Progress and Prospects for Genetic Modification of Nonhuman Primate Models in Biomedical Research

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Abstract

The growing interest of modeling human diseases using genetically modified (transgenic) nonhuman primates (NHPs) is a direct result of NHPs (rhesus macaque, etc.) close relation to humans. NHPs share similar developmental paths with humans in their anatomy, physiology, genetics, and neural functions; and in their cognition, emotion, and social behavior. The NHP model within biomedical research has played an important role in the development of vaccines, assisted reproductive technologies, and new therapies for many diseases. Biomedical research has not been the primary role of NHPs. They have mainly been used for safety evaluation and pharmacokinetics studies, rather than determining therapeutic efficacy. The development of the first transgenic rhesus macaque (2001) revolutionized the role of NHP models in biomedicine. Development of the transgenic NHP model of Huntington's disease (2008), with distinctive clinical features, further suggested the uniqueness of the model system; and the potential role of the NHP model for human genetic disorders. Modeling human genetic diseases using NHPs will continue to thrive because of the latest advances in molecular, genetic, and embryo technologies. NHPs rising role in biomedical research, specifically pre-clinical studies, is foreseeable. The path toward the development of transgenic NHPs and the prospect of transgenic NHPs in their new role in future biomedicine needs to be reviewed. This article will focus on the advancement of transgenic NHPs in the past decade, including transgenic technologies and disease modeling. It will outline new technologies that may have significant impact in future NHP modeling and will conclude with a discussion of the future prospects of the transgenic NHP model.

Key Words: animal model; human diseases; nonhuman primates; transgenesis

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Introduction

The search for a better animal model to capture human physiological and disease conditions has been the goal of biomedical research for decades. This is the key to achieving medical advancement that ultimately benefits patients. Although model systems such as drosophila, *C. elegans* and zebrafish have played important roles in biomedicine, such as the discovery of the regulatory role of noncoding RNA in healthy and disease conditions (Ambros, 2003; Ambros, 2008), this review will focus on the mammalian model systems, specifically nonhuman primates (NHPs). While there are numerous model systems available for researchers, mammalian models (rodents, etc.) remain in the mainstream and are currently the most favored species to advance our knowledge in basic biology, physiology, human diseases, and the development of novel therapeutics (Ambros 2003; Gilley et al. 2011; Golding et al. 2006; Grossniklaus et al. 2010; Hauschild et al. 2011; Hitz et al. 2009; Marsh et al. 2003; Stieger et al. 2009; Tessanne et al. 2012; Tyska et al 2000; von Horsten et al. 2003; Yang et al. 2008a; Zeiss 2010; Zschemisch et al. 2012). The development of genetic and molecular techniques such as pronuclear microinjection (Hogan 1994), embryonic stem cell (Evans 1996; Evans 2008; Nichols et al. 1990; Thomson et al. 1998; Thomson and Marshall 1998), and gene targeting (Joyner et al. 1989; Koller and Smithies 1989; Zijlstra et al. 1989) in the 1980s revolutionized the platform for conducting biomedical research. This led to a new era in animal modeling, specifically in rodents, that allowed the dissection of genetic components, gene functions, and regulatory networks in healthy and diseased conditions (Chan et al. 2001; Dawson et al. 2008; Golding et al. 2006; Jinnah et al. 1990; Kang and Grossniklaus 2011; Melo et al. 2007; Rubinsztein 2002; Sasaki et al. 2009; Sommer et al. 2012; Sun et al. 2008; Tessanne et al. 2012; Vaitukaitis 1998; Yang et al. 2008a). Since the first transgenic mice were created in the 1980s, thousands of genetically modified mice have been created. Transgenic mice that carry genetic defects known to cause human diseases have been the prime interest. In general, transgenic rodents or animals can be categorized by expression patterns, which include: Overexpression, Gene targeting: (knock-in, knock-out, and knock-down), Conditional expression, and Inducible expression. Although an

expression pattern could be manipulated by strategies such as the selection of promoters, tetracycline inducible system, Cre-lox conditional expression system, and artificial chromosome (AC), the transgene has to be inserted into a genome in order to achieve long term expressions that can be passed to the next generation through the germ cells. Although most of the currently available genetic engineering techniques have been successfully used for the generation of genetically modified rodents, the rodent model does not always recapitulate human conditions (Elsea and Lucas 2002; Gilley et al. 2011; Rice 2012). Due to physiologic differences between rodents and higher primates, such as life span (Gilley et al. 2011), brain size and complexity (Chen et al. 2000; Gilley et al. 2011; Hsiao et al. 1996; Polymeropoulos et al. 1997; Yang et al. 2008a) and motor repertoire (Courtine et al. 2007; Rice 2012), as well as the availability of cognitive behavioral testing (Bachevalier et al. 2011; Bachevalier et al. 2001; Bachevalier and Nemanic 2008; Ewing-Cobbs et al. 2012), NHPs are considered one of the best animal models; especially for complex disorders that correlate with aging, cognitive behavioral function, mental development, and psychiatric dysfunctions. In addition to neural psychiatric related disorders, metabolic function (Hogstedt et al. 1990; O'Sullivan et al. 2012; Smith et al. 1978), reproductive physiology (Hewitson et al. 2002; Kundu et al. 2013; Obaldia et al. 2011; Wolf 2009), and immunology (Gallo et al. 1989; Thomas et al. 1982) are other areas of research where the NHP model has been widely used.

Although the application of NHPs in biomedicine has a decades long history, NHPs have been primarily used in pharmacokinetic (Glogowski et al. 2012; Kanazawa et al. 1990; Kao et al. 2006; King and Dedrick 1979; Liu et al. 2009; Lutz et al. 1984) and toxicity studies (Jarvis et al. 2010; Lee et al. 1994), specifically in drug development, physiological response, and efficacy studies of new treatments. Studies on the understanding of normal physiological functions (Gallo et al. 1989; Kundu et al. 2013; O'Sullivan et al. 2012; Wolf 2009) and chemical induced conditions mimicking human disease conditions (Blesa et al. 2012; DeLong and Coyle 1979; Vezoli et al. 2011) are also areas of research in which the NHP model plays a key role. The creation of the first transgenic NHP in 2001 (Chan et al. 2001) and subsequent development of the transgenic NHP model of Huntington's disease (HD) in 2008 (Yang et al. 2008a) revolutionized the traditional role of NHPs in biomedicine to the new frontier of modeling human genetic disorders. The interest in NHP models has increased in the past decade because of reports on the new creation of transgenic NHPs, application of transgenic technology in different primate species (marmoset, cynomolgus monkey, and more) (Niu et al. 2010; Sasaki et al. 2009; Sun et al. 2008), and the establishment of new primate research centers and consortiums in different countries. Nonetheless, the hope is to recapitulate human disease conditions through NHPs, not only physiologically, but also genetically by the creation of transgenic NHPs using the latest genetic engineering tools; advancing the understanding of disease pathogenesis and finding new therapeutics for human diseases.

Unlike NHPs, rodents, or other model systems such as *C-elegans* or flies, are often used in the frontline of technology development, and as a concept model for new technology and drug development. In theory, genetic engineering techniques established in rodents can be translated into NHPs. However, ethical concerns such as the use of higher primates in research, limitations on the supply of NHPs, and their high cost are often the factors for consideration in the development of a genetically modified NHP model. To overcome these barriers, highly efficient methods are necessary for creating transgenic NHPs, because the number of animals required in the process is a major limiting factor and engenders ethical concerns. For example, it took two decades to create transgenic NHPs after the first transgenic mouse was created, simply because a traditional pronuclear microinjection approach is not as efficient (0.5-4% in livestock such as pig and 20-25% in mice) when compared to retroviral or lentiviral vectors (90%-100%) (Chan et al. 2001; 2002; Niu et al. 2010; Niemann and Kues 2003; Sasaki et al. 2009; Sun et al. 2008; Yang et al. 2008a). The development of a more efficient and safer lentiviral vector system created a transgenic HD monkey in 2008, and was followed by the creation of transgenic marmosets and pigtail macaques that expressed a green fluorescent protein (GFP) gene as a proof of principle (Niu et al. 2010; Sasaki et al. 2009; Yang et al. 2008a).

The advancement of the transgenic NHP model has been driven by the availability of high efficiency genetic tools. Although only a handful of transgenic monkeys have been reported, the application of the transgenic NHP model in biomedical research is just beginning. The commitment of the National Institutes of Health to the National Primate Research Centers in the United States, the recent development of the Japanese consortium of a marmoset model for neuroscience research, and the aggressive development of primate research in China and other nations shows the importance of the transgenic NHP model. The transgenic NHP model is expected to become an important model system, specifically in translational and preclinical studies as well as basic research.

Key Technologies Leading to Successful Genetic Manipulation in NHPs, and the Latest Technologies That May Impact Future Development in Genetically Modified NHP Models

Most transgenic NHPs, including germline (Chan et al. 2001; Niu et al. 2010; Sasaki et al. 2009; Sun et al. 2008; Yang et al. 2008a) or focal transgenesis (Kordower et al. 2000; Mittoux et al. 2000; Palfi et al. 2007), are primarily generated by viral vectors and overexpression of the gene of interest. Due to the limitation of the genetic engineering tools that are currently available, transgenic NHP modeling

has focused on dominant genetic diseases such as HD (Chan et al. 2001; Yang et al. 2008a), while others working with transgenic NHPs reported only the over expression of GFP (Niu et al. 2010; Sasaki et al. 2009; Sun et al. 2008). Gene targeting technology has been successfully used for developing a functional knock-down and functional knock-out rodent models of human diseases. This process uses homologous recombination in embryonic stem cells (ESCs), followed by blastocyst injection to create chimeric rodents (Bertelli et al. 2009; Lin et al. 2001; Woodman et al. 2007), or by nuclear transplantation to create cloned rodents (Rideout et al. 2002; Wakayama 2007; Wakayama and Yanagimachi 2001). A similar technique has not been reported in the creation of transgenic NHPs.

Since the report on the creation of Dolly (Wilmut et al. 1997), the first mammalian species cloned by somatic cell nuclear transplantation (SCNT) in 1997, the scientific community has embraced the exciting era of animal cloning. Ideally, somatic cells such as skin fibroblasts can be genetically modified, selected, characterized, and followed by SCNT. In theory a colony of cloned animals with identical genetic backgrounds, and perhaps phenotypes, could be established (Hauschild-Quintern et al. 2013; Hauschild et al. 2011; Schnieke et al. 1997; Yang et al. 2007) for the production of valuable pharmaceutical products. A colony could also be used for the creation of animal models that recapitulate human disease conditions that are used for studying disease pathogenesis and developing novel therapeutics. Although SCNT has been relatively successful in rodents such as mice (Rideout et al. 2002; Wakayama 2007) and livestock such as pig, cattle, and sheep (Hauschild et al. 2011; Meissner and Jaenisch 2006; Tessanne et al. 2012; Wilmut et al. 1997), a SCNT cloned NHP has not been achieved (Mitalipov and Wolf 2006; Simerly and Navara 2003). Tremendous amounts of effort and resources have been invested in the development of SCNT in NHPs; discouraging results have hampered the interest in creating cloned NHPs. While the development of SCNT continues in a less aggressive manner, the search for alternative approaches in creating functional knock-down or functional knock-out NHPs by novel gene targeting technologies has increased. Gene targeting in somatic cells followed by SCNT is an ideal approach for creating a NHP model of recessive genetic diseases or dominant negative genetic diseases. New genetic tools such as small hairpin RNA (shRNA), zinc finger nuclease (ZFNs), and Transcription Activator-Like Effector Nucleases (TALENs) have opened a new era of genetic engineering, and added new strategies for creating transgenic animals. Gene targeted transgenic rodents and livestock have been successfully created by these methods (Carbery et al. 2010; Golding et al. 2006; Hauschild-Quintern et al. 2013; Hauschild et al 2011; Tessanne et al. 2012; Whyte and Prather 2012; Zschemisch et al. 2012). Translation of these technologies into NHPs is foreseeable in the future, while optimization in terms of targeting efficiency is inevitable before the production of transgenic NHPs.

Several approaches have been attempted for the creation of transgenic NHPs. Among these methods, transgenesis, mediated by viral vectors, specifically lentiviral vectors, has been the most successful method (Chan et al. 2001; Niu et al. 2010; Sasaki et al. 2009; Yang et al. 2008a). Transgenesis by perivitellie space delivery of viral vector into oocytes, or early preimplantation embryos (zygotes or two to four cell embryos), was first reported in the creation of transgenic cattle at an unprecedented efficiency of close to 100% (Chan et al. 1998). Today, all reported transgenic NHPs were created by lentiviral vector mediated gene transfer in oocytes, or early preimplantation embryos, except the first transgenic NHP, *ANDi*, who was created by using a retroviral vector (Chan et al. 2001). Lentiviral vector is one of the most effective methods for gene transfer in mammalian cells, including neural cells that are generally difficult to achieve by other gene transfer methods (Kordower et al. 1999; Naldini 1998; Pfeifer 2004; Yang et al. 2008b; Zufferey et al. 1998). Pseudotyped lentiviral vector is capable of infecting all cell types with a lipid membrane, unlike retroviral vector that primarily targets actively dividing cells (Naldini 1998; Naldini et al. 1996; Pandya et al. 2001). Various species of transgenic NHPs have been generated by lentiviral vector, including rhesus macaques, cynomolgus monkeys, and marmosets (Chan et al. 2001; Niu et al. 2010; Sasaki et al. 2009; Yang et al. 2008a). It seems that lentiviral mediated gene transfer will continue as the main delivering vehicle for creating transgenic NHPs, while incorporation of new technologies such as shRNA, ZFN, and TALENs are foreseeable.

In addition to lentiviral mediated gene transfer, sperm mediated gene transfer (SMGT) developed in the 1980s has been attempted to create transgenic NHPs (Chan et al. 2000a; Chan et al. 2000b; Chan et al. 2000c). Instead of co-incubation of sperm with naked DNA, followed by artificial insemination in domestic species (Lavitrano et al. 1989; Lavitrano et al. 2013), NHP sperm were incubated with plasmid DNA, followed by intracytoplasmic sperm injection (ICSI) (Chan et al. 2000a; Chan et al. 2000c). Although this approach did not result in transgenic NHPs, a similar approach has been successfully used for creating transgenic mice (Perry et al 1999). In theory, SMGT is a perfect approach for creating transgenic animals using simple fertilization steps with genetically modified spermatozoa. However, the inconsistent gene transfer rate (5-60%) shown in the production of transgenic pigs suggests (Lavitrano et al. 2006) that further investigation is needed for optimizing the procedures for NHPs. In fact, the latest development of induced pluripotent stem cell (iPSC) technologies, and the subsequent derivation of germ cells such as spermatids (Easley et al. 2012), may open a new door for SMGT. Mature spermatozoa have been generated using grafted spermatogonia (Hermann et al. 2012). By combining different technologies as described, primary cultures such as skin fibroblasts can be genetically modified followed by in vitro differentiation to spermatids, testicular transplantation, recovering transgenic spermatozoa for ICSI, followed by embryo transfer. Transgenic spermatids can be used for ICSI directly followed by embryo transfer.

The generation of chimeric embryos made by blastocyst injection of genetically modified ESCs is commonly used

for creating gene-targeted rodents (Xia et al. 2006; Zimmer and Gruss 1989). The first challenge in gene targeted-rodents is to determine successful germline transmission, followed by an extensive breeding program to establish a line of transgenic mice with a stable and defined genotype. In theory, a similar approach can be achieved in NHPs, but this theory is not without concerns and challenges. It takes three to four years to reach pubertal age, and five to six months of gestation time in rhesus macaque (Chan et al. 2001; Chan 2004; Chan et al. 2002; Yang et al. 2008a). In addition, the rhesus macaque is a seasonal breeder, reproductively active in spring and winter. The rhesus macaque normally carries a singleton. These facts further hinder the breeding process. In this case, New World monkeys like the marmoset may be a better choice because of their short pubertal age and non-seasonal breeding cycle (Okano et al. 2012; Sasaki et al. 2009). Tachibana and colleagues reported successful production in regards to creating chimeric monkeys by aggregation of early embryo (Tachibana et al. 2012) and support the notion that the generation of transgenic chimeric monkeys is a possible approach. Unlike rodents, NHP ESCs injected into blastocysts cannot integrate into the inner cell mass and develop chimeric embryos (Tachibana et al. 2012). Nonetheless, chimeric animals, including rodents and livestock, have been generated for decades; mice are the only species that adapted to the technology successfully with significant impact on the field. Therefore, chimeric technology in NHP is interesting, but its application in developing a transgenic NHP model may not be an ideal approach. The level of chimerism varies among individuals and requires extensive characterization steps and breeding.

SCNT, one of the most anticipated approaches for creating transgenic animals, has great promise for creating identical transgenic NHPs for modeling human genetic diseases and the development of novel therapeutics. A cohort of genetically identical animals, (with similar if not identical clinical phenotypes) with a genetic defect linked to human disease, is an ideal model for drug development. Because of the negative reports in creating cloned NHPs in the past two decades (Mitalipov and Wolf 2006; Simerly et al. 2003; Simerly and Navara, 2003), the progress in SCNT has been significantly hampered. The rise of new technologies has further reduced the interest and effort in developing SCNT in NHPs. Although reports on deriving ESCs from SCNT embryos has drawn considerable attention to the fact that personal stem cells can be generated, there are ethical concerns in using an oocyte as the host remains (Byrne et al 2007). The development of iPSC technology has once again shifted the research direction in personal medicine away from SCNT (Takahashi et al. 2007; Takahashi and Yamanaka 2006). Questions on whether iPSCs are different from ESCs (Hyun et al. 2007), and if the origin of iPSCs influences clinical outcome (Fairchild 2010; Kadereit and Trounson 2011; Kim et al. 2010; Suarez-Alvarez et al. 2010), remains the focus of research. Personal stem cells derived by reprogramming of a patient's own cells is the best cell source for cell replacement therapy (Blin et al. 2010; Hwang et al. 2010; Lunn et al. 2011; Perrier and Peschanski 2012; Tucker et al. 2011). It seems that the interest in SCNT derived personal stem cells will continue to fade, but the importance of developing an identical or cloned transgenic NHP model for human diseases remains strong. From an animal model perspective, specifically in drug discovery research, a cohort of animals with identical genetic background and perhaps similar, if not identical, clinical phenotypes would be a unique resource for precise and accurate determination of therapeutic efficacy of novel treatments: without or with minimal influence of genetic variation. Nevertheless, the failure of a traditional SCNT method in NHP suggests the fundamental differences between species, and that a new strategy is necessary to overcome the barrier.

While the development of a transgenic NHP model has been focused on modeling disease progression, pathogenesis, and its potential preclinical application, a pluripotent stem cell model provides a unique in vitro platform for drug discovery research and the development of gene and cell based therapy (Maury et al. 2012; Saha and Jaenisch 2009; Tiscornia et al. 2011). Therapeutic efficacy can be determined in transgenic NHPs such as HD-NHP (Carter and Chan 2012; Chan et al. 2010; Yang and Chan 2011; Yang et al. 2008a). The latest development of iPS technology has not only led to new hope in personal medicine, but has stimulated the development of personal cell based therapy (An et al. 2012; Carter and Chan 2012; Chan et al. 2010; Consortium 2012; Marchetto et al. 2011; Tucker et al. 2011). iPSCs derived from human patients develop cellular phenotypes relevant to diseases (An et al. 2012; Consortium 2012; Cooper et al. 2010; Livesey 2012; Maury et al. 2012; Sanchez-Danes et al. 2012; Tiscornia et al. 2011; Young and Goldstein 2012), and the rise of interest in cell based therapy continues (Abdel-Salam 2011; An et al. 2012; Cooper et al. 2012; Hwang et al. 2010; Marchetto et al. 2011). The successful genetic correction of HD phenotypes in iPSCs further suggests the potential of gene and cell based therapy for genetic disorders such as HD (An et al. 2012). As potential gene and cell based therapy for treatment of human diseases evolves, there is an urgent need for a preclinical animal model to validate these findings and evaluate their long-term safety and efficacy (Carter and Chan 2012; Perrier and Peschanski 2012). The combination of a transgenic NHP model and iPS technology may create a novel preclinical model system for developing personal medicine in higher primates, and could lead to new insights into translational medicine that may facilitate and accelerate clinical application in human patients (Carter and Chan 2012; Perrier and Peschanski 2012).

The success in creating a transgenic HD NHP proved the principle of modeling human inherited genetic disease using NHPs (Putkhao et al. 2013; Yang et al. 2008a). The search for more effective methods to create functional knock-down and functional knock-out NHP models for dominant negative and recessive genetic disorders is increasing, due to the limitations of currently available animal models. While SCNT is an ideal method for creating a gene targeted animal

model, as described in the previous section, disappointing results in the generation of SCNT NHPs has driven the search for an alternative approach. In the new era of genetic engineering, novel technologies have evolved including: gene silencing by small interference RNA (siRNA) (Hitz et al. 2009; Raymond et al. 2010; Seibler et al. 2007; Seibler et al. 2005; Van Pham et al. 2012; Xia et al. 2006), gene targeting by ZFNs (Carbery et al. 2010; Ellis et al. 2012; Kobayashi et al. 2012; Passananti et al. 2010; Strange and Petolino 2012), and TALENs (Cermak et al. 2011; Liu et al. 2012; Mahfouz et al. 2011; Sung et al. 2012). These novel genetic engineering tools open new opportunities in NHP modeling of human genetic diseases, not only in dominant genetic disorders, but also recessive and dominant negative genetic diseases. A NHP model of genetic disorders caused by the loss of gene function or haploinsufficiency was practically impossible until the recent development of small hairpin RNA (shRNA), ZFN, and TALENs. Functional knock-down (partial loss of function or haploinefficiency) can be achieved by silencing of the target gene of interest by shRNA. In gene silencing by shRNA, the targeted gene is functionally competent with a reduced functional transcript, depending on the efficacy of shRNA-mediated degradation. Stable integration of shRNA into the genome for constitutive expression, or in an inducible manner is necessary. Targeted disruption of a specific gene will lead to functional knock-down or haploinefficiency. If one allele is disrupted while functional knock-out results, or if both alleles are disrupted by ZFN or TALENs. Unlike shRNA, ZFN creates a permanent gene disruption, the challenge is to efficiently achieve a gene-targeting event at an early embryonic stage to avoid mosaicism that could affect functional knock-down, or functional knock-out efficacy in subsequent generations. Although these methods have shown great success in reducing functional protein in vitro and in transgenic rodents as well as livestock (Carbery et al. 2010; Hauschild-Quintern et al. 2013; Tessanne et al. 2012; Whyte and Prather 2012; Zschemisch et al. 2012), the application in NHPs remains an exploratory area with great prospects, but not without challenges. Similar to other techniques described previously, the major obstacle in translation into NHP is to achieve high delivery and targeting efficiency so a gene targeted NHP model of human disease can be generated in a cost-effective manner with a minimal a number of animals, reducing ethical concerns.

Gene silencing by shRNA is no different than a traditional transgenic approach of overexpressing shRNA specifically targeted to the gene of interest. Lentiviral mediated transgenesis is a good choice for creating transgenic NHPs expressing gene specific shRNA. Its silencing efficiency relies on rigorous selection of shRNA in vitro, prior to the creation of gene targeted NHPs. In case of gene disruption by ZFN and TALENs, ZFN and TALEN pairs will bind to the targeted DNA sequence in early embryos and induce double-stranded break and repair, creating deletion or rearrangement of the targeted DNA sequence. Today, gene targeted rats and mice have been created using pronuclear or cytoplasmic microinjection of ZFN or TALEN mRNA (Sung et al. 2013; Zschemisch et al. 2012), or by direct injection of the ZFN proteins (Gaj et al. 2012). In addition to mRNA injection, gene targeting in embryonic stem cells, or skin fibroblasts by overexpression of the ZFN or TALEN pairs followed by blastocyst injection or SCNT, have been successfully used for the creation of gene targeted pigs and mice (Hauschild-Quintern et al. 2013; Hauschild et al. 2011). Gene targeted large animals created by microinjection of ZFN and TALEN mRNA have not been reported. Although high targeting efficiency by using ZFN and TALENs followed by SCNT has shown to be an effective method for the creation of functional knock-out pigs (Hauschild-Quintern et al. 2013; Hauschild et al. 2011; Whyte and Prather 2012), a similar approach does not translate into NHPs because SCNT remains the major road block. Once again the same challenge of efficiency arises. One possible approach is overexpressing the ZFN and TALEN using a traditional delivery and expression approach such as lentiviral vector. Additionally, adeno associated virus (AAV) mediated ZFN pairs expressions have also been suggested as a potential strategy for gene therapy (Ellis et al. 2012; Lombardo et al. 2007; Rahman et al. 2013).

Genetically Modified NHP Model of Human Inherited Genetic Diseases: Pros and Cons of Modeling Human Disease with Transgenic NHP

Although a tremendous amount of global effort has been channeled into the development of transgenic NHP research, the concept of transgenic NHP modeling of human inherited genetic diseases is still at the infancy stage. With the support of the United States National Institutes of Health, pioneering research has led to the development of the first transgenic NHP, "*ANDi*", followed by the first report of a transgenic NHP model of HD (Yang et al. 2008a). Together with the fast development of a transgenic marmoset model in Japan (Okano et al. 2012; Sasaki et al. 2009) and the increase of primate research infrastructures in China (Niu et al. 2010; Sun et al. 2008), the popularity of the transgenic NHP model is quickly increasing, and its impact on future advancement of biomedicine is expected.

A transgenic HD monkey model, the first and only reported transgenic NHP model of human disease, is a good example to present the pros and cons of modeling human disease with transgenic NHP. The points for consideration when developing transgenic NHP human disease model will also be discussed.

Huntington's disease is an autosomal dominant inherited neurodegenerative genetic disease that is caused by the expansion of the polyglutamine (polyQ; CAG) repeats in exon1 of the Huntingtin gene IT15 (*HTT*) gene (Group 1993). CAG repeat lengths over thirty-nine results in pathological HD. A negative correlation has been shown between repeat length and age of onset and lifespan (Lee et al. 2012;

Roos 2010; Ross and Shoulson 2009; Walker 2007). Patients with the longer CAG repeat lengths exhibit very severe symptoms of HD starting in adolescence. HD is categorized into juvenile and adult forms, based on CAG repeat lengths that result in a distinct course of clinical manifestations (Andrich et al. 2007; Geevasinga et al. 2006; Rasmussen et al. 2000; Ribai et al. 2007; Roos 2010; Ross and Shoulson 2009; Ruocco et al. 2006). HD is a devastating neurological disorder that progressively impacts motor, cognitive, and psychiatric functions as the patient ages (Crook and Housman 2011; Ho et al. 2003; Paulsen et al. 2006; Roos 2010; Ross and Shoulson 2009). Although a genetic test is available, treatments are currently limited to symptomatic management of the patients' symptoms.

It is well accepted that NHPs are one of the best model systems for neuroscience research and modeling human neurological disorder (Okano et al. 2012; Yang and Chan 2011; Yang et al. 2008a). In the case of HD, the progressive impact on motor functions: such as chorea, dystonia, and fine motor control can be evaluated in NHPs with a battery of tests that are well established for NHPs (Bachevalier et al. 2011; Bachevalier et al. 2001; Bachevalier and Nemanic 2008; Ewing-Cobbs et al. 2012). Additionally, the Unified Huntington's Disease Rating Scale (UHDRS) (Group 1996) is one of the standard clinical assessments used to evaluate the presence and severity of motor symptoms, and the psychiatric dysfunctions that accompany the disease. The Huntington's Disease Primate Model Rating Scale (HDPMRS) is a modified version of the UHDRS that was developed for HD monkeys that primarily focuses on motor deficits (Yang et al. 2008a) as an indicator for monitoring the progression of HD. Although an HD rodent model develops motor impairment such as dystonia and abnormal gait, differences in motor repertoire and anatomical features in rodents have limited the assessment of fine movement ability. Due to the difference in the organization of the motor systems and behavior among rodents (Elsea and Lucas 2002; Gilley et al. 2011; Rice 2012), NHPs, and humans, the translation of potential interventions using a rodent model may not be sufficient for accurate evaluation (Elsea and Lucas 2002; Gilley et al. 2011; Rice 2012). Furthermore, differences in neuro-anatomy and the development of corticospinal tracts have significant impact on the development of fine motor abilities (Courtine et al. 2007). New World monkeys such as squirrels and marmosets can be easily trained to use their hands, because of the projection patterns of their corticospinal tracts, and because their nonprimary motor cortical areas are similar to humans. Old World monkeys, like the rhesus macaque, are considered better suited for evaluating fine motor skills compared to New World monkeys (Courtine et al. 2007; Lemon et al. 2004; Shimazu et al. 2004). In cases of HD, as the disease progresses, locomotion and fine motor abilities become significantly impaired. In order to capture progressive fine movement deficits during the course of HD, as well as evaluate therapeutic efficacy of potential intervention, a NHP model of HD is critical for accurate assessment when using the battery of sophisticated tests that are available for NHP. Using New World monkeys has the following advantages: smaller size, shorter pubertal age, non-seasoning breeding cycle, and a higher twin pregnancies rate that facilitates a faster generation time of genetically modified monkeys and downstream breeding process (Okano et al. 2012; Sasaki et al. 2009). Old World monkeys, such as rhesus macaques, have been the major primate model in biomedical research because of the well established knowledge about their physiological and disease conditions, and the tools (cognitive behavioral tests) that are available for comparative studies with humans (Bachevalier et al. 2001; 2011; Chan 2004; Courtine et al. 2007; Han et al. 2009; Kanazawa et al. 1990; Kordower et al. 2000).

While motor impairment is one of the earliest clinical signs for diagnosis, cognitive decline, and psychiatric disturbances often precede the onset of motor dysfunction (Ho et al. 2003; Paulsen et al. 2008; Peavy et al. 2010; Stout et al. 2011; Tabrizi et al. 2012; Tabrizi et al. 2011; Vaccarino et al. 2011). A battery of tests to measure the development of emotional responses, social interactions, cognitive, and motor skills are well established in NHPs (Bachevalier et al. 2011; Bachevalier et al. 2001; Bachevalier and Nemanic 2008; Ewing-Cobbs et al. 2012) and have been adapted in HD-monkeys to assess neural developmental milestones and their correlation with HD progression. In addition to cognitive behavioral assessment, one of the early clinical features of HD is progressive brain atrophy (Paulsen et al. 2006; Paulsen et al. 2010). Non-invasive MRIs provide an optimal quantitative tool for determining anatomical and functional changes that may associate with disease progression. In addition to structural changes, disruptions of white matter integrity and connectivity (Sritharan et al. 2010; Vandenberghe et al. 2009; Weaver et al. 2009) have also been reported in HD as the disease progresses. Behavioral manifestations in HD patients are often the first evidence of underlying neuropathologic developments; the combination of neurobehavioral assessment and correlation with MRI imaging is critical for establishing a full picture and timeline in regards to brain development and changes. Correlation with other longitudinal measurements including molecular profiles can be constructed throughout the course of the disease.

In addition to clinical assessments, molecular profiling studies using peripheral blood, cerebral spinal fluid, and post-mortem brain tissues are also areas of strong interest in the search for potential biomarkers and therapeutic targets (Borovecki et al. 2005; Cha 2007; Runne et al. 2007; Tabchy and Housman 2006). Recent studies have found abnormalities in lymphocytes, which include an elevated level of oxidative DNA damage (Morocz et al. 2002) and an increased number of apoptotic monocytes (Bergman et al. 2002). The alteration of the gene expression pattern specifically observed in lymphocytes may be used as diagnostic information complementary to clinical evaluation and as a biomarker indicating the progression of the disease. The HD monkey model is a powerful platform for the longitudinal monitoring of genome-wide expression profiles, including noncoding RNAs (microRNAs) and metabolomic profiling,

used to advance our knowledge of HD molecular pathogenic cascades. While similar longitudinal studies are ongoing through two major programs, TRACK-HD (Tabrizi et al. 2012) and PREDICT-HD (Paulsen et al. 2008), information gained from longitudinal studies that parallel human studies in HD monkeys are important for future preclinical application of HD monkeys when monitoring disease progression, and determining therapeutic efficacy of new therapeutics.

Similar to other neurological disorders such as Alzheimer's and Parkinson's disease, HD is a complex systemic disorder that progresses as an individual ages. While most of the prior clinical studies are cross-sectional studies using a cohort of selected pre-symptomatic (prodromal) or symptomatic patients for comparison studies, the importance of longitudinal studies has increased (Paulsen et al. 2008; Tabrizi et al. 2012; Tabrizi et al. 2011). A Discrepancy with the overestimation of the sensitivity of measurements in cross-sectional studies also suggests the importance of unbiased longitudinal studies for precise interpretation of the results, and for determining possible clinical applications (Hobbs et al. 2010a; Hobbs et al. 2010b; Solomon et al. 2008; Tabrizi et al. 2012; Tabrizi et al. 2011). Longitudinal studies on a cohort of prodromal, pre-symptomatic patients, or animal models using a variety of clinical assessments such as battery of cognitive behavioral tests, MR imaging, molecular profiling of accessible tissues, like peripheral blood and CSF throughout the course of HD development will be critical for accurate assessment of disease progression and the establishment of a timeline of disease milestones based on clinical assessments and molecular markers. One of the most important bottlenecks in preclinical studies is the lack of animal models that recapitulate human disease conditions with precise and well defined clinical progression referenced by multiple clinically relevant assessments for determining therapeutic efficacy. The fast development in gene and cell therapy such as those ongoing developments in HD will benefit greatly with the use of transgenic HD monkeys for promoting clinical translation.

Future Developments and Prospects in Genetically Modified NHP Models in Biomedicine

The advancement of a transgenic NHP model aligns with the increasing interest in translational medicine indicated by the establishment of the National Center for Advancing Translational Sciences (NCATS). The importance of an animal model that allows for not only evaluating pharmacokinetic and toxicity, but also determining the efficacy of novel therapeutic approaches, breaks the bottleneck for the translation from bench to bedside. Besides the criteria for selecting an appropriate model system that could maximize the outcome, awareness of the importance of longitudinal study has increased in the biomedical field, both in research and clinical studies (Bateman et al. 2012; Morris et al. 2012; Paulsen et al. 2008; Tabrizi et al. 2012). While there is no perfect animal model; drosophila, zebrafish, C-elegans, rodents, pigs, and NHPs are all unique model systems that can address unique questions that can help with advancing biomedicine. It is important to match specific research questions with the best, and most appropriate, animal model.

This review has focused on the latest advancement in the NHP model, specifically a transgenic NHP model of human diseases. Along with the advancement of the latest molecular tools and new concepts for therapeutics such as small molecules, targeted gene knock-down, and the development of iPSCs for potential personal cell therapy; the need for an animal model that recapitulates human disease conditions, not only physiologically, but also genetically, is more important than ever for assessing therapeutic efficacy. With the increased awareness of the importance of longitudinal studies as demonstrated by the latest report on longitudinal studies in HD (Paulsen et al. 2008; Tabrizi et al. 2009; Tabrizi et al. 2012; Tabrizi et al. 2011), animal models such as NHPs that simultaneously allow longitudinal clinical assessment using multiple clinical measurements are a crucial component in translational medicine. The role of a transgenic NHP model of human diseases in preclinical study is expected to increase in the near future. The advancement in molecular tools such as ZFN and TALENs will open new doors for modeling a wide-spectrum of human genetic diseases without limitation to dominant genetic disorders.

In addition to the foreseeable impact of a transgenic NHP model in preclinical study, increased interest in basic research is also expected. NHPs are an important model species for understanding neural computation, cognition, and behavior. NHPs can also be used for probing the circuit-level basis of human neurological and psychiatric disorders, due to the greater similarity between the NHP brain and the human brain. (Elsea and Lucas 2002; Gilley et al. 2011; Okano et al. 2012; Rice 2012; Sasaki et al. 2009; Yang et al. 2008a). To ideally resolve how complex functions emerge from the activity of diverse cell types, one should be able to perturb the activity of genetically, specified cell types and neural pathways in the primate brain in a temporally precise fashion. Over the past few years, the ability to optically perturb specific brain regions, neural types, and pathways through an optogenetic approach by genetic expression of light inducible reagents such as archaerhodopsin from *Halorubrum strain* TP009 (ArchT), and channelrhodopsin-2 (ChR2) has revolutionized the field of neuroscience (Boyden et al. 2005; Deisseroth 2012). Using optogenetic technology to influence the activity of specific groups of neurons in the NHP brain in a reliable and systematic way will help us to understand how neuronal circuits function in primates under normal and pathologic conditions. Recent studies in NHPs involving a combination of optical neural controls, fMRI, and cognitive behavior tests already suggest the potential role that a NHP model could play in mapping functional brain networks by induced changes in behavioral and neural networks (Boyden et al. 2005; Chaudhury et al. 2013;

Deisseroth 2012; Gerits et al. 2012; Han et al. 2009; Kravitz et al. 2010; Tsunematsu et al. 2011; Tye et al. 2013). With the combination of a transgenic NHP model of human disease and optogenetic technology, precise neural networks in healthy and diseased conditions can be dissected systematically. This would open up new horizons in understanding how neural circuits function in higher behaviors, and in brain pathologies.

Conclusion

This review discusses the latest developments in a transgenic NHP model, and the approaches that have, or may have, a potential impact in the future advancement of genetic modification of the NHP genome and the creation of a better model of human diseases. We have used the HD monkey as an example to lay out the logic behind the development of a transgenic NHP model. While detailed discussion on pathogenesis and clinical advancement of HD are not the focus of this review, readers that are interested in those areas can see the references provided (Klempir et al. 2006; Paulsen et al. 2008; Ross and Shoulson 2009; Rubinsztein 2002; Stout et al. 2011; Tabrizi et al. 2012). Although an HD monkey is the only reported transgenic, NHP model of human inherited genetic disease, the author is aware of ongoing efforts in the development of transgenic marmoset and macaque models for Alzheimer's and Parkinson's, as well as other neurological diseases. In addition to modeling human disease, the development of optogenetic tools and applications for NHPs, perhaps in a NHP model of human diseases, will lead to a new approach for dissecting the neural network in a systemic fashion. Nonetheless, it is an exciting time in transgenic NHP modeling. The development of a transgenic NHP model will continue to thrive and advance biomedical research in finding the cure for human diseases.

Acknowledgments

All animal procedures were approved by the Emory University Institutional Animal Care and Use Committee (IACUC). Animal research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to the principles stated in the *Guide for the Care and Use of Laboratory Animals* prepared by the National Research Council. Yerkes National Primate Research Center (YNPRC) is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). YNPRC is supported by the National Center for Research Resources P51RR165 and is currently supported by the Office of Research and Infrastructure Program (ORIP)/OD P51OD11132. AWSC is supported by ORIP/NIH (RR018827), NINDS (NS064991) and the American Recovery and Reinvestment Act Fund.

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