Stem Cell Transplantation

Biology, Processing, and Therapy

Edited by
Anthony D. Ho, Ronald Hoffman, and Esmail D. Zanjani

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Preface

The continuing enthusiasm for and controversy around stem cell research has been spurred by the establishment of human embryonic stem cell lines in 1998. This technology has opened up novel avenues for tissue engineering in organ transplantation. Never in the history of biomedical research have scientific discoveries stirred up such tremendous repercussions on a global scale. Stem cells have been compared to the "fountain of youth", that mankind has searched for since time immemorial. It has been speculated that out of stem cells, we might be able to produce all sorts of replacement parts for regenerative medicine.

Despite this world-wide enthusiasm and efforts, major fundamental issues have remained unresolved. For embryonic stem cells, the challenges are tumorogenesis, rejection by the host immune system, transmission of pathogeneic agents during cultivation, in addition to the continuing ethical debate. For adult stem cells, initial results intended to demonstrate the plasticity potentials have been severely challenged. Some of the initial experiments were not reproducible and others have demonstrated that nuclear or cell fusions might account for most of results interpreted to be due to transdifferentiation. In addition adult stem cells, if identifiable, are of such miniscule amount to be of no clinical relevance.

Nevertheless, stem cells derived from the adult bone marrow, i.e. hempatopoietic stem cells, have been used in the clinic already for almost 40 years for patients with leukaemia and hereditary immuno-deficient diseases. Within this time, blood stem cell transplant has evolved from an experimental therapy into standard of care for specific types of myelo- and lymphoproliferative disorders. Progress was, however, gradual and incremental and many groups have contributed. This development has shown that stem cell research requires resources, commitment and team work.

To bring stem cell technology into clinical practice for regenerative medicine, a thorough understanding of the basic principles underlying stem cell regeneration and regulation of self-renewal versus differentiation is absolutely essential. Research efforts in the next years should focus on the cellular and molecular mechanisms regulating "stemness" and the decision process involved in differentiation. Only through a fundamental understanding of these principles can we be able to acquire the power to manipulate a stem cell's destiny. This volume, Frontiers in Stem Cell Transplantation, deals with all the above mentioned challenges.

Part 1 focuses on basic stem cell biology with an introductory chapter on clinical potentials of stem cells. This is followed by a chapter each on the epigenetic control of hematopoietic stem cell fate and the impact of micro-RNAs on stem cell biology and medicine.

Part 2 focuses on standardization and quality assurance of stem cell preparations with chapters on novel mobilization based on a precise understanding of the SDF-1α/CXCR4 pathway in stem cell lines derived from umbilical cord blood and bone marrow and the challenges associated with genetic manipulation of hematopoietic stem cells.

Part 3 focuses on the strategies which are on the threshold to clinical applications: large animal models testing the plasticity of human marrow-derived stem cells, a unique murine blastocyst model for studying transdifferentiation, animal models testing the potentials of MAPC, and mesenchymal stem cells as vehicles for genetic targeting. The last and fourth is on novel strategies using adult stem cells within clinical trials. Mesenchymal stem cells might serve as a unique immunomodulator, and this is dealt with in chapter 15. The clinical practice and the evidence for adoptive immunotherapy in hematologic malignancies are summarized in chapters 14 and 16.

Heidelberg, Chicago, Reno, April 2006
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Part I
Stem Cell Biology

1
Clinical Potentials of Stem Cells: Hype or Hope?

Anthony D. Ho and Wolfgang Wagner

1.1
Introduction

The present enthusiasm for and controversy around stem cell research began with two breakthroughs: (i) the successful cloning of "Dolly" by Ian Wilmut, Keith Campbell and coworkers in 1997 [1]; and (ii) the establishment of human embryonic stem cell (ESC) lines by the laboratory of James Thomson in 1998 [2]. Without any doubt, these technologies have opened up novel avenues for tissue engineering and organ transplantation [3]. Never in the history of biomedical research have scientific discoveries spawned such tremendous repercussions on a global scale. The ability to rejuvenate or even replace defective organs and the tissues of the human body has been a centuries-old dream. Stem cells have demonstrated their potential to develop into practically all types of specialized cells and tissues in the body, and have therefore been compared to the "fountains of youth" that mankind have searched for since time immemorial. Recent discoveries using both adult and embryonic stem cells as starting cell populations have led to speculations that out of such "raw material" we might be able to produce all sorts of replacement parts for regenerative medicine. Hopes are high that many age-related degenerative disorders such as heart disease, Parkinson's disease, diabetes, and stroke could some day be cured by stem cell therapy.

1.2
What are Stem Cells?

All life forms begin with a stem cell, which is defined as a cell that has the dual ability to self-renew and to produce progenitors and different types of specialized cells in the organism. For example, in the beginning of human life, one fertilized egg cell – the zygote – becomes two, and two becomes four [4]. In these early stages, each cell might still be totipotent – that is, a whole organism can be derived out of each of these cells. Within 5 to 7 days, some 40 cells are formed which

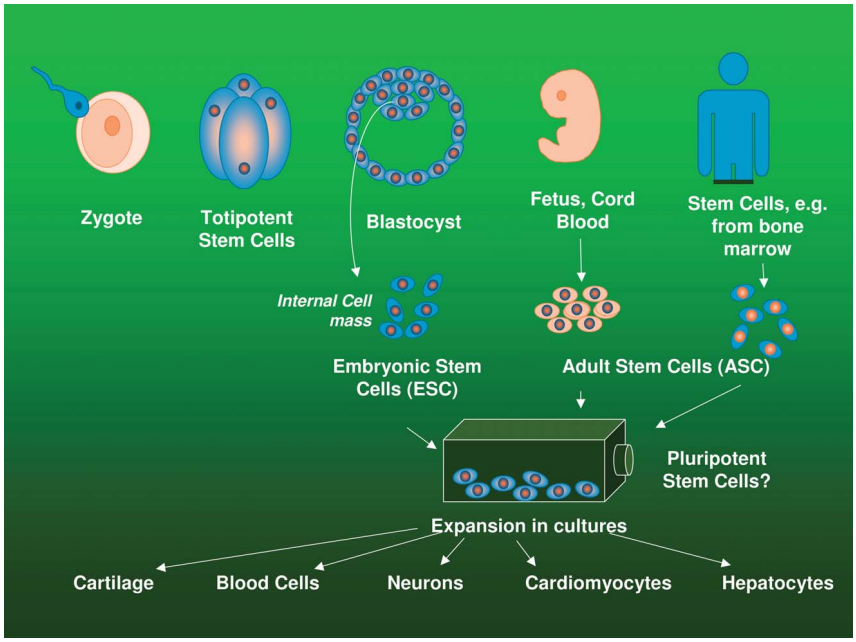

Figure 1.1 Sources for embryonic and adult stem cells.

build up the inner cell mass, surrounded by an outer cell layer forming subsequently the placenta. At this stage, each of these cells in the inner cell mass has the potential to give rise to all tissue types and organs including germ cells – that is, these cells are pluripotent (Fig. 1.1). Ultimately, the cells forming the inner cell mass will give rise to the some 10^{13} cells that constitute a human body, organized in 200 differentiated cell types [5]. Many somatic, tissue-specific or adult stem cells are produced during fetal development. Such stem cells have more restricted ability than the pluripotent ESC and they are multipotent – that is, they have the ability to give rise to multiple lineages of cells. These adult stem cells persist in the corresponding organs to varying degrees during a person's whole lifetime.

1.3 Stem Cells and Regeneration

Lower life forms have amazing prowess of regeneration which mammals and especially humans woefully lack [6]. Upon decapitation, planaria (e.g., a flatworm) will regenerate a new head within 5 days. Hydra, a small tubular freshwater animal that spends its life clinging to rock, is able to produce two new organisms

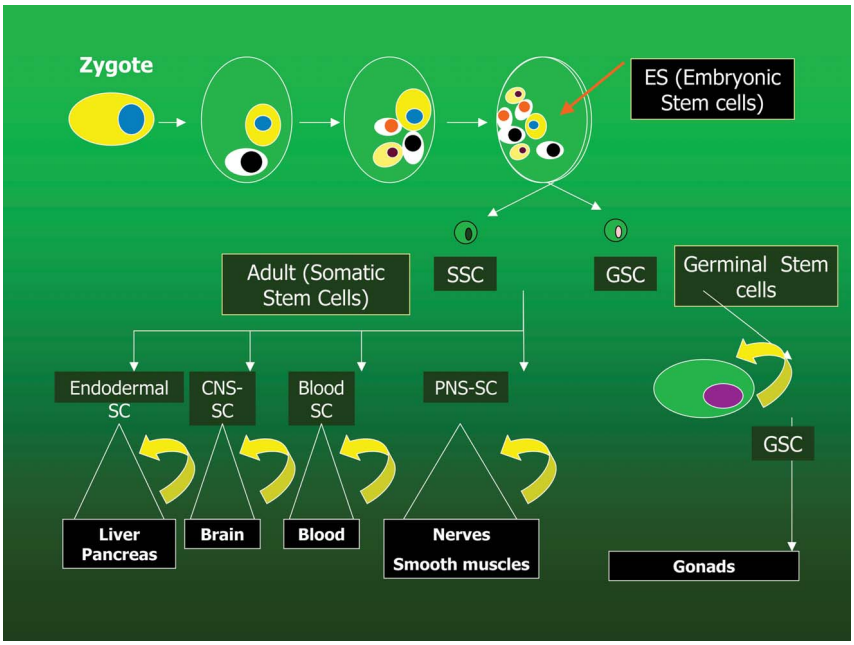

Figure 1.2 Embryonic stem cells (ES) are derived from 5- to 7-day-old embryos and are pluripotent. Pluripotent stem cells can also be derived from germinal stem cells (GSC) and possibly from some somatic (adult) stem cells (SSC). During embryonic development, tissue-specific stem cells (SC) give rise to the mature, differentiated cell types that constitute the specific organs with special functions.

within 7–10 days when its body is halved. After losing a leg or the tail to a predator, a salamander will recover with a new limb or tail within a matter of days.

Mammals pay a high price for climbing up the evolutionary ladder, and have lost comparable regenerative power. Those animals with staggering regenerative potentials are either in possession of an abundance of stem cells, or they can convert specialized cells into stem cells on demand. For example, it has been estimated that some 20 % of the planaria consists of stem cells, while hydra is a "kind of permanent embryo" [6]. Salamanders use a completely different mechanism; when they need a new limb or tail, they convert an adult differentiated cell back to an embryonic undifferentiated one. These cells then gather at the site of a severed organ and form a blastema, which regenerates the missing part. An understanding of the cues and molecules that enable the stem cells to initiate self-renewal, divide, proliferate, and then differentiate to rejuvenate damaged tissue might be the key to regenerative medicine.

To a limited extent, humans can rejuvenate some types of tissue, such as the skin and the bone marrow, but are nowhere near as proficient. The regenerative power is associated with an adequate presence of stem cells in these organs – that

is, epidermal stem cells in the skin and hematopoietic stem cells (HSCs) in the bone marrow (Fig. 1.2). Moreover, regenerative potential of the skin and marrow declines with age [7, 8]. An understanding of how ESCs differentiate into various tissues and how adult stem cells can be coaxed to replace damaged tissue could therefore hold promise for cell replacement of tissue repair in many age-related degenerative disorders.

1.4 Adult and Embryonic Stem Cells

In 1998, the group of James Thomson reported on the establishment of human ESC lines. Human ESCs used for research have been extracted form embryos created by *in-vitro* fertilization. Some 40 cells forming the inner cell mass at day 5–7 after fertilization are transferred to a culture dish lined with feeder cells. After culturing and replating for several months, these cells might maintain their self-renewing ability without differentiating into specialized cells, and give rise to ESC lines that could, in theory, replicate for ever [9–11]. Thus, ESCs have the potential to form most – if not all – cell types of the adult body over almost unlimited periods.

As mentioned above, the adult body has a small number of adult or somatic stem cells in some tissues and organs [12–14]. Such adult stem cells (ASCs) have been known to possess the ability to regenerate the corresponding tissue from which they are derived. Hematopoietic stem cells (HSCs), for example, continuously regenerate the circulating blood cells and cells of the immune system during the life span of the organism. Based on animal models, many studies have recently claimed that ASCs might exhibit developmental potentials comparable to those exhibited by ESCs [14]. More recent reports, however, have severely challenged the interpretation of the initial results, suggesting the "plasticity potential" or "trans-differentiation" of ASCs [15–18]. Hence, ASCs have the ability to regenerate the tissue from which they are derived over the lifespan of the individual, while ESCs have the potential to form most, if not all, cell types of the adult body over very long periods of *in-vitro* cultivation. ESCs seem to demonstrate unlimited potential for growth and differentiation. The use of ES-derived cells for transplantation, however, is associated with hazards and ethical controversies. In animal studies, undifferentiated ESCs can induce teratocarcinomas after transplantation, and they have been shown to be epigenetically instable. Pre-culturing of immature ESCs in conditions that induce differentiation along a specific pathway might reduce the risk of tumor genesis. Animal studies have also shown that only donor ESCs after a specific differentiation stage would be accepted by a fully grown animal. ESCs must be primed towards a predefined differentiation pathway before transplantation. Such cultures are likely to contain a variety of cells at different stages of development, as well as undifferentiated ESCs. Purification of the cell preparation is necessary before clinical use could be considered.

1.5
In the Beginning was the Hematopoietic Stem Cell

The concept of stem cells was introduced by Alexander Maximow in 1909 as the common ancestors of different cellular elements of blood [19]. It took, however, almost another 60 years – that is, in 1963 – before McCullough and his coworkers provided unequivocal evidence for the existence of stem cells in the bone marrow [20, 21]. In a murine model, their series of experiments demonstrated that, first of all, cells from the bone marrow could reconstitute hematopoiesis and hence rescue lethally irradiated recipient animals. Second, by serial transplantations, they have established the self-renewal ability of these cells. When cells from the spleen colonies in the recipients were harvested and re-transplanted into other animals that received a lethal dose of irradiation, colonies of white and red blood corpuscles were again found in the secondary recipients. Based on these experiments, HSCs were defined as cells with the abilities of self-renewal as well as multilineage differentiation. This discovery marked the beginning of modern-day stem cell research. Only in recent years have other somatic stem cells been identified in tissues with a more limited regenerative capacity, such as the liver and the brain [22, 23].

The first *successful* attempts of using bone marrow transplantation as a treatment strategy for patients with hereditary immunodeficiency or acute leukemias were performed during the late 1960s [24–27]. The original idea was to replace the diseased bone marrow with a healthy one after myeloablation. Without the benefits of present-day knowledge of immunology and supportive care, morbidity and mortality rates associated with the treatment procedure were then high [27]. Nevertheless, the results were considered encouraging as compared to those obtained with conventional treatment options. Bone marrow transplantation has in

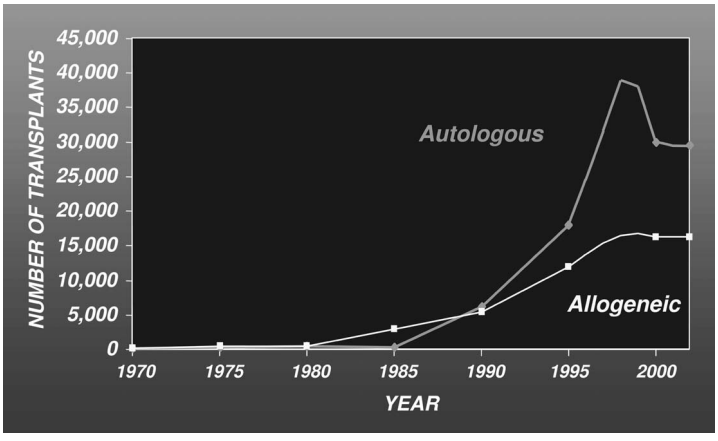

Figure 1.3 Annual numbers of blood and bone marrow transplants worldwide (1970 to 2002), as registered by the International Bone Marrow Transplant Registry.