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Transmission and Death Rates in Transgenic Mice Containing Growth Hormone Receptor Gene

Kim, H. J. and D. I. Jin†

Department of Applied Biological Sciences, Sun Moon University

성장호르몬수용체 유전자를 지닌 형질전환생쥐의 세대전달율 및 치사율

김 현 주 · 진 동 일†

선문대학교 응용생물과학부

ABSTRACT

To study the signaling effect of growth hormone (GH) *in vivo* on animal physiology, transgenic mice containing GH Receptor (GHR) gene fused to metallothionein promoter were produced by DNA microinjection into one-cell stage embryos. Three founder mice were produced with transgenic mice with approximately 4~6 copies of GHR genes and transgene was transmitted into the progeny. The founder mice were mated with normal mice to produce F_1 mice, and intergation and transmission of transgene were checked by polymerase chain reaction and Southern blot methods. Transmission rate of GHR transgenic mice were 20~50% in F_1 generation and 50% in F_2 generation which means that some founder mice were mosaic and transgene in F_1 mice was transmitted to F_2 progeny with Mendelian ratio. Death rate of GHR transgenic mice after birth was about 10~30% in F_1 and F_2 progenies indicating that GHR gene may affect death of transgnenic progeny.

Ⅰ. INTRODUCTION

The GHR was first cloned from rabbit and human liver cDNA libraries (Leung et al., 1987) and has now been cloned from liver cDNA libraries of rat, rabbit, mouse, cow, pig, sheep, and chicken (Kelly et al., 1993). The regulation of GHR has been studied under several different situation of growth failure in rats. Chronic diabetes (Postel-Vinay et al., 1980), fasting (Postel-Vinay et al., 1982), and streptozotocin-induced diabetes (Baxter et al., 1980) are associated with a decrease in GHR in liver. A putative growth hormone receptor from rabbit liver and the growth hormone binding protein from rabbit serum have the same amino-terminal amino-acid sequence, indicating that the binding protein corresponds to the extracellular hormone-binding domain of the liver receptor (Leung et al., 1987). The GH has several major activities. It interacts with GH receptors in adipose cells and brings about an increase in free fatty acids by increase lipolysis. GH interacts with receptors on the cell membranes of liver, kidney, and muscle

† Corresponding author : Department of Applied Biological Sciences, Sun Moon University

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and causes the release of somatomedins (IGFs) into circulation. Somatomedins are mitogenic (growth stimulatory) for many types of somatic cells and are of great importance in growth. Growth hormone acts directly on liver cells (Vergani et al., 1997). GHR mRNA has been detected in liver, adipose tissue, small intestine, heart, skeletal muscle, brain and testis (Mathews et al., 1989; Frick et al., 1990). The signaling mechanisms initiated by binding of GH to GHR are complex. Identification and characterization of GHR in signaling pathways should provide a greater understanding of how GH elicits its diverse on body growth and metabolism.

Although some of transgenic animals containing growth-related genes were stimulated in growth and enhanced in food conversion, severe detrimental effects on the transgenic animals were also observed (Pursel et al., 1989). In this study GHR gene was used for the production of transgenic mice to evaluate the effect of GHR gene on animal physiology.

Ⅱ. MATERIALS AND METHODS

1. Vector Construction

The pMT-LCR (locus control region) expression vector 2999 (pMT5 ′ 3 ′) contained the mMT-I promoter and 650bp of hGH 3 ′ untranslated region and poly A signal. pMT5 ′ 3 ′ has mouse MT locus with 10kb of 5′ hypersensitive (HS) site and 7kb 3′ hypersensitive site. The unique Nru I site of pMT5 ′ 3 ′ which cut is inserted cDNA of GHR. Transgenes were linearized with Ksp I for microinjection into embryos.

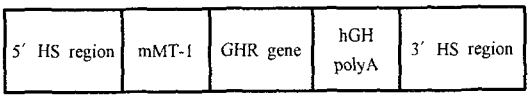

Fig. 1. Construction of MT-GHR gene.

2. Production of Transgenic Mice

The FVB/n female mice over 6 weeks used in this study. Transgenic mice with GHR genes were generated by the microinjection of cloned DNA into fertilized eggs, followed by embryo transfer into pseudopregnant foster mothers. Transgenic mice were outbred with non-transgenic mice to produce F_1 and F_2 progenies. The mice were maintained on a 14-h light, 10-h dark lighting cycle.

3. DNA Isolation

DNA was isolated from whole newborn mice tail. Tissue was incubated for overnight at 55℃ in Proteinase K/DNA extraction buffer (50mM Tris/ 100mM EDTA/10% SDS). The homogenate was extracted twice in PCI (phenol: chloroform: isoamyl alcohol=25 : 24 : 1) and once CI (chloroform: isoamyl alcohol = 24 : 1). DNA precipitation was carried out precipitation with a 2 volume excess of 100 % ethanol and 0.1 volume of 3M sodium acetate and was air-dry about 5 minutes and diluted in $T_{10}E_1$ buffer (10mM Tris-HCl/ 1mM EDTA).

4. Detection of Transgene with PCR Amplification

PCR amplification was performed with the Single Block TM system (ERICOMP Corp). PCR was carried out in 20 μl reaction volume containing 100ng/ μl of DNA solution, 2mM of the dNTP, 10 pM of the primer (sense and antisense primer), and the 1 unit of Taq polymerase (Promega Corp.). The primer pair used for detection of transgene forward primer 5'- GACGTCCAATCACGTGTCGA-3′ and reverse was 5′ -TTATTAGGACAAGGCTGGT-3'. PCR cycling was for 1 min at 94℃, 1 min at 57 ℃, and 1 min at 72℃ for 35 cycles. Also extra 5 min at 94℃ was allowed for separation of double strands at the beginning and an extra 7 min at 72 ℃ was allowed for extension and the end of amplification. After PCR, genomic DNA was

separated in 2% agarose gel electrophoresis. The size of the fragment amplified from cDNA should 580 bp (GHR gene).

5. Southern Blot Hybridization

The genomic DNA (10 to 20 ug) was digested with EcoR I and electophoresed 1 % agarose gel for overnight in 1 × TAE buffer with EtBr (0.5 μg /ml). The gel was denatured in denaturation solution (0.2mM NaCl/0.6mM NaCl) for 40 min with shaking at RT and then neutralized in neutralization solution (1mM Tris/0.6mM NaCl) for 1 hr. The DNA fragments were transferred to Nylon transfer membrane (Schlecher & Schull Corp.) for overnights in 20 × SSC (1 × SSC: 0.15 M NaCl/0.015 M Sodium citrate) by capillary method. The membrane was rinsed with 2 × SSC and baked in 80℃ for 2 hrs. The membrane was prehybridized in hybridization buffer at 42℃ for 2 hrs and added ^{32}P-labeled probe for overnight at 42℃. Probe (containing IGF-1R or GHR cDNA) was purified with QIAquick nucleotide removal kit, probe was labeled with ^{32}P by random primer methods. The blot were washed 2 × SSC/0.1% SDS for 30 min at 68℃ twice and exposed the filter to X-ray film 16 to 24 hrs at −70℃ with an intensifying screen.

Ⅲ. RESULTS AND DISCUSSION

1. Identification of Transgenic Mice

Transgenic mice with GHR were screened primarily by PCR. Fig. 2 showed those transgenic mice with GH receptor gene were screen PCR and amplified size was 550 bp. As shown Fig. 3, the copy number of transgene was estimated 4 to 8 copies by Southern blotting analysis and detection size was 1.3 kb following EcoR I digestion. It is also common for a transgene locus to contain multiple copies of the transgene, arranged in a head-to-tail array. Transgene integration efficiency

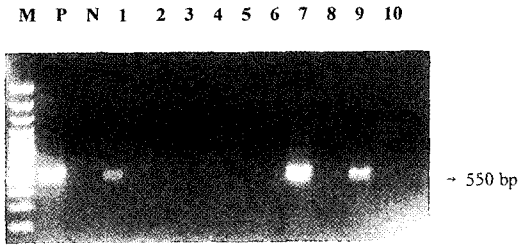

Fig. 2. PCR screening of transgenic mice with GHR gene; M: 100-bp ladder marker, P: positive control, N: negative control,Lane 1, 7, 9: transgenic mice with transgene, Lane 2, 3, 4, 5, 6, 8, and 10: non-transgenic mice without transgene.

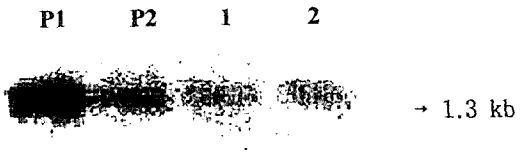

Fig. 3. Identification of transgenic mice with GHR gene by SouthernBlot analysis; P1: positive control (900 pg loaded), P2: positive Control (90 pg loaded), Lane 1, 2: transgenic mice with transgene.

is low and transgenes are expressed in only about half of transgenic lines, though some specific transgenes are expressed in a higher proportion. For detection of transgene, PCR and Southern blot method were used in this study. The PCR is a technique for the *in vitro* amplication of specific DNA sequences by the simultaneous primer extension of complimentary strands of DNA. Southern blotting was used for identification of transgene and presumption of copy number.

2. Transmission Rate of Transgene and Death Rate

In Table 1, transmission rate of transgenic mice with GHR genes in F_1 progeny was 20~50 % while

Table 1. Transmission rate of transgenic mice with GHR gene in progeny

Line	Sex of F_0	No. of Transgenics/F_1 progeny(%)	No. of Transgenics/F_2 progeny(%)
1	Male	3/ 9 (33.3)	9/20 (45)
2	Male	5/10 (50)	3/7 (43)
3	Female	4/20 (20)	8/21 (38)

that in F progeny was about 40~45%. Transgenic mice that are mosaic occur frequently. Mosaicism in the original transgenic Myk-103 female was initially detected as infrequent transmission of the pMK insert to her offspring and was proved by the finding that her female descendants show enrichment for pMK sequences and transmit the insert to half of their offspring. Wager et al. (1983) also observed mosaicism in a transgenic mouse, but the extent of mosaicism was not uniform from tissue to tissue, perhaps as a result of integration at a later stage of development. Integration after first cleavage could result in a mosaic animal with the foreign DNA in only some cells. In the mouse, a few cells are recruited between the 16- and 64-cell stage to form the inner cell mass- these cells are primarily destined to be the embryo, while the remainder of the cells, called trophectoderm, develop into the fetal placenta (Palmiter et al., 1984). In mosaics resulting from delayed integration the transgenic cells are usually distributed to both the trophectoderm and the inner cell mass, but sometimes to only one of these two cell types (Wilkie et al., 1986).

As shown Table 2, death rate of F_1 transgenic mice with GHR gene (27.8%) was higher than F_2 progeny (13.2%). Data of transmission rate of transgenic mice with GHR gene (Table 1) did not contain data of death rate. Death rate (before 3 weeks after birth) of transgenic mice may be the

Table 2. Death rate of transgenic mice with GHR geneLine

Line	No. of Death progeny/ F_1 progeny	No. of Death progeny/ F_2 progeny
GHR	10/36(27.8%)	4/30(13.2%)

phenotype of transgenic mice.

In 1980's, transgenic mice with GH-overexpression was produced, body weight was twice than control mice. But these mice have diverse pathological pattern, for instance, premature aging, infertility, libido etc. Molecular research concern with siganl transduction was very important for understanding of growth pattern and physiological action (Palmiter et al., 1982; 1983). Mice made transgenic for human (Palmiter et al., 1982), rat (Palmiter et al., 1983) or bovine GH (McGrane et al., 1988) have accelerated growth rates and adult body weights reaching 100 % of the body weight of their normal littermates. In female mammals, GH acting directly or via stimulation of IGF-I can influence sexual maturation, ovarian cycle, ovulation, fertility, and lactation. High concentration of GH production also results in female infertility. Female infertility in transgenic mice expressing the MThGH, MTrGH, and MTbGH is suppressed whereas male fertility appears normal (Hammer et al., 1986). By Orian et al. (1989), a large number of mice show liver abnormalities and the MT-GH1 construct does not result in infertility in female transgenic mice expressing high levels of GH. Liver size (under high levels of serum GH in transgenic mice) was between 20% and 40% larger than in sham mice (Orian et al., 1989). Overexpression of growth hormone in transgenic mice is associated with various degrees of impairment of female reproductive functions (Cecim et al., 1995).

Ⅳ. 요 약

본 연구는 growth hormone receptor(GHR) gene의 동물생리에 미치는 영향을 연구하기 위해 metallothionein promoter와 GHR gene을 이용하여 생쥐의 1-cell 수정란에 DNA 미세주입법에 의해 형질전환생쥐를 생산하였다. 세마리의 형질전환생쥐가 생산되었는데 DNA 분석결과 4~8 copy의 GHR 유전자를 지닌 것으로 확인되었다. 이들 세마리의 GHR 형질전환생쥐를 정상 형질전환생쥐와 교미시켜 F_1과 F_2 새끼를 생산하였는데 이들의 전달율은 F_1에서 20~50%였고 F_2에서는 약 50%를 나타내어 모자익형태로 유전자가 정착되었음을 확인할 수 있었다. 3주령까지의 사망률은 F1과 F2 새끼에서 약 10~30%를 나타내어 GHR 유전자의 발현이 형질전환생쥐의 초기 사망에 영향을 미치는 것으로 나타났다.

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