Available online at www.sciencedirect.com
SciVerse ScienceDirect
Journal of Genetics and Genomics 40 (2013) 67−73
JGG

REVIEW

Genetically Modified Pig Models for Human Diseases

Nana Fan [a,b], Liangxue Lai [a,*]

[a] *Key Laboratory of Regenerative Biology, South China Institute for Stem Cell Biology and Regenerative Medicine, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510530, China*
[b] *School of Life Sciences, University of Science and Technology of China, Hefei 230027, China*

Received 29 May 2012; revised 10 December 2012; accepted 30 December 2012
Available online 8 January 2013

ABSTRACT

Genetically modified animal models are important for understanding the pathogenesis of human disease and developing therapeutic strategies. Although genetically modified mice have been widely used to model human diseases, some of these mouse models do not replicate important disease symptoms or pathology. Pigs are more similar to humans than mice in anatomy, physiology, and genome. Thus, pigs are considered to be better animal models to mimic some human diseases. This review describes genetically modified pigs that have been used to model various diseases including neurological, cardiovascular, and diabetic disorders. We also discuss the development in gene modification technology that can facilitate the generation of transgenic pig models for human diseases.

KEYWORDS: Pig; Transgene; Gene targeting; Human disease model

INTRODUCTION

Genetically modified animal models provide important insights into the pathogenesis of human diseases. These models are also essential in developing new drugs. Classical mouse models have promoted significant developments in biomedicine. However, mice and humans are different in many aspects, including physiological traits and gene expression. Thus, mouse models cannot sufficiently mimic human diseases in some cases and large animal models remain urgently needed (Verma et al., 2011; Walters et al., 2011; Li and Li, 2012). Pigs have been recently used as models for human diseases because they are more similar to humans than mice in terms of anatomy, neurobiology, cardiac vasculature, gastrointestinal tract, and genome (Bendixen et al., 2010). Pigs also present fewer ethical and economic issues compared with primates.

Genetically modified mice can be easily obtained through genetic modification in embryonic stem (ES) cells followed by chimeratechnology. No pig ES cells have passed the crucial test of germ line contribution until now. Genetic modification in pigs is more difficult than in mice. The first method used to create genetically modified pig, pronuclear microinjection, is inefficient. Only about 1% of the injected zygotes can produce transgenic pigs (Uchida et al., 2001). In addition, the endogenous genes of animals cannot be specifically altered by this technique (Lai and Prather, 2003). The most popular method for modifying pig genes is the genomic modification of somatic cells followed by somatic cell nuclear transfer (SCNT). Using this method, a number of random genetically modified pigs have been cloned and used as human disease models. However, only a few specific genetically modified (knockout and knockin) pigs have been obtained by this method due to the limited proliferation competency and extremely low frequency of DNA homologous recombination in somatic cells (less than 10^{-6}) (Yang et al., 2011).

Zinc-finger nuclease (ZFN) technology has recently emerged as a powerful tool for genome editing (Whyte and Prather,

* Corresponding author. Tel/fax: +86 20 3201 5346.
E-mail address: Lai_liangxue@gibh.ac.cn (L. Lai).

http://dx.doi.org/10.1016/j.jgg.2012.07.014

2012). The success of ZFN technology for endogenous gene targeting in pigs was first reported by Yang in 2011. The efficiency of gene targeting in primary pig somatic cells increased from less than 10^{-6} to 4% and above (Yang et al., 2011). ZFN technology has provided a high-efficiency platform with which to generate gene-targeted pigs.

Improvements in methods to generate genetically modified pigs have led to an increase in the number of pig models for human diseases.

Here, we review the genetically modified pig models for human diseases that have been produced thus far to our knowledge. The genetically modified pigs described in this review are listed in Table 1.

GENETICALLY MODIFIED PIG MODELS FOR NERVOUS SYSTEM DISEASES

Neurodegeneration is the umbrella term for the progressive loss of structure and function of neurons (Okouchi et al., 2007). Many neurodegenerative diseases, which include Huntington's disease, Alzheimer's disease, and spinal muscular atrophy (SMA), are caused by genetic mutations that allow the generation of transgenic animal models for these diseases. Several gene-modified mouse models have been generated and play important roles in understanding the pathogenesis of neurodegenerative disorders. However, most of these mouse models show no apoptosis of neurons or overt neurodegeneration in brains. Pig brain is gyrencephalic and more similar to the human brain in terms of anatomy, growth, and development than rodent brains (Lind et al., 2007). In addition, pig has a long lifespan, which is advantageous in studying the neurodevelopment and development process of neurodegenerative diseases. Thus, pigs are considered more suitable models for human neurodegenerative diseases.

Huntington's disease

Huntington's disease (HD) is an autosomal-dominant neurodegenerative disease characterized by progressive degeneration of neurons and movement disorder, as well as cognitive decline and psychiatric problems (Reiner et al., 2011). HD is caused by an expansion of CAG triplets at the 5′ end of the Huntington (*htt*) gene, which encodes an expanded polyQ tract (> 36 glutamines) in the N-terminal of the huntingtin (HTT) protein. As early as in 2001, a transgenic pig model for HD was produced by pronuclear microinjection (Uchida et al., 2001). However, the development of behavioral and neuropathological symptoms of HD in the transgenic pigs remains unclear.

Nine years after the first HD pig model, transgenic HD pig models that expressed N-terminal (208 amino acids) mutant HTT with an expanded polyglutamine tract (105Q) were produced using the SCNT method (Yang et al., 2010). Postnatal death, dyskinesia, and chorea-like movement were observed in some mutant *htt* transgenic pigs. Transgenic HD pigs displayed typical apoptotic neurons with DNA fragments in their brains, similar to human HD. In contrast, mouse models expressing the same transgene often failed to replicate apoptosis. These findings suggest that the pig model is more similar to humans than mouse in terms of neuropathology for HD.

Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurological disease of the brain that leads to the irreversible loss of neurons and cognitive functions and ultimately leads to dementia and

Table 1
Genetically modified pig models for human diseases

Human disease	Gene modification	Technique	Reference
Huntington's disease	Mutant Huntington (*HTT*)$^{+}$	PM	Uchida et al., 2001
	Mutant Huntington (*HTT*)$^{+}$	SCNT	Yang et al., 2010
Alzheimer's disease	Amyloid precursor protein (*APP*) K670Nt/M671L^{+}	SCNT	Kragh et al., 2009
Spinal muscle atrophy	Survival motor neuron (*SMN*)$^{+/-}$	SCNT	Lorson et al., 2011
Cardiovascular disease	Peroxisome proliferator-activated receptor-γ (*PPAR-γ*)$^{-/-}$	SCNT	Yang et al., 2011
	Endothelial nitric oxide synthase 3 (*eNOS3*)$^{+}$	SCNT	Hao et al., 2006 Whyte and Laughlin, 2010
	Catalase (*CAT*)$^{+}$	SCNT	Whyte et al., 2011
	Apolipoprotein CIII (*ApoCIII*)$^{+}$	SCNT	Wei et al., 2012
Diabetes mellitus (MODY3)	Hepatocyte nuclear factor-1 homeobox A, dominant negative ($HNF1\alpha^{dn}$)$^{+}$	SCNT	Umeyama et al., 2009
Diabetes mellitus type 2	Glucose-dependent insulinotropic polypeptide receptor ($GIPR^{dn}$)$^{+}$	Lentivirus injection	Renner et al., 2010
Retinitis pigmentosa	Rhodopsin, mutant P347L (*RHO* P347L)$^{+}$	PM	Petters et al., 1997
	Rhodopsin, mutant P23H (*RHO* P23H)$^{+}$	SCNT	Ross et al., 2012
Stargardt-like macular dystrophy type 3	Mutant elongation of very long chain fatty acids-4 (*ELOVL4*)$^{+}$	PM and SCNT	Sommer et al., 2011
Breast cancer	Breast cancer associated gene 1(*BRCA1*)$^{+/-}$	SCNT	Luo et al., 2011

PM: pronuclear microinjection; SCNT: somatic cell nuclear transfer.