Stem cells: hype or hope?

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Stem cells undergo self-renewal and differentiate into multiple lineages of mature cells. The identification of stem cells in diverse adult tissues and the findings that human embryonic stem cells can be proliferated and differentiated has kindled the imagination of both scientists and the public regarding future stem cell technology. These cells could constitute an unlimited supply of diverse cell types that can be used for cell transplantation or drug discovery. The new options raise several fundamental ethical issues. This review gives an overview of the scientific basis underlying the hope generated by stem cell research and discusses current ethical and funding regulations.

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▼ Pluripotency denotes the ability of one cell to develop into any kind of differentiated cell present in the body. This review deals with pluripotent stem cells derived either from early embryonic or adult tissues. It does not discuss cells that are already committed and have become progenitors of specific cell lineages.

Three pluripotent cell types have been established from human tissue: embryonic carcinoma cells (EC cells), embryonic germ cells (EG cells) and embryonic stem cells (ES cells). Human EC lines are derived from the undifferentiated stem cell components of germ cell tumors [1,2]. Compared to human ES cells they seem to have less differentiation capacity *in vivo* and are usually aneuploid [3]. They could serve as a model from which one can gain an understanding of signals that promote cell differentiation, but because of their karyotype, EC cells are not suitable for clinical applications.

EG cells are isolated from the genital ridges of the developing embryo [4], typically at 5–9 weeks post-fertilization for humans. By contrast, human ES cells are derived from embryos aged 4–6 days, which corresponds to a pre-implantation stage. These cells are harvested from the inner cell mass (ICM) of the blastula. Human ES cells have been shown to keep a stable diploid karyotype after propagation in culture, and because of their expression of a high level of telomerase activity (which delays senescence) they possess a long-term proliferative potential, making them suitable for unlimited expansion in culture. When plated on mouse embryonic fibroblast feeder cells, human ES cells can be maintained in an undifferentiated state and proliferate [5–7]. In the absence of growth factors [8], or following withdrawal of fibroblast growth factor-2 (FGF-2) from the medium [9], human ES cells can differentiate into a wide variety of cell types. Upon transplantation into immunodeficient mice, undifferentiated ES cells differentiate into all three EG layers [5].

In the media, ES cells have been proposed as a potential source of donor tissue for transplantation therapies that could lead to cures for a wide range of diseases and conditions, such as diabetes, heart failure and diseases of the nervous system [in particular brain and spinal injuries, multiple sclerosis (MS), as well as Parkinson's and Alzheimer's diseases]. A report from the National Institutes of Health (NIH; Bethesda, MD, USA) recently stated: 'This class of human stem cell holds the promise of being able to repair or replace cells or tissues that are damaged or destroyed by many of our most devastating diseases and disabilities.' (NIH, July 2001; http://www.nih.gov/news/stemcell/scireport.htm). Thus, human ES cells are connected with a great deal of hope based on their (theoretical) ability to proliferate indefinitely and their inherent capacity to develop into virtually any type of tissue desired. A cause for concern is that ES cells also present the risk of developing into teratomas (benign tumours containing cell types from the endo-, ecto- and mesoderm) following transplantation [7,10]. However, two recent studies revealed no histological evidence of teratoma formation 1–8 weeks after human ES cells were transplanted into the lateral cerebral ventricles of newborn mice [8,9].

Most of the available data on ES cells has so far been obtained from mouse cells, which

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were first isolated in 1981 [11,12]. Mouse ES cells have been shown to differentiate *in vitro* into a variety of tissues from all three EG layers: cardiomyocytes, skeletal muscle, smooth muscle [13–15], melanocytes [16], hematopoietic progenitors, endothelial cells [17], neurons [18], glia [19] and pancreatic islet cells [20,21]. Mouse ES cells have also unveiled their full potential to develop into cells from all germ layers after injection into a blastocyst [22]. Because of their ability to differentiate into many different cell types, ES cells are a convenient model system for the study of mechanisms underlying lineage specification. However, organogenesis still does not occur to a significant degree *in vitro*.

Use of ES cells in animal models of human disease

Diabetes

Current diabetes drug therapies do not provide sufficiently tight control of blood glucose to avoid late-stage complications. Transplantation approaches using the whole donor pancreas or isolated islet-cell transplantations (only 10% of grafted patients are insulin-independent after one year in successful programs) are limited by a shortage of donors, major surgery and long-term immunosuppression, which might even counteract islet cell function [23]. Although attempts to generate islets *in vitro* from adult human pancreatic duct cells have been successful, the ability of these cells to restore blood glucose *in vivo* remains unproven [24]. Therefore, alternative cell sources are sought. Recently, Lumelsky and coworkers succeeded in generating cells that produce insulin and other pancreatic endocrine hormones from mouse ES cells [21]. The authors describe a five-step technique to turn mouse ES cells into cell clusters that bear some resemblance to pancreatic islets. Upon glucose stimulation these cells release insulin, albeit only low levels that, after transplantation into diabetic mice, did not exceed 2% of the normal production. Nevertheless, diabetic mice receiving this type of cell transplant survived longer and maintained their body weight, despite the low insulin production [21]. Using a lineage selection system in mouse ES cells, Soria and coworkers produced an insulin-secreting cell line that normalizes blood glucose when transplanted into streptozocin-induced diabetic mice [20].

Engineering ES cells to produce an abundant source of insulin-producing cells for transplantation in diabetes is one of the major applications for ES cell technology, but there is still a long way to go. First, cells need to be better characterized with regard to their response to glucose, and insulin production needs to be higher. Second, similar results have to be obtained reproducibly in human ES cells. Interestingly, there is already one report published on the generation of insulin-producing cells from human ES cells [25]. However, the cell population achieved was not homogenous and contained only low numbers of insulin-secreting cells. Moreover, so far, glucose responsiveness has not yet been reported for these cells and there are no transplantation studies in animals using insulin-producing human ES cells. An additional problem is that juvenile diabetes is probably an autoimmune disease [26]. Therefore, the autoimmune process might attack the transplanted cells, leading to graft failure.

So, although the field of stem cell transplantation for diabetes has tremendous potential, there are still many basic scientific issues to address before clinical trials can be seriously considered.

Demyelinating diseases

Demyelination is the major pathology in diseases such as MS and leukodystrophies and is also a feature of spinal cord injury. There is evidence from animal experiments, although scarce, that transplanted myelinogenic cells (glial cells producing myelin) can remyelinate the damaged axons and restore function [27]. Glial cell transplantation could, therefore, provide a therapeutic strategy for chronic demyelinating disorders. Cells can be obtained either from peripheral nerves or from multipotential stem cells, which have been expanded and committed to oligodendrocyte lineage before transplantation. In a recent study, ES cell-derived oligodendrocyte precursors were transplanted into a rat model of myelin disease. The cells efficiently myelinated axons in both the brain and spinal cord [19]. Furthermore, Liu and coworkers have reported that oligodendrocytes derived from mouse ES cells can remyelinate axons *in vitro* [28]. After transplantation into the spinal cord of rats with chemically induced demyelination or myelin-deficient shiverer mutant mice, grafted cells migrated into the host tissue and differentiated mainly into mature oligodendrocytes, which produced myelin. However, the authors do not provide data to suggest improved neurological function in these animals [28].

These results obtained with mouse ES cells should stimulate trials with human cells in animal models of demyelinating disease. Although the animal experiments suggest that it might be possible to successfully transplant such cells from different sources, the clinical application of these cells in human disease is not straightforward. The first clinical trial began at Yale University School of Medicine (New Haven, CT, USA) in July 2001. One patient suffering from MS received an autograft of peripheral myelin-forming cells (Schwann cells), obtained from the sural nerve, into a demyelinated plaque in the right frontal lobe (www.myelin.org/schwann_cells.html). This is part of a safety study, which also addresses whether the graft can

survive, but clinical benefit is not expected. This pioneering clinical trial highlights two fundamental questions: first, many demyelinating diseases, such as MS, are multifocal and it is a difficult surgical task to target several sites. In addition, migration patterns of transplanted cells are not predictable. Second, the pathogenesis of MS has been suggested to involve an autoimmune component [29,30] and, in an analogy to the situation for juvenile diabetes, the grafted tissue might be attacked by the autoimmune process.

For the reasons listed previously, the best candidate for stem cell transplantation in demyelinating diseases could be focal cases with pathology, such as isolated optic neuritis or transverse myelitis. However, results from the clinical studies using primary cells are needed before too much optimism should be raised about stem cell transplantation in demyelinating disorders.

Spinal cord injuries

Spinal cord injury is a major source of morbidity that often affects young individuals. Degenerative diseases of the spinal cord, such as amyotrophic lateral sclerosis, are less common but often lead to premature death. Mouse ES cells have been grafted into the injured spinal cord of immunosuppressed rats. Surviving cells developed into neurons, oligodendrocytes and astrocytes and supported partial recovery of motor function in the hind limbs that were affected by the spinal damage [31]. However, no clinical trial has demonstrated significant functional effects of grafting primary embryonic neural tissue to the spinal cord in humans. Therefore, the development of a stem cell-based therapy for spinal injuries is unlikely to be imminent, and will not be discussed in any further detail here.

Parkinson's disease

Parkinson's disease (PD) is so far the only disorder that has been treated successfully with transplantation of embryonic brain tissue [32]. Thus, it is likely that ES cell technology will be applied to this disease relatively soon. Therefore, we will discuss the application of stem cell therapy in PD in some detail. In PD, dopaminergic neurons in the substantia nigra – a region in the ventral midbrain – slowly die, causing a striatal dopamine deficit and a severe movement disorder. Transplants of dopamine neurons obtained from primary embryonic mesencephalic tissue can partially reverse many of the symptoms of PD [33,34].

A major practical limitation for clinical neural transplantation trials in PD is the need to use multiple donors for each patient [35]. This is partly because of an excessive cell death in the transplants, which tends to be 70–90% and occurs during graft preparation or within the first week after implantation [36]. Even when several measures are taken to inhibit death of the grafted neurons the survival rate typically does not exceed 40% [36]. For this reason, there are attempts to develop alternative sources of donor tissue, such as porcine xenografts, various sources of dopamine-producing autografts and stem cell transplants [35]. The most attractive alternative would be to use a stem cell that could be proliferated in an unlimited fashion and then differentiated into a dopamine-producing cell with a full repertoire of neuronal features (i.e. long-distance axonal growth, efferent and afferent synapse formation and regulated dopamine release).

So far, mouse ES cells have been differentiated successfully into dopaminergic neurons *in vitro*. It has been demonstrated that 7–30% of all the neurons derived from ES cells can be induced into dopaminergic neurons [37–40]. Recently, undifferentiated mouse ES cells were found to develop into fully differentiated dopamine neurons after transplantation into the stratium in a rat PD model. The ES cell-derived dopamine neurons reversed drug-induced motor asymmetry in the immunosuppressed hemiparkinsonian rats. Although these data are promising, 25% of the grafted animals exhibited teratoma-like structures arising from the grafted cells, and in 16% of the transplanted rats the implants contained cells expressing mesodermal markers [41]. These findings indicate that several safety aspects of the technology need to be addressed before neural grafts derived from ES cells can be considered for clinical trials.

An interesting recent development was the observation that dopaminergic neurons could be differentiated successfully from an ES cell line after somatic nuclear transfer in mice [42]. This technology opens up the possibility of grafting completely histocompatible cells to patients in the future and is a good example of so-called 'therapeutic cloning'. However, the approach raises at least two issues. First, many regard somatic cell nuclear transfer in human cells to be unethical [43] because it involves manipulation of the genome of human cells that have the potential to develop into a whole individual ('human cloning'). Second, although most cases of PD are not considered to be inherited, it is possible that there are genetic factors that predispose the dopaminergic neurons to death and that, for this reason, it is undesirable to transfer the patient's own genome into the grafted cells.

The results describing dopamine neurons obtained from mouse ES cells show promise regarding the possibility of a clinical application in PD. Further encouragement comes from two recent papers reporting the differentiation of human ES cell-lines into various types of neurons, including a dopaminergic phenotype [8,9], following grafting to

Table 1. Current stem cell policy worldwide (see Refs [71,72] and http://www.esf.org)

Countries	Regulations
Australia, Spain, Israel, Singapore	Both derivation and use of stem cells from spare embryos permitted
Austria	Embryo research prohibited, creation only for ensuring pregnancy
Canada	2001 Human Reproductive and Technology Bill: bans cloning but allows research on leftover human embryos before 14 days of development
China	Derivation and use of stem cells from embryos created for research permitted, *de facto* policy
Finland	Legislation from November 1999 forbids creation of embryos for the purpose of research
Germany	Embryo Protection Law: it is illegal to derive cells from an embryo for research or diagnosis. However, the import of human ES cell lines is allowed
Ireland, Norway	Any research on *in vitro* embryos, stem cells, cloning is forbidden
Italy	No specific law. Italian health minister gave support to a report published in December 2000 by a group of scientific experts that supports cloning of human embryos for the derivation of stem cells for therapeutic purposes
The Netherlands	September 2000: allows research on supernumerary human embryos but not the creation of embryos for research purposes
Sweden	Stem cells can be derived from embryos until age 14 days. Creation of embryos for research purposes and cloning is not permitted. Enforced from 3 October 2001
Switzerland	Swiss national ethical committees are considering allowing the use of already-derived ES cells
USA	August 2001: research on existing ES cell lines allowed, derivation of new cell lines from existing embryos permitted, creation of embryos for research purposes prohibited. Applies only to publicly funded research, not private sector research

neonatal mice. Taken together with observations that dopamine neurons can be obtained from mouse ES cells, it is possible to envisage clinical trials with differentiated human ES cells in PD in the not-too-distant future.

Ischemic heart disease

Human ES cells grown as embryoid bodies display spontaneously contracting regions. Cells in these regions express cardiac muscle-specific antigens and transcription factors [44]. Cardiac muscle cells derived from human ES cells might constitute a cell source for transplantation in patients suffering from ischemic heart disease or myogenic heart disorders [44]. However, there are also reports in the literature that adult-derived stem cells can differentiate into cardiac tissue and repopulate regions damaged by myocardial ischemia [45,46].

Differences between human and mouse ES cells

Most of the available experimental data on ES cells has been obtained in mice. Although these data provide hope that similar success can be achieved with human ES cells, there are clearly species-differences between these cell types, which could make transfer into the clinical arena difficult.

Human ES cells are isolated from the inner cell mass of the blastula stage of an embryo. The embryos are usually obtained from infertility clinics, where they represent leftover embryos that are stored frozen after *in vitro* fertilization trials. These human cells are much more difficult to propagate than mouse ES cells because they divide more slowly (mouse doubling time is 12 h, whereas for human cells it is 23 h). They also differ from murine ES cells in the expression of certain markers and probably some of their signaling pathways [47]. Human ES cells could only previously be grown on a feeder layer in serum or with FGF-2 and, unlike mouse ES cells, they do not respond to leukemia inhibitory factor (LIF) [5,6]. A recent report suggests that human ES cells can also proliferate on artificial substrates in the presence of conditioned media [48].

Only a relatively small number of human ES cell lines have been characterized and described in the literature [5–7]. The present culture protocols for human ES cells have limitations. For example, the cells do not always survive well after being re-dissociated from a culture and transferred into a new culture dish (so-called 'passaging of cells'). In addition, spontaneous differentiation readily occurs and it is difficult to achieve clonal growth of human ES cells, which means it could be difficult to obtain pure cell populations for clinical use. When injected into immunodeficient mice, undifferentiated human ES cells have been found to form benign teratomas with tissue representing derivatives from all three germ layers [5]. In general, differentiation of human ES cells appears to be

more complete *in vivo* than under in *vitro* conditions. Cell signals that regulate embryonic pattern formation are still being elucidated and, therefore, it will not be trivial to devise protocols that promote the differentiation of a defined cell type.

Stem cell ethics

Although human ES cells have a great therapeutic potential, they are also considered a difficult ethical issue. Because transplantation of cells or organs obtained from adults post-mortem is considered acceptable in most Western cultures, harvesting cells for transplantation purposes from embryos destined for destruction is often viewed differently. This has prompted many governments to revisit their existing regulations (see Table 1) and create special rules for embryo-derived stem cells. In August 2001, US President Bush declared funding for ES cell research acceptable for pre-existing human ES cell lines. However, research involving the creation of new cell lines is not permitted for public funding. Worldwide, it is estimated that there are 71 human ES cell lines available (see Table 2). However, research on the existing cell lines will be limited by lack of characterization and availability. The decision has been debated widely and many experts question whether these cell lines will be sufficient to meet the demands on research in this field [49].

Table 2. Existing cell lines (see http://www.nih.gov/news/stemcell/index.htm, Stem Cell Registry)

Name	Numbers of existing cell lines reported to the National Institutes of Health
BresaGen, Athens, GA, USA	4
CyThera, San Diego, CA, USA	9
ES Cell International, Melbourne, Australia	6
Geron Corporation, Menlo Park, CA, USA	7
Göteborg University, Göteborg, Sweden	19
Karolinska Institute, Stockholm, Sweden	5
National Center for Biological Sciences/Tata Institute of Fundamental Research, Bangalore, India	3
Reliance Life Sciences, Mumbai, India	7
Technicon-Israel Institute of Technology, Haifa, Israel	4
University of California, San Francisco, CA, USA	2
Wisconsin Alumni Research Foundation, Madison, WI, USA	5

This table lists the numbers of human ES cell lines that meet the scientific and ethical criteria set up by the Administration of US President Bush to qualify the cells for possible future research using funding from the National Institutes of Health (Bethesda, MD, USA).

Adult-derived stem cells

During the embryonic development that follows the blastula stages when ES cells are harvested, the three germ-layers ectoderm, mesoderm and endoderm are formed. The progeny of ES cells then segregate into groups of more organ-specific progenitor cells, which gradually mature into adult somatic cells [50].

Stem cells have now been identified in a variety of adult tissues, such as bone marrow, blood, skin, liver, muscle and even the adult brain. These adult-derived stem cells (ASCs) are pluripotent and have the ability to self-renew. Furthermore, in contrast to ES cells, they are easily accessible and devoid of serious ethical issues because they can, for example, be harvested from the patients themselves. This would also make immunosuppressive treatment after transplantation unnecessary.

It appears that ASC have a broader capacity to differentiate into different cell types than was originally thought. An overview of different ASC types and their transdifferentiation capacity is given in Table 3. Thus, brain-derived ASCs can regenerate the entire hematopoietic system in lethally irradiated mice [51] and differentiate into several diverse cell-type tissues when injected into mouse or chick blastula [52]. Furthermore, brain-derived ASCs can differentiate into myocytes [53,54]. A new type of stem cell, found in skin tissue, has recently been shown to differentiate into neurons *in vitro* [55]. Another fascinating cell source of ASCs is the bone marrow. Bone marrow-derived cells can differentiate into chondrocytes, osteoblasts and adipocytes [56–58], skeletal muscle cells [58,59], liver cells [60] and endothelial cells [61], and can restore muscle dystrophin expression [62]. The amazing versatility of these cells has raised hope for the treatment of ischemic heart disease. Bone marrow from mice injected systemically [46] or into the myocardium [45] of mice after myocardial ischemia formed proliferating myocytes and vascular structures. Kocher and coworkers demonstrated similar findings for human bone marrow-derived angioblasts, which can revascularize infarcted myocardium in animal models and improve cardiac function [63]. A recent report has taken bone marrow ASC research one major step further. Grafted single bone marrow stem cells repopulate not only the

Table 3. Transdifferentiation potential of adult stem cells

Tissue sources	Type of research	Donors	Recipient	Potentials of differentiation
Brain	*In vivo*	Mouse Human	Mouse or chick blastocysts *scid/bg* mice	Multi-tissues [52] Skeletal muscles [53] Blood cells [51]
Brain	*In vitro*	Mouse Human		Skeletal muscles [53,54]
Bone marrow	*In vivo* (i.a., i.c., i.p., i.s.i., i.v., i.v.i.)	Mouse Human Rat	Irradiated mice New born mice Ischemic rats Ischemic mouse heart	Neurons [69,70,73–75] Oligodendrocyte [67] Microglia [67,70] Macroglia [67] Astrocytes [67-70,75] Hepatic cells [60,76] Epithelial cells [64] Myocardium [45] Skeletal muscles [59,62] Endothelium [61,63]
Bone marrow	*In vitro*	Mouse Rat Human		Neurons [65,66] Astrocytes [65,66,68] Adipocyte [57,58] Chondrocyte [57,58] Osteocyte [57,58]
Skin	*In vitro*	Mouse		Neurons [55] Astrocytes [55] Oligodendrocyte [55]
Muscle	*In vitro*	Mouse		Blood cells [77]
Fat tissues	*In vitro*	Human		Muscle, bone, cartilage [78]

This table summarizes the capacity of various sources of adult-derived stem cells to differentiate into different types of cells.
Abbreviations: i.a. intra-arterial; i.c., intra-cerebral; i.p., intra-peritoneal; i.s.i., intra-striatal injection; i.v., intra-venous; i.v.i. intra-ventricular injection.

whole bone marrow, forming all blood cell lineages, but also differentiate into specific cell-types in multiple organs of irradiated mice: lung, skin, stomach and intestine [64]. Bone marrow can also give rise to brain tissue. Bone marrow ASCs differentiate into cells expressing neuronal markers *in vitro* [65,66], and upon intravenous transplantation they exhibit markers of glia [67,68]. When injected systemically, bone marrow ASCs can migrate into the brain and develop into cells expressing neuronal markers [69,70]. If bone marrow ASCs are indeed able to differentiate into neurons this would open up an important potential cell source for transplantation. However, data need to be reproduced and it has to be proven that these cells are neurons by both morphological and functional criteria.

In short, it is clear that ASCs and ES cells share some similarities, such as the ability to self-renew and to give rise to specialized cells and tissues. However, it is also obvious that there are distinct features of ASCs and ES cells. It appears that ES cells have a higher ability to proliferate. One ES cell can be generated in large quantities in culture conditions, but in most cases it is difficult to maintain ASC cells to proliferate without becoming specialized (differentiated). In addition, ES cells have a much higher capacity to differentiate than ASCs. Available evidence suggests that ASCs cannot give rise to as many different specialized cell types as ES cells. In culture conditions, ES cells form clumps of cells that can differentiate spontaneously to generate many cell types. This property has not been observed for cultured ASCs. In particular, if undifferentiated ES cells are transplanted *in vivo*, teratomas, which contain a mixture of several cell types, can develop. Thus, this could constitute a major problem with regard to taking ES cells to a clinical transplantation in the future. It is not clear whether similar problems might occur if pluripotent ASCs are grafted and, so far, teratomas have not been reported with experimental ASC transplants. In conclusion, each source of stem cells bears both advantages as well as disadvantages.

Future perspectives

There are several potential applications of ES cell and ASC technology in human medicine: basic embryological research, functional genomics, growth factor and drug discovery, toxicology and cell transplantation. For this we will need to grow stem cells on a large scale, to introduce genetic modifications into them and to direct their differentiation. To guide different stem cells into the desired lineage requires the identification of factors that direct their differentiation. Currently, science is still lacking important knowledge in many of these areas and patience is needed. The general public and financial investors should not expect immediate clinical breakthroughs from these cells. However, they should not lose hope too quickly, before basic research has investigated the full potential of these exciting cells.

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