Chimeric Animal Models in Human Stem Cell Biology

Joel C. Glover, Jean-Luc Boulland, Gabor Halasi, and Nedim Kasumacic

Abstract

The clinical use of stem cells for regenerative medicine is critically dependent on preclinical studies in animal models. In this review we examine some of the key issues and challenges in the use of animal models to study human stem cell biology—experimental standardization, body size, immunological barriers, cell survival factors, fusion of host and donor cells, and in vivo imaging and tracking. We focus particular attention on the various imaging modalities that can be used to track cells in living animals, comparing their strengths and weaknesses and describing technical developments that are likely to lead to new opportunities for the dynamic assessment of stem cell behavior in vivo. We then provide an overview of some of the most commonly used animal models, their advantages and disadvantages, and examples of their use for xenotypic transplantation of human stem cells, with separate reviews of models involving rodents, ungulates, nonhuman primates, and the chicken embryo. As the use of human somatic, embryonic, and induced pluripotent stem cells increases, so too will the range of applications for these animal models. It is likely that increasingly sophisticated uses of human/animal chimeric models will be developed through advances in genetic manipulation, cell delivery, and in vivo imaging.

Key Words: chicken embryo; chimera; human; imaging; nonhuman primate (NHP); rodent; stem cells; ungulate; xenotransplantation

In Vivo Animal Models: A Prerequisite for Successful Translational Stem Cell Biology

The development of regenerative medicine—the use of stem cells to replace diseased, damaged, or destroyed tissue—began at least several millennia ago, with the earliest recorded human skin grafts documented in Sanskrit texts from 3000 BC (Hauben et al. 1982), although at that time the concept of the cell, let alone the stem cell, was unknown. The witting use of stem cells began several decades ago with the first attempts at bone marrow transplantation. Today, stem cells are touted as a future mainstay of medicine, with the potential for replacement of virtually any cell type in the human body. But realizing this potential in the clinic poses substantial challenges, including the fact that most organs comprise in vivo environments that are far more complex than those of the blood or skin. Thus, along with the many hurdles inherent in coaxing stem cells to generate the right types of cells and with sufficient purity to enable their rational use for replacement therapy, getting stem cells and their progeny to the right places in complex tissues usually requires extensive in vivo testing in experimental animal models.

We examine some of the principal challenges stem cell biologists face in studying stem cells in vivo and describe a variety of animal models that have served as host systems for the xenotypic transplantation of human stem cells. Because our backgrounds are in neuroscience, we draw chiefly, but not exclusively, from studies of neural stem cells and neural disease and injury models.

The Challenge of Moving from in Vitro to in Vivo Models

Most stem cell research still revolves around in vitro models, for good reason. In vitro studies are cheaper, faster, easier to standardize and measure and therefore easier to replicate, and can involve large volumes of cells that greatly facilitate the study of gene expression and molecular mechanisms. But the relative simplicity of in vitro systems is also their major shortcoming as a tool for translational research: it is very difficult to predict, on the basis of in vitro behavior, what stem cells are capable of doing in vivo, where the cellular and molecular environments are more complex and dynamic, and where individual differences in genetic background, metabolism, diet, and endocrine physiology can create unforeseen and difficult to predict interactions. In the following sections we describe some of the principal problems facing stem cell biologists when they make the transition from in vitro to in vivo studies of human stem cells.

Standardization of in Vivo Models

The concept of the stem cell niche (the cellular and molecular microenvironment surrounding the stem cell) is of paramount

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importance for understanding the way stem cells are regulated. Numerous studies in diverse tissues have shown that stem cells are dependent on dynamic molecular signals from associated cells and extracellular matrices for their survival, proliferation, and the proper differentiation of their progeny (Scadden 2006). Such signals can be replicated to some extent in vitro, particularly through the use of 3-dimensional matrix cultures, but the richness of a physiological niche is probably rarely achieved in this way. In vivo transplantation provides physiologically complete but also more complex niches that are difficult to monitor and control. Strategies to standardize animal models include the use of isogenic strains (Festing and Fisher 2000), the use of transgenic animals in which niche components are molecularly defined (Guezguez and Bhatia 2008), cotransplantation of genetically modified niche-related cells, and the use of embryonic hosts in which niches are developmentally staged and defined (Sigurjonsson et al. 2005).

Size

The replenishment and repair of tissues by endogenous stem cells involves proliferation and often migration along specific pathways. The size of organs obviously influences the extent to which these processes affect the regenerative process and is therefore important to consider when extrapolating from animal models to humans. The growth of axons of stem cell–derived neurons in an embryo model or a small rodent involves distances measured in millimeters, whereas in humans the comparable hurdle would be measured in many tens of centimeters. Similarly, the ability of neural stem cells to migrate across an entire brain hemisphere in a mouse would get them only a fraction of the way across a single cortical gyrus in the human brain. Striking examples of stem cell–mediated repair in animal models must therefore be tempered by the scaling factors involved in translating to the human body. For example, embryonic stem cell (ESC[1])–derived motoneurons have been implanted into rat spinal cords and their axons coaxed to grow to limb muscles, but these results required the deposition of molecular chemoattractants along the trajectory to the muscles (Deshpande et al. 2006). Achieving this in a human limb or the human diaphragm muscle would present a much bigger challenge.

Immunological Barriers

The problem of immunological barriers occurs in any transplantation scenario, whether it involves cells or organs. Xenotypic or allogeneic transplantation can trigger host-versus-graft and, in the case of hematopoietic or lymphoid cells, graft-versus-host reactions, both of which can abrogate the beneficial effects of the transplant. Although ESCs may not induce full-blown immune responses, their differentiated progeny typically do (Batten et al. 2007; Bonde et al. 2008; for reviews, Boyd et al. 2005; Boyd and Wood 2009). Several strategies have been developed to avoid these deleterious reactions, including immunosuppression in normal hosts, the use of genetically immunodeficient animals as hosts (Thomsen et al. 2008), the use of autologous stem cells, the use of stem cells genetically modified to diminish or eliminate major histocompatibility complex (MHC)–based interactions and signaling (Batten et al. 2007; Drukker and Katz. 2002), and the desensitization of the host immune system to xenotypic stem cells (Bonde et al. 2008; Kelly et al. 2009).

Survival of Implanted Stem Cells

Even when immunological barriers are overcome, survival of human stem cells implanted in animals is not guaranteed, as is evident from numerous studies in which the number of cells that survive is substantially lower than the number of cells injected. It is highly likely that, beyond immune-mediated cell destruction, mismatches in the requirements for and availability of trophic support lead to the apoptosis of stem cells and their progeny. Such mismatches may arise because of species differences, in the case of xenotypic transplantation, but also because of regional differences in the trophic environment in an organ or tissue. For example, it has become clear in recent years that glial cells in different brain regions have different molecular and functional characteristics that differentially influence the behavior of homotypic and heterotypic neurons (Le Roux and Reh 1995; Petit et al. 2001; Yeh et al. 2009). Overcoming the problem of inadequate trophic support will require more knowledge about such regional differences in all organs that are targets for stem cell implantation. This is a research area whose surface has hardly been scratched.

Potential Fusion of Implanted Human Stem Cells with Host Cells

The notion of transdifferentiation by somatic stem cells transplanted into ectopic tissues has fueled substantial controversy (Eisenberg and Eisenberg 2003). One of the counterarguments was the possibility that atypical differentiation might be due to the fusion of implanted stem cells with host cells, generating heterokaryotypic cells whose site-specific characteristics would reflect the amalgamation of the host cell phenotype into the implanted cells. Cell fusion is normal in some tissues, but otherwise quite rare. Nevertheless, it has occurred at low frequency in several stem cell implantation experiments (Alvarez-Dolado et al. 2003; Terada et al. 2002; Ying et al. 2002), and for this reason nearly all stem cell implantation studies now include some kind of control for cell fusion.

It is critically important to ascertain and characterize any contribution of cell fusion before considering the use of stem cell implantation in a standardized treatment scenario. This is true even if cell fusion, through the combination of host and donor properties, is potentially beneficial—for example, if the fused cell gains the survival advantage of the host cell's

[1]Abbreviations used in this article: ESC, embryonic stem cell; HSC, hematopoietic stem cell; PET, positron emission tomography

autologous immunological status while maintaining the donor cell's tissue replenishment properties.

Methods to test for cell fusion include preimplantation genetic tagging, postimplantation karyotyping and/or genotyping, and postimplantation phenotyping. These approaches involve a postmorten assessment, so the development of a test method that could be used noninvasively in vivo (e.g., linking the expression of an in vivo–detectable donor reporter gene to the presence of host genes) would be a useful advance.

Finding and Tracking Implanted Human Stem Cells

The location and tracking of stem cells are indispensable for characterizing the fate of stem cells, especially in vivo. For this reason we provide here a more comprehensive treatment of this particular topic.

Invasive or Postmortem Techniques

Many possibilities exist for tagging stem cells so that they can be identified after implantation. Most methods typically entail invasive, often postmortem, assessment—for example, through the prelabeling of stem cells with a variety of intracellular or genetic markers (e.g., fluorescent dyes, quantum dots, reporter genes, and nucleotide analogues such as 5-bromo-2-deoxyuridine or BrdU) for later histologic identification, or immunohistochemical identification using species-specific antibodies. But progressive dilution of the marker through cell division affects most dyes as well as BrdU, limiting their utility to situations in which postimplantation proliferation is not excessive. BrdU labeling has been used to assess the migratory capabilities, integration, and differentiation of human neural stem cells injected in the brains of monkeys (Bjugstad et al. 2005, 2008; Redmond et al. 2007). Although it is believed that stem cells retain more BrdU than somatic cells because they divide more slowly, a dilution of BrdU at each mitosis does occur (for review, Yan et al. 2007). In addition, BrdU is released from apoptotic or necrotic cells and taken up by surrounding cells, thereby creating false positives, a problem that occurs with any other marker that could be taken up by other cells after unintended release from the implanted cells. For this reason, genetic markers, such as beta-galactosidase or fluorescent proteins, are often preferred, but these require the genetic manipulation of the stem cells, which may have unforeseen consequences on cell differentiation, and the marker proteins may also be downregulated in response to in vivo environments. An alternative approach that is independent of dilution or downregulation is to use karyotyping by fluorescent in situ hybridization (FISH), based on either species or gender differences.

Noninvasive in Vivo Techniques

Tracking cells noninvasively in vivo provides the advantage of longitudinal analysis in individual animals, thus increasing the analytical power of an experiment and providing insight into dynamic events that would otherwise be more difficult to comprehend and assess. This is an area that is developing rapidly, and the number and sophistication of options are increasing.

Genetic tagging with fluorescent or bioluminescent proteins. Many types of stem cells can be stably transfected, using different types of viruses, with genes encoding fluorescent proteins (Gavrilescu and Van Etten 2007; Kume et al. 2000; Larocca et al. 2002; Rappa et al. 2004). The first fluorescent protein to be used, green fluorescent protein (GFP), has been complemented in recent years by a variety of related fluorescent proteins with different excitation/emission properties, allowing all sorts of fluorescence combinations (Pakhomov and Martynov 2008). The various fluorescent proteins can be visualized noninvasively in vivo in small animals, within limits related to tissue depth, sensitivity, and the spatial resolution of the imaging system. In a recent study, human glioma cell lines (U87, U251, U373) were stably transfected with red fluorescent protein (RFP) and transplanted into the brains of immunodeficient mice engineered so that all nucleated cells expressed an enhanced variant of GFP called eGFP (Niclou et al. 2008). This method made it possible to show that the U87 cells, unlike the other cell lines, cannot infiltrate brain tissue in vivo.

Although transduction with fluorescent proteins appears to be a robust way to tag stem cells, researchers have reported discrepancies in the expression levels of fluorescent proteins in vitro and in vivo (Kurre et al. 2002; Rosenzweig et al. 2001). For example, monkey bone marrow cells transfected with GFP were strongly GFP-positive in vitro, but when transplanted back into animals the blood showed only low circulating levels of GFP-positive cells, a result that the authors interpreted as a strong downregulation of the GFP expression. In our own studies we have seen numerous examples of human cells with initially strong GFP expression in vitro that lose the GFP expression to such an extent in vivo that it is detectable only by immunohistochemical amplification (unpublished results).

In vivo whole animal imaging has been developed more extensively using bioluminescent proteins, particularly luciferase, an enzyme that generates light upon reaction with the substrate D-luciferine. In a recent study (van Amerongen et al. 2008), the luciferase gene was placed under the control of the promoter of the collagen 1 (α2 chain) gene in transgenic mice. Bone marrow cells from these mice were injected intravenously into host mice in which endogenous bone marrow cells had been eliminated by gamma irradiation. Four weeks after the transplantation, when the injected cells had repopulated the host bone marrow, a permanent ligation of a coronary artery was performed. After delivery of D-luciferine to the mice by intraperitoneal injection, a luciferase signal was found originating exclusively from the heart where bone marrow cells had differentiated to collagen 1 (α2 chain)–expressing myofibroblasts at the ischemic site.

Whole animal imaging of fluorescent or bioluminescent proteins requires the use of high-sensitivity CCD (charge-coupled device) cameras or photodiode arrays. Higher-resolution images of brightly fluorescent cells are possible with in vivo two-photon microscopy but the visualization depth is much more limited. A recent study in mice addressed the organization of hematopoietic stem cells (HSCs[1]) in their niche at the cellular level in vivo by combining conventional and multiphoton confocal microscopy (Lo Celso et al. 2009). The authors simultaneously detected bone (by second harmonic generation, a property of crystals to emit at half of the wavelength used to illuminate them), GFP-labeled osteoblasts (expression restricted to the collagen α1 chain promoter), blood vessels (by imaging nonspecifically targeted quantum dots injected into the bloodstream just before imaging), and DiD- or DiI-labeled HSCs grafted after gamma-irradiation suppression of the host HSCs. In this way they were able to visualize HSCs in their niche and determine that when the cells are closely associated with bone and osteoclasts they proliferate and expand whereas when separated from osteoclasts they produce differentiated progeny.

Tagging with radiochemical and magnetic resonance labels. The limitations inherent in detecting labeled stem cells at depth using fluorescent or bioluminescent approaches, even in small animals, have prompted the use of other imaging modalities for which animal size is not an issue. These alternatives include positron emission tomography (PET[1]) and magnetic resonance imaging (MRI) (Table 1).

PET and the related SPECT (single photon emission computed tomography) involve the detection of gamma rays emitted by radiochemicals. Several approaches for labeling stem cells enable their tracking with PET or SPECT, including direct labeling with radionuclides and transfection with reporter genes that bind or promote uptake of radiochemicals (Acton and Zhou 2005; Zhang et al. 2008). Both PET and SPECT, which permit detection of signals that originate at any depth in the organism, are routinely used clinically for medical imaging of human patients. PET is more sensitive than SPECT, but the latter is cheaper and easier to implement. The principal disadvantage for the use of PET or SPECT in animal studies is that the spatial resolution is relatively low, with a theoretical limit of about 1 to 2 mm (Jacobs and Cherry 2001). SPECT and PET have nevertheless been successfully used to visualize rat cardiac stem cells for several days in vivo after intramyocardial injection, demonstrating proof of principle (Terrovitis et al. 2008).

MRI involves the detection of energy that is first absorbed by magnetic dipoles when they are caused to resonate in tissue and then released when the dipoles relax. Because hydrogen atoms are natural dipoles, differences in hydrogen content (e.g., correlated with water content) can be imaged directly in tissue. However, for the purposes of imaging stem cells, magnetic particles are introduced into the cells to provide sharp magnetic discontinuities. Several approaches are effective for labeling stem cells with magnetic particles. For example, metalloproteins such as the iron-binding proteins transferrin or ferritin can be overexpressed in cells by transfection or receptor-mediated endocytotic uptake via the transferrin receptor (TfR). An increase in iron accumulation also results from the overexpression of TfR itself, which has been used to detect tumorigenic fibrobasts (Gilad et al. 2007). A combined overexpression of TfR and ferritin in mouse neural stem cells led to iron accumulation that was nontoxic and detectable with MRI but with low contrast (Deans et al. 2006). Researchers recently produced a transgenic mouse ESC line in which they stably introduced the human ferritin heavy chain (Liu et al. 2009), permitting the differential detection of teratomas derived from the transgenic ESCs as opposed to those derived from control ESCs.

A more effective approach in terms of MRI contrast is to label stem cells with iron oxide particles, of which several types are available, varying in size, coating, and iron oxide content (for reviews, Slotkin et al. 2007; Sykova and Jendelova 2007). Superparamagnetic iron oxide particles (SPIOs) were first introduced as MRI contrast agents (Mendonca Dias and Lauterbur 1986; Renshaw et al. 1986). SPIOs are nanoparticles on the order of 50 to 150 nm in diameter; ultrasmall superparamagnetic iron oxide particles (USPIOs) are about 30 to 50 nm in diameter, and monocrystalline iron oxide nanocompounds (MIONs) range from 100 to 200 nm in diameter. In addition to these nanometer-sized particles, micrometer-sized particles of iron oxide (MPIOs, ranging from about 1 to several microns in diameter) have been developed and shown to be more efficient for MRI detection than SPIOs, due to their higher density of iron (Shapiro et al. 2005). MPIOs can be coated with various fluorescent components embedded in a polystyrene matrix to facilitate combination with fluorescence microscopy, and can also be coated with streptavidin to allow the specific targeting of MPIOs to cell surface antigens bound by biotinylated antibodies (Shapiro et al. 2007).

Various types of stem cells take up iron oxide particles. A particular advantage of MRI over PET is its substantially higher spatial resolution—mouse hepatocytes labeled with MPIOs and grafted into the spleen of a host mouse have been detected at the single cell level (Shapiro et al. 2006). A major disadvantage, however, is that cell division dilutes the iron particles such that after several cell cycles the cells may contain so little iron that they are no longer detectable. False positives represent another problem that can arise when stem cells die and release iron particles that host cells can then sequester.

PET and MRI are complementary approaches, with PET having a higher sensitivity but lower spatial resolution than MRI. Recently, the best of both worlds has been achieved with the development of a 3D PET detector deployed in an MRI machine, allowing for simultaneous PET/MRI detection (Judenhofer et al. 2008). It is likely that future developments in both labeling techniques and combinatorial imaging modalities will revolutionize the tracking of single stem cells in vivo over extended periods of time.

Table 1 Comparison of imaging approaches relevant to studies of human stem cells in animal models[a]

Imaging type	Lateral resolution	Sensitivity	Detection depth	Invasiveness
Postmortem	150-350 nm*	Single molecule	Unlimited	High
Whole animal	2-20 μm*	Femtomole	10-15 mm	Noninvasive
Multiphoton microscopy	150-350 nm*	Tens of molecules	0.5 mm	Low to moderate
PET/SPECT	1-2 mm	High**	Many centimeters	Noninvasive
MRI	10-100 μm	Low**	Many centimeters	Noninvasive

*Depends on excitation wavelength and numerical aperture.

**Comparing PET to MRI.

***But with depth limitation as indicated.

[a]MRI, magnetic resonance imaging; PET, positron emission tomography; SPECT, single photon emission computed tomography.

[b]+++ = common to most laboratories; ++ = available in some laboratories; + = available only at dedicated centers.

Chimeric Animal Models Commonly Used to Study Human Stem Cells

Rodents

Advantages and Disadvantages

Small rodents have been a mainstay of biomedical research for many years. With the discovery of the SCID (severe combined immune deficiency) mutation in the early 1980s (Bosma et al. 1983), immunodeficient mice and rats became invaluable tools for xenotransplantation (as well as allogeneic and even syngeneic transplantation). Several immunodeficient mouse strains are available, making the mouse a versatile model for the in vivo study of stem cell biology (reviewed in Thomsen et al. 2008).

Rodents offer several advantages relative to other mammalian models: they are small (among other things, their small size facilitates noninvasive in vivo imaging), relatively inexpensive, and easily handled. In addition, the number of transgenic rodent strains is increasing, providing a potentially unlimited toolbox of genetically defined in vivo models for the study of human stem cells.

There are also, however, several disadvantages. The larger size of many human organs, and the greater complexity of some, such as the human brain, does not permit a direct translation of experimental studies in rodents to human clinical trials. For instance, rodents have better chances of recovering locomotion after spinal cord injuries than humans, probably due to differences in endogenous recovery mechanisms and the degree of cortical control involved but probably also related to the smaller size of the spinal cord (NRC 2005). Therefore, functional recovery and physiological processes in rodents must be evaluated with great caution before extrapolating to humans. In the case of neural cell replacement therapy larger animal models may offer more appropriate conditions. Furthermore, since rodents have much shorter life spans than humans it is difficult to evaluate the long-term effects of stem and progenitor cell transplants. One great concern, particularly with embryonic stem cells, is tumor formation; ESCs have the potential to generate complex teratomas in vivo, some of which may become cancerous (Przyborski 2005; Thomson et al. 1998).

Rodent Models Used to Study Human Stem Cells

The earliest experiments in which human stem or progenitor cells were transplanted into rodent models involved the study of human hematopoiesis. The first reports of successful human cell transplantations into SCID mice were in the late 1980s (McCune et al. 1988; Mosier et al. 1988). In 1992 came the milestone discovery that human bone marrow cells injected intravenously in SCID mice could repopulate the host bone marrow (Lapidot et al. 1992). Soon after, researchers showed that the repopulating cells made up a small, phenotypically distinguishable fraction of the human bone marrow, leading to the phenotypical definition of the hematopoietic stem cell in humans (Bhatia et al. 1997; Larochelle et al. 1996). Since then, immunodeficient rodents have been invaluable in further characterization of human hematopoietic stem and progenitor cells.

Immunodeficient rodents quickly became attractive models for studies beyond hematopoiesis. The ability to study human embryonic and adult stem and progenitor cells in vivo opened up new potential avenues for treatment of neurodegenerative diseases and central nervous system injuries. Studies using the xenotypic transplantation of human neural stem/progenitor cells into the rodent central nervous system started in the early 1990s and produced several important insights into human neural stem cell biology. One of the earliest studies reported that human telencephalic neuroblasts transplanted into the lesioned striatum of adult rats (in an animal model of Parkinson's disease) integrated

Animal size	Temporal dynamics	Cost	User-friendliness	Typical availability of facilities[b]
Unlimited	None	Low to moderate	Moderate	+++
Small rodent or equivalent	Low	Moderate	High	++
Unlimited***	High	Moderate	Low	++
Limited only by instrument size	Low	High	Low	+
Limited only by instrument size	Moderate	High	Low	+

and projected axons along some of the major fiber tracts (Wictorin et al. 1990). Later in the decade, a rat model revealed that human neural progenitors can incorporate into the developing brain and differentiate into cells of multiple neural phenotypes (Brüstle et al. 1998). By transplanting human ESC-derived neural progenitors into the lateral ventricles of newborn mice investigators showed that these cells have the capacity to form physiologically patent neurons in vivo (Reubinoff et al. 2001; Zhang et al. 2001). A more recent study found that human ESC-derived oligodendrocyte progenitor cells transplanted into the injured spinal cord of adult rats contributed to remyelination and improved locomotion (Keirstead et al. 2005), laying the cornerstone for the first clinical trial using human ESCs in spinal cord injury patients, scheduled to start in 2009 (Alper 2009).

Rodent-human chimeras have also been used in the study of other organ-specific stem cells, for example in the study of liver disease and toxicology. Recently, chimeric mice with humanized livers were established by transplanting human hepatocytes into SCID transgenic mouse lines (reviewed in Katoh et al. 2008).

Ungulates

Advantages and Disadvantages

Sheep, goats, and pigs provide several important advantages for xenotransplantation studies. Their large size and physiological similarity to humans permit the use of surgical and other procedures on the same scale as used in the clinic as well as an easier translation to human physiology and pathology (Brevini et al. 2008; Ourednik et al. 2001). Their large size also facilitates not only the expansion of implanted human cells to numbers sufficient for biochemical analysis but also multiple samplings over time. Physiological similarity between pigs and humans is sufficiently great that organ transplantation from pigs to humans has become a viable option actively pursued for a variety of organs (Lu et al. 1994).

Similarity at the cellular level is also greater than between rodents and humans. For example, pig neural stem cells are molecularly more similar to human neural stem cells than are those of mice (Baizabal et al. 2003); and in human and ungulate homologues of certain proteins, such as trophic and colony-stimulating factors, molecular similarity is high enough that they can be used interchangeably (Verfaillie et al. 2000; Zanjani et al. 1994). Because the relevant ungulates are domesticated, they are relatively well standardized genetically. In addition, recent advances in reproductive cloning permit the production of isogenic lines, although relative to rodents the logistics involved are more demanding. The genomes of several ungulates have been sequenced, making transgenic approaches also possible (Campbell et al. 1996; Wilmut et al. 1997). Breeding procedures are highly developed, which facilitates the implantation of human stem cells into ungulate fetuses, thus avoiding immunological barriers (Flake and Zanjani 1997; Zeng et al. 2006). And the longer life spans of ungulates enable long-term evaluation of the safety and efficacy of potential stem cell therapies (Dall et al. 2002; Vodicka et al. 2005).

Among the disadvantages of these species, size limits the options available for whole animal imaging. Moreover, maintenance and handling are proportionately more expensive than for smaller animals.

Ungulate Models Used to Study Human Stem Cells

Sheep and goats have been used extensively since the 1960s in preclinical studies of bone marrow transplantation, in particular for the xenotransplantation of human stem cells into sheep and goat fetuses. The first stable xenograft of

human fetal hematopoietic stem cells into a sheep fetus took place in the early 1990s (Zanjani et al. 1991). Since then, this model has been used to test the proliferative kinetics and differentiation potential of a variety of human stem cells, including subpopulations of umbilical cord blood cells, fetal liver HSCs, bone marrow–derived HSCs (Almeida-Porada et al. 2007; Michelini et al. 2008; Narayan et al. 2006; Zanjani et al. 1994), mesenchymal stem cells (Airey et al. 2004; Almeida-Porada et al. 2002), and neural stem cells (Almeida-Porada et al. 2005). The use of goats for similar studies of human HSCs began somewhat later (Huang et al. 2002; Zeng et al. 2005, 2006). In pigs, most efforts have focused on transplants from pig to human, including whole organs and fetal progenitor cells to treat Parkinson's disease (Schumacher et al. 2000). Nevertheless, there have been several attempts at xenotransplanting human stem cells into pigs, including transplants of human umbilical cord blood stem cells into pig fetuses (Fujiki et al. 2003) and of human renal progenitor cells into pig kidneys (Hammerman 2004).

Nonhuman Primates

Advantages and Disadvantages

An obvious advantage of using nonhuman primates is that they are physiologically and morphologically closely related to humans and thus provide one of the most easily validated animal models for the study of human biology. Their reproductive physiology is also advantageously similar to that of humans but by the same token can pose an inconvenience due to long gestation times (relative to smaller animals) and thus possible temporal constraints on experiments. The size and social behavior of most primates necessitate expensive housing facilities. Large body size also demands a scaling-up of technical procedures and instrumentation, which can be an advantage for procedures originally designed for humans but a disadvantage with procedures designed for rodents. Ethical issues are more serious, with respect to both the high level of cognitive development in primates and species conservation issues. In addition, affective relationships that can develop between the animal and the researcher can be problematic. Nevertheless, nonhuman primates have become important animal models in stem cell research primarily because of their close relationship to humans.

Nonhuman Primate Models Used to Study Human Stem Cells

Parkinson's disease. Nonhuman primates represent one of the most relevant and widely used animal models for studying Parkinson's disease (see Joers and Emborg 2010 in this issue). This is primarily because the neurotoxic effects of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) on nigrostriatal dopaminergic neurons are very similar in monkeys and humans and the resultant motor abnormalities in monkeys closely resemble those seen in MPTP-poisoned humans and in humans with idiopathic Parkinson's disease (Burns et al. 1983; Gerlach and Riederer 1996; for review, Jenner 2003). Rodents, on the other hand, are more resistant to MPTP neurotoxicity (Gerlach and Riederer 1996; for review, Jenner 2002).

Researchers have extensively tested conspecific (monkey-to-monkey) transplantation of fetal dopaminergic progenitor cells and ESC-derived dopaminergic neurons (e.g., Takahashi et al. 2009; Wang et al. 2007). Working from this platform, the implantation in MPTP-treated monkeys of human stem cells and stem cell–derived neurons has become an important step in preclinical trials for stem cell–based replacement strategies for Parkinson's disease (reviewed in Richardson et al. 2008). Implants of both human neural stem cells (Bjugstad et al. 2005, 2008; Redmond et al. 2007) and human neural progenitor cells (Emborg et al. 2008) in MPTP-treated monkeys have survived, migrated along appropriate pathways, and produced behavioral improvement.

Spinal cord injury. Although rodents are the most commonly used species in studies of spinal cord injury, they are far from ideal models of human injuries for several reasons, including a higher inherent capacity for behavioral recovery, a much smaller spinal cord, and the lack of fine motor control of the hands and digits, which is severely compromised in humans and other primates after injury to the corticospinal tract. For these reasons, several nonhuman primate species have been used to study spinal cord injury, including the tiny marmoset (*Callithrix jacchus;* about the size of a small rodent), the macaque monkey (*Macaca fascicularis*), and the rhesus monkey (*M. mulatta*). Many of these studies have focused on dorsal column lesions and their effects on hand function (reviewed in Darian-Smith 2007), a few have addressed molecular and cellular mechanisms of axon growth arrest (Fouad et al. 2004; Freund et al. 2006; Ho and Tessier-Lavigne 2006), and more recently studies have begun to test the effects of injection of human stem cells. Reports indicate that human neural stem and progenitor cells improve functional recovery in the marmoset after contusion injuries of varying degrees (Iwanami et al. 2005) and that human mesenchymal stem cells augment neurogenesis and functional recovery in the rhesus monkey (Deng et al. 2006). Nonhuman primate models are likely to receive increasing attention as the basic cellular and molecular biology involved in spinal cord injury is better understood in rodent models.

Chicken Embryo

Advantages and Disadvantages

The chicken embryo has a long history as a model for xenotypic transplantation, dating back at least to the early 1900s in work using the extraembryonic chorioallantoic membrane as a tissue platform for investigating tumor cell growth (Murphy and Rous 1911). The chorioallantoic membrane was used extensively to study human tumors for many decades

and remains to this day a favorable system for investigating tissue explants from different species and organs (Vogel and Berry 1975). Xenotransplantation into embryonic tissue per se was pioneered by Nicole Le Douarin and colleagues, who began in the early 1970s to use xenotypic transplantation of quail tissue into chicken embryos to study regional fate in the central and peripheral nervous system and other structures (Le Douarin 1973). The key advantages of the chicken embryo for studies such as these include low cost, easy handling, the ability to time incubations and stage embryos accurately for experimental standardization, high accessibility for a variety of surgical implantation procedures, and the lack of a developed immune system and thus no rejection of xenotypic grafts. The disadvantages are that chicken embryos are very small and thus poor models for human organs in terms of size, and they have a number of physiological and anatomical peculiarities not found in humans, although the basic organization of many organs is similar to that of human organs; in particular, the organization of the brain stem and spinal cord is very similar to that in mammals, and the fact that chickens use bipedal locomotion has suggested a greater similarity to human locomotion than for quadrupedal rodents (Jacobson and Hollyday 1982). Chickens are currently not suitable for routine transgenic modification, although genetic manipulation is possible in a variety of embryonic tissues using in ovo electroporation or virus-mediated transfection of DNA constructs (Ishii et al. 2004; Krull 2004).

Use of the Chicken Embryo to Study Human Stem Cells

The accessibility of the chicken embryo and its lack of immune response has made it a popular model for xenotransplantation, particularly in the case of homotopic transplants of mouse embryo structures, whose subsequent development as an integral part of the chicken embryo can then be studied ex utero (e.g., Mitsiadis et al. 2003), but also more recently in the case of human stem cells. A principal approach is to use this to test the differentiation potential of stem cells in defined embryonic tissue niches (Goldstein 2006). For example, investigators have induced human ESCs to form neural crest stem cells and then grafted them into the chicken embryo neural crest, where they integrate and give rise to multiple neural crest derivatives (Jiang et al. 2009; Lee et al. 2007a); and another recent study found that human sacrococcygeal teratoma cells grafted into the neural crest behave like epiblast-derived stem cells (Busch et al. 2009). In addition, research has shown that the neural crest environment influences and in some cases reprograms metastatic human melanoma cells toward a nonmetastatic phenotype (Hendrix et al. 2007; Kasemeier-Kulesa et al. 2008). Human ESC-derived neurons have been transplanted into the neural anlage of the chicken embryo and shown to differentiate into appropriate neuronal phenotypes, an important step in establishing neural cell lines for replacement therapy of a variety of neurological diseases such as motoneuron diseases (Lee et al. 2007b; Wichterle et al. 2009). Research on the plasticity of human somatic stem cells in the chicken embryo has revealed that (1) adult human dental pulp stem cells (which are believed to derive from the neural crest) differentiate anatomically and molecularly into neurons after implantation in the mesencephalic neural tube of the chicken embryo (Arthur et al. 2008), and (2) adult human HSCs from bone marrow generate functional neurons capable of impulse generation and the formation of synaptic connections after implantation into the spinal neural tube of the chicken embryo (Sigurjonsson et al. 2005).

Stem cells from adult human olfactory mucosa have been shown to give rise to multiple cell types in the chicken embryo, demonstrating their multipotency (Murrell et al.

Table 2 Comparison of animal models described in this article[a]

Species	Size	Cost	Current feasibility of genetic manipulation	Suitable for whole animal imaging	Similarity to human	Level of ethical concern[b]
Mouse	Small	Low	Very high	All modalities	Organ-specific, moderate	Moderate
Rat	Small	Low	High	All modalities	Organ-specific, moderate	Moderate
Pig, sheep, goat	Large	High	Moderate	PET, MRI	Organ-specific, high	Moderate
Nonhuman primate	Small to large	Very high	None	PET, MRI (all modalities for marmoset)	Generally high	High
Chicken embryo	Very small	Very low	Low	All modalities	Organ-specific, low to moderate	Low

[a]MRI, magnetic resonance imaging; PET, positron emission tomography

[b]As gauged by degree of protection conferred by animal research regulatory agencies and level of use in Western society.

2005). Mesodermal derivatives also have been generated from human stem cells. Human ESC-derived hematoendothelial progenitors colonize blood-forming organs in the chicken embryo (Park et al. 2009), and adult human mesenchymal stem cells form blood vessels in the chorioallantoic membrane (Jadlowiec et al. 2005). The chicken embryo has also been used to study interactions between human glioma cells and adult human skin-derived stem cells (Pisati et al. 2007). In principle, the chicken embryo provides a convenient platform for the in vivo characterization of any type of human stem cell in interactions both with host chicken tissues and with other human cells in combinatorial implants. The chicken embryo thus represents one of the most versatile animal models available for studying human stem cell biology.

Conclusions

The choice of animal model for chimeric studies of human stem cells hinges primarily on the methods to be used and the desired proximity to clinical translation. The cost of animal husbandry may also be decisive. Smaller animals are relatively cheap to maintain and are advantageous for a variety of experimental approaches, especially with respect to in vivo imaging and tracking. Their size and short lives, however, are major disadvantages in extrapolating results to the scale of the human body. Larger animals present nearly the opposite scenario—more easily scaled to the human body but more costly to maintain and more challenging for many experimental approaches, particularly certain imaging approaches. To an extent some of the imaging challenges inherent in using larger animals may be circumvented through the use of noninvasive MR and PET imaging, and future improvements in these imaging modalities are likely to increase the prospects of successful tracking in large animals. With respect to transgenic manipulation, small rodent models are still superior, but transgenics will probably play an ever greater role in the future use of large animal models. Table 2 provides a summary comparison of the different animal models.

Since no single animal model is ideal, a rational approach to their use for chimeric studies of human stem cells would arguably involve related studies in more than one model. Initial experiments in small, relatively inexpensive, and versatile animal models provide a good platform for moving to larger animal models with greater relevance for preclinical studies. Clinical researchers, who might naturally gravitate toward the large, and basic researchers, who for a variety of reasons might prefer the small, can benefit from joining forces in designing multimodel studies to accelerate the translation of animal research to human applications.

As the use of human somatic, embryonic, and induced pluripotent stem cells increases, so too will the range of applications for these animal models. It is likely that increasingly sophisticated applications involving human/animal chimeric models will emerge through advances in genetic manipulation, cell delivery, and in vivo imaging, all immensely exciting developments in their own right. In the context of human/animal chimeric studies they represent a means to a greater end: the eventual use of human stem cells to treat human disease.

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