Establishment of transgenic mice carrying the gene of human nuclear receptor NR5A2 (hB1F)

Shui-Liang Wang, Hua Yang, You-Hua Xie, Yuan Wang, Jian-Zhong Li, Long Wang, Zhu-Gang Wang, Ji-Liang Fu

INTRODUCTION

Nuclear receptor (NR) is a superfamily of eukaryotic transcription factors that are crucial for gene regulation and development. Members of this superfamily include receptors for steroid and non-steroid hormones as well as a large number of orphan receptors whose regulatory ligands have not been identified. The aim of this study was to establish an hB1F transgenic mouse model to promote the functional study of hB1F.

METHODS: Transgene fragments were microinjected into fertilized eggs of mice. The manipulated embryos were transferred into the oviducts of pseudopregnant female mice. The offsprings were identified by PCR and Southern blot analysis. Transgene expression was analyzed with RT-PCR and Western blot analysis. Transgenic founder mice were used to establish transgenic mouse lines. The F1 and F2 mice were identified by PCR analysis.

RESULTS: Seven mice were identified as carrying copies of transgene. RT-PCR and Western blotting results showed that the transgene was expressed in heart, liver, lung, kidney and stomach in one of the transgenic mouse lines. Genetic analysis of the transgenic mice demonstrated that the transgene was integrated into the chromosome at a single site, and was transmitted stably.

CONCLUSION: In this study we established an hB1F transgenic mouse model, which will facilitate the investigation of the biological function of hB1F in vivo.

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Abstract

AIM: Human hepatitis B virus enhancer II B1 binding factor (hB1F) was cloned and characterized as a novel member of the Ftz-F1 (NR5A) nuclear receptor subfamily. Although progress has recently been made, its biological function remains largely unidentified. The aim of this study was to establish an hB1F transgenic mouse model to promote the functional study of hB1F.

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Identification of transgenic mice

Founder (G0) mice were identified by PCR and Southern blotting analysis. For PCR, DNA was extracted from tails with Genomic DNA MINIPREP kit (Sangon Inc, China). PCR amplification was performed with hB1F primers (P1: 5’-CCGACAATGTGACATGGAA-3’ and P2: 5’-CTGCTGCGGTAGTTACA-3’) and pcDNA3 primers (P3: 5’-ATGCCGTTGCGCTCTATG-3’ and P4: 5’-CCGCTCCATCCGAGTA-3’) which would produce 300 bp and 1333 bp fragments from mice carrying the transgene, respectively. For Southern blotting, genomic DNA was digested overnight with HindIII and subjected to electrophoresis on a 1.0% agarose gel. DNA was transferred onto nylon membrane (MILLIPORE Co., Ltd, London, UK). Hybridization was performed under a stringent condition with a randomly-primed (α-32P)-labeled hB1F probe.

Expression of the transgene

One of the transgenic mouse lineages was used to study the expression of transgene. Total RNA was isolated from tissues with the TRIzol reagent (Invitrogen, CA, USA), according to manufacturer’s instructions. First strand cDNA was synthesized by reverse transcription (Promega, USA). Semiquantitative RT-PCR reactions were performed using primer pairs 5’-CCGACAATGTGACATGGAA-3’ and 5’-CTGCTGCGGTAGTTACA-3’ for hB1F cDNA, and 5’-AATTTTGCAATTTGCGAGGAG-3’ and 5’-TGTGAGGAGAGTGCTCAATG-3’ for mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA, which resulted in the generation of 300 bp and 600 bp products, respectively. PCR was performed for 30 cycles at 94°C for 1 min, at 57°C for 1 min, and at 72°C for 1 min. PCR products were electrophoresed on 1.5% agarose gels. Signals were quantified by density analysis of the digital images using BioStar image software, version 2.

For Western blotting, protein samples from tissues were prepared according to the protocols by the manufacturer (Santa Cruz Biotechnology, Inc., WA, USA). 50 µg protein of each sample was electrophoresed on 10% SDS-polyacrylamide gel and transferred to PVDF membrane. Membranes were blocked with 5% (w/v) non-fat milk in Tween-TBS (TBST) overnight at 4°C and incubated with anti-Mouse primary antibody (Sigma, USA) at a dilution of 1:1500 in TBST for 2 h at room temperature. Membranes were washed three times with TBST and incubated with a secondary antibody (horseradish peroxidase-conjugated anti-mouse IgG) at a dilution of 1:2000 at room temperature for 1 h. Immunodetection was carried out with an enhanced chemiluminescence kit (Amersharm Pharmacia Biotech, USA).

Transmission of transgene

To study the transmission of transgene in mice, transgenic founder mice were mated to normal C57 mice to produce the next generation (F1). PCR and Southern blotting were used to identify the F1 transgenic mice which were confirmed by Western blotting analysis. The transgenic fragment containing the full length hB1F cDNA was microinjected into the male pronucleus of 653 fertilized oocytes of F1 hybrids between C57 and CBA mice. The injected eggs were implanted into the oviducts of 24 pseudo-pregnant foster mothers, of which 13 mice became pregnant and gave birth to 97 offsprings. Eleven offspring mice were identified to carry the hB1F cDNA as demonstrated by PCR analysis (Figure 1), seven out of which were further confirmed by Southern blotting analysis (Figure 2). The ratio of transgene integration was 11.3% and 7.2%, respectively, by PCR and Southern blotting analysis.

RESULTS

Establishment of hB1F transgenic mice

The transgene fragment containing the full length hB1F cDNA was microinjected into the male pronucleus of 653 fertilized oocytes of F1 hybrids between C57 and CBA mice. The injected eggs were implanted into the oviducts of 24 pseudo-pregnant foster mothers, of which 13 mice became pregnant and gave birth to 97 offsprings. Eleven offspring mice were identified to carry the hB1F cDNA as demonstrated by PCR analysis (Figure 1), seven out of which were further confirmed by Southern blotting analysis (Figure 2). The ratio of transgene integration was 11.3% and 7.2%, respectively, by PCR and Southern blotting analysis.
Genetics of transgenic mice

To establish the transgenic mouse lineages, founder mice were mated to C57 mice to produce F1 mice. Among 128 mice of the first generation, 60 were identified as carrying hB1F cDNA transgene by PCR analysis (Figure 5). The ratio of transgene transmission was 46.9%. The F1 mice from the same founder were mated each other to produce the F2 mice. 45 out of 64 F2 mice were hB1F transgenic mice, with a ratio of transgene transmission of 70.3%. These results showed that the inheritance of hB1F transgene was in accordance with Mendel’s laws, and the transgene was integrated into the chromosome in a single site and could be transmitted stably.

DISCUSSION

hB1F (NR5A2) is a novel member of the FTZ-F1 nuclear receptor subfamily. It was originally cloned based on its interaction with hepatitis B virus (HBV), enhancer II B1 element[6]. It has been shown to be a critical regulator of HBV gene expression and replication. Recent studies have revealed that hB1F is mainly involved in the regulation of cholesterol related gene expression in the hepatic-intestinal system. hB1F and its mouse homolog mLRH-1 are essential for the regulation of cholesterol and bile acid homeostasis, which may ultimately reveal the yet unidentified biological functions of hB1F.

In conclusion, we reported here the successful generation of a transgenic mouse model expressing the orphan nuclear receptor hB1F (NR5A2). Future studies will focus on the physiological and pathological changes in this mouse model using powerful analytic methods e.g. microarray comparisons of gene expression profiles between normal and transgenic mice.

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