New perspectives in stem cell research: beyond embryonic stem cells

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Abstract

Although stem cell research is a rather new field in modern medicine, media soon popularized it. The reason for this hype lies in the potential of stem cells to drastically increase quality of life through repairing aging and diseased organs. Nevertheless, the essence of stem cell research is to understand how tissues are maintained during adult life. In this article, we summarize the various types of stem cells and their differentiation potential *in vivo* and *in vitro*. We review current clinical applications of stem cells and highlight problems encountered when going from animal studies to clinical practice. Furthermore, we describe the current state of induced pluripotent stem cell technology and applications for disease modelling and cell replacement therapy.

Introduction

Scientists have observed that in some tissues such as skin, blood and intestinal epithelium, the lifespan of fully differentiated cells is short and cells of this status do not renew themselves. This has led to the concept that there is a pool of stem cells in these tissues that has an extensive self-renewal capacity and is able to generate daughter cells that then undergo differentiation. These adult stem cells (ASCs) can only generate a range of cell types of the tissue in which they reside and are thus called multipotent. In addition to ASCs, there are stem cells with an even broader differentiation potential, so-called embryonic stem cells (ESCs). ESCs can be isolated from embryos and maintained in culture without undergoing differentiation. They are

able to generate all cell types of the body, but not the umbilical cord, trophoblasts and associated structures, thus they are unable to generate a functional organism. These cells are described as being pluripotent. The characteristics of different sources of stem cells are summarized in Table 1.

Embryonic stem cells

Mouse ESCs are derived from the inner cell mass of the blastocyst before it would have implanted in the uterine wall. Their isolation in 1981 by Sir Martin J. Evans and Matthew Kaufman (1) and independently by Gail R. Martin (2) has contributed extensively to our knowledge of pluripotency and differentiation of stem cells. This finding has revolutionized science by providing a platform for generating knockout mice, which have now become a standard research tool. In 1998, James Thomson and his team reported the first successful derivation of human ESC lines (3), thus extending the great potential of ESCs by providing the opportunity to develop stem cell-based therapies for human disease. However, this finding has also caused disquiet, as these cells were derived from *in vitro*-fertilized human embryos that in theory would have the potential to produce a human being. However, besides ethical issues associated with the use of human embryos, these are a very limited cell source hindering large-scale application for potential treatment regimes. A further disadvantage of ESCs is their unlimited proliferative capacity; this could cause tumour formation upon transplantation (so-called teratomas). Also, ESCs would not be immune-compatible with a heterologous patient, due to their different genetic origin, thus further restricting their therapeutic potential. Thus, several methods to generate patient-matched ESCs have been developed. These techniques are based on the discovery that adult cells can, in some circumstances, be forced to switch lineages by nuclear reprogramming.

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	ESC	ASC	iPSC
Accessibility of stem cells	Very limited*	Unlimited	Limited*
Stem cell expansion potential	Indefinite	Low/High**	Indefinite
Ethical concerns related to stem cells	High	No concerns	Low
Risk of GVHD	High	Low/No risk***	No risk
Risk of cancer formation	High	No risk	High
Immuno-compatibility	No match	High/Full match***	Full match
Differentiation potential	Pluripotent	Multipotent	Pluripotent

*Access to ESC is limited by low number of human embryos. Also there are limitations in efficient productions of ESC and iPSC.

**Expansion potential of ACS is limited due to spontaneous differentiation of cell *in vitro* depending on their age and origin (e.g. Lineage-committed neural, hepatic or pancreatic stem cells have a very limited proliferative capacity while umbilical cord-derived mesenchymal stem cells have very high proliferative capacity).

***Allogenic ASC immuno-compatibility depends on HLA match of the cells donor. In case of autologous ASC (e.g. cord blood or bone marrow) the HLA match is full.

Table 1. Characteristics of different sources of stem cells for their use in research and therapy.

Nuclear reprogramming and induced pluripotent stem cells

Somatic cells can be reprogrammed to a pluripotent state by injecting the nucleus of an adult cell into an enucleated oocyte (4,5) [for a review refer to (6)]. This leads to redirection of the somatic cell nucleus by the host cytoplasm. After several cell divisions, the reprogrammed cell forms a blastocyst, which is a genetic match with the nuclear donor (mitochondrial DNA however is of the recipient – present in the oocyte cytoplasm). Up to now, human somatic cell nuclear transfer as it is called, is severely limited as it is very resource-intensive. Also, the technique tends to cause some cell damage and therefore is very inefficient. As an alternative to using oocytes, ESCs can be used for reprogramming human somatic nuclei (7). Unfortunately, this method is also rather inefficient and cannot be used for therapeutic applications due to the level of tetraploid cells obtained.

These studies on somatic cell nuclear transfer clearly demonstrated that adult cells can be reprogrammed to a pluripotent state. Recently, the first report of inducing pluripotency in adult cells by direct reprogramming has led to the emergence of a new research field – induced pluripotent stem cells (iPS cells). In 2006, Takahashi and Yamanaka screened 24 genes important for maintaining pluripotency and self-renewal in ESCs and found that retroviral transduction of only four of these genes sufficed to reprogram somatic cells to a pluripotent state (8). Soon after this report demonstrating reprogramming of murine fibroblasts, iPS cells have been derived from multiple murine and human somatic cell types (9,10); it is now even possible to generate iPS cells from patients with specific diseases (11–13). Such cell lines will help to establish *in vitro* disease models and may lead to discovery of new drugs. While generation of iPS cells is technically

undemanding, reprogramming is still a slow and inefficient process consisting of largely unknown events. Studies using various sets of reprogramming factors and different cell sources have reported different reprogramming efficiencies. Methods of factor delivery also heavily influence efficiency of iPS derivation. The main drawback of viral factor delivery has been use of retroviral vectors that integrate into the cell's genome, which results in cell dysfunction or might contribute to tumorigenesis. Thus, various non-integrating approaches have been developed (14–18); yet these approaches remain inefficient. Various small molecules and soluble factors that enhance the reprogramming process have been identified, but besides their lack of permanent genome modification and ease of use, they exert broad and non-specific effects that could lead to deregulation of gene expression [review on generation and techniques for reprogramming various cell types, (19)]. However, development of new reprogramming techniques is very important for future clinical applications of iPS cells, as they greatly enhance safety of iPS cells for therapeutic purposes.

Adult stem cells

ASCs are more suitable for clinical purposes, as they are ethically acceptable and readily available from many tissues. Furthermore, ASCs can be used for autologous treatment of degenerative, traumatic and congenital diseases as they can be purified from a patient's own body thus avoiding complications of immunity. The best-described ASCs are haematopoietic stem cells (HSC), found in the bone marrow niche, and in umbilical cord blood (UCB), first identified by Ernest McCullough and James Till (20). Alternatively, they can be purified from peripheral blood after mobilization from bone marrow by administering granulocyte colony-stimulating factor (G-CSF) with or

without CXCR4 antagonist (21). HSCs are capable of differentiating into all myeloid and lymphoid lineages. Whether they can also differentiate into non-haematopoietic lineages is still controversial, as non-HSCs may also account for some cell differentiability plasticity observed *in vitro*.

Mesenchymal stem cells (MSCs) are another prominent type of multipotent stem cell, capable of differentiating into various mesodermal lineages, including osteoblasts, chondroblasts, adipocytes, fibroblasts, neuronal cells or myocytes. MSCs are present in many organs, but are specially abundant in the bone marrow and UCB. MSCs can be expanded *ex vivo* and modified to acquire specific properties. They have been shown to enhance regeneration in a wide range of damaged tissues and are also readily available from individual patients; thus they are being examined in increasing numbers of medical applications.

UCB, amniotic fluid and placenta are immediately available sources of stem cells. The unique populations of ontogenetically young adult stem cells derived from these tissues are very powerful resources for ASC-based therapy. The non-invasive nature of collection and their easy storage make these ASCs a very potent and readily accessible cell source for allogeneic/autologous transplantation. Their prospective use is currently under wide examination and their status is summarized in (22,23).

Stem cell therapy today and in the future

Even though stem cell research is a novel field in science, stem cells have been in clinical use for decades. Haematopoietic stem cell transplantation is the oldest and most widely available stem cell therapy (24). It is very successful, because HSCs do not need to be expanded *ex vivo* prior to transplantation and there is no need to reconstitute complex organ architectures. Allogeneic HSC transplantation is a common procedure used to treat bone marrow failure. It is also widely used to prolong life of patients with hematopoietic diseases such as leukaemia, thalassaemias, sickle cell disease or myeloid hypoplasia (e.g. anemia or neutropenia). Autologous HSC transplantation is also performed to reconstitute the immune system in immunocompromised patients after radiation and/or chemotherapy. UCB is an accepted alternative to HSCs, as it can be harvested easily without any impact on the donor, stored in UCB banks, has a low risk of viral contamination and reduces potential for graft-versus-host disease.

MSCs are an interesting cell source for allogeneic stem cell therapy, as they are not only multipotent, but they also have immunosuppressive properties that minimize risk of immune rejection. Again, Wharton's jelly of the umbilical cord and the placenta, are very accessible sources of MSCs. HSCs and MSCs can be used not only to treat

disorders of the hematopoietic system. Further major diseases such as neurodegenerative disease can be treated with the use of MSCs. In human cell transplant derived animal models of various neurodegenerative diseases, massive loss of neurons is partially compensated by spontaneous neurogenesis involving neural stem cells (NSCs) (25,26). However, this intrinsic repair process alone cannot restore central nervous system (CNS) function. Thus the use of exogenous stem cells may boost regeneration of affected tissue [for a review refer to (27)]. Indeed MSCs and HSCs seem to migrate towards a site of injury within the CNS. While a direct role for these cells in neuronal replacement is still actively debated, undifferentiated stem cells could contribute to CNS regeneration through intrinsic neuroprotective capacities such as production of neurotrophic factors, stimulation of neurogenesis, reduction of neuroinflammation or even non-neuronal cell replacement [elegantly reviewed in (28)]. In the case of Parkinson's disease, a neurodegenerative disease affecting dopaminergic neurons, stem cell transplantation has been performed successfully leading to partial alleviation of Parkinson symptoms. In this respect, several stem cell types have already been tested: MSCs (29), olfactory mucosa-derived NSCs (30), human ESC-derived dopaminergic neurons (31) and iPS cells (32). These procedures indicate that human clinical trials with various stem cell types are likely to emerge soon. For example, a breakthrough in adult stem cell transplantation has been reported recently by a pan-European team from the universities of Barcelona, Bristol, Padua and Milan. A tissue-engineered trachea (windpipe), utilizing the patient's own stem cells, has been successfully transplanted into a young woman suffering from failing airways. This treatment provided the recipient with a functional windpipe and did not provoke immune rejection commonly seen with conventional transplanted organs (33).

Stem cell therapy for cardiac disease is also very promising, as current therapies can only delay progression of disease but cannot provide a cure for heart failure. Just as in the case of natural regeneration of brain cells, regenerative potential of heart muscle is insufficient to compensate its severe loss caused by myocardial infarction or other defects in myocardial integrity. Several cell types have been tested for their potential in cardiac therapy. Initially, skeletal myoblast were tested, but they did not differentiate into cardiomyocytes. Therefore, they cannot integrate functionally into the myocardium. HSCs have been transplanted, but there is still controversy over whether they could differentiate into cardiomyocytes *in vivo*. Furthermore, improvements in cardiac function upon HSC transplantation were rather small. In this respect, MSCs are more promising as they can differentiate into cardiomyocytes and are less immunogenic than

HSCs. Endogenous cardiac stem cells (CSCs) seem to be the gold standard for regenerating heart muscle, but no clinical data using CSCs are yet available. For a review on stem cell therapy for cardiac disease and future directions, please refer to (34).

Diabetes mellitus is a further major health problem. One of its forms is an autoimmune disease, which causes destruction of pancreatic insulin-producing β cells in islets of Langerhans. Exogenous insulin administration has remained the standard treatment for type I diabetes for nearly 90 years, but it often was not able to prevent long-term complications of the disease. Pancreas and islet transplantation potentially could cure diabetes, but they carry major risks for the patient despite the technical difficulty and health care costs. On the one hand, whole pancreas transplantation has good efficiency, but involves highly complicated surgery. On the other hand, islet transplantation carries the need for lifetime immunosuppression and the risk of long-term graft dysfunction. Thus, the search for other sources of β cells suitable for transplantation has begun. Human ESCs have been differentiated *in vivo* into insulin-secreting cells after implantation into mice, but teratoma formation in these animals remains a safety concern (35). In a promising study by Zhou *et al.*, differentiated murine pancreatic exocrine cells could be reprogrammed into insulin-secreting cells which responded to hyperglycaemia *in vivo* (36). Human MSCs have also been successfully transplanted into diabetic immunodeficient mice, decreased hyperglycaemia and increased insulin levels and higher β cell numbers were observed (37). Two studies on newly diagnosed type I diabetes mellitus patients have demonstrated prolonged insulin independence after autologous non-myeloablative HSC transplantation (38,39). These approaches to generate new β cells for clinical applications raise hope for new diabetes treatments.

While ASCs are already used to help treat various diseases, first clinical studies on use of pluripotent stem cells are just beginning. The biotechnology company 'Geron Corp.' is the first to conduct a clinical trial approved by the Food and Drug Administration (FDA) of the USA, using an ESC-based product to treat complete spinal cord injury [based on (40)]. Unfortunately at this moment, the trial is on hold as a fraction of the animals developed cysts at the injury site. While this first trial may pave the way for further ESC-based treatments, iPS cells have a major advantage over ESCs; they can be derived from the same patient for whom disease treatment is being sought. This should obviate the need for immunosuppression upon transplantation and circumvent any ethical issues derivation of ESCs faces. Several elegant studies have already proven the potential of iPS cells to treat disorders. In a humanized sickle cell anaemia model, mice have been

rescued after transplantation of haematopoietic progenitors derived from autologous iPS cells with a genetically corrected β -globin locus (41). In a further report, iPS cells were differentiated into neurons that functionally integrated into the host brain upon transplantation into foetal mouse brain. In addition, such cells were able to improve behaviour in a rat model of Parkinson's disease (32). In another study, injection of undifferentiated iPS cells into diseased myocardium of mice led to multilineage repair (42). Scientists from the Center for Regenerative Medicine in Barcelona have generated patient-specific iPS cells from Fanconi anaemia patients that can, upon correction of the genetic defect, give rise to healthy haematopoietic progenitors (43). Furthermore, skin fibroblasts of a patient with homozygous β -0-thalassaemia have been reprogrammed into iPS cells, which could be differentiated into haematopoietic cells that synthesized haemoglobin (44). There is no doubt that these studies demonstrate the enormous therapeutic potential of iPS cells, but there are still major hurdles to overcome before iPS cell-based treatments could be used in the clinic in broad applications.

The best cell type in terms of safety and efficiency for reprogramming still has to be determined. Risk of pluripotent stem cells to cause teratomas after transplantation has to be eliminated by excluding them from *in vitro*-differentiated cultures. Those pure populations of disease-relevant cells then would have to be transplanted into patients by safe and effective methods that still have to be established. Reprogramming efficiency must be increased and generation of iPS cells must be scaled up and standardized to allow high-throughput drug screens and toxicity tests, as well as fast availability for an increasing number of patients. Finally, comprehensive pre-clinical trials are needed to evaluate safety and efficacy of 'clinical grade' iPS cells.

In conclusion, new clinical trials of HSCs, MSCs and NSCs for treatment of various pathologies other than already-established transplantation therapies, demonstrate the diversification of stem cell research. Newly evolving pluripotent stem cell therapies harbour enormous potential for treatment of currently incurable diseases. First clinical data on therapeutic effectiveness of pluripotent stem cells will be available in the near future, but benefits on a larger population of human patients are still some way off. Nevertheless, the remarkable progress of iPS cell technology towards clinical implementation in the last 3 years indicates that personalized stem cell therapy will undergo a revolution that will be thrilling to observe.

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