem cells, using techniques that do not require the destruction of an embryo and can be widely accepted.

3. Multipotent adult stem cells.

They are also referred as somatic or progenitor stem cells. They are derived from the ESCs differentiation and can differentiate into tissue-specific cell types. During development they are stored in specific niches, are involved in organogenesis and coexist in different tissues with the differentiated cells. Their nomenclature emerges from the tissue where they are present, eg mesenchymal, endothelial, neural stem cell, etc. [20].

The discovery of multipotent stem cells in adult organisms caused great excitement, since on the one hand they are a generation source of autologous cells for transplantation therapies, without the immunological rejection risk of transplanted allogeneic donor cells on the other hand they outweigh the ESCs ethical concerns.

Their role is reparative, replenishing cells which are aging or apoptosing maintaining the normal recycling of organs such as blood, heart, breast, etc. [21,22,23]. Their number is small but it is important that they can be isolated from the umbilical cord blood [24]. The reasonably accurate study of their differentiating potentiality is now the subject of many
investigations [25].

The research on somatic stem cells, although they do not exhibit ESCs pluripotency, has been funded and has progressed much further than ESCs, because it circumvents the ethical concerns. The use and also their clinical application have been known for decades by the successful treatment of leukemia and other malignant conditions of the hematopoietic system, by the transplantation of hematopoietic or mesenchymal stem cells of the bone marrow [26.27]. However, the biggest hopes for the use of somatic cells in a variety of nosologic conditions, in the context of regenerative medicine, emerge from researches demonstrating that there is the possibility, through re-programming pathways, that the ESCs pluripotency get reinstalled in the adult stem cells.

4. Developments – milestones in stem cells research.

Since 1950 already, the research was focused on hematopoietic stem cells (HSC) and its potential to cure leukemia and other malignant conditions of the hematopoietic system while the concept of embryonic stem cells remained theoretical. Since then, important discoveries have shed light on our understanding of stem cells biology (both embryonic and adult), which have been isolated, cultured in vitro, undergone genetic manipulation and are used now in modern therapeutics.

4.1 From HSCs to the mouse ESCs.

The knowledge that radioactive radiation can eliminate the production of all blood cell types, from bone marrow, urged three Canadians researchers, Ernest A., McCullough and James Till, to try restoring the production of blood cell lines, by injecting bone marrow cells of a healthy donor. Their experiments in lethally irradiated mice [28] demonstrated, in 1963, that the injection of a small number of bone marrow cells from healthy donors was sufficient,
in order to return the production capacity of all three blood cell lines to the irradiated laboratory animal. Going further, after they managed to genetically label the donor cells, they localized them in cell colonies in the spleen of the transplanted animal. So the first tangible experimental evidence emerged, that there was a stem cell which could be differentiated into cells of the white, red and megakaryocytic line. The isolation of this adult multipotent stem cell (HSC), was achieved years later, however, the foundations of stem cell research and also the efforts to locate the most primordial pluripotent embryonic stem cell had already been established.

It took several years of research and as lately as in 1981, eighteen years after Till’s announcement, pluripotent stem cells were isolated and the term embryonic stem cell (ES) [29] was introduced. Experiments with mice embryos, conducted by Martin Evans, Matthew Kaufman, and Gail Martin, led for the first time to the isolation of a stem cell which was cultured and differentiated into a variety of cell types of different germ layers. There were now the first experimental data on pluripotency versus HSC multipotency, but most important was the fact that there was the possibility of studying, on an experimental level, factors that stimulate proliferation, prevent differentiation or determine the development and the fate of the cell. In fact, a new approach for studying the mammals’ development was started in order to draw conclusions which will be used for the benefit of modern therapeutics.

4.2 HSCs and the first human ES cell line.

Alongside the researches on mice stem cells, researches in humans were also developed. In 1968, at the University of Minnesota, a case of combined immunodeficiency was successfully treated by transplantation of bone marrow cells [30.31]. The severe defect in B and T lymphocytes in this syndrome, leads to immune deficiency and virtually to a complete lack of any immune response to any kind of infection. The transplantation of bone
marrow cells obtained from its sibling brother in the patient restored the lesion, indicating that the transplantation of histocompatible bone marrow cells may restore blood cell lines lesions.

The discovery of HSCs in the umbilical cord blood in 1978 [32] and the ease with which they are approached, collected and preserved, gave a new impetus to treating diseases of the hematopoietic system by HSCs transplantation. So since 1988, transplantations of stem cells collected from the umbilical cord blood, are in continuous clinical practice.

The parallel research on the isolation and cultivation of pluripotent ESCs against HSCs with committed differentiating ability, yield fruits in 1998, when from human blastocysts, at the University of Wiskonsin, James Thomson isolated ESCs which were cultured, maintained their pluripotency and differentiated into cells of all three germ layers [33]. This specific cell line, which even today is in function, is one of the most important discoveries in stem cells. It gave the opportunity of *in vitro* studying the developmental biology which cannot be studied in the intact human embryo, the potentiality of testing new drugs and it simultaneously highlighted the differences between human ESCs and mouse ESCs. Furthermore, the study and clarification of the mechanisms controlling differentiation became accessible, which is a prerequisite for the directed differentiation of ESCs in order to differentiate into specific types of functional cells, the manufacture of which could be used for tissue replacement in a multitude of disorders.

Despite the significant benefits which could emerge from the research founded by this discovery, serious ethical conflicts arising from the necessary destruction of human embryos has been hindered further investigation. Thus, therapeutic cloning and the production pluripotent cell derivatives, was the necessary alternative research route to the stem cells study.

**4.3 Cloning – Parthenogenesis.**
The first cloning of a mammal, which led the scientific community to major controversies, was accomplished in 1996. Dolly, the sheep, is the first cloned mammal created through the process of nuclear transfer (SCNT) by Ian Wilmut and Keith Campbell, at the Roslin Institute in Edinburgh, Scotland [34.35]. In SCNT, from a fertilized cell, the nucleus is removed and in its place the nucleus of a somatic cell is transferred, which is reprogrammed by the cytoplasm. The cell with the transplanted somatic nucleus proliferates, forming a morula which is becoming a blastocyst and continues towards the creation of an integrated organism.

The cloning of Dolly triggered the interest for cloning human embryos. The first announcement of such an effort was made on October 31, 2001, at the Institute of Advanced Cell Technology in Worcester [36]. The first four human embryos were unsuccessfully cloned. The scientists who participated in the project, insisted on claiming that therapeutic cloning aims at creating cells or tissues - using the genetic material of a patient - in order to treat a disease and has nothing to do with reproductive cloning aiming at creating an integrated organism, implanting the cloned embryo in the uterus of a volunteer mother. Despite of the serious legal and ethical issues raised by cloning with SCNT, the experiments have continued and are ongoing, using mainly adult skin fibroblasts easily isolated with a simple skin biopsy. Stemagen Corporation in Canada announced the development of human cloned blastocysts following SCNT with adult fibroblasts [37]. Although it was not possible to extract stem cells from the inner cell mass of the blastocysts, through the experiment it was first demonstrated that adult nuclei are reprogrammed by the human oocytes to generate cloned human blastocysts.

In order to develop ESCs that would be more acceptable by those opposed to the production of ESCs from cloned embryos, scientists have turned their attention to the production of blastocysts through parthenogenesis, i.e. using only an ovum without additional
genetic material. The oocytes are collected during their maturation cycle in a diploid stage, they are activated, under specific conditions and are beginning to proliferate like normal embryo. If diploid spermatocytes are used respectively, we talk about androgenesis. By this technique, pluripotent autologous stem cells can be produced, which after their induction into a specific cell line, they can be transplanted to the patient without problems.

This technique, which produces ESCs without embryonic destruction, exhibits tremendous progress and is almost used in parallel to SCNT, to produce blastocysts parthenogenetically.

Despite the fact that SCNT and parthenogenesis do not require the traditional embryonic destruction for obtaining ESCs, the view that the process "is involving the creation of human lives in the laboratory merely to destroy them for the alleged benefit of others" [37], continues to exist. Thus, the production of induced pluripotent stem cells (iPS) seemed to be expected.

4.4 Induced pluripotent stem cells (iPS).

The production of pluripotent stem cells, through a re-programming of adult somatic nuclei at an earlier embryonic stage, seemed to outweigh the ethical and legal dilemmas of human cloning.

The innovative research of Shinya Yamanaka, at the University of Kyoto in 2006, showed that mice fibroblasts can be re-programmed to an embryonic stage [38]. The pluripotency has been ensured through retroviral transfer of genes, responsible for self-renewing capacity of stem cells.

The production of iPs cells with relatively simple laboratory techniques and the use of adult nuclei, indicate the ability to generate iPs cells by a specific, eg Parkinsonian patient in order for their self-transplantation to lead to a replacement of the lesioned cells due to
disease. However, the use of iPs cells cannot be considered safe, since they have a 20% possibility of oncogenic activity risk. The use of retroviral vectors integrating genes and the absence of c-myc oncogene [39], have reduced the oncogenicity of the produced iPs cells and constitute a significant technical progress in the ongoing research.

4.5 Human stem lines not requiring embryonic destruction.

In 2005 at Kingston University in England, the isolation of cells from the umbilical cord with an immunomagnetic technique which were very similar to ESCs was announced [40]. They were called embryonic-like stem cells (CBEs) and expressed ESCs markers in the culture. They probably represent a special category placed in a more primitive state than adult stem cells and can be used as an alternative to ECSs, without ethical constraints and with clinical application potential.

Two years later, in 2007, stem cells have been isolated from the amniotic fluid which have been cultivated and expressed ESCs and adult stem cells markers [41]. The cells have been obtained with no significant risk to the mother or the embryo and differentiated into cells of all three germ layers. The finding of some amniotic stem cells of the Y chromosome in DNA indicates that they originate from the male embryo, not the mother. The discovery of these cells was an important step in stem cells research, despite of the fact that they could be regarded as a complete substitute of ESCs.

Ongoing research on the production of ESCs without embryonic destruction, led to the development of human ES cell lines through the cultivation of individual cells obtained from embryos, using the technique of genetic pre-implantation diagnosis [42]. The cells cells have been cultured in an optimal developmental environment and continued to develop normally. At the blastocyst stage they were frozen. From the inner cell mass, ESCs have been isolated which expressed all pluripotency markers, exhibited self-renewing capacity and genetic
stability. They also differentiated into cells of all three germ layers. The Advanced Cell Technology Institute believes that these results may put an end to all ethical concerns arising from the necessity of embryonic destruction for the production of ESCs.

5. Conclusions.

The ability of stem cells to differentiate into virtually any cell type, is theoretically leading to the assumption that their clinical applications could be unlimited. Although there have been important steps in ESCs research, our knowledge is essentially still rudimentary. Future expectations to be able to treat many, even incurable at present, diseases are enormous. Probably, through ESCs research, answers to the mysteries of the genetic development might be provided and we might be able to treat genetic defects. In any case, their use must be safe, effective, ethical and should only aim to human protection.

References


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