25 Stem Cell Technology

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INTRODUCTION

■ Significance of Stem Cell Technology

Advances in stem cell biology over the past decade have given rise to new biotechnologies that will be used in a range of pharmaceutical applications including creation of cellular tools for studying the origins and progression of disease, development of phenotypic screens for use in drug discovery, development of cellular assays of drug toxicology and metabolism, and production of cell-based medicinal products for clinical use in various forms of "cell therapy." A phenotypic screen makes use of an assay that detects a cellular response such as a change in cell morphology, or another downstream event, rather than an initial signal transduction event at the plasma membrane. Stem cells are of particular value in this regard because they can be expanded and then differentiated, providing an unlimited source of mature differentiated human cells (such as neurons or cardiomyocytes). These cells offer the opportunity to develop high-throughput plate-based assays to detect features such as the rate and amplitude of beating of heart cells, or a functional response of a specific subtype of mature neurons, generating potentially more informative assays than current cell-based assays.

■ What Is a Stem Cell?

The fundamental property of a stem cell is the capability to multiply and to give rise to a variety of differentiated cells, but the general term "stem cell" is used in several contexts, each important for different reasons, as shown in Table 25.1. Adult, embryonic, mesenchymal, and induced pluripotent stem cells are currently the subject of intense investigation. Stem cells differ in the breadth of mature cell phenotypes to which they can give rise, and they are characterized by their potency as defined in Table 25.2. Before the practical applications of stem cells can be fully realized, in both the research laboratory and clinic, it will be necessary to understand in detail how to control their differentiation towards mature postmitotic phenotypes.

■ Adult Stem Cells

Adult (or tissue) stem cells are now known to be present in many if not all individual organs in adults and are generally thought to be "multipotent," meaning they can give rise to the cells found in their organ of origin, but not in other organs (Fig. 25.1). The identification of adult stem cells in human tissues has necessitated a repositioning of basic tenets of some biological sciences, most notably in neuroscience, where the prevailing view was that no new neurons were born in humans after birth (Zhao et al. 2008). Adult stem cells are rare and they cannot always be isolated and grown in culture. Even when they can be grown in culture, usually they cannot be grown indefinitely. In tissues, they exist in a defined, organized environment of supporting cells that define the architecture of the "stem cell niche" (Scadden 2006). For example, in the bone marrow there are many hematopoietic stem cell niches, each of which contains stromal cells to support the function of hematopoietic stem cell (HSC). Each HSC is capable of producing the progenitors of all types of blood cells (Taichman 2005). Differentiation of HSCs has been studied extensively and is now well understood (Fig. 25.2), but at present conditions that allow HSCs to be maintained and expanded in vitro have not been established. A hallmark of adult stem cells is their ability to "self-renew" and undergo asymmetric cell division. This means that when they divide they usually give rise to two different cells, one an identical stem cell and the other a partly differentiated progenitor cell (Fig. 25.2), a process that occurs in a polarized

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Type of stem cell	Origin	Characteristic potential	Practical uses
Adult (tissue) stem cells	Exist in small number in many tissues, often in a well-defined and supportive niche	Multipotent: give rise to cells of the relevant tissue or local environment	Some adult stem cells can be expanded in vitro (e.g., neural stem cells). Characterization of phenotype is challenging
Mesenchymal stem cells (MSCs)	A collective term for stem cells sourced from stromal or connective tissue (bone marrow, adipose tissue, or umbilical cord tissue)	Yet to be fully determined. MSCs can differentiate into cells of connective tissues, e.g., chondrocytes, osteoblasts, and adipocytes, but they have also been reported to give rise to many other unrelated cell types	MSCs from specific sources are in clinical development for several applications (e.g., see www.mesoblast.com)
Cord blood-derived MSCs	A specific source of MSCs. Extracted at birth from umbilical cord blood	Yet to be fully determined. The hope is that they will be a source of many cell types for individual patients	Private cell banks are already established for cryopreservation of cord blood samples
Embryonic stem cells	Result from in vitro culture of the inner cell mass (embryoblast)	Pluripotent: have the potential to produce all cell types of the adult organism	Vital source of differentiated cells for research and possibly cell therapy
Induced pluripotent stem (iPS) cells	Derived by reprogramming of somatic cells (often skin fibroblasts) taken from an adult biopsy	Pluripotent, although methods for full reprogramming are still in development	May allow pluripotent cells from individuals to be obtained without the need for fertilized human eggs

Table 25.1 ■ Origin, characteristics, and uses of stem cells.

Totipotent (or omnipotent) cells	Can differentiate into all embryonic and extraembryonic cell types (i.e., in humans they give rise to the fetus and the placenta)
Pluripotent cells	Can differentiate into all three germ cell types (endoderm, mesoderm, or ectoderm) and subsequently into all embryonic cell types
Multipotent cells	Can differentiate into closely related cells, such as all cells in a particular organ. Typical of adult tissue stem cells
Oligopotent cells	Can differentiate into a restricted closely related group, such as a hematopoietic progenitor cell that can produce a subset of blood cell types
Unipotent cells	Have the property of self-renewal but can only give rise to cells of their own phenotype, such as muscle stem cells

Table 25.2 ■ Definitions of cell potency.

manner controlled by the niche. The common pattern in adult tissues is that the resulting progenitor cells, sometimes referred to as "transit amplifying" cells, are capable of expansion by symmetric division and can subsequently differentiate to form the various cell types needed for repair or replenishment of the relevant tissue. Such mechanisms are well documented in tissues that are regenerated continuously in the adult, such as the epithelia of the skin, intestine and other mucosal tissues, and the bone marrow (Lander et al. 2012). It has now become apparent that similar processes are also found in organs that are not continuously replenished, such as the brain. The realization that adult stem cells are present in many organs offers the possibility that repair and regeneration could be stimulated and controlled in degenerative diseases by drug therapy, but whether this will be possible remains to be seen. In the brain, neural stem cells have been identified in the subventricular zone and in the dentate gyrus (part of the hippocampus) (Alvarez-Buylla et al. 2001), but whether they are present in other regions of the brain remains to be investigated.

Adult stem cells have been used since the 1950s to treat cancers of blood cells, as one of the components of bone marrow transplants (Santos 1983). This procedure involves whole body irradiation to kill malignant cells in multiple myelomas and leukemia. The patient then receives a bone marrow transplant, not in itself a stem cell product, but the transplant contains a few HSCs which subsequently home to the bone marrow stem cell niches and begin to replenish the blood (Fig. 25.3). Rejection and graft-versus-host disease are

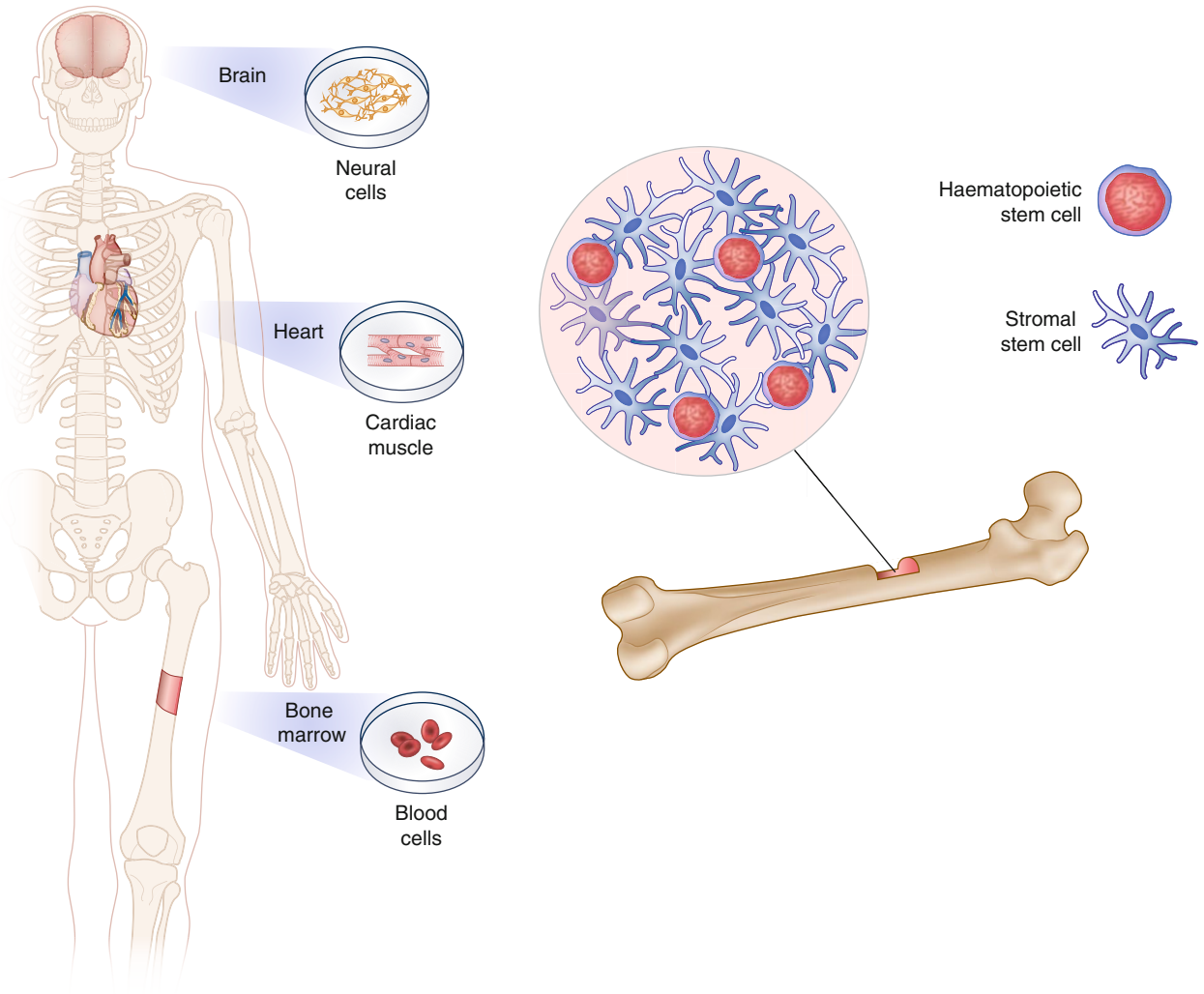

Figure 25.1 ■ Adult stem cells are present in many tissues in specific stem cell niches, giving rise to a specific group of cells found in the relevant tissue. The examples shown have been studied in detail but adult stem cells, yet to be defined, may be present in many other tissues.

still threatening complications of this form of therapy, but its practice can now be considered to be routine.

Mesenchymal stem cells (MSCs), sometimes called multipotent stromal cells, have generated considerable interest in recent years for cell therapy applications (Bianco et al. 2008). MSCs can be isolated from bone marrow, adipose tissue, and umbilical cord tissue (from the particularly rich source of Wharton's jelly and also from umbilical cord blood). Because cord blood can be sampled, frozen, and banked at birth, this source of MSCs has been identified as a potential source of cells for use in a regenerative capacity in later life. There are now several private companies that offer personal cell banking services, and public cord blood banks that supply pooled cord blood samples for clinical use. Whether cord blood banking will prove to be useful remains to be seen. MSCs have been reported to differentiate into various phenotypes (including chondrocytes, osteoblasts, and adipocytes) as well as other phenotypes, suggesting that MSCs have wider potential than one would expect. MSCs have been investigated in preclinical models of many applications and have been reported to home to damaged tissues and tumors from the vasculature. MSCs are in clinical development using direct injection for treatment of bone and joint diseases, heart disease, for repair of muscle and ligament damage, and even for repair of ischemic brain tissue. Which of these applications will prove to be successful is difficult to predict at this stage of clinical development.

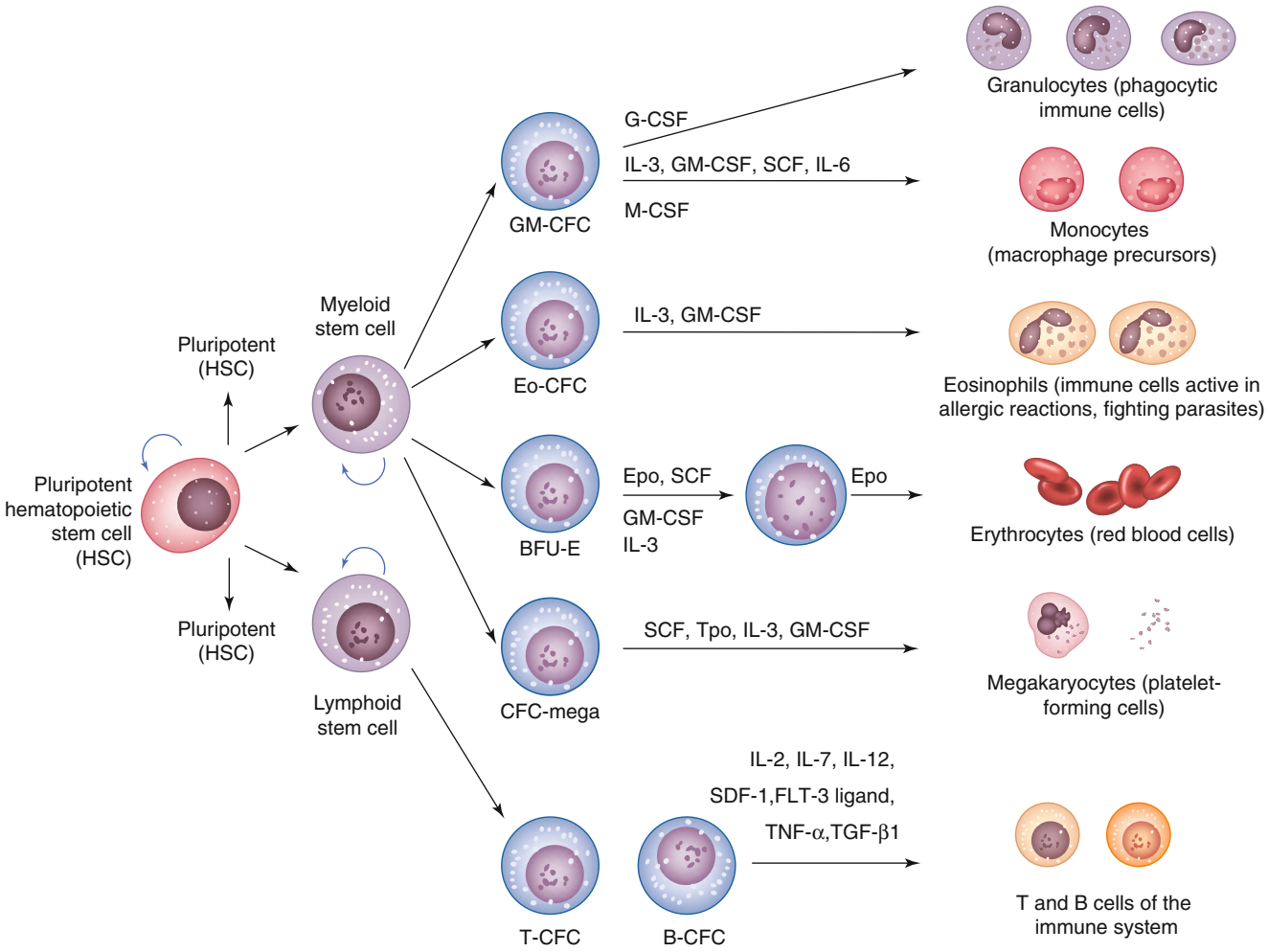

Figure 25.2 ■ Asymmetric division of adult hematopoietic stem cells (HSCs), to produce myeloid or lymphoid stem cells, further differentiation to form mitotic progenitors, and subsequently under the control of specific growth factors and cytokines, to form fully differentiated blood cells. The differentiation pathways of the hematopoietic system are better characterized than those of other tissues, but the pattern of differentiation is typical of other tissues.

■ Embryonic Stem Cells

During the earliest stages of mammalian development, soon after egg and sperm combine, the resulting diploid cells are said to be "totipotent," i.e., they can give rise to both the embryo and placental tissue. At the blastocyst stage of embryogenesis (day 5 in humans), the "inner cell mass" or "embryoblast" is compacted and separated from the surrounding "trophoblast." The latter combines with the maternal endometrium to form the placenta. The inner cell mass can be extracted and grown in vitro as embryonic stem (ES) cells, which can give rise to all three germ cell types (mesoderm, endoderm, and ectoderm), and therefore potentially any cell type found in the adult (Fig. 25.4). Mouse ES cells were first isolated in 1981 (Evans and Kaufman 1981; Martin 1981), but it took until 1998 for a similar procedure to be described allowing human ES cells to be grown in culture (Thomson et al. 1998). ES cells can now be grown for many cell divisions, limited only by genetic damage that occurs by mutation after extensive culture. The pluripotency of ES cells can be demonstrated in mice by injecting cells into a fertilized egg, resulting in the production of chimeric mice (i.e., mice made up of cells derived from both the donor and the injected ES cells). This process has been used routinely over the past 20 years to produce transgenic mice for research purposes. Human ES cells are usually identified by their ability to produce a teratoma (a tumor containing cells from all three germ cell types) when cells are injected into immunodeficient mice, but this not as robust a method for validation of pluripotency.

■ Maintenance and Differentiation of ES Cells in Culture

Mouse ES cells were first grown as compact colonies on a feeder layer of mouse embryonic fibroblasts, in media

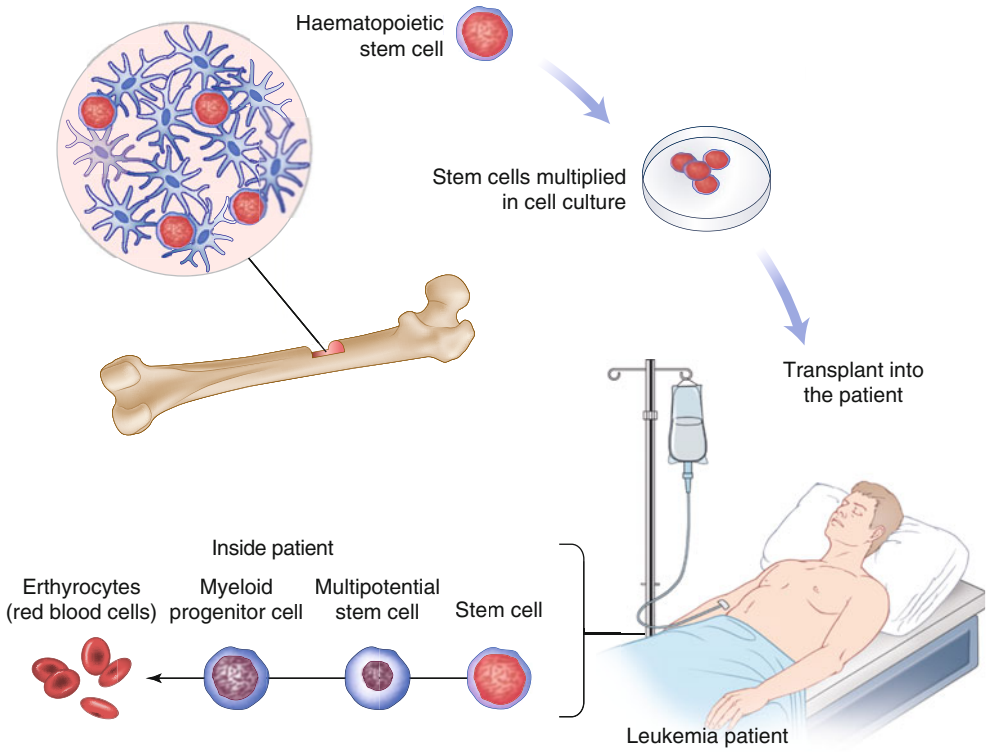

Figure 25.3 ■ Schematic representation of bone marrow transplantation, a form of stem cell therapy that was first used over 50 years ago. The transplant contains hematopoietic stem cells from the donor. These cells repopulate niches in the recipient bone marrow.

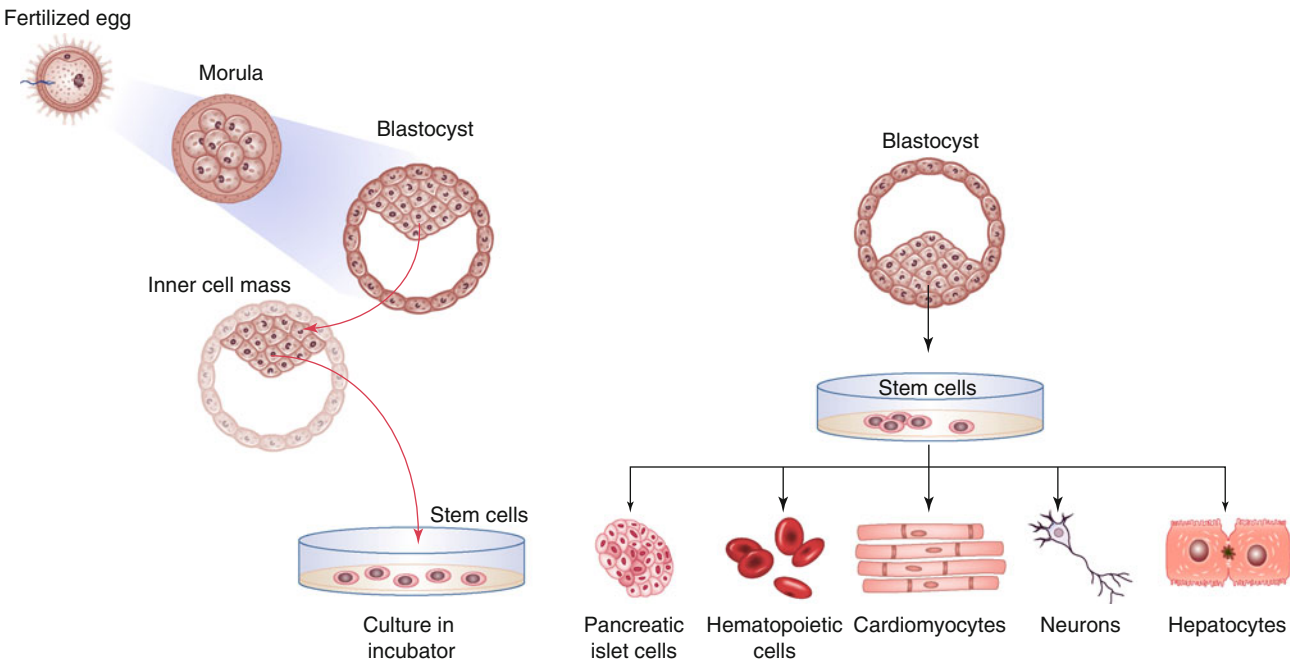

Figure 25.4 ■ Extraction of the inner cell mass of the blastocyst gives rise to embryonic stem cells (ES cells), which have the capacity to differentiate into all 200+ somatic cell types found in the adult human.

containing leukemia inhibitory factor (LIF) and fetal bovine serum. Efforts to simplify culture methods soon established that the feeders could be substituted with gelatin-coated culture plates, though differentiation occurs to some extent in the absence of the feeder layer. The vital component in serum was found to be bone morphogenetic protein (BMP). Thus, mouse ES cells can be grown in chemically defined medium with LIF and BMP4 (Ying et al. 2003). Human ES cells are grown in the presence of high concentrations of basic fibroblast growth factor (FGF2) and are unresponsive to LIF (Levenstein et al. 2006). The difference in responsiveness between mouse and human ES cells has been extensively studied and debated. The two methods of derivation may result in isolation of cells from slightly different stages of development. Human ES cells are thought to resemble cells from the later epiblast stage. More recently, it has been demonstrated that mouse ES cells can be maintained and grown very efficiently in the presence of small molecule inhibitors of mitogen-activated protein kinase (MEK1/2) and glycogen synthase kinase-3β (GSK-3β). This medium changes their phenotype slightly to what may be represent a "ground state" for mouse ES cells (Ying et al. 2008). A better understanding of the ground state and how this relates to human ES cells will be an important step forward and will allow human ES technology to be reproduced more effectively.

The technical challenge, now that human ES cells can be maintained and expanded, is to develop robust methods to control and direct ES cell differentiation, so that human cells of any desired phenotype can be obtained (Keller 2005; Murry and Keller 2008). In the context of cell therapy, it is also important to ensure that no undesired cells are present in a product for clinical use, such as undifferentiated cells, or cells that are capable of dedifferentiation, either of which could cause tumor formation after implantation. This science is immature at present and will remain a priority for investigation for several years. Thus, far attention has focused on the differentiation of human ES cells towards products that could be of obvious use for cell therapy, e.g., midbrain dopaminergic neurons for Parkinson's disease, cardiomyocytes for reinforcement of damaged heart tissue, and pancreatic β-islet cells for implantation in Type I diabetes. From a fundamental view, to improve our understanding of cell phenotype, for screening of small molecules that modulate cell function, and for disease modeling, it will be important to research ways of producing many other cell types. At present, fine tuning of differentiation programs is beyond our control. Differentiation usually results in mixed populations of cells. For example, neural differentiation can be induced quite effectively, but the result of further differentiation is a mixed population of cells that often include both neurons and glia, and the neurons are comprised of a variety of neuronal subtypes. Timing, duration, and concentration of exposure to specific morphogens are of critical importance to the outcome and will need to be optimized in each case.

■ Cell Therapy: The Broader Context

The potential of stem cells as a source of products for cell therapy needs to be understood in the context of alternative approaches to cell therapy and transplantation. A few cell therapies are already in clinical use. There are long-standing clinical practices that involve cell transplantation, including bone marrow transplants, and in vitro fertilization. In addition the FDA and EMA have both approved cell therapy products for niche applications. The chondrocyte product ChondroCelect (TiGenix) is currently the only cell therapy product approved in Europe by the EMA. Provenge (Dendreon) is a patient-specific cell therapy for prostate cancer immunotherapy that has been approved by the FDA. In this case, a sample of the patient's own white blood cells is treated with an engineered fusion polypeptide to produce a vaccine for reinjection. The intention is to deliver a cancer vaccine to professional antigen-presenting cells, i.e., dendritic cells. These applications of cell therapy are outside the scope of this chapter and will not be discussed in detail, but their development has done much to define a framework for development of stem cell-based products and their evaluation by regulatory agencies.

The term "regenerative medicine" is often used to describe the current interest in repair, restoration, or replacement of damaged tissue. The strong interest in use of stem cell-derived products is based on their potential to expand and differentiate in vivo, giving them the potential to participate actively in repair of damaged tissue. This is desirable but will require a complete understanding of the fate of stem cells, early and late progenitors, and differentiated cells after transplantation in each specific clinical application. This science is in its infancy at present. Differentiated cells that are incapable of dividing or further differentiation may also have useful roles in cell therapy and could be used to secrete protective or regenerative proteins (i.e., growth factors or cytokines) to the local environment ("gene therapy by cell therapy," cf. Chap. 24).

Another fundamental consideration in all forms of cell therapy is the distinction between autologous cell therapy (when the donor is also the recipient) and allogeneic cell therapy (when the donor cells are delivered to one or more different recipients). Allogeneic therapies and xenogenic therapies (those derived from animal sources) introduce immunological complications that need to be managed.

The general interest in stem cell therapies and regenerative medicine around the world has allowed unregulated practice of cell therapy to develop in some countries. This is a major concern for stem cell scientists, because treatments are being offered in the absence of any proven efficacy. In addition there is suspicion that the products in use have been manufactured with insufficient attention to quality control. Patients are travelling to private clinics and paying large sums of money for unproven treatments, creating a phenomenon that has been referred to as "stem cell tourism." It is very important that patients are warned of the dangers of falling prey to unethical operations. An up-to-date source of information on private clinics and stem cell tourism is available at the website of the International Society for Stem Cell Research (www.isscr.org).

IMMUNOLOGICAL CONSIDERATIONS IN CELL THERAPY

The potential application of cellular products derived from ES cells in cell therapy is limited by graft-host rejection issues, as with all therapeutic strategies based on transplantation, unless the transplant is derived from "self." Administration of drugs to suppress the immune response is standard practice for patients undergoing transplantation, but with immunosuppression come side effects and uncertainty. The hope is that induced pluripotent stem (iPS) cell technology (see below) may overcome rejection problems but it is too early to be sure at this stage. Another approach is to bank a collection of ES cell lines that allows selection of a matched HLA haplotype or a close match (Lui et al. 2009). It has been estimated that with a bank of 70–100 ES cell lines, a partially matched ES cell line can be chosen that is adequate for each recipient.

An alternative, particularly when a match cannot be found, is to produce ES cells for individual patients, by somatic cell nuclear transfer (SCNT) (Wilmut et al. 2002). This process, also known as "therapeutic cloning," involves implantation of a cell nucleus from the patient (i.e., genomic DNA extracted from a skin biopsy) into a human egg which has undergone removal of its own DNA. The environment in the enucleated egg is able to reprogram the DNA from the patient, removing epigenetic marks and restoring the DNA to an embryonic state. The development of an inner cell mass in the egg, after a period of incubation, allows extraction of ES cells that have the patient's exact genotype. These cells could be used subsequently for production of implants for cell therapy (Fig. 25.5). SCNT is also the first step in the process by which animals are cloned by "reproductive cloning," which involves implantation of the engineered egg into a surrogate mother (Fig. 25.6) (Campbell et al. 1996). Reproductive cloning of humans is illegal but is also likely to be impractical. It is known from experience with animal cloning that SCNT is an inefficient process. Most eggs that have undergone SCNT are unable to completely reprogram the donor DNA, and as a result the surrogate pregnancy is usually unproductive. Even when the pregnancy comes to term, the cloned offspring is known to carry many epigenetic marks that may compromise normal development, and the famous sheep, "Dolly," the first large animal to be cloned by way of SCNT, is known to have had several developmental defects (Wilmut et al. 2009). Second-generation animals, produced by mating a clone with another parent, are usually unaffected by such defects, indicating that SCNT is much less efficient than the natural process of reprogramming of DNA in a fertilized egg. Given that defects are known to occur after SCNT, the subsequent derivation of cells for clinical uses might also be prone to failure due to defects in ES cell differentiation. There is insufficient data available at this stage to judge whether this will be a limitation in practice. There are significant ethical concerns that have limited the practice of SCNT. A human egg donor is required, and unless the process becomes more efficient, women who are prepared to donate eggs would need to provide several eggs to produce a single ES cell line. There is concern that women could be exploited, particularly women from low economic backgrounds, and as a result SCNT is not supported by government funding at present in most countries. A restricted number of ES cell lines have been produced using spare eggs from in vitro fertilization programs, but the status of SCNT remains a controversial topic and is subject to legal constraints that vary from country to country. An alternative source of cells for regenerative medicine in the future may be umbilical cord blood stem cells, which are now being banked at childbirth, at least in private practice. Whether cord blood cells can be harnessed to produce all cell phenotypes is not clear at present. However, many of the ethical issues surrounding SCNT, and uncertainty of cord blood stem cell potency, may become irrelevant if the promise of induced pluripotent stem (iPS) cells can be realized.

IPS CELL TECHNOLOGY

In 2006 stem cell scientists were surprised by a remarkable discovery that has revolutionized the field and its potential practical application. Two Japanese scientists reported that mouse skin fibroblasts could be reprogrammed to produce pluripotent cells by forcing expression of just four genes (*Sox2, Oct4, Klf4,* and *cMyc*) using lentiviral vectors (Takahashi and Yamanaka 2006). A year later similar methods were

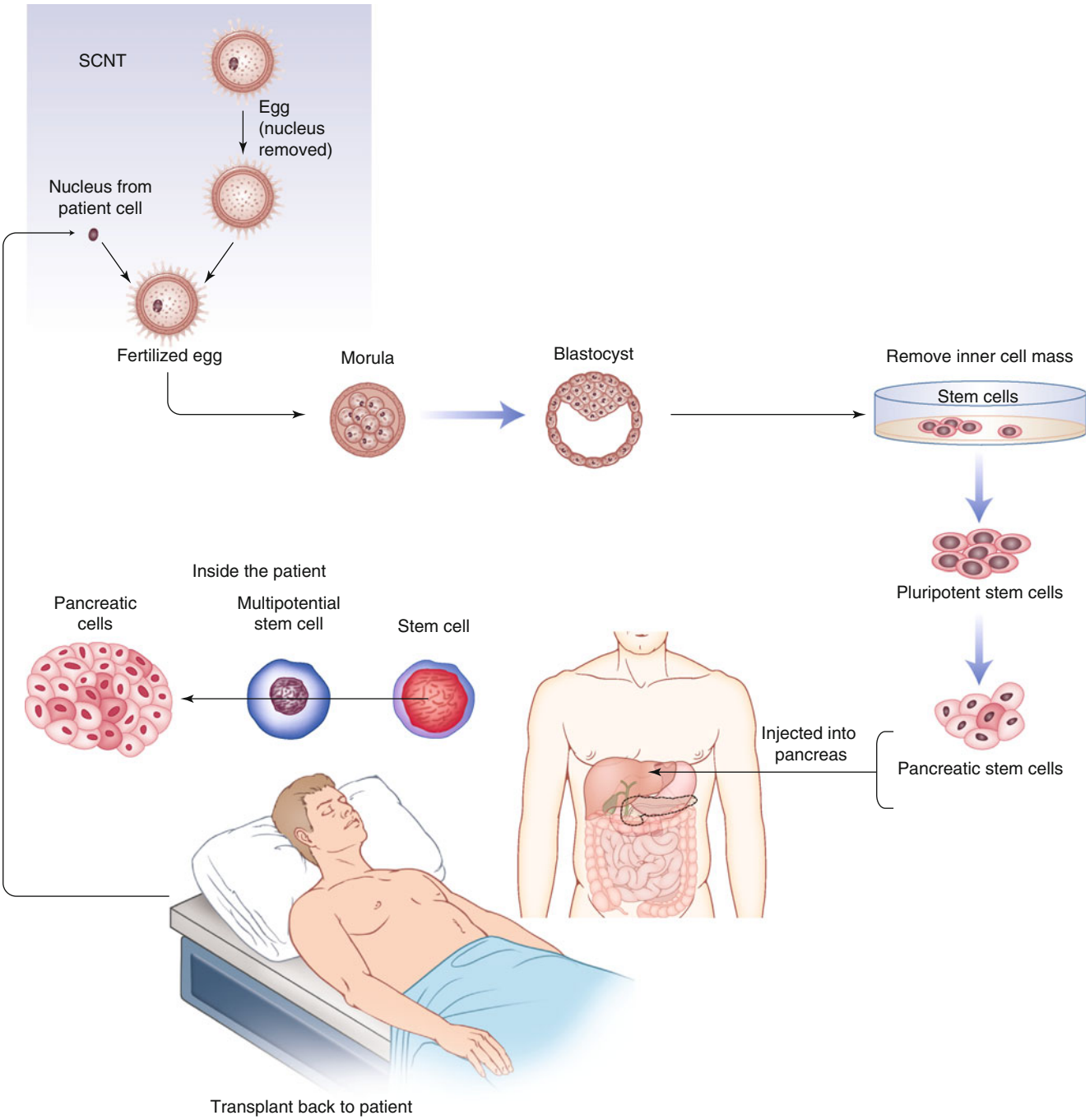

Figure 25.5 ■ Schematic diagram of the production and clinical use of cell therapies derived using somatic cell nuclear transfer (therapeutic cloning). The example given is for possible treatment of Type I insulin-dependent diabetes.

published for production of iPS cells from human fibroblasts (Takahashi et al. 2007; Yu et al. 2007). This indicated that patient-specific pluripotent stem cells could be produced without the need for human eggs, using cells extracted from a simple skin biopsy. The significance of this discovery to regenerative medicine cannot be overestimated. Over the last 5 years, the iPS cell field has exploded with activity, and the technology is now in use in hundreds of stem cell biology laboratories around the world. The four genes initially identified can be partly substituted by alternatives, and several experiments have shown that integrated lentiviral constructs can be avoided to reduce safety concerns, by using nonviral plasmids (Jia et al. 2010), microRNAs (Yang et al. 2011), protein transduction, and even by substituting some of the factors with

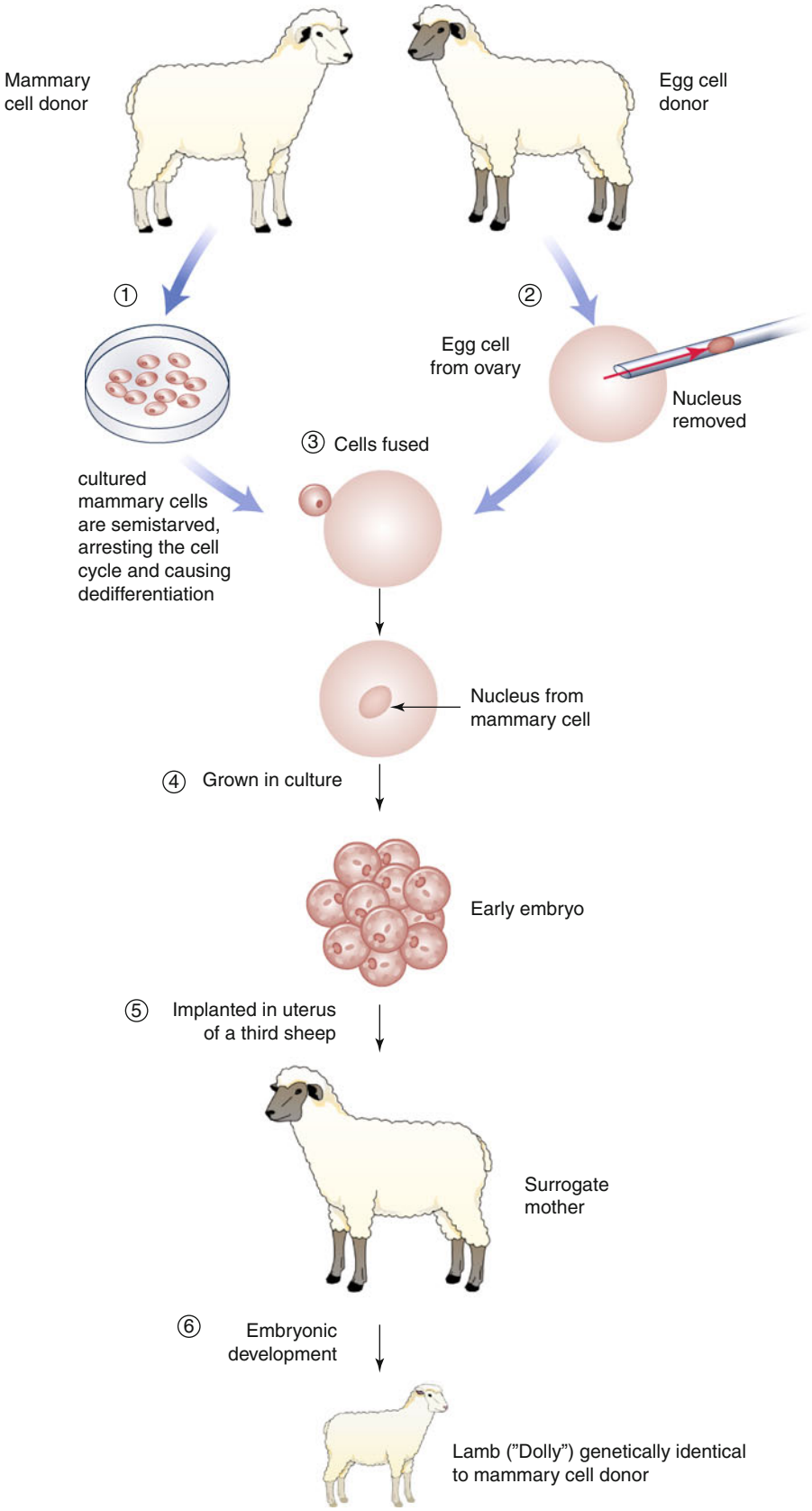

Figure 25.6 ■ Schematic diagram of the concept of reproductive cloning, as used to produce the cloned sheep "Dolly".

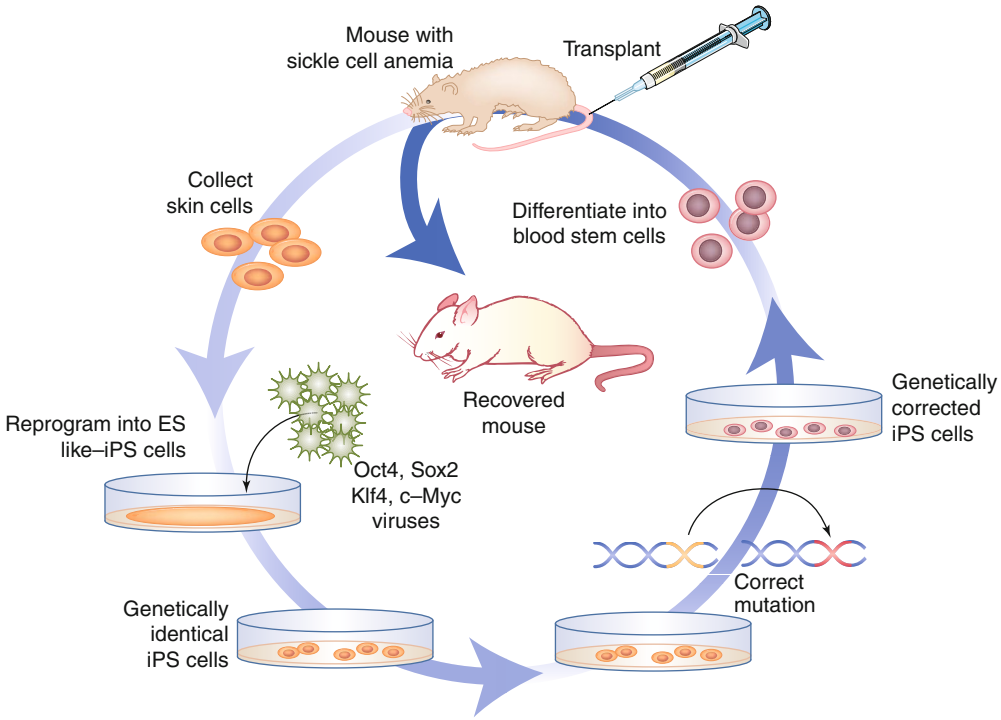

Figure 25.7 ■ Method used to produce iPS cells, correct a genetic defect responsible for sickle cell anemia, and implant the corrected stem cells into mice to cure sickle cell anemia in an animal model.

small molecules (Yuan et al. 2011). Often the safer alternative methods work with reduced efficiency but nevertheless produce the same result. The technology is still in the early years of its development, but if it delivers its potential, iPS technology will have profound effects on the understanding of disease, correction of genetic defects, and cell therapy. Already iPS cells have been used to correct defects in mouse models of Parkinson's disease (Hargus et al. 2010) and to cure a model of sickle cell anemia in mice (Fig. 25.7) (Hanna et al. 2007).

Considerable effort has been directed at investigating how iPS cells differ from ES cells and investigating whether reprogramming is complete enough to produce truly pluripotent cells. True pluripotency is difficult to demonstrate unequivocally in human iPS cells so the development of methods to measure the extent of reprogramming will be important for practical applications. There are indications that iPS cells can have chromosomal defects and are not fully reprogrammed (Chin et al. 2010). Female human iPS cells appear to maintain the inactivated X chromosome that was present in the skin fibroblasts, though this has not been a problem with mouse iPS cells (Tchieu et al. 2010). In a recent report in mice, iPS cells induced an immune response in a genetically identical host from which the cells were derived (Zhao et al. 2011). The mechanisms causing this immunogenicity need to be studied in more detail to investigate whether this is a widespread problem. It is possible that the above unfavorable reports may be the result of inadequate control over reprogramming. Studies of the properties of iPS cell generated from multiple laboratories will address these important issues in the coming years.

DIRECT REPROGRAMMING

During the last 2–3 years, forced expression of genes has been used to convert fibroblasts directly into unrelated differentiated cells, including neurons (Ambasudhan et al. 2011; Wernig et al. 2008) and cardiomyocytes (Burridge et al. 2012). The technique used is analogous to that used to derive iPS cells, except that genes associated with the desired somatic cell are expressed instead of pluripotency genes. The realization that cellular phenotypes can be transformed in this way has been met with astonishment and is certainly breakthrough technology. It raises the possibility that interconversion could be performed in vivo, though it does not allow for expansion of cells in preparation for an implant. However, direct reprogramming of fibroblasts to neural stem cells, as reported in 2012 (Han et al. 2012; Thier et al. 2012), may be a short cut to neurons. This approach may offer some advantages over production of neurons by way of iPS cells.

USE OF PRODUCTS DERIVED FROM STEM CELLS IN CELL THERAPY

There is much work to be done to develop effective cell therapies. From a clinical perspective, it is not always clear what type of cell needs to be implanted, where or

how and how many cells are needed for a clinically relevant dose. For example, the major symptoms of Parkinson's disease are caused by loss of A9 dopaminergic neurons that project from the substantia nigra, releasing dopamine at synapses in the striatum. To reduce or replace this loss of A9 neurons by cell therapy could be achieved in at least two ways. Firstly, the cells of the substantia nigra could be supported with an implant of neural stem cells, which appear to protect the remaining neurons in animal models. This effect has been termed the "chaperone" effect (Redmond et al. 2007) and may be the result of secretion of supporting factors such as brain- and/or glial-derived neurotrophic factors (BDNF and GDNF) from the implant. The alternative or perhaps complementary approach would be to replace the dying neurons with new dopaminergic neurons. In this case the neurons would need to be integrated and form useful interactions with the relevant neural pathways in the brain. If the strategy is to provide new neurons, should the cells be neural stem cells, progenitors, dopaminergic precursors or mature dopaminergic neurons, or a mix? Are any dopaminergic neurons effective or must they have the exact phenotype of A9 neurons? Should the cells be implanted into the striatum or the substantia nigra or both? Will the damaged local environment be toxic to the implant? If so, what can be done to prepare the tissue for an implant? These are all questions that will need to be addressed as the technology progresses.

Each opportunity for cell therapy raises specific questions that will need to be addressed before such therapies can be used in a widespread manner. Several diseases and traumas of the CNS, other than Parkinson's disease, are under investigation as targets for cell therapy. These include Huntingdon's disease, Alzheimer's disease, and stroke (Lindvall and Kokaia 2010). In animal models of Alzheimer's disease, neural stem cell implants improved cognitive defects, probably by secretion of BDNF and protection of forebrain cholinergic neurons (Blurton-Jones et al. 2009). This may represent a similar mechanism of action and outcome to the studies carried out in models of Parkinson's disease in primates (Redmond et al. 2007). In stroke and neurotrauma, the most important objectives may be to reduce inflammation in the short term, and a gene therapy approach delivered by cell therapy may be a good option. There is considerable activity in cardiac cell implantation (Freund and Mummery 2009). In cardiac cell therapy for myocardial infarction, the treatment would ideally provide structure, strength, and elasticity, to regenerate heart muscle function. However, it is vital that cardiomyocyte implants are integrated with the heart conduction system and do not result in ectopic beating of heart tissue, which could be life-threatening. The extent of productive integration and reduction in scar tissue in the heart has been limited thus far and will need to be improved. In another busy research field, which aims to devise a cell therapy to treat Type I (and potentially Type II) diabetes, the search is on to find a mechanism to replace glucose sensitive, insulin-producing pancreatic β-islet cells. A simple injection of islet cells into the pancreas is unlikely to succeed because the implanted cells would be placed in the same destructive environment that led to loss of the patient's own islet cells, usually by an autoimmune reaction. In this case new islet cells are being tested in an encapsulated form, effectively in a polymeric delivery system, so that the cells are spared from immediate destruction (Kroon et al. 2008). This approach may also allow the implant to be removed if there are safety concerns after implantation. It can be anticipated that many other therapeutic opportunities are currently under investigation and will be explored in the future. Perhaps the most significant advances have been made in construction of implants for treatment of macular degeneration, which causes blindness due to retinal damage (Idelson et al. 2009; Schwartz et al. 2012). Clinical studies have commenced, using injection of retinal pigment epithelial cells derived from ES cells into the eye, with early signs of success.

DISEASE MODELING AND DRUG DISCOVERY

Whether or not iPS cells have a future in cell therapy, they will undoubtedly have a bright future in disease modeling and in drug discovery (McKernan et al. 2010; Rowntree and McNeish 2010). Since iPS cells can be expanded in a similar manner to ES cells and can be differentiated to obtain mature somatic cells, it is now possible to obtain differentiated cells with the exact genotype of patients who are suffering from specific diseases. This is particularly exciting in relation to neurological and neurodegenerative diseases of the central nervous system (CNS). Generally, CNS diseases can only be studied using postmortem tissue, when the brain tissue is too badly damaged to extract useful information on the origins and progression of the disease. Using iPS technology it is now possible to compare neurons from unaffected and diseased patients before the disease has emerged (Fig. 25.8). The ability to expand pluripotent cells prior to differentiation means that a sufficient number of cells can be generated to consider developing plate-based screening assays. Over the past 2 years, this strategy has been used to study neurons derived from patients with schizophrenia (Brennand et al. 2011), Rett syndrome (Marchetto et al. 2010), and Alzheimer's disease (Israel et al. 2012). Defects were observed in all cases, and markers of disease were upregulated in the

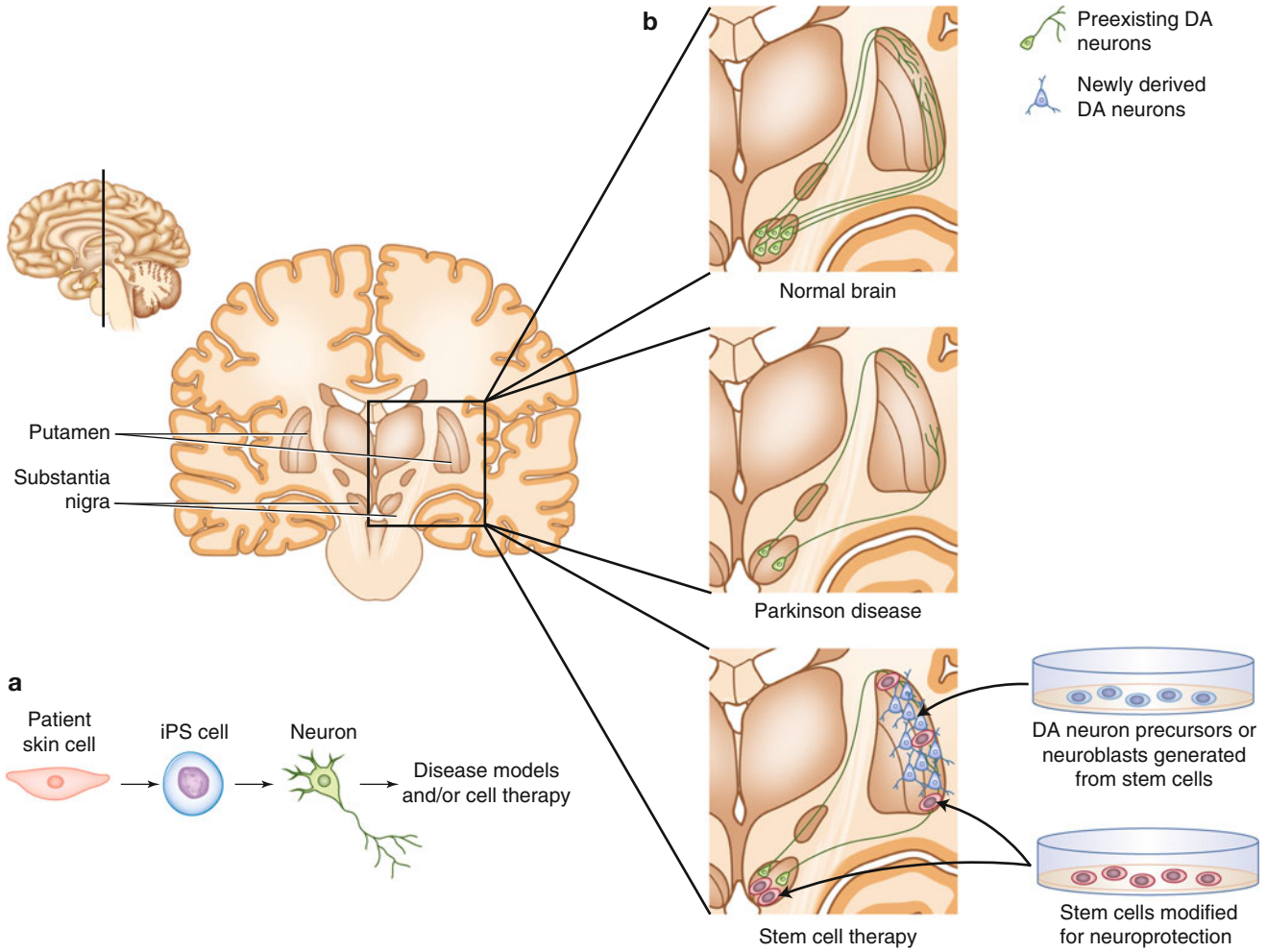

Figure 25.8 ■ (**a**) Derivation of iPS cells from patients suffering from disease, such as Parkinson's disease (PD) has the potential to lead to a new generation of cell culture disease models, allowing a comparison of functional properties and phenotype of patient-derived neurons with control neurons. (**b**) ES cells, iPS cells, or corrected patient-derived iPS cells could be used for cell therapy, shown here for PD. Loss of A9 dopaminergic neurons in the substantia nigra could be prevented by protective implants into the substantia nigra as well as implantation of precursor cells into the striatum.

patient-derived cells. Remarkably, defects in synapse formation in schizophrenia-derived cells were reversed by exposure to antipsychotic drugs, illustrating how such cells will be valuable for drug discovery and also suggesting that antipsychotic drugs may act to modulate synaptic connections between neurons, as well as producing short-term benefits.

Pharmaceutical companies are beginning to explore the use of stem cell-derived assays to study specific functional responses in screening experiments (Pouton and Haynes 2007). Assays of this type require more skill and effort to set up, qualify, and validate than simple cell-based assays, such as stably overexpressed receptor models in immortalized cells. But the rewards could be the discovery of more relevant hits and lead compounds with improved activity in vivo. A phenotypic response in a cell line that has the appropriate signaling systems expressed at appropriate levels is likely to be a smarter approach to drug discovery than use of existing screening assays. The availability of appropriate disease models using iPS technology will add value and may have the power to differentiate between desired activities in diseased cells and side effects in normal cells.

CANCER STEM CELLS

An added consequence of advances in the understanding of stem cell biology, particularly in relation to adult stem cell biology, has led to the realization that many cancers, particularly leukemia (Majeti et al. 2009) and solid tumors of epithelial tissue, may have their origins in mutations and familial polymorphisms in tissue stem cells. Many authors have suggested that the failure of cancer chemotherapy can be explained by its inability to kill slowly dividing cancer stem cells.

This has become a hotly debated topic in cancer biology. Much of the argument concerns the difficulty in establishing the proportion of cells within a tumor that have the capacity to give rise to a new tumor, when transplanted into a healthy animal. The need to use tumor xenografts in immunodeficient mice makes this a difficult assay to interpret when examining human tumors. The transplanted cells may be unable to give rise to a tumor because the local environment of the transplant is not appropriate, being the wrong species and most likely the wrong tissue. Nevertheless, there is mounting evidence that many tumors are initiated in stem or progenitor cells. To accept the cancer stem cell model implies a paradigm shift and implies that the target of drug therapy to eradicate the disease should be the cancer stem cell. Some cancers may arise not from adult stem cells, but from a downstream progenitor cell. This appears to be the case for some prostate cancers (Wang et al. 2009) and also basal cell-like "triple negative" breast cancer (Lim et al. 2009), which has the appearance of a basal tumor but in fact arises from a mammary progenitor cell. Different approaches to drug therapy of cancer are certain to emerge from research on cancer stem cells, but at present it is not clear how these cells can be targeted effectively. Study of gene expression in cancer stem cells, for example, by transcriptomics (cf. Chap. 8) using DNA array technology, may identify specific targets for drug discovery, either to inhibit cancer stem cell activity or to cause their differentiation into "normal" cells. For example, the invasive brain tumor, glioblastoma multiforme, could potentially be treated using a strategy that reduced the cancer stem cell phenotype to that of normal glial cells, as an alternative or additive to traditional chemotherapy.

REGULATORY ISSUES

Within the US Food and Drug Administration (FDA), the responsibility for regulation of cell therapy products lies with the Center for Biologics Evaluation and Research (CBER). Cell therapy products are defined by the FDA as "human cell, tissue, and cellular and tissue-based products (HCT/Ps)." Although there is a considerable amount of activity in research and development, both in the academic and commercial sectors, no stem cell therapy or gene therapy products have been approved by the FDA (June 2012). To monitor activity, review data, and anticipate future needs, the FDA operates the Cellular, Tissue and Gene Therapies Advisory Committee. Transcripts of meetings can be viewed at the FDA website, which provides a useful insight into current thinking on regulatory aspects of regenerative medicine. Details of the product development and approval process for HCT/Ps are posted at the CBER site (www.fda.gov/biologicsbloodvaccines/cellulargenetherapyproducts). At the clinical trials stage, cell therapy products require an Investigational New Drug Application (IND- clinical trials) and for product approval, a Biologics License Application (BLA- marketing) (see the PHS Acts 42 USC 262 and 21 CFR 1271). European Union countries and some other countries in Europe are guided by the European Medicine Agency (EMA – www.ema.europa.eu/ema), which drafts guidelines for cell therapies in a product category called advanced therapies. This includes gene therapy, cell therapy, and tissue-engineered products. The majority of cell therapy products will be classified as Advanced Therapy Medicinal Products (ATMP) in Europe and will therefore be regulated by medicinal product Directive 2001/83/EC and Regulation EC (No 1394/2007). They will require a clinical trial application (CTA), which is the responsibility of the appropriate national competent authority and subsequently will submit a Marketing Authorization Application (MAA) to the EMA. The Committee for Advanced Therapies (CAT) at the EMA is responsible for evaluation of product license applications and makes recommendations to the Committee for Medicinal Products for Human Use (CHMP). A landmark first gene therapy product (Glybera) was approved by the EMA in 2012.

Both the FDA and EMA, and other regulatory agencies around the world, are grappling with new paradigms in terms of the balance between risk and benefit. Risks in cell therapy are difficult to anticipate, so risk-benefit analysis will be a considerable challenge to committees going forward. There are also complex safety issues relating to the quality control of cell therapy products, which will require examination by regulatory authorities (Herberts et al. 2011). Each product will require detailed assessment on a case-by-case basis. Publicly Available Specification documents published by the British Standards Institute (BSI PAS 83:2012; BSI PAS 84:2012, and BSI PAS 93:2011) are freely available and provide valuable guidelines on quality control and development of cell therapy products.

CONCLUDING REMARKS

There is a general view that advances in biomedical science, including stem cell biology, will be important stimuli for change in our approach to many diseases and healthcare in general. Practical application of stem cell technology will require highly trained practitioners, both at the technical level and with regard to advising and counselling patients. Pharmaceutical scientists and pharmacists will be important members of the team of professionals that deliver these changes in healthcare, a challenging and exciting prospect. Much can be learned from the R&D processes used

by traditional biotech (e.g., during development of therapeutic monoclonal antibodies and vaccines). Pharmacists can play a key role in development of stem cell therapies, as many applications are conceived by academic groups and small spin-off companies, who do not necessarily know how to translate a concept into a medicinal product. Pharmacy professionals can provide valuable experience in relation to the application of the principles of GLP, GMP, and GCP.

SELF-ASSESSMENT QUESTIONS

■ Questions

1. What is the difference between embryonic and adult stem cells?
2. What are the possible advantages in using embryonic stem cell technology in drug discovery?
3. How is somatic cell nuclear transfer carried out and what are the problems with this technique?
4. What are induced pluripotent stem cells and why are they important?
5. Which diseases could potentially be treated with cell therapy?
6. What problems could arise in use of stem cell-derived products for cell therapy?

■ Answers

1. Embryonic stem cells are grown in vitro after extraction of the inner cell mass from a blastocyst. Adult stem cells are found in vivo in many tissues, usually in the specialized environment of a stem cell niche, that support their asymmetric cell division.
2. Cell culture models used in drug discovery are often immortalized cells that are used to assay for receptor activation using stably transformed cells. The advantage of embryonic stem cells is that they can be expanded in a pluripotent state and then encouraged to differentiate into specialized mature somatic cells. These fully differentiated cells are likely to express the appropriate signaling systems which will allow a sophisticated functional experiment to been designed. This approach will result in more powerful data on the efficacy of drug candidates.
3. Somatic cell nuclear transfer (SCNT) involves the injection of a donor genome into an enucleated egg, such that the embryo develops as a clone of the donor genome. This allows embryonic stem cells to be derived using the donor genome and in principle allows implantation into the uterus of a recipient female leading to pregnancy. There are ethical problems concerned with supply of fertilized human eggs and also technical problems caused by incomplete reprogramming of the donor nucleus.
4. iPS cells are produced by transient expression of pluripotency genes in somatic cells, leading to reprogramming to form pluripotent cells resembling embryonic stem cells. The production of iPS cells allows pluripotent cells to be obtained from a patient without the need for SCNT. iPS cells could be used to derive differentiated cells for implantation therapy or to produce models of disease.
5. A variety of diseases may 1 day be treatable with cell therapy. Examples of current "test beds" for cell therapy are Parkinson's disease, myocardial infarction, and macular degeneration. A number of other neural conditions are under investigation including Huntingdon's disease, Alzheimer's disease, stroke, and spinal injury.
6. One of the concerns with stem cell-based therapy is the possibility that rare pluripotent or multipotent cells in the implant could give rise to tumors. Thus, the quality control of the implant will be of paramount importance. Often, in particular in treatment of CNS diseases, it is not clear whether a progenitor, precursor, or fully mature cell should be implanted. Careful preclinical work will be needed in each clinical indication to establish the most effective approach. Where the strategy is designed to replace a cell that is lost in a particular disease, the environment into which the implant is placed may not be supportive of cell survival and integration. In general, attention will need to be paid to repairing the tissue to provide a protective environment for the implant.

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