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The potential impact of new generation transgenic methods on creating rabbit models of cardiac diseases

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ABSTRACT

Since the creation of the first transgenic rabbit thirty years ago, pronuclear microinjection remained the single applied method and resulted in numerous important rabbit models of human diseases, including cardiac deficiencies, albeit with low efficiency. For additive transgenesis a novel transposon mediated method, e.g., the Sleeping Beauty transgenesis, increased the efficiency, and its application to create cardiac disease models is expected in the near future. The targeted genome engineering nuclease family, e.g., the zink finger nuclease (ZFN), the transcription activator-like effector nuclease (TALEN) and the newest, clustered regularly interspaced short palindromic repeats (CRISPR) with the CRISPR associated effector protein (CAS), revolutionized the non-mouse transgenesis. The latest gene-targeting technology, the CRISPR/CAS system, was proven to be efficient in rabbit to create multi-gene knockout models. In the future, the number of tailor-made rabbit models produced with one of the above mentioned methods is expected to exponentially increase and to provide adequate models of heart diseases.

Contents

1. Overview of the cardiac disease transgenic rabbit models created with pronuclear microinjection

Since the creation of the first transgenic rabbit thirty years ago (Hammer et al., 1985), pronuclear microinjection remained the single applied method and resulted in numerous important rabbit models of human diseases, with a variable (see Table 1), but in general low efficiency (Duranthon et al., 2012). These transgenic rabbit models were emerging as one of the most relevant experimental model systems for cardiovascular diseases. The lipid metabolism and the way atherosclerosis develops in rabbits are similar to that of the human (Fan et al., 2015). Since atherosclerosis and ischemic heart disease are among the leading causes of death

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Table 1
Cardiac disease transgenic rabbit models created with pronuclear microinjection.

Overexpressed mutant or wild type proteins and transgenic efficiencies[a]	References
Models for lipid metabolism and atherosclerosis	
• Hepatic lipase	(Fan et al., 1994)
• Apolipoprotein B100 A: 0.25; B: 10%	(Fan et al., 1995)
• 15-Lipoxygenase	(Shen et al., 1996; Shen et al., 1995)
• Matrix metalloproteinase	(Liang et al., 2006; Yamanaka et al., 1995)
• Apolipoprotein A1 A: 1.0%; B: 11%	(Boullier et al., 2001; Duverger et al., 1996)
• Lecithin-cholesterol acyltransferase	(Hoeg et al., 1996)
• Apolipoprotein E2	(Huang et al., 1997)
• Apolipoprotein E3	(Fan et al., 1998)
• Lipoprotein lipase A: 0.53%; B:8.3%	(Fan et al., 2001; Ichikawa et al., 2004)
• Apolipoprotein A A: 0.71%; B: 11.7%	(Fan et al., 2001; Kitajima et al., 2007; Rouy et al., 1998; Senthil et al., 2005)
Apolipoprotein A2	(Koike et al., 2009)
Apolipoprotein CIII	(Ding et al., 2011)
• Vascular Endothelial Growth Factor (VEGF) A: 0.33%; B:4.37%	(Kitajima et al., 2005; Liu et al., 2007)
• Matrix metalloproteinase [MMP]-12	(Liang et al., 2006; Yamada et al., 2008)
• Plasma phospholipid transfer protein	(Masson et al., 2011)
• C-reactive protein (CRP)	(Matsuda et al., 2011)
Models of altered sarcomeric proteins	
• β −MyHC-Q^{403} (β-myosin heavy chain) (R400Q) A:0.65%; B:21%	(Marian et al., 1999; Patel et al., 2001; Senthil et al., 2005)
• cTnI-G146Cardiac troponin I (R146G)	(Sanbe et al., 2005)
• α-MyHC (α-myosin heavy chain)	(James et al., 2000; Suzuki et al., 2009)
Models of inborn arrhythmogenic disease long QT syndrome	
• KCNQ1/KvLQT1 (KvLQT1-Y315S, LQT1, loss of IKs)	(Brunner et al., 2008; Odening et al., 2008)
• KCNH2/HERG (HERG-G628S, LQT2, loss of IKr)	(Brunner et al., 2008; Odening et al., 2008)
• KCNE1/minK (KCNE1-G52R, LQT5, altered IKs) A: 0.80%; B: 10.5%	(Major et al., 2016)

[a] Transgenic efficiencies are indicated where relevant data were published A: % transgenic of total injected and transplanted and B: % transgenic of total born alive.

in developed countries, most transgenic rabbit models were created in this area of cardiovascular diseases. The transgenic rabbit models of lipid metabolism and atherosclerosis established to date are listed in Table 1. Rabbit models of atherosclerosis and myocardial infarction are described in this issue by *Baumgartner et al.* and were recently reviewed by (Fan et al., 2015). Transgenic rabbit models have been established for hypertrophic cardiomyopathy (HCM), a relatively common inherited heart disease, that can lead to serious ventricular arrhythmias and is the most frequent cause of sudden cardiac death in young people (Decker et al., 2009). Mutations of all known sarcomeric proteins, the structural components of heart can cause HCM. The protein composition of the rabbit heart is closer to humans than rodents: In the human heart the β-myosin heavy chain (β−MyHC) comprises 90% of the total myofibrillar myosin, in the rabbits heart similarly to humans, 80% is made of β−MyHC, in contrast to mouse heart, the other myosin isoform, α-myosin heavy chain (α-MyHC) predominates with 95% (Marian, 2005). Different transgenic mouse models were created, among which none reproduced left ventricular hypertrophy, the hallmark of HCM (Marian, 2005). The first rabbit model of HCM was created by (Marian et al., 1999) with pronuclear microinjection carrying a common point mutation of β-myosin heavy chain R400Q (Table 1). Transgenic rabbits recapitulated the phenotype and primary abnormalities observed in human carriers: myocyte disarray and interstitial fibrosis, along with left ventricular hypertrophy. The usefulness of this transgenic rabbit strain in translational studies was underlined later, when it was used to prove, that simvastatin treatment induces regression of hypertrophy and fibrosis (Patel et al., 2001) and to evaluate the effect of atorvastatin in preventing cardiac hypertrophy (Senthil et al., 2005). Another transgenic rabbit model was created with pronuclear microinjection of the mutant cardiac troponin I (cTnI-R146G). R146G is a missense mutation causing HCM in human carriers (Sanbe et al., 2005). The cTnI-G146 rabbits recapitulated the phenotype of human HCM, including cardiac hypertrophy, myocyte disarray, interstitial fibrosis, and enhanced myofibrillar Ca^{2+} sensitivity. In order to better understand the role of the myosin isoforms, the α-myosin heavy chain was overexpressed in the transgenic rabbit heart, where it reached 15−40% of the total myofibrillar myosin, contrary to the normal adult human ventricle, where it is composed of 5−10% (James et al., 2000). The incorporation of 40% alpha-MHC led to greater myofilament power production and more rapid cross-bridge cycling, which facilitate ejection and relengthening during short cycle intervals, and thus protected against tachycardia-induced cardiomyopathy (Suzuki et al., 2009).

Another emerging area of cardiac disease models are the so called inborn arrhythmogenic diseases such as long QT syndrome (LQTS). Relevant transgenic rabbit models created with pronuclear microinjection are reviewed by *Lang and Odening* in this issue and listed in Table 1. In brief, transgenic LQTS rabbit models selectively overexpressing dominant-negative pore mutants of the human KvLQT1 (KvLQT1-Y315S, LQT1, loss of IKs) or HERG channels (HERG-G628S, LQT2, loss of IKr) in the heart completely mimic the human LQTS phenotype with QT prolongation, spontaneous sustained pVTs and SCD (Brunner et al., 2008; Odening et al., 2008). The first LQT5 transgenic rabbit model expressing a mutant human minK (KCNE1-G52R) protein exhibits increased cardiac repolarization instability and may thus be very useful for proarrhythmia studies on drugs challenging “silent” LQTS subjects clinically (Major et al., 2016).

2. Potential advantages of transposon mediated additive transgenesis

DNA transposons were primarily developed for gene therapeutic aims, but turned out to be applicable in mammalian transgenesis as well. DNA transposons are mobile genetic elements, which can integrate into the genome of the host cell by a simple “cut and paste” mechanism. The newly developed transposon vectors enable to cut out the transgene of interest flanked by

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