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REVIEW

Transgenic Nonhuman Primate Models for Human Diseases: Approaches and Contributing Factors

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ABSTRACT

Nonhuman primates (NHPs) provide powerful experimental models to study human development, cognitive functions and disturbances as well as complex behavior, because of their genetic and physiological similarities to humans. Therefore, NHPs are appropriate models for the study of human diseases, such as neurodegenerative diseases including Parkinson's, Alzheimer's and Huntington's diseases, which occur as a result of genetic mutations. However, such diseases afflicting humans do not occur naturally in NHPs. So transgenic NHPs need to be established to understand the etiology of disease pathology and pathogenesis. Compared to rodent genetic models, the generation of transgenic NHPs for human diseases is inefficient, and only a transgenic monkey model for Huntington's disease has been reported. This review focuses on potential approaches and contributing factors for generating transgenic NHPs to study human diseases.

KEYWORDS: Nonhuman primates; Disease model; Transgenesis

1. INTRODUCTION

Transgenic approaches are improving the scope of biotechnologies in the agricultural industries for increasing production or introducing disease resistance in domestic animals (Karatzas, 2003; Kuroiwa et al., 2004), as well as in biomedical research aimed at producing disease animal models. The main approach for generating transgenic animals is first to introduce foreign DNA directly into cell lines or into germ cells such as oocytes, spermatozoa or embryos, and then to obtain transgenic offspring by assisted reproductive technologies (ARTs). The first transgenic mouse was born in 1974 (Jaenisch and Mintz, 1974). Up to now, thousands of transgenic mouse disease models have been produced, constituting an important tool for understanding the pathogenesis of genetic diseases and developing effective therapies. Nevertheless, rodents are not optimal models for humans due to major species differences in their physiology, anatomy, biochemistry and genetics. In contrast, nonhuman primates (NHPs), such as rhesus monkeys, are very similar to humans in all these respects. However, a major limitation to widespread use of NHPs as models for human diseases is the difficulty of generating transgenic animals. The first transgenic monkey was born in 2001 (Chan et al., 2001), which opened a potential new era of transgenic NHP applications.

Progress in producing transgenic NHPs was last reviewed about ten years ago (Wolfgang and Golos, 2002). Potential approaches and the importance of transgenic NHPs in neurodegenerative disease studies have also been reviewed (Chan, 2004). However, few transgenic NHPs have been born in recent years. In this review, we summarize the progress made in the field of transgenic NHP disease models in the past

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decade. We also describe approaches for producing transgenic NHPs, contributing factors and problems to be overcome in the future.

2. TRANSGENIC APPROACHES IN NHPs

2.1. Retroviral vector introduction

The basic procedure in retroviral vector introduction is to recombine the target gene into a retroviral vector and then to produce high titer virus. Then, virus is injected into the perivitelline space of oocytes to enable the foreign gene to integrate into the host DNA. As reported for the first transgenic monkey named ANDi (for "inserted DNA," in a reverse transcribed direction), the foreign gene green fluorescent protein (*GFP*), enclosed by a pseudotyped vesicular stomatitis retroviral virus envelope glycoprotein (GVSV-G) vector, was injected into the perivitelline space of mature rhesus monkey oocytes. GFP expression was observed in ANDi's hair shafts, toenails and placenta (Chan et al., 2001).

2.2. Self inactivating (SIN) lentiviral vector

At the end of 2001, Wolfgang and colleagues published their progress in producing transgenic monkeys. Blastocysts resulting from *in vitro* fertilization (IVF) were transduced with a SIN lentiviral vector carrying an enhanced GFP (eGFP) and transferred into recipient females. Two live offspring were born, and placentas from all conceptuses showed the expression of the reporter transgene (eGFP) (Wolfgang et al., 2001).

Transgenic NHPs with germline transmission were first reported in 2009 (Sasaki et al., 2009). A SIN lentiviral vector in sucrose solution was injected into marmoset embryos. After embryo transfer, transgenic marmosets that expressed the transgene in several organs were born. Importantly, they achieved germline transmission of the transgene, and the transgenic offspring developed normally.

2.3. Simian immunodeficiency virus (SIV)-based vector

In 2010, Niu and colleagues developed an improved methodology for the production of transgenic rhesus monkeys, making use of a SIV-based vector that encodes eGFP and a protocol for infection of early cleavage-stage embryos (Niu et al., 2010). Two living infant monkeys that stably expressed eGFP were produced, indicating the usefulness of SIV-based lentiviral vectors for the generation of transgenic monkeys and improved efficiency of transgenic technology in NHPs.

2.4. Modified lentivirus vector to construct mutant genes

In 2008, a transgenic rhesus monkey model of Huntington's disease (HD) that expresses polyglutamine-expanded HTT was produced (Yang et al., 2008a, 2008b). In this experiment, a lentiviral-based vector "pFUW" (F: human immunodeficiency virus-1 flap element (HIV-flap); U: ubiquitin promoter; W: woodchuck hepatitis virus post-transcriptional regulatory element (WRPE)) was used to construct a mutant htt and GFP lentiviral vector. Lentiviruses carrying exon 1 of the human *HTT* gene with 84 CAGs (HTT-84Q) and the *GFP* gene under the control of human polyubiquitin-C promoter were microinjected into the perivitelline space of monkey metaphase-II-arrested oocytes followed by IVF, *in vitro* culture and embryo transfer into surrogate females. Hallmark features of HD, including nuclear inclusions and neurophil aggregates, were observed in the brains of the HD transgenic monkeys. Additionally, the transgenic monkeys showed important clinical features of HD, including dystonia and chorea (Yang et al., 2008a, 2008b).

Procedures for making transgenic mice have become routine, allowing foreign genes to be inserted into a target locus. However, it is hard for these approaches in the current transgenic NHPs to prevent random integration of transgenes. In other words, we have not obtained any intrinsically transgenic NHPs. The reason is that, until now, transgenic NHPs were all produced depending on retroviral or lentiviral vectors, both of which belong to the retroviridae virus, the enveloped double-stranded (ds) RNA virus (Goff, 2001). Due to the presence of the endogenous homolog in the target cell genome, a recessive gene defect that requires mutation at both alleles will not be achieved. Therefore, the retroviridae viruses cannot overcome the random integration of transgenes (Chan, 2004).

2.5. Other potential methods for producing transgenic NHPs

As the genome sequencing projects for various animal species are completed, it has become feasible to perform precise genetic modifications. Many methods have been successfully employed in farm and laboratory animals (Kues and Niemann, 2011; Yu et al., 2011). The approach in NHPs should be simple compared to the viral vector methods described above, indicating that more methods should be developed in the future for achieving transgenesis in NHPs. Consequently, we anticipate that more and more genetic modification methods will be established in these animals.

2.5.1. Pronuclear microinjection

Pronuclear microinjection is the most used and effective method in generating transgenic animals (Wall, 2001). The fundamental principle is to inject an alien gene or gene fragments into a pronucleus of an oocyte undergoing fertilization. In this way, the foreign gene will integrate fully into the genome of the embryo and fetus *via* the DNA replication process (Cozzi et al., 2009; Gardiner and Teboul, 2009). This method, a conventional transgenic technique, does not need viral vector. Although transgenic mice, rabbit, sheep and swine were produced by utilizing pronuclear microinjection (Hammer et al., 1985a, 1985b), no transgenic NHP has been obtained using this method. This method is highly inefficient and costly. Efficiencies of $<1\%$ required microinjection and transfer of thousands of embryos to