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CONCISE REVIEW

Human–Monkey Chimeras for Modeling Human Disease: Opportunities and Challenges

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The search for a better animal model to simulate human disease has been a ''holy grail'' of biomedical research for decades. Recent identification of different types of pluripotent stem (PS) cells and advances in chimera research might soon permit the generation of interspecies chimeras from closely related species, such as those between humans and other primates. In this study, we suggest that the creation of human–primate chimeras—specifically, the transfer of human stem cells into (non-ape) primate hosts—could not only surpass the limitations of current monkey models of neurological and psychiatric disease but would also raise important ethical considerations concerning the use of monkeys in invasive research. Questions regarding the scientific value and ethical concerns raised by the prospect of human–monkey chimeras are more urgent in light of recent advances in PS cell research and attempts to generate interspecies chimeras between humans and animals. While some jurisdictions prohibit the introduction of human PS cells into monkey preimplantation embryos, other jurisdictions may permit and even encourage such experiments. Therefore, it is useful to consider blastocyst complementation experiments more closely in light of advances that could make these chimeras possible and to consider the ethical and political issues that are raised.

Keywords: pluripotent stem cells, interspecies chimera, naive pluripotent stem cells, disease modeling, neurological disease, psychiatric disease

New Approaches to an Old Problem

Neurological and psychiatric diseases are a devastating problem, causing profound human suffering and disease burden worldwide. The World Health Organization estimates at least 1 billion people are affected by neurological disease; the number is expected to increase considerably in the future [1]. Neurological disorders are also a major cause of mortality and comprise 12% of total deaths globally. Currently available treatment options are often fruitless. Failure rates for experimental central nervous system drugs are higher compared with other classes of drugs [2]. One possible roadblock is the inadequate quality of existing animal models, which impedes elucidation of disease mechanisms and development of new treatments.

One strategy for modeling of neurological disease is to generate disease-relevant cell types from patient-specific induced pluripotent stem (iPS) cells [3–6]. The defining features of human iPS (hiPS) cells—indefinite self-renewal in culture and capacity to differentiate into any cell type—in principle, allow for access to an endless supply of disease-relevant cells. Furthermore, iPS cell-derived cells are genetically identical to the source patients. When combined with advances in generating neural cell types by directed differentiation of hiPS cells, researchers have probed disease-specific effects on a relevant cell type. This experimental strategy has been successfully applied to establish correlations between patient-specific genetic mutations and abnormal neuronal phenotypes for a range of highly penetrant neurological diseases [7,8].

Classical strategies for modeling neurological disease using patient-specific iPS cells involve differentiation in a dish. However, in vitro differentiation possesses critical limitations, including the failure to achieve functional maturation of in vitro-generated human PS cell derivatives that tend to exhibit immature, fetal-like features. Moreover, current methodologies are not compatible with the production of complex three-dimensional tissues, preventing scientists from assessing connectivity and systems-level functionality of disease-specific cells. Consequently, new approaches are required to generate more sophisticated hiPS cell-based models of neurological disease.

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The limitations of in vitro disease modeling underscore a need to return to the in vivo approaches. For example, it is now possible to establish mice within which a significant percentage of host glia are patient derived [9]. Indeed, a recent study generated hiPS cell mouse chimeras using glial progenitor cells derived from iPS cells from patients with childhood-onset schizophrenia [10]. These mice were reported to exhibit unusual behaviors, which included increased anxiety, antisocial traits, and perturbed sleep. A similar approach has been applied to modeling Huntington's disease [11]. However, one challenge for modeling neurological and psychiatric conditions using rodents as a host animal is knowing whether the model faithfully recapitulates signs of the disease. The use of nonhuman primates (NHPs) as host animals may generate more interpretable data.

Because of their similarities to humans, NHPs are essential models for studying neurological disease. Nonetheless, there remain neurological characteristics unique to humans. Knowledge of relevant convergent and divergent features must be inextricably linked to the choice of approach for studying neurological and psychiatric diseases. While NHP models for neurological disease have existed for decades, rapid advances in CRISPR/Cas9-mediated genome editing are altering how we model disease using NHPs [12–14]. For example, proof-of-principle experiments applying CRISPR/Cas9 to early primate embryos have generated knockout monkeys for the PPARG and RAG1 loci [15].

Nonetheless, there exist three hurdles associated with use of CRISPR/Cas9-mediated genome editing. First, while CRISPR/Cas9 accurately cleaves its target genomic loci, it also possesses off-target activity that can induce undesired genetic changes. Second, off-target or delayed Cas9 activity can also cause genetic mosaicism, where an animal is composed of cells with different genotypes. A third technical challenge is creating NHPs with mutations at multiple loci. Overcoming this obstacle may be achieved by unifying genome editing with somatic cell nuclear transfer-based cloning of monkeys, which will in principle enable generation of transgenic NHPs by using donor nuclei from cultured gene-edited cells [16].

Given the limitations posed by CRISPR/Cas9 approaches, we believe new advances in hiPS cell research may provide a fruitful alternative method for creating appropriate NHP models of neurological diseases. It is now broadly accepted that in vitro pluripotency manifests as a continuum of different cellular states [17]. At one polar extreme is the naive state, which reflects unrestricted cellular potency. At the other end is the primed state, where cells are poised for differentiation [18]. The key distinction between naive and primed PS cells is the ability to generate chimeras—the capacity to contribute to all three germ layers when injected into a preimplantation embryo [18,19]. While rodent PS cells are in a naive state because they can form chimeras, conventional PS cells in primates and human are likely to reside in a non-naive pluripotent state as primate PS cells cannot form chimeras when introduced into preimplantation embryos [20].

In recent years, interest has grown in understanding the xenogeneicity of PS cells—the capacity of PS cells from one species to contribute to the embryos of another species [21]. Rodent PS cells are xenogeneic as one can reproducibly generate interspecies chimeras between mice and rats [22]. To our knowledge, the introduction of human PS cells into embryos of other species has not given rise to live interspecies chimeras [21,23]. It has been suggested that matching developmental stage of donor cells and host embryos is essential for chimera formation [24]. To match donor cell stage with host embryos, recent studies have attempted to reset the developmental stage of human PS cells toward a naive state with the aim of conferring chimera competency. While the precise constellation of molecular and biological characteristics that identify a naive or primed PS cell is contentious, naive PS cells—at least in rodents—possess a unique ability to form chimeras. Therefore, a discussion of human naive and primed pluripotency is germane because the availability of chimera-competent human PS cells is an essential reagent for generating interspecies chimeras. However, attempts to generate interspecies chimeras using human naive-like cells and either mouse or pig host embryos have produced very low rates of chimerism [21,23]. These initial results suggest that interspecies chimerism with naive-like human PS cells is limited, which may reflect species barriers beyond matching developmental timing. Nonetheless, while modest, the existence of any human–pig cross-species chimerism provides hope that it may be possible to achieve significant levels of human chimerism in large animals [21].

These data provoke questions regarding why the levels of chimerism observed in these experiments are very low. One interpretation is the developmental stage of donor cells and host embryos has not been sufficiently matched [24–26]. This could be because naive pluripotency has not been appropriately instated in donor cells [17,27]. In contrast, host blastocysts at the time of embryo injection may not be developmentally synchronized with putative donor naive cells [28]. In this regard, the nature of chimera competency in primates may fundamentally differ from the rodent paradigm, such that currently defined ''naivete'' does not correspond to chimera-forming ability. Preliminary evidence suggests that ''intermediate'' types of PS cells, rather than naive-like types, apparently possess a higher capacity to chimerize embryos of other species [21,29]. Finally, the evolutionary distance between donor and host species may play a role [30]. The existence of rat–mouse chimeras suggests that the results obtained when introducing human PS cells into mouse and pig host embryos will differ if host embryos from more closely related species such as NHPs were used [21,22]. To develop effective strategies to lower species barriers, it may be instructive to study chimerism in early-stage human–monkey embryos cultured to post-implantation stages to identify barriers to human–nonprimate interspecies chimera formation [30].

In short, transformative advances in stem cell and chimera research necessitate revisiting the questions surrounding human–monkey chimeras.

Considering Human–Monkey Chimeras: Modeling Neurological and Psychiatric Disease

Despite the advantages offered by genetically engineered NHPs, current NHP models for neurodegenerative diseases are so limited as to require consideration of the benefits of human chimeras. Below, we describe certain deficiencies of different NHP models where production of NHPs with high-grade human chimerism could allow more faithful modeling

of human disease (Table 1). In Table 1, we have provided a side-by-side comparison of the differences between different approaches for modeling neurological and psychiatric diseases using NHPs, including a direct comparison of the pros and cons of transgenic versus interspecies chimeric approaches to disease modeling. A key limitation of the transgenic approach is that our understanding of the genetic roots of certain diseases remains primitive. For example, although some genes responsible for familial Alzheimer's disease (AD) are known, the genetic bases for the more common ''sporadic'' disease are poorly understood.

Indeed, among primates, humans appear to be the only species that manifest the complete clinical and pathological sequela of AD [31]. Currently, the NHP model that best approximates AD uses aged primates to study changes in the brain and behavior associated with AD. However, this model possesses deficiencies. One problem is that while aging primates undergo a cognitive decline, the hippocampus, a memory-associated region, is relatively spared in aged NHPs, despite manifesting severe neuronal loss in AD patients. Having an ApoE4 allele is correlated with earlier onset for AD [32]. One might also pose the possibility of generating a transgenic monkey carrying human ApoE4, a risk allele for AD, and a disease-causing mutation in amyloid precursor protein as a potential transgenic model for AD. While such a model will prove useful, it is important to note that the ApoE4 allele has a weak or no obvious effect on AD for individuals from certain ethnicities [33,34].

To address such insufficiencies, a monkey containing neural tissues derived from AD patients could yield insights into the apparently unique human susceptibility to AD. Furthermore, by applying a strategy called interspecies blastocyst complementation, one could potentially generate specifically targeted regions of chimeric brains that are entirely human derived [21,22]. In this method, donor PS cells of species A are injected into the embryos of species B that are organogenesis disabled. If disabling organogenesis is lethal for embryos of species B, the resulting chimera will possess the missing organ completely derived from species A. This strategy has been used to generate rat pancreas in mouse and vice-versa, and efforts to apply the same strategy to generate human organs in large animals are underway [21,22]. In theory, one could generate a human stem cell-derived hippocampus by injecting xenogeneic human PS cells into a monkey embryo that is hippocampus disabled. Such an experiment could prove useful for modeling neurological conditions with human-specific biological features. Indeed, the recent development of neural blastocyst complementation in mice suggests the feasibility of translating this approach to human-NHP chimeras [35].

Another example pertains to modeling of Parkinson's disease (PD). The best NHP model of PD uses 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to selectively kill dopaminergic neurons, the primary cell type lost in PD [36,37]. Nonetheless, while the MPTP model reproduces most PD

Table 1. Approaches for Modeling Neurological and Psychiatric Disease Using Nonhuman Primates

Model type	*Aging*	*Injury/toxin*	*Transgenic*	*Chimera*
Example	Parkinson's disease (PD) Alzheimer's disease (AD)	MPTP model of Parkinson's disease. 3-nitropropionic acid model of Huntington's disease. PCP model of schizophrenia.	Transgenic Huntington's disease model	Not available
Pros	Can serve as partial model for neurodegenerative disease	Can recapitulate most, but not all, disease hallmarks.	Study function of disease-associated genes and noncoding regions in genetically defined system. For monogenic disease, may mimic pathology more accurately than chemical lesions.	Study patient cells in human-like setting for modeling disease, drug screening. Modeling of polygenic diseases. Study human-specific features. Model inherited disease for which causative mutations are unknown.
Cons	Aging is not the same as neurodegenerative disease. Accordingly, differences from corresponding human neurodegenerative disease (eg, for modeling AD, cognitive decline with age, but neuronal loss lacks similarity with that observed in AD).	Does not recapitulate all disease hallmarks, particularly mechanism of cell death. Money-to-monkey variation. Does not replicate human condition as faithfully as genetic models.	Difficult to model polygenic disease, multiple risk alleles of small effect. Off-target effects of CRISPR/Cas9. Mosaicism from delayed and/or multiple cleavage events from Cas9 injection into early embryos. Cannot model inherited disease where causative mutation unknown.	Difficult to control chimerism. Likely monkey-to-monkey variation. Uncertain effects on animal welfare.

MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PCP, phencyclidine.

hallmarks, it also has limitations. A major limitation is that the MPTP-based mechanism of cell death differs from the mechanism that occurs in PD—it does not address why people develop PD. Using transgenic NHPs to model diseases with multiple genetic loci containing ''risk variants'' will be challenging. While there are multiple genes associated with a small percentage of patients with familial variants of PD, most are not. Attempting to model the nonfamilial cases in which multiple genetic loci, especially of small effect, cooperate to predispose to PD will be very difficult using transgenic primates. Therefore, human–monkey chimeras containing tissues derived from PD patients could offer unique advantages compared with both chemical lesion and transgenic-based approaches.

Finally, using transgenic NHPs to model psychiatric disorders whose genetic etiology is complex and poorly understood will be challenging [38]. It is noteworthy that NHP models for psychiatric conditions, such as bipolar disorder (BP) and schizophrenia, either do not exist or if existent, reveal little about disease pathophysiology. For example, while the phencyclidine model for schizophrenia models some of the memory deficits, despite a clear genetic loading, currently available models possess deficiencies [39]. In contrast, using human–monkey chimeras, one could study the function of patient cells carrying multiple genetic risk variants in a human-like setting. For example, in the case of BP, for which no NHP model exists and for which the genetic bases remain poorly understood, one could generate a human–monkey chimera with neural tissue derived from a patient with BP and study the brain and behavior of such an animal. Human–monkey chimeras may prove indispensable for modeling of polygenic diseases.

The creation of human–monkey chimeras raises some additional ethical questions. To those already opposed to monkey research, human–animal chimeras may pose no interesting new issues. However, some who accept existing monkey research may worry that a human–monkey chimera would be capable of enhanced suffering, or that it could, by meeting some yet-to-be-specified mental criterion, qualify for special status that would render further experimentation on it unethical. Whether chimerism in portions of the monkey brain would affect cognition or emotion is unknown. The issue is complicated by the fact that no human–monkey chimera can have any chance at life at all, except as a research subject; it may be that, if the chimera has a life that is not too burdensome, there may be fewer objections to it being created in the laboratory. Finally, the burden on chimeras of experimentation needs to be weighed against the possible benefits of any given line of research, alternative methods to achieve the same goals, and compared also with the continuing burden of disease, and indeed of experimentation, on humans.

A Cautious Path Forward

As new experimental methods for studying aspects of the human brain continue to develop, provocative questions will be raised [40]. Given advances in the stem cell field and chimera research, there exists a need to discuss the scientific merits and ethical concerns that accompany human–monkey chimera formation by scientists, ethicists, and the general public.

In the near term, we recommend that steps be taken as follows:

(i) Support transparent research to study the true nature of chimera-competent and xenogeneic pluripotency in humans and other primates, and to understand the xenogeneic barrier. A step-by-step approach would improve methodology and identify pitfalls. Before making human–monkey chimeras, it will be prudent to first pursue the generation of interspecies between NHP species. It will be instructive, for example, to introduce xenogeneic PS cells from great apes into monkey host embryos. It will also be useful to investigate the merits of complementary experimental strategies, such as introducing apoptosis-disabled PS cells into preimplantation embryos. Such experiments could inform how to control human PS cell derivative contributions to the chimeric monkey brain.

(ii) Closely monitor the welfare of all initial human–monkey chimeric models of neurological and psychiatric disease. The transfer of disease-specific human stem cells is likely to affect research animals in ways that compromise rather than enhance their normal capabilities and health [41]; thus, all human–monkey neurological chimera research should be independently reviewed and monitored to ensure compliance with appropriate animal welfare standards.

(iii) Require independent review. A determination must be made that the necessary minimal number of monkeys will be used to answer a meritorious research question for which there exists no reasonable alternative approach.

(iv) Harmonize differing chimera research guidelines issued by the U.S. National Academy of Sciences (NAS) and the International Society for Stem Cell Research (ISSCR). Although the ISSCR guidelines permit NHP blastocyst complementation experiments pending stem cell ethics review, the NAS guidelines do not.

(v) Learn from the example of mitochondrial replacement therapy and human germ line editing. Funding agencies should create forums in which experts from the scientific and bioethics communities can deliberate along with the public about risks and rewards of using such technology. It will be essential to engage the public in addressing the implications of human–monkey chimera formation.

In summary, recent advances in generating chimera-competent PS cells and chimera research raise the prospect of human–monkey chimeras, presenting new possibilities for biomedical and translational research as well as difficult policy challenges. The existence of biologically and clinically relevant differences between human and primates may justify the use of human–primate chimeras to more accurately model human disease. These conditions include but are not limited to AD and psychiatric disorders such as BP and schizophrenia. However, animal welfare considerations call for caution and clear reasoning regarding scientific necessity. Data from these experiments may possibly lead to new ethical arguments against advancing to more complete chimeric experiments. Realizing the promise of human–monkey chimera research in an ethically and scientifically appropriate manner will require a coordinated approach. The field of human–monkey chimera research will need the support of governments, research institutions, and private foundations. If successful, the development of human–monkey

chimera technology may expand the breadth of chimera research from the laboratory toward potential clinical benefits for patients with serious neurological and psychiatric disorders.

Author Disclosure Statement

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