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Generation of lentiviral transgenic rats expressing Glutamate Receptor Interacting Protein 1 (GRIP1) in brain, spinal cord and testis

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Abstract

In neuroscience, rats have several advantages over mice as a model organism. For instance, behavioral experiments are more advanced and the larger size of the brain is better suited for surgical manipulation and biochemistry. Furthermore, the vascular physiology of rats is considered closer to human, providing clinical relevance. Because transgenesis rates achieved by conventional pronuclear injection are extremely low (0.2–3.5%), the availability of transgenic rats in neuroscience is limited. Lentivirus infection is an efficient way to integrate exogenous genes into the genome of a one-cell embryo to generate transgenic animals. We report here the generation of synapsin I promoter driven GRIP1-transgenic rats using lentiviral transgenesis. GRIP1 was chosen as a transgene because it interacts with AMPA receptors and is involved in glutamate receptor signaling. From a single infection experiment, 45% of the offspring carried the transgene and 40% achieved germ-line transmission. The expression of GRIP1 was observed at low levels in brain, spinal cord and testis. Interestingly, one transgenic copy lacked a 147 bp fragment in the GRIP1 coding region most likely caused by alternative splicing of genomic lentiviral RNA. Co-immunoprecipitation from rat brains showed that transgenic GRIP1 is in complex with the endogenous GluR2 subunit of AMPA receptors. These results indicate that functional transgenic GRIP1 protein is expressed in rat brain using lentiviral vectors containing a human synapsin I promoter. Tissue specific lentiviral transgenic rats will be a powerful tool for various applications in modern neuroscience.

Keywords: Lentiviral vectors; Viral gene transfer; Synapsin promoter; AMPA receptor; GRIP1/ABP; Transgenic rats

1. Introduction

Mouse is the first choice model organism for genetic manipulation in mammals. Well established methods are available to generate transgenic mice using microinjection of exogenous DNA into the pronucleus of fertilized eggs. In addition, efficient homologous recombination in ES cells facilitates the targeted manipulation of the mouse genome that is also known as generation of "knockout" or "knockin" animals. The main reason why mice are amenable to transgenic microinjection is because their oocytes have an easily visualized pronucleus and show a high efficiency of genomic integration of injected DNA (Jaenisch, 1988). Importantly, pluricompetent ES cell lines are available in only limited species. In fact, there is no ES cell line established for rats and blastocyst cultures in vitro which support normal development of rat embryos.

Rats are advantageous over mice for neuroscience research in many respects. Historically, they have been the rodent of choice for studies of brain anatomy and development, behavior, neuropharmacology, and brain biochemistry (Gill et al., 1989; Jacob, 1999). In addition, the larger size of rat head and brain (∼5-fold) makes them more convenient

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for chronic in vivo imaging or in vivo electrophysiological recording after surgically mounting small equipments on to the skull (Feng et al., 2000; Mehta et al., 2000; Helmchen et al., 2001; Li et al., 2005).

The generation of 'conventional' transgenic rats by DNA microinjection has been achieved, but the process is inefficient (Charreau et al., 1996). Only 0.2–3.5% of the injected embryos develop into rats expressing the transgenes (Pinkert, 2002). A breakthrough came with the use of lentivirus-based vectors to transfer genes into one-cell embryos (Lois et al., 2002; Pfeifer et al., 2002). These studies demonstrated efficient generation of transgenic mice and rats by viral-mediated gene transfer. The integrated provirus is not silenced during development like oncoretroviral vectors, and the lentiviral transgene is effectively expressed in mouse and rat (Lois et al., 2002; Pfeifer et al., 2002). In addition to high efficiency, lentiviral transgenesis is less invasive to embryos, and technically less demanding. Moreover, because lentivirus vectors can infect a wide range of vertebrate cells, they are potentially useful for creating transgenic animals in numerous species, including birds, cats, pigs, non-human primates, etc. (Pfeifer, 2004). Even without extension to other vertebrate animals, the ability to conveniently manipulate genes in rats would have a huge impact on biomedical research.

So far most studies have generated lentiviral transgenic animals carrying ubiquitously expressing promoters (Hamra et al., 2002; Lois et al., 2002; Pfeifer et al., 2002; Hofmann et al., 2003, 2004; McGrew et al., 2004). For neuroscience applications, it would be useful to develop ways to restrict expression of transgenes specifically to the brain. Multiple promoters are available that target transgene expression to specific brain cells, such as neurons, astrocytes and oligodendricytes (Wells and Carter, 2001). Studies comparing neuronal promoters have suggested that synapsin I might be a useful promoter based on both neuronal-specificity and high-level expression (Kugler et al., 2001; Dittgen et al., 2004). The synapsin I promoter drives expression in postnatal neurons (Sauerwald et al., 1990; Hoesche et al., 1993) and has been used in several germline transgenic studies in mice (Heumann et al., 2000).

Until now most lentiviral transgenic studies report the generation of lentiviral transgenic animals carrying reporter molecules such as the green fluorescent protein (GFP) or β-galactosidase to visualize the transgenic expression patterns (Hamra et al., 2002; Lois et al., 2002; Pfeifer et al., 2002; Hofmann et al., 2003, 2004; McGrew et al., 2004). We are interested to express neuronal regulators that can be used to either reflect or modify functions in synaptic plasticity, neuronal morphology or animal behavior. GRIP1 was chosen as a transgene because it plays significant role in glutamate receptor signaling. Originally cloned as an AMPA receptor binding protein (Dong et al., 1997; Srivastava et al., 1998; Wyszynski et al., 1999), GRIP1 has been mostly studied in the context of trafficking and synaptic stabilization of AMPA receptors (Song and Huganir, 2002; Bredt and Nicoll, 2003). Recent evidence suggest that GRIP1 is also important for trafficking and delivery of other membrane proteins, such as EphB receptors (Hoogenraad et al., 2005) and extracellular matrix protein Fras1 (Takamiya et al., 2004).

Here we describe a lentiviral strategy to generate transgenic rats that have tissue-specific expression of GRIP1 protein, driven by human synapsin I promoter. Approximately 45% of the rats contained the transgenic DNA and 40% of the rats transmit the GRIP1 transgene through germline. The transgenic GRIP1 expression occurred at low levels in testis, spinal cord and brain and co-immunoprecipitates with AMPA receptors. Together these results show that lentiviral vectors containing the synapsin I promoter can be used to generate tissue-specific transgenic expression in rats.

2. Results

One of the major limitations of the lentiviral vector is its "small capacity". It is considered that insertion of ∼8 kbp between the two LTRs impairs viral packaging (Kootstra and Verma, 2003). This prevented us from using the CaMKII promoter (8.0 kbp) that is known to be forebrain-specific in transgenic mouse (Mayford et al., 1996). After testing neuron-specific tubulin (500 bp), human synapsin I (450 bp), rat synapsin I (5.0 kbp) and Fugu αCaMKII (3.5 kbp) promoters, we identified the 450 bp fragment of the human synapsin I promoter as ideal for high and neuron-specific expression in hippocampal cultures. Thus, we used this promoter for further experiments.

Rat GRIP1 cDNA was cloned in frame with a HA- and FLAG-tag sequence and inserted downstream of the synapsin I promoter, generating the vector named FhS-GRIP1-W (Fig. 1A). Lentiviral particles were collected from the culture medium of HEK293T cells that are co-transfected with FhS-GRIP1-W, VSVG, and Δ8.9 as described (Lois et al., 2002). Infection of lentivirus derived from FhS-GRIP1-W in dissociated hippocampal primary cultures resulted in the expression of the full length GRIP protein at the expected molecular mass (∼125 kDa) (Fig. 1B) specifically in neuronal cells (Fig. 1C). The transgenic GRIP1 staining in hippocampal neurons shows bright punctuate signals in the cell body, axons and dendrites (shaft and spines), very similar to endogenous GRIP1 staining (Burette et al., 1999; Dong et al., 1999) (Fig. 1C). Thus, these data show that the synapsin I promoter drives neuronal expression of GRIP1.

Next, we infected single-cell rat embryos (about 50) by microinjecting concentrated viral particles (10^6 infectious unit/μl) into the perivitelline space that lies between the zona pellucida and the cell membrane. Twenty undamaged injected embryos were selected and immediately implanted into the ovarian duct of a pseudopregnant female and carried to term. Southern blot analysis of the offspring (11 animals) revealed that five founders (45%) carried at least one copy of the transgene (Fig. 1D). These data are comparable to the efficiency of FUW-GFP (FUGW) lentivirus injections in rat embryos (Lois et al., 2002).