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Strategies for Improving Animal Models for Regenerative Medicine

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Abstract

The field of regenerative medicine is moving toward translation to clinical practice. However, there are still knowledge gaps and safety concerns regarding stem cell-based therapies. Improving large animal models and methods for transplantation, engraftment, and imaging should help address these issues, facilitating eventual use of stem cells in the clinic.

Introduction

In this Forum, we discuss the current status, challenges, and major directions for future development of animal models to facilitate the use of stem cells in regenerative medicine. The variety of stem cell sources and a wide spectrum of potential applications make the development of universal recommendations and guiding principles very challenging, yet certain common themes and possible solutions are emerging that can increase the predictive validity of animal models for regenerative medicine. This report is based on discussions that

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took place at a recent NIH workshop on this topic (http://dpcpsi.nih.gov/orip/documents/summary_of_the_improving_animal_models.pdf).

Animal Models for Stem Cell-Based Regenerative Medicine: Mice versus Large Animal Models

The discovery of mouse embryonic stem cells (ESCs) in 1981 revolutionized the study of developmental biology, and mice are now used extensively to study stem cell biology. However, there are limitations to their application as models for regenerative medicine. Mouse models do not reproduce in full certain human disease conditions. For example, laboratory mice are insulin resistant and prone to cancer and renal failure. They are relatively obese and hypertensive due to the constant access to food. In comparison to humans, mice have small body size, short lifespan, and substantially different physiology. Significant progress has been made in the creation and use of humanized mice. There are, however, disadvantages to using the current strains, including complications in reproducing standard chimeras, limitations in choices of human cell types that can be used for xenotransplantation, residual host immunoreactivity, and problems with translating conditioning regiments between species. The evolutionary distance between donor and recipient animals will also affect the survival of transplanted stem cells due to species differences in trophic properties of tissues.

Larger animal species, which were critical for developing hematopoietic stem cell therapies, often have an enhanced ability to predict clinical efficacy relative to mice. The utilization of large animal models is expected to increase and, therefore, further development of large animal stem cell technologies will also be required. It will be critical to select the large animal that is most appropriate for each potential therapy in humans. The pig has emerged as one of the best examples of a large animal model currently being used to study human genetic diseases (review in Kuzmuk and Schook, 2011). Even without genetic modification, minipigs and full-size breeds have been widely used for studying infectious diseases, cardiovascular disease and atherosclerosis, wound healing, digestive processes, diabetes, ophthalmology, and some cancers, as well as providing organs for xenotransplantation. The value of pigs as biomedical models has been enhanced over the last decade by targeting specific genomic sites for modification. Swine disease models created by targeted genetic engineering include those for cystic fibrosis, Alzheimer’s and Huntington’s disease, retinitis pigmentosa, hyperlipoproteinemia, and muscular dystrophy. Recently, creation of humanized pigs has been reported (Suzuki et al., 2012), as well as improved preclinical disease models suitable for testing stem cell therapies in pigs (Giraud et al., 2011; McCall et al., 2012). Most of this work has been enhanced through access to the swine genome sequence and the use of inbred minipigs (http://www.nsrrc.missouri.edu/strainavail.asp). Work in swine will complement nonhuman primate research for neurological treatments when analyzing recovery of fine motor skills or impact on cognitive function. This would be facilitated by equivalent advances in primate transgenesis, like the recent monkey models of Huntington’s disease.

Major challenges remain, however, for using large animals in stem cell research. For example, there is limited availability of species-specific reagents, such as antibodies and

growth factors, and fully annotated expression microarrays. Authenticated ESCs have been difficult to generate from large domestic species, such as dog, swine, cattle, sheep, and goats. This has been obviated in part by creation of induced pluripotent stem cells (iPSCs) from these species by standard reprogramming technologies. There is a lack of centralized resources where cells can be characterized and stored, reagents made available, and databases maintained for the wider biomedical community. If these barriers can be overcome, well-characterized large animal stem cells can provide an appropriate choice of animal models for particular human disease conditions and medical applications. These studies will complement the use of mice, leading to more comprehensive studies that can then be applied to humans.

Animal Induced Pluripotent Stem Cells as Emerging Models for Human Therapeutic Applications

The field of stem cell research experienced a dramatic new direction with the isolation of iPSCs from humans and mice. Several studies on various animal systems suggest that the basic pluripotency network appears to be conserved among different species, allowing derivation of iPSCs from a variety of large animal species, including pigs, monkeys, dogs, and several others (Plews et al., 2012). As for the human, iPSCs from ungulates and monkeys are of epiblast type, and fibroblast growth factor 2 and activinnodal signaling systems are critical for their growth in culture and for maintenance of pluripotency. Animal iPSCs should be of value as a renewable source of cells for testing safety after progenitor cells are introduced as a tissue graft and for testing surgical and related technologies required before human trials can proceed. Many of the challenges that face human iPSC research apply to animal iPSCs as well, including issues related to safety, efficiency, and differentiation potential.

Several problems remain before the potential of nonrodent animal iPSCs can be realized. Cell lines must be characterized in more detail, chimerism tested, and reprogramming increased in efficiency and speed in order to enhance genome integrity. Cell surface markers can be inconsistent among various lines and cell populations may be heterogeneous, probably reflecting different stages of reprogramming. Efficient derivation of animal iPSC lines requires further development of technologies for generating the cells, preferentially avoiding gene integration and potential risks of tumorigenesis.

An important aspect of animal studies is the ability to test immune responses to iPSCs and their derivatives. A number of animal studies have reported that iPSCs can form teratomas and other types of tumors in immunodeficient, allogeneic, syngeneic, and xenogeneic recipients due to the inability of the host to reject teratoma-inducing cells. The innate immune system appears capable of dealing with small numbers of undifferentiated iPSCs that might be present in grafts, despite efforts to eliminate them. The ability of adaptive and innate immune reactions to weaken engraftment of syngeneic stem cell transplants is another important aspect of the host reaction that can affect the efficiency of cell transplantation. There is an urgent need to test immune reactions against therapeutic iPSC derivatives and a small number of potentially tumorigenic cells. Larger animal species have important advantages as model systems in this regard since their immunophysiology is closer to

humans than to rodents. Like humans, larger animal models have developed as outbred populations over time, thus shaping genomic adaptations. Rodent models will remain important tools for immunological discovery and proof of concept, whereas larger animal models will be critical for successful translation for clinical applications of iPSCs.

iPSCs from patients have the potential to help identify the molecular and cellular basis of human disease by “disease-in-a-dish” modeling. Though this field is still in its infancy, reports thus far indicate the general ability of iPSCs to recapitulate certain cellular abnormalities of the corresponding cells from patients with various Mendelian disorders. The ability to model low-penetrance phenotypes, late-onset disorders, and genetically complex disorders, however, remains to be proved. Animal model systems may help solve some of these problems because transplantation experiments can be performed using the animal as a host. The use of allogeneic iPSCs in animal model systems or xenotransplantation of human stem cells in immunocompromised or humanized animals should facilitate analysis of disease phenotypes that require cellular interactions in the tissues. Animal iPSCs can have certain advantages for testing hypotheses regarding the influence of environmental or epigenetic components of disease first identified in cell culture. The effects of exposures and genomic modifications can be tested at the level of the tissue, organ, and the whole animal, preserving interactions among distinct cell types in vivo. Use of animal systems also facilitates experimental design by providing controls with matching genetic background, age, gender, and exposure history.

Improving Stem Cell Transplantation: Engraftment and Imaging

Two different approaches can be taken for stem cell-based therapies. The first is transplantation, in which stem cells, or derived progenitor or differentiated cells, are delivered directly to the body. The second approach relies on activation of endogenous stem and progenitor cells or somatic cells reprogrammed in situ. Studies in animal model systems and in humans indicate significant cell death after transplantation and limited engraftment and differentiation of transplanted stem cells. Retention and survival of transplanted cells must be improved. One of the likely reasons for low levels of cell survival and engraftment is the absence of the proper cellular environment and substrate for engraftment. Attempts have been made to provide the transplanted cells with biomaterial carriers and signaling molecules, which will prevent cell death and will help to re-establish proper cell interactions. Among techniques that are currently under development to address these problems in animal models are genetic modification of the cells and the use of tissue-engineering techniques involving three-dimensional biodegradable scaffolds, additional types of support cells, and other bioactive molecules. Various nanomaterials have been explored to control stem cell behavior such as adhesion and differentiation by modulating biomimetic characteristics and mechanical properties.

An important goal of stem cell-based therapies for treatment of damaged or diseased tissues is to establish proper connections between cells or with extra-cellular signaling molecules. This has been challenging for cardiovascular repair and even more complicated for stem cell-mediated transplantation in the retina or brain, where appropriate synaptic connections must be created within the three-dimensional tissue structure. Knowledge of the homing and

niche signals that mediate the activation, migration, and integration of stem cells to damaged tissues is limited. Cytokines, growth factors, adhesion molecules, cell-cell contacts, and small metabolic products are active players in these processes. These mechanisms may vary among different animal species and should be investigated and evaluated in a search for the most appropriate model for a particular application. Progress has been made, for example, in the use of synthetic and natural guide materials, nerve guide coatings, topographical cues, special scaffolds, and support cells to guide nerve cell engraftment (Bell and Haycock, 2012). Pharmacologically active microcarriers, such as biodegradable and noncytotoxic poly lactic-co-glycolic acid microspheres covered with extracellular matrix molecules, have been successfully tested in animal models of Parkinson's disease and infarcted myocardium in rats and mini-pigs, respectively.

Cellular imaging is a high priority for basic research and clinical translation in regenerative medicine. Imaging is important for several applications, including guiding and verifying the accuracy of cell injection, tracking cell migration, survival, and behavior, evaluating off-target effects, and monitoring long-term cell engraftment. Reagents should be optimized that exhibit quantitative biosensing properties and functional readouts that can report changes in cell conditions, such as activation, differentiation, or injury. These parameters in most cases cannot be obtained from patients via direct sample extraction, in distinction to animal studies. The development of noninvasive imaging approaches to monitor transplanted stem cell behavior is critical for future translation to clinics.

Significant advances have been achieved in the use of imaging modalities such as single-photon emission computed tomography (SPECT), positron emission tomography (PET), and MRI to monitor noninvasively transplanted cells in myocardium, skin hair follicles, the CNS, bone marrow, mammary glands, and retina, as well as other organs in rodents. The development of imaging techniques in large animals is becoming increasing important, due to the application of protocols and equipment more relevant to the clinical setting. Among complications are the need for equipment accommodating larger species, limited tissue penetration, and the outbred nature of large animals, which may require more animals and longer analysis time. Large animals will also demand additional knowledge of their biology and careful monitoring of physiological parameters during imaging procedures. MRI has been used to detect nanoparticle-labeled cells in large animals in several studies, but the approach was limited by label leakage and uptake by macrophages. Cells have also been labeled with a variety of radionuclides. Limitations of several isotopes were relatively short half-life and effects on cell viability. Good results were obtained by using a PET-herpes simplex virus thymidine kinase-reporter system. Stable expression for an extended period of time was reported recently with PET or SPECT after administration of radiotracer in combination with expression of a transgenic sodium iodide symporter (NIS) in a pig model of myocardial infarction (Templin et al., 2012). Another successful study has been reported in pigs using iPSC-derived endothelial cells labeled with [^{18}F]- fluorodeoxyglucose for PET imaging and iron particles for colocalization with MRI (Gu et al., 2012). New, more sophisticated PET/SPECT reporter genes will require addressing potential oncogenicity and immunogenicity of transplanted cells. No single imaging technique provides all of the desired information, permitting single-cell detection in an animal model. However, current

techniques should allow optimization of procedures and on future improvement should satisfy most needs, thus facilitating cell tracking in humans.

How Animal Models Can Address the Potential Challenges of Stem Cell Therapy

The challenges associated with unique properties of stem cells that must be understood before moving to clinical applications have been identified and continue to grow. Among these are genetic instability, high mutation rate and tumorigenic potential, epigenetic memory of differentiated iPSCs, and the immune response after stem cell transplantation. Some of these concerns became evident during experiments when animal stem cells were tested and should be examined rigorously for human stem cells. Importantly, animal models will be critical to evaluate new potential hazards and to address these problems (Frey-Vasconcells et al., 2012). The standard use of preclinical animal models for studies that include adsorption, distribution, metabolism, excretion, and toxicology is mandated by the US Food and Drug Administration. The validated processes and regulatory requirements are well established for these studies. However, even in these investigations, there are issues as to whether the standard models are appropriate for cell-based therapy. For example, biodistribution with pathology analysis using sequential sections of the entire body may not be an appropriate model for a localized cell transplant that is not expected to survive in an allogeneic setting. A number of special considerations should be taken into account when designing tumorigenicity studies, such as the animal species for testing, the type and condition of therapeutic stem cells or their by products, in vivo survival time, and potential distribution and migration of the transplanted cells. Several concerns were reported based on mouse studies that employed a variety of cell types, including mesenchymal stem cells (MSCs). Extensive preclinical data using MSCs from larger animal species (pigs, dogs, and sheep) for transplantation as well as clinical studies have not detected a major risk for tumor growth. The following reasons were proposed for the discrepancies between preclinical and clinical studies at least in regard to MSCs and their potential applications for treatment of heart disease: substantial differences in mouse and larger animals and human cell behavior in culture, differentiation capacities of particular cell preparations, sensitivity to triggers of transformation, and properties of the immune surveillance systems (Hatzistergos et al., 2011). Very limited information is available regarding other types of transplanted cells and animal model species to make a valid selection of the most predictable model. Rigorous studies should be conducted for each application to evaluate the oncogenic risk of the use of stem cell products in the most appropriate model system, especially large animals.

Conclusions

Insufficient knowledge of human stem cell biology and the absence of animal models that precisely recapitulate particular human disease phenotypes, with comparable organ size and physiology, are currently significant limitations for the progress of regenerative medicine. Improvement of existing rodent models, as well as development and characterization of stable stem cell lines and disease models from larger animal species, will take place in concert with other innovative approaches, such as microsystem tissue engineering and in

vitro modeling of human disease pathways by using iPSCs from affected individuals. These various approaches are complementary and are highly relevant to the future of stem cell-based regenerative medicine. However, it is also clear that extant models need to be modified, new models developed, and rigorous standards of preclinical evaluation followed to maximize their research value. There is no single, perfect animal model that can completely predict the outcome of clinical trials. The challenge is to collect relevant and sufficient information from as many models as are required to make an informed decision regarding the potential benefits and risks to patients.

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REFERENCES

Bell JH, Haycock JW. Tissue Eng. Part B Rev. 2012; 18:116–128. [PubMed: 22010760]

Frey-Vasconcells J, Whittlesey KJ, Baum E, Feigal EG. Stem Cells Transl Med. 2012; 1:353–358. [PubMed: 23197814]

Giraud S, Favreau F, Chatauret N, Thuillier R, Maiga S, Hauet T. J. Biomed. Biotechnol. 2011; 2011:532127. [PubMed: 21403881]

Gu M, Nguyen PK, Lee AS, Xu D, Hu S, Plews JR, Han L, Huber BC, Lee WH, Gong Y, et al. Circ. Res. 2012; 111:882–893. [PubMed: 22821929]

Hatzistergos KE, Blum A, Ince T, Grichnik JM, Hare JM. Circ. Res. 2011; 108:1300–1303. [PubMed: 21617132]

Kuzmuk, KN.; Schook, LB. Pigs as a model for biomedical sciences. In: Rothschild, MF.; Ruvinsky, A., editors. The Genetics of the Pig. Second Edition. Wallingford, UK: CAB International; 2011. p. 426-444.

McCall FC, Telukuntla KS, Karantalis V, Suncion VY, Heldman AW, Mushtaq M, Williams AR, Hare JM. Nat. Protoc. 2012; 7:1479–1496. [PubMed: 22790084]

Plews JR, Gu M, Longaker MT, Wu JC. J. Cell. Mol. Med. 2012; 16:1196–1202. [PubMed: 22212700]

Suzuki S, Iwamoto M, Saito Y, Fuchimoto D, Sembon S, Suzuki M, Mikawa S, Hashimoto M, Aoki Y, Najima Y, et al. Cell Stem Cell. 2012; 10:753–758. [PubMed: 22704516]

Templin C, Zweigerdt R, Schwanke K, Olmer R, Ghadri JR, Emmert MY, Müller E, Küest SM, Cohrs S, Schibli R, et al. Circulation. 2012; 126:430–439. [PubMed: 22767659]