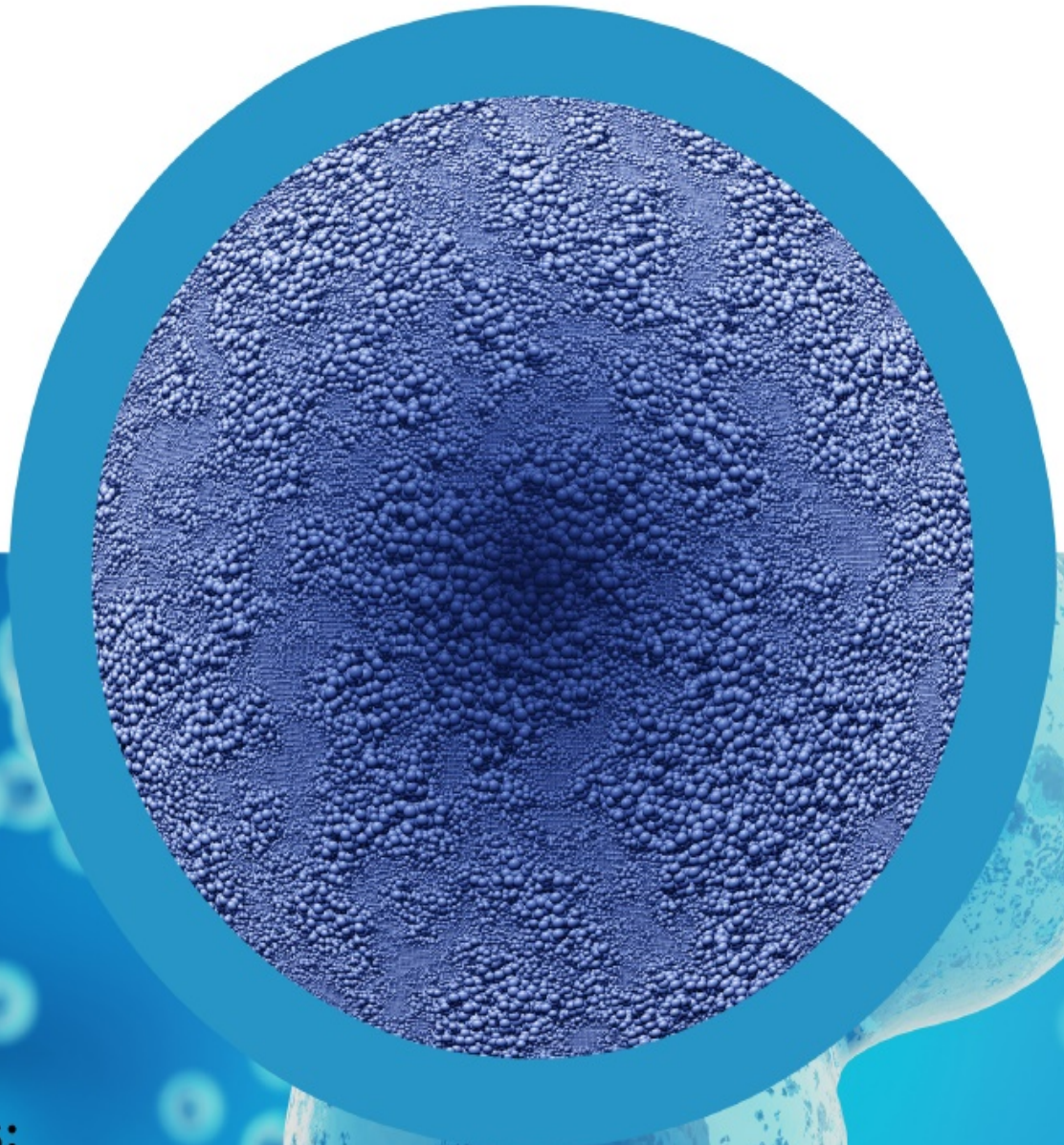
eISBN: 978-1-68108-437-4
ISBN: 978-1-68108-438-1

TOWARD THE FUTURE:
THE NEW CHALLENGES OF THE CELL THERAPY AND POTENTIAL OF REGENERATIVE MEDICINE



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Bentham Books

Toward the Future: The New Challenges of the Cell Therapy and Potential of Regenerative Medicine

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eISBN (Online): 978-1-68108-437-4

ISBN (Print): 978-1-68108-438-1

First published in 2017.

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FOREWORD

As a woman first, and as passionate researcher second, I was always attracted by what did represent Marie Curie and her breakthroughs for the entire scientific world. One of my favorite quotes is:

"We must not forget that when radium was discovered no one knew that it would prove useful in hospitals. The work was one of pure science. And this is a proof that scientific work must not be considered from the point of view of the direct usefulness of it. It must be done for itself, for the beauty of science, and then there is always the chance that a scientific discovery may become like the radium a benefit for mankind".

Marie Curie

For me this means that as researcher we must find passion in what we make to trigger the "out of the box" thinking and to realize the unimaginable. When I was a student I meet two distinguished researchers ***Francesco Zinno***, and ***Nicola Daniele*** whom introduced me with the main goals of the hematopoietic stem cells (HSCs) manipulation processes. Cell therapy helped the mass implementation of one the breakthrough that changed the way of making medicine, the regenerative medicine. Regenerative medicine involves the use of some of the most advanced therapeutic technologies of the 21st century. We can define this branch of medicine as methods to replace or regenerate human cells, tissues or organs in order to restore or establish normal function with the use of cell therapies, tissue engineering, gene therapy and biomedical engineering techniques [1]. The rapid pace of the supporting science is likely to see its application across ever increasing fields of clinical practice. This book's main aim is to ponder on the importance of the regenerative medicine under the success that this area was given by stem cell research, and cell- and gene-based therapy. To start this journey, authors decided to give a general picture of the biology of human stem cells and their classification, to introduce the reader with their unique and extraordinary properties. After that, they continued with a typology-based structured analysis of the potentiality of these cells to upgrade the therapeutic treatments.

They explain better the actual use of the stems cells and stems cells-like for regenerative therapeutic purpose such as:

- Cell-therapy approaches in neurologic areas such as Parkinson's disease;
- Retinal repair;
- Optic nerve regeneration;
- Reconstruction of the tissues in cases of burn;

- Bladder repair;
- Liver diseases;
- Angiogenesis and cardiac repair.

Authors continued with the researches that aim to reach and overstep the actual bounders of the "personalized medicine" to underline the stage of evolution and the infinite possibility of this new outstanding way of thinking on medicine. This book outline an attractive promotion of the present and future benefits of mankind from the discovery of stems cells and their use on the regenerative medicine. The more we know about stems cells the more we approach with the potentiality of human body to heal its self.

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PREFACE

This eBook entitled “Toward the Future: The New Challenges of the Cell Therapy and Potential of Regenerative Medicine” comprises chapters written by the leading experts in this field that provide state-of-the art information about the developments in important selected areas of Regenerative Medicine.

Regenerative Medicine is the process of creating living, functional tissues to repair or replace tissue or organ function lost due to age, disease, damage, or congenital defects.

This field holds the promise of regenerating damaged tissues and organs in the body by stimulating previously irreparable organs to heal themselves.

Regenerative Medicine also empowers scientists to grow tissues and organs in the laboratory and safely implant them when the body cannot heal itself.

Importantly, Regenerative Medicine has the potential to solve the problem of the shortage of organs available through donation compared to the number of patients that require life-saving organ transplantation.

Regenerative Medicine is also one of the fastest growing biomedical industries in the world because patients are being cured of diseases that were once incurable. Moreover, this field represents a new paradigm in human health because the vast majority of treatments for chronic and life-threatening disease focus on treating the symptoms, not curing the disease.

In fact, there are few therapies in use today capable of curing or significantly changing the course of a disease.

Stem cell therapy, when combined with immune and gene therapy, shows even greater potential to cure diseases. This new combination of regenerative cell therapies will open a new age of medicine, forever changing how it is practiced.

We would like to express our gratitude to all the Authors for their excellent contributions. We would also like to thank the entire team of Bentham Science Publishers, particularly Dr. Humaira Hashmi and Prof. Atta-ur-Rahman for their excellent efforts. We are confident that this Volume will receive wide appreciation from students and researchers.

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CHAPTER 1

Biology of Human Stem Cells

Silvia Franceschilli*

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Abstract: Stem cells are always regarded as cells with unique and extraordinary properties. SCs are able to self-renew and differentiate into specialized cells and this is a great advantage to maintain homeostasis in the body. The cells can divide with two different strategies and they are influenced by intrinsic and extrinsic factors of their microenvironment in which there are: the niche. The internal signals are represented by the genetic information of the cells, the external signals come instead from the microenvironment and they are physical or chemical signals. Stem cells are classified into embryonic stem cells and adult stem cells. Embryonic stem cells are derived from the inner cell mass of the blastocyst, these have great potential and over the years researchers have studied their properties and the importance of keeping them in appropriate culture conditions. Adult stem cells are found in a large number of tissues and have the very important role to replace damaged cells in living tissue. SCs can also be classified according to their potential, so they can be defined as totipotent, pluripotent, multipotent and they can differentiate respectively in a decreasing number of specialized cells of the body.

Keywords: Adult stem cells, Division, Embryonic stem cells, Mesenchymal stem cells, Multipotent stem cells, Niche, Pluripotent stem cells, Stem cells, Totipotent stem cells.

INTRODUCTION

Stem cells (SCs) have unique features because they are an undifferentiated kind of cells, that have capacity to renew themselves and that can turn themselves into many different cells types with specific functions. SCs have the important role to

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maintain the homeostasis in the body because in some organs they can renew, maintain or replace damaged tissues. In other organs they also can divide under special conditions [1]. The potential of SCs consists of dividing themselves without limits to renew cells and tissues, and the cells that resulted from this division can remain stem cells or become specialized type of cells [2]. The ability of SCs to "self-renew" or to generate differentiated cells can be defined by some signals derived from the special microenvironment of stem cells that is defined as "niche". In 1978, Schofield developed the hypothesis of this environment that could maintain the proprieties of stem cells [3, 4]. In the niche, cells can be influenced by internal and external signals to divide themselves by two different mechanisms: symmetric or asymmetric strategy [5]. Asymmetric division is characterized by the generation of a daughter with stem-cell fate and a cell that differentiates into different types. This mechanism is useful because of the production of two products with a single division but it can be considered a problem because it is not able to expand the number of stem cells. Over the years a lot of studies were carried out to describe the mechanisms that rule this type of division [6 - 9] and today it is possible to describe two different types of them: intrinsic or extrinsic mechanism. The second type of division is the symmetric one, that is defined by the ability of stem cells to divide themselves symmetrically to generate two stem cells or two differentiated cells. This type of division can be considered useful during wound healing and regeneration [10].

During the differentiation SCs lose their special condition of unspecialized cells, and this process is controlled by several steps and it is lead by internal and external signals. The internal signal consists of the information that is contained in the DNA and the external is represented by physical or chemical signals coming from the microenvironment. The combination of these elements regulates the behavior of stem cells [1].

There are different types of SCs, that can be classified into two groups: embryonic and adult stem cells [2]. According to their potential of differentiation, stem cells can be classified as totipotent, pluripotent or multipotent stem cells. Totipotent stem cells are capable to generate all the body because of their high capacity for differentiation. Pluripotent stem cells have the ability to form about 200 kinds of differentiated cells but not an organism, and finally multipotent stem cells can

define cells of specific tissue. Haemopoietic stem cells can be considered multipotent stem cells because they can form any blood cells but not other tissues [11].

Embryonic Stem Cells

In 1981 researchers discovered how to isolate embryonic stem cells from mouse embryos. At the end of nineties, in 1998, scientists described a method to derive stem cells from human embryos and how to grow them in culture [12 - 14]. They were defined human embryonic stem cells. In the early steps of embryogenesis, 3 or 5 days after the fertilization, the blastocyst is formed. It is composed by three parts, the trophoblast, an hollow cavity inside and finally the inner cell mass (ICM). This cell mass is composed by a special type of cells, that have the extraordinary potential to differentiate into a significant number of specialized cell types [15]. Embryonic stem cells can be maintained in culture in their undifferentiated state for a long time but they require appropriate conditions because gene expression and property of cells can be influenced by the environment [16, 17]. These cells are characterized by the expression of some specific transcription factors such as OCT3/4, NANOG and SOX2 [18]. The transcription factors OCT4, SOX2, NANOG control the expression of genes including other transcription factors such as STAT3, HESX1, FGF-2 and TCF. Moreover these transcription factors control signaling elements that are necessary to maintain the stem cell state and they repress some genes that would stimulate differentiation [19]. Embryonic stem cells retain the ability to differentiate themselves into many types of cells representing the three germ layers consisting of endoderm, mesoderm and ectoderm [20].

Adult Stem Cells

Human tissues are able to adapt themselves to different environmental conditions and to use a certain plasticity to survive in different circumstances. This is possible thanks to the presence of adult stem cells [21]. These cells are undifferentiated and they are among differentiated cells in tissues or organs and they live in specific areas defined as “niches” [1 - 22]. Niche is sensitive to the different hormonal signals or those coming from the microenvironment and can

CHAPTER 2

Hematopoietic Stem Cells: Identification, Properties and Interest for Clinical Applications

Nicola Daniele[*], **Francesco Zinno** and **Federica Tomassetti**

Cryolab, Parco Scientifico Università Tor Vergata, Roma, Italia

Abstract: The hematopoietic stem cells (HSCs) are a population responsible of the hematopoiesis's process; they have the characteristic to repeatedly divide or they can mature to generate different cell types, through the process of hematopoiesis. In this regenerative process, the cells are organized in a hierarchical structure: at the summit there are the hematopoietic stem cells and to the base, there is the progeny in differentiation. Hematopoietic cells commissioned to a particular hematic spinneret can be induced to convert themself in cells of the different spinner; another important feature of HSC is plasticity, that is the potential differentiation, thanks to which the cells are capable to undertake phenotypic and functional characteristics of other organs or tissues.

The process of hematopoiesis is regulated by numerous external and internal factors which operate on transcriptional level; this factors can also interact with each other.

Recently, knowledge about HSCs increases more and more; which allows their application also in clinical scope, to permanently treat serious pathologies.

Keywords: CD34+, Differentiation, Hematopoietic stem cells, Plasticity, Transplant.

INTRODUCTION

Hematopoietic stem cells are responsible for the making and turnover of all corpuscular blood's elements; all blood cells originate in fact to pluripotent

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Nicola Daniele (Ed.)

hematopoietic stem cells (PHSC) which constitute 0 0,1% of the nucleated cells in the bone marrow;

Basically, the main characteristics of these cells are:

- the ability to self-renew, or to replicate for many cell cycles;
- non-specialization, as not being mature cells can not carry out any kind of position within the organization;
- may accrue as a result of specific molecular signals and develop themself through a process of differentiation.

Generally these cells are in a quiescent state and remain undifferentiated for indefinite time; in response to various urges they interrupt the quiescence to begin their cycle of mitotic division [1].

Normally, very few HSCs are delegated to the regeneration of hematopoietic cells at any one time; the other remain in the G_0 phase of the cell cycle. In fact we know that the blood cells have a limited survival, around 120 days. Their brief existence is linked to the ability to make the most of their task of defense against infection and transport oxygen to tissues; for this reason they must be constantly replaced with new differentiated cells that are fully functioning [2].

This regeneration process takes the name of hematopoiesis and starts from the stem cells present in bone marrow, which divide mitotically and / or differentiate into two classes of multipotent hematopoietic stem cells: the first, the colony forming unit S (CFU- S), will give the cells of the myeloid lineage, including erythrocytes, granulocytes, monocytes and platelets; the second, the colony forming unit- LY (CFU- Ly), is a precursor of the cells of the lymphoid lineage, comprising B and T lymphocytes.

After numerous studies and observations, different antigens have been identified on the outer surface of these cells; on the presence or absence of these molecules, hematopoietic stem cells of man can be divided into two separate classes: cells CD34+ / HLA-DR+ / CD38-, which can divide themselves and became in different type of blood cells, and CD34+ / HLA-DR- / CD38- which can be differentiated in hematopoietic precursors and stromal cells [3].

Identification of the HSC and Role of the CD34 Antigen

With the aim to study more details of the hematopoietic stem cells, it was necessary to identify and isolate them from all other stem cells and from mature cells. Most of the time the problem that scientists encounter encountered in the identification of these cells, it was the lack of instrumentation. The absence of appropriate methods and technologies to support, have been a major obstacle to the study of this type of cells.

The overcoming of certain limits, in fact, lead to the discovery of the CD34 antigen subsequently; the application of new knowledge about its expression, have proven extremely useful to be able to identify exactly hematopoietic stem cells, in opposition to those already differentiated. In addition the HSC, before maturing, have an increased degree of differentiation and a decrease in the self-renewal capacity, the multipotency and the proliferative potential.

As mentioned earlier, the hematopoietic stem cells express different antigens on their outer surface; among which the most significant is the CD34; it is a trans-membrane glycoprotein which is expressed by the cells only in certain stages of development, when these cells are still immature. The distribution clonal of this antigen varies according to the different moments of cellular development. Consequently, the progenitor cells express the highest levels of CD34, while in cells that are maturing the presence of CD34 receptors on the cell surface decreases drastically [4].

Discoveries and studies in the field of hematopoietic stem cells, were made possible thanks to methods of cell separation using several techniques, including:

- the marking of the cells with monoclonal antibodies (mAb) which, being specific for certain antigens, put in evidence the presence or absence of the receptors on the cell surface, temporarily took under consideration;
- separation with marbles immunnomagnetiche;
- the high-affinity chromatography, based on the interaction between avidin and biotin;
- and finally the flow cytometry.

CHAPTER 3

Stem Cell Therapy Applications: The Challenge of Regenerative Medicine

Fulvia Fraticelli*

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Abstract: Regenerative medicine is a new concept of developing medicine. This field of science concerns reconstruction and repair of damaged tissues and organs. In physiological conditions, their integrity and functionality are ensured by the presence of adult stem cells that maintain and renew effectively all cell types. When there are injuries resulting from different causes, its essential to reconstitute the original structure and this represents the goal of regenerative medicine. The aim is to employ stem cells in clinic as cell therapy. For obtaining this result, its necessary to know the biology of stem cells and then its essential to have technologies useful to regenerate them in culture and allow their vitality. Today stem cells are a therapeutic reality: their potentiality helped in healing many kinds of diseases and restore the health of patients. With the progress of research and the development of new therapy protocols, it will be possible to expand stem cell therapy to different specialized areas of pathology.

Keywords: Cell therapy, Hematopoietic stem cells, Mesenchymal stem cells, Regenerative medicine, Stem cells.

INTRODUCTION

The discovery of stem cells has been one of the greatest revolutions in medical field because, thanks to their typical characteristics, they are used currently in clinical practice. From the earliest research, the extraordinary ability of these cells to self-renew and at the same time, differentiate themselves into unlimited cell types has been noticed. This plasticity has been employed in therapy for treatment

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care and complete resolution, sometimes, of several diseases. The advancement of knowledge, the multidisciplinary study of pathology and the availability of new methodologies for investigation of cells contributed to a radical change in medicine. Until a few years ago, diseases were treated with traditional approaches, essentially the pharmacologic ones. But, in recent times, a new field of medicine has developed: the field of regenerative medicine. The bone marrow contains mainly two different kinds of cell: hematopoietic stem cells (HSCs) and stromal ones, also called mesenchymal stem cells (MSCs).

Hematopoietic stem cells have an undeniable therapeutic value, because they are able to repair and regenerate also non hematopoietic tissues, so their clinical use concerns different fields of pathology. The main use of HSCs in therapy consists in transplantations, although current studies are proving that they are applied in heart diseases, in bone and cartilage alterations, cancer, neurodegenerative impairment and so on [1].

The model of stem cells is the hematopoietic type, derived from bone marrow, peripheral blood and umbilical cord. But also mesenchymal stem cells are important in therapy: they have different origins but, according to several properties of renewal and differentiation, they can be used in cell therapy and regenerative medicine in a specific way [2].

MSCs have a great potentiality in clinic, because they differentiate in several populations, like vascular, endothelial, muscular, and non-mesodermal ones. So, they can be used for tissue repair especially thanks to secretion of growth factors and cytokines. They should be able to work as inhibitors of cancer proliferation, switching off inflammatory reactions, checking immune responses and so reestablishing integrity of tissues [3].

In addition to conventional medicine, nowadays it is trying to extend the application of stem cells as replacement therapies for several different diseases. In particular, the use of induced pluripotent stem cells (IPS) seems to be promising. They are taken from patients and are capable of differentiating into various cells, applying the technology of reprogramming somatic cells. Using cells coming from pluripotent stem cells, its possible to realize a cell therapy for many different

body systems. Availability of a stock of specific cell types is the most important condition for regenerative medicine to perform and so realizing specific therapies for individual pathologies [4].

The basic requirement for application of stem cells in clinic consists in the employment of substrates on which growth, regeneration and self-renewal are ensured. Thanks to these materials, its possible to have many types of substances for maintenance of human pluripotent stem cells. In this way, regenerative medicine will be realized and a stem cell based therapy will be achieved. A large variety of materials exist: the main characteristics being the promotion of self-renewal, pluripotency and expansion. The recommendable substrate for clinical use would be inexpensive, easy to manipulate, rapid to use, safe and easily sterilized. Synthetic polymers, synthetic peptides, hydrogels and extracellular matrix proteins are some of the substrates currently available for an optimal clinical appliance [5].

Hematopoietic Stem Cells in Therapy

Hematopoietic stem cells are very useful in therapy, since they are able to regenerate and reproduce many specific cells. Their expansions in culture allow to obtain easily a large amount of materials, in particular blood cells for hematological disorders. As a result of new techniques, it was possible to create HSCs from embryonic stem cells (ESCs) and IPS. The natural growth, development and survival of these cells must be reproduced *in vitro* for making best use of HSCs in clinic. The most important application includes transplants and cell-based therapies [6].

Hematopoietic stem cells transplantation represents the gold standard treatment for blood diseases. Thanks to the regeneration carried out by stem cells, its possible to achieve engraftment and survival, which are essential for the success of transplants. Since there is a network of signals which cooperate to ensure the functionality of cells within stem cells niche, the comprehension of multiple interactions is primary for using cells in therapy. By analyzing specific pathways and using *in vitro* technologies, the success of transplantation using HSCs from ESCs and IPS can be increased. In this way, some of the problems related to

CHAPTER 4

Pluripotent Stem Cells: Basic Biology and Translational Medicine

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Abstract: The derivation of human embryonic stem cells in the last decades, made possible by the parallel and growing development of *in vitro* fertilization and embryo cryopreservation technologies, have opened the door for regenerative medicine. The study of cell replacement in loss of function diseases has received further impulse by the derivation of induced pluripotent cells less than 10 years ago. Currently, pluripotent cells are extensively employed in disease modeling, toxicology testing, and drug discovery. Phase I clinical trials with both embryonic and induced pluripotent cells derivates have been underway for a few years now, and initial results have been published recently. As the field of regenerative medicine moves forward at an impressive pace, we aim to review the origin and characteristics of the different kind of pluripotent stem cells, their potential use in key translational areas, and the challenges and opportunities that we face for their integrated use in a modern and personalized medicine.

Keywords: Cell therapy, Differentiation, *In vitro* disease modeling, *In vitro* drug screening, *In vitro* embryo culture, Induced pluripotency, Pluripotent stem cells, Regenerative medicine.

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THE CONCEPT OF PLURIPOTENCY

A chapter on the promises of translational medicine in the field of stem cell biology, promises brought about by our ability to harness pluripotent cell, would not be starting off on the right foot without a brief definition of what pluripotency is.

Etymologically, the term pluripotency derives from the union of the Latin words "*totus*" which means entirely or all, and "*potens*", which means having ability or power. Something pluripotent is therefore something with the ability of being, or becoming, all. When applied to cell biology, the term pluripotent acquires a more specific meaning and describes the capability of a cell to *divide indefinitely* ***while*** *maintaining the ability to differentiate into all cell types of an organism*. In this "while" resides the unique power of pluripotent cells. In biology, in fact, we can find several examples of cells that are able to divide indefinitely, from cancer cell lines, to primary lines immortalized by viral transduction. In the same way, there are examples of cells which are capable of differentiating in several cell types, like for instance the progenitors of an organ or tissue, or even into all the cell types that form an individual, like the fertilized oocyte or zygote. However, these states of pluripotency are not sustained over time, and in fact are present only for a few hours/cell divisions in the life of an organism. When left to their own devices, naturally occurring pluripotent states tend to differentiate quickly to a more differentiated, less potent state.

How is pluripotency tested? From its definition, it is clear that the ideal pluripotent cell should be able to give rise to a complete individual, however a series of proxy tests for differentiation potential have been devised. The most stringent proof of pluripotency, so far only available in mice, is given by the tetraploid complementation assay [1]. This test is based on the observation that when tetraploid (4n) and diploid (2n) cells are mixed together in a preimplantation mouse embryo, the tetraploid cells will segregate to the trophectoderm, leaving the diploid ones to form the inner cell mass (ICM). It is possible to create tetraploid early embryos by fusion of the two blastomeres of a 2-cell embryo. Diploid cells to be tested for pluripotency are added to the 4n cells, and a chimeric embryo is reconstructed. Because of the selective segregation of 4n cells, the

resulting pup will be formed entirely from the cells to be tested, and if a fertile individual is born, pluripotency is proven with the highest degree of confidence. A less stringent variation of the tetraploid supplementation test is the simple chimera formation test. A chimera is an individual which is made up by more than one genetically distinct cells. When injecting pluripotent cells into the blastocele of a developing embryo, some of them will be incorporated into the ICM, and will give rise to part of the developing individual. The degree and quality of this integration is usually taken as an indication of the level of pluripotency. Interspecies chimeras between human pluripotent cells and mice embryos were not produced until a few years ago, but are a technical possibility. Nonetheless, due to both legal and ethical concerns, the most common and accepted proof of pluripotency in human cells is the teratoma test. Teratomas are benign tumors which may be composed by derivatives of the three embryonic layers; teratomas are usually found in newborn or developing feti, but can be also discovered accidentally in adult individuals. In stem cell research, a teratoma is produced, intentionally, by injecting a pellet of cells of putative pluripotent potential into an immunologically suppressed host, usually a SCID mouse. Injection sites can vary, from subcutaneous to intratesticular to sub capsular in kidney and liver. Usually, if the tested cells are indeed pluripotent, a teratoma will form within 8 to 10 weeks. A less stringent *in vitro* test of pluripotency is through the production of so called embryoid bodies (EBs). EBs are produced when pluripotent cells are removed from an undifferentiated milieu, and allowed to aggregate and grow, usually in suspension. Pluripotent cells in this condition tend to form aggregates that differentiate in an undirected way, and presenting, often, cell populations which are precursors of more differentiated cell types and tissues. Cells that are not pluripotent, but that can still differentiate into some cell types (called multipotent) can also differentiate to a certain degree in EBs, thus forming EBs alone is not considered a stringent test of pluripotency.

Regardless of how pluripotency is tested, it takes both a cell population able to differentiate into all cell types of an organism, as well as an *in vitro* system which is able to override the differentiation signals to maintain a pluripotent cell population. While such an efficient *in vitro* culture system for human cells has been developed in the last 20 years and is still under improvement in the present

CHAPTER 5

Exploiting the Role of Hematopoietic Stem Cell Transplantation as a Cure of Hematological and Non Hematological Diseases

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Abstract: This chapter describes hematopoietic stem cells and their therapeutic uses to cure otherwise lethal, malignant and non-malignant diseases. Here we analyze the biological characteristics of different hematopoietic stem cell sources and how they are mobilized, collected, selected from the patient himself for autologous transplantation, or from matched or mismatched, related or unrelated donors for allogeneic transplantation [1 - 3]. Hematopoietic stem cell transplantation has been implemented for decades and has undergone many improvements over the years [3]. Today it is a safe, feasible option for selected patients and it still remains the only cure for a wide range of malignancies or non-malignant diseases despite advances in understanding disease genetics and biology. Moreover, with improvements in conditioning regimens and graft manipulation [2, 4], cells can be transplanted to enhance immune reconstitution and reduce relapse, which are the most common cause of transplant failure [2, 4]. Given the immunological modulation and anti-leukemic effect of these cells, conditioning regimen can be reduced and transplantation is now extended to elderly patients who are more susceptible to drug toxicity.

Keywords: Autoimmune diseases, Hematological diseases, Immunology, Solid tumors, Stem cell transplantation, Stem cells.

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INTRODUCTION

The discovery of several types of stem cells (SCs) that have different roles in human tissue creation and regeneration, makes them potentially promising as treatment for degenerative diseases such as myocarditis [5], Alzheimer's and Parkinson's disease, and other degenerative neurological disorders and autoimmune diseases [6], for which currently available therapies are ineffective in the long term. *In vitro* and *in vivo* human SCs have demonstrated great capacities for tissue regeneration and immune system modulation [7]. The regenerative capacity of hematopoietic stem cells (HSCs) from bone marrow (BM), peripheral blood (PB) and umbilical cord blood (UCB) [8, 9] has been known for decades. Over time technological advances in harvesting SCs, separating sub-populations with specific functions, and conservation have become more and more specialized and automated, so that today, worldwide, SC therapy is safe and successful for a wide range of human diseases like, for example, acute leukemia (AL), multiple myeloma (MM), severe aplastic anemia (SAA), thalassemia major and other hemoglobin diseases, immune-deficiencies and some solid tumors. This chapter will focus on HSC properties and their therapeutic uses, allogeneic (from a donor) and autologous (self) transplantation and future perspectives.

Hematopoietic Stem Cells

Hematopoiesis initially occurs in long and flat bones, and then in vertebrae, the sternum, ribs and iliac crests [10]. Based on an average adult blood volume of about 5 liters, each day an adult human produces 2 x 10^{11} erythrocytes, 1 x 10^{11} leukocytes and 1 x 10^{11} platelets which all derive from HSCs. Primitive, multi-potent HSCs are rare, occurring at a frequency of 1 in 10,000 to 1,000,000 BM cells, are quiescent in the steady-state, and at any one time, only a fraction enter the cell cycle to proliferate and give rise to different progenitors. They gradually lose one or more developmental potentials and ultimately become committed to a single cell lineage, which matures into the corresponding blood cell type. With the advent of monoclonal antibodies and flow cytometry, HSCs and their progenitors are characterized by the presence or absence of specific surface markers and by the ability to efflux fluorescent dyes [11]. The typical HSC marker is CD34, an

integral trans-membrane glycoprotein with a molecular weight of 105-120 Kd (354 amino acids) which is expressed in 1-3% of BM cells, in 0.01-0.1% of PB cells, and in 0.1-0.4% of UCB cells [12]. Identifying BM stem and progenitor cells, CD34 is a maturation, but not a differentiation marker. Neither a receptor nor a signal transduction molecule, it is involved in SC homing and adhesion processes [13]. Synthesis of monoclonal antibodies against the CD34 antigen has provided the means for developing immune-magnetic systems to separate HSCs from blood. Given the paucity of CD34+ HSCs, hematopoietic growth-factors like Granulocyte-Colony Stimulating Factor (G-CSF) are administered to patients before autologous SCT (ASCT), and to donors before allogeneic SCT [14]. In the BM, hematopoietic growth-factors expand the SC compartment and alter adhesion to the stroma. CD34+ hematopoietic progenitors increase in number to the point that they leave the BM microenvironment and pour into the bloodstream (mobilization), where they are easily collected by leukapheresis. Selection procedures separate ontogenetically more immature progenitors, characterized by self-renewal and multi-lineage differentiation capacity, which are able to reconstitute the recipient's hematopoietic system [14]. Reconstitution depends on HSC "homing" as they migrate in the recipient from PB to the BM microenvironment. SC homing is a multi-step process: 1) Rolling on vascular endothelium. PBSCs (for example after a graft infusion) weakly bind adhesion molecules (selectins E and P) which are constitutively expressed by the vascular endothelium. These weak links cyclically create and break, allowing cells to roll on endothelium surface [15]. 2) Activation. After interaction with vascular endothelium, SCs that do not express the CXCR4 receptor detach from endothelium and return into circulation, while those expressing the CXCR4 receptor are activated by CXCL12 and SDF1, cytokines produced by BM endothelial cells. Links with adhesion molecules LFA-1/ICAM-1 and VLA-4/VCAM-1 [16] are stimulated to block SCs on the endothelial surface. 3) Migration from blood vessel to tissue. VLA-4 and VLA-5 integrins interact with fibronectin in the extracellular matrix, facilitating CXCR4+ stem cell migration through endothelial barrier fenestrations and penetration across the underlying basal lamina. 4) Homing to the BM microenvironment. SCs finally reach the BM niche. This microenvironment, which maintains SC properties and quiescence, is populated by various types of cells, adhesion molecules, chemotactic and growth

CHAPTER 6

Human Induced Pluripotent Stem Cells-Based Strategies: New Frontiers for Personalized Medicine

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Abstract: Recent advanced protocols on cell reprogramming for the generation of human induced pluripotent stem cells (hiPSCs) has improved the comprehension of the pathogenic mechanisms and the development of new drugs. In fact, disease-specific pluripotent stem cells offer an ideal platform for both cell and gene therapy protocol applications and represent a good possibility for new and personalized pharmacological treatments. Without any doubt, the most innovative therapies are those which provide a site specific gene correction, and are suitable to those diseases for which a drug's therapy is not available. In the last decade have emerged ZFNs, TALENs, and the CRISPR/Cas9 system, tools for genome engineering, consisting of a sequence-specific DNA-binding domain and a non-specific DNA cleavage domain, that allow to correct mutated genes *in vitro*.

In this chapter, we focus on hiPSCs as a target cells for gene manipulation: new strategies as Zinc-finger nucleases, TALENs and CRISPR/ Cas9 have been developed to maximize the efficiency of genome editing protocols on human reprogrammed cells. Indeed, humanized iPSCs-based disease model systems exploit an individualized cell-based platform that has unlimited growth potential for novel regenerative strategies and clinical therapeutics, along with companion diagnostics, to predict and prognosticate the molecular basis of various human diseases.

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Keywords: CRISPR/Cas9, Gene editing, HESCs, hiPSCs, Human diseases, TALENs, ZFN.

INTRODUCTION

Human embryonic stem cells (hESCs) are pluripotent stem cells obtained from the inner cell mass of embryos at the blastocyst stage [1]. These cells have the unique feature to proliferate unlimited *in vitro*, preserving pluripotent state, moreover they can also differentiate into three germ layers and their derivatives adult tissues. For this reason, hESCs have significant potential for regenerative medicine, even if ethical issues regarding their origin have hindered the clinical application [2]. Thus, fetal, perinatal and adult stem cells can be an alternative source and a new option for regenerative medicine [3, 4]. Unfortunately, immune rejection and differentiation capacities are often not both satisfied by these types of stem cells, making their use inappropriate [5, 6]. An alternative and promising new possibility is the generation of induced pluripotent stem cells (hiPSCs) obtained from adult cells of patients affected by human diseases [7]. hiPSCs play the part of adult and embryonic stem cells, recapitulating the pathological phenotypes and the etiopathology of the diseases *in vitro*. In this way these cells have revolutionized the approaches for understanding the molecular and functional mechanisms of many diseases [8]. Moreover, hiPSCs are a valuable instrument for cell replacement therapy offering an important clinical implication, thanks to their ability to give rise to many disease-relevant cell types. Currently, degenerative diseases are treated with small molecule therapeutics and surgical interventions, looking for alternative therapeutic as iPS technology [9]. In fact the generation of pluripotent cells from patients with developmental or degenerative disorders allows the repopulation of injured or degenerated tissues, thanks to their potential to form *in vitro* relevant somatic lineages [10]. Moreover, patient-specific iPSC-derived systems represent platforms to assess personalized pharmacological therapy of the patient's disease symptoms and to test *in vivo* cell-based repair/modulation of their disease profile. Lastly, disease-specific hiPSCs represent a good target for gene therapy approaches. A new method named 'genome editing' has been recently developed and largely used in the studies of functional genomics, transgenic organisms and gene therapy [11]. Genome editing consists in specific nucleases, which can create *in vitro* site

changes in the genomes through a specific DNA-binding domain and a non-specific DNA cleavage domain. Subsequent correction of mutated genes induces established insertions, deletions or substitutions at the loci of interest.

Relatively new approaches have been developed, such as Zinc-Finger Nucleases (ZFNs), Transcription Activator- Like Effector Nucleases (TALENs) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated (Cas) protein 9 system.

ZFNs are engineered nucleases [12], containing a custom Cys2-His2 DNA-binding motif and a DNA catalytic module of the FokI restriction endonuclease. Other popular genome editing platforms are represented by TALENs [13], which are derived from a natural protein of plant pathogenic bacteria Xanthomonas. The DNA-binding domain of TALENs contained between 13 and 28 repeats, each of which is composed of 33–35 amino acids. Two hypervariable amino acids known as the repeat variable diresidues (RVDs) are responsible for DNA binding. Recently, also CRISPR/Cas9 system provides a promising addition to ZFNs and TALENs for genome editing [14]. CRISPR/Cas9 is based on small RNA for sequence-specific cleavage. Because only programmable RNA is necessary to create sequence specificity, CRISPR/Cas9 is easily adaptable and develops very quickly [15, 16]. All the above discussed, gene edit approaches can also be used to induce specific mutations in hiPSCs, converting wild type sequence to mutated one.

In this chapter, we will focus on hiPSCs as a target for gene manipulation. Novel strategies focused to increase the efficiency of genome editing protocols on human reprogrammed cells will be also discussed.

1. GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSCS)

In 2007, a series of follow-up experiments has been performed in which human adult cells has been reprogrammed into hiPSCs by Shinya Yamanaka's lab. Nearly simultaneously, a research group led by James Thomson achieved the same result. The reprogramming to pluripotent cells has been done through the exogenous induction of four transcription factors such as Octamer- Binding

CHAPTER 7

Stem Cells for Therapeutic Delivery of Mediators and Drugs

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Abstract: This chapter discusses alternative methods for drug delivery. Specifically, we focused on the use of mesenchymal stem cells (MSCs) and to show how these stem cells can be available as off-the-shelf cells. The advantages of MSCs are their unique immune properties and the ability of these cells to migrate to areas of inflammation such as tumors. In addition, MSCs are easy to harvest with several fold expansion, as well as little to no ethical concern. Although MSCs are similar by phenotype, the effectiveness from each source needs to be compared for homing to the desired organ/tissue, intercellular communication and the delivery of non-coding RNA through secreted exosomes. A major advantage of MSCs is the ease by which they can become available as off-the-shelf cells containing the drugs or RNA for immediate transplant to patients. There is little concern that MSCs will linger for a prolonged period because the clinical and experimental evidence indicate that allogeneic MSCs (off-the-shelf) can be readily cleared by the immune system. The chapter discusses why there is an immediate need for cellular delivery of drugs, given the cumbersome regulation and confounds of current single drug trials.

Keywords: Breast cancer, Cancer stem cell, Connexin, Cytokines, Drug delivery, Exosomes, Mesenchymal stem cells, miRNA, Non-coding RNA.

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INTRODUCTION

The current method to select a drug to treat diseases is generally based on a drug that has undergone costly clinical trials in systematic phases. The trial is accompanied by an overwhelming large package of the application to comply with the respective regulatory bodies. These trials are generally performed with clear inclusion and exclusion criteria for specific populations. This clearly indicates that the clinical trials have begun with a bias and, this bias could continue to marketing when those exclusion criteria may not be relevant.

The United States of America (USA), which has a diverse patient population with respect to ethnicity, is quite clear that all ethnic groups and sexes should be included in the clinical trials. This message is adhered by institutional regulatory boards that oversee the trials, with careful review in the justification for the proposed inclusion and exclusion criteria. Due to these regulations and the availability of diverse ethnic groups in the USA, the system, although not perfect, the clinical trials in the USA are generally inclusive with respect to ethnicity.

The importance of including individuals with different ethnic backgrounds seems to be overlooked as the cost of clinical trials increase, leading to the drug developers seeking countries that could conduct the trials at less cost. The question is not the integrity of the trial but the ability to conduct the trials at reduced cost, although at the expense of ethnic diversity. This is usually not deliberate but to conduct cheaper clinical trials in counties in which the general population is ethnically homogeneous. Despite these shortcomings, the drugs are eventually approved and used for all ethnic backgrounds.

Another issue currently in medicine is the over-use of the term ‘precision medicine’. This is generally considered as the development of treatments to specifically suit the patient, despite similar diagnosis. It is assumed that this type of treatment will consider the varied polymorphisms in human but it is not clear if a large number of parameters are, or will be considered when the treatments are designed. Basic biologists and clinical scientists in general, are not open to include mathematicians and engineers in their teams. These latter individuals can develop simulation models that can be tested by biologists. This type of ‘tunnel

vision' of those involved in clinical trials has led to a relatively small window of soaring profits for the makers of the drugs. However, this could results in questionable long-term efficacy for the patients.

To reiterate the potential harm by excluding ethnic diversity in a drug trial pertains to the many failures of the drug. At this time, it is left to the individual physicians to fill the gap. This is rarely done since a controlled trial is costly. In some cases there are cooperative bodies that share the data from different patients within a country and with international bodies. However, it should be noted that the cost needed to fix the problem that was created early has to be paid by someone, and in most cases, by higher cost of the same drugs or others.

The field of stem cells is evolving as a different method of drug delivery. This article will use a model of breast cancer (BC) to discuss how stem cells can be developed for drug delivery. The premise is to understand how the microenvironment facilitates the disease, in this case, cancer dormancy or metastasis. The information will then identify drug targets. The targets will be blocked by drugs or small non-coding RNA (ncRNA) within stem cells. This method might eliminate the broader issue of ethnic diversity but would not eliminate the problem.

This article focuses on mesenchymal stem cells (MSCs), mostly due to the ease in obtaining large amounts, reduced ethical issues, and the unique ability of MSCs to be used given across allogeneic barrier [1]. We will also discuss the types of communications that could occur that will allow the MSCs to transfer the drugs. We will focus on RNA delivery since this is a growing field in therapeutics. The data has identified MSCs as good cellular vehicle to deliver small ncRNA or their antagomiRs.

Mesenchymal Stem Cells (MSCs) - Background

Since this review is focused on MSCs, we will present a brief background on these stem cells. This section will also include the advantages of MSCs, in particular, their use across allogeneic barrier, generally referred as `off-the-shelf' stem cells. MSCs are ubiquitously present in adults and in fetal tissues. In adults, MSCs are predominantly found in the bone marrow and adipose tissues. MSCs

SUBJECT INDEX