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Growth Rates of Transgenic Mice Containing Growth Hormone Receptor Gene

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ABSTRACT

Transgenic mice containing GH Receptor (GHR) gene fused to metallothionein promoter were analyzed to evaluate effect of GHR expression on growth *in vivo*. Three founder mice lines contained copies of GHR transgene and transmitted these genes into F_1 and F_2 progenies. The mRNA expression of transgene was identified using RT-PCR with GHR genes in tissues. To analyze the effects of transgenes on growth performance, body weights of pups were measured at 4, 10 and 14 weeks after birth. The body weight of transgenic mice was higher compared with that of non-transgenic control mice regardless of sex ($P<0.05$). Body weights between transgenic and non-transgenic mice were increased with aging. Overall, GHR transgenic mice tended to grow about 10 to 15 % faster than non-transgenic mice without any pathological defects.

(Key words : Transgenic mice containing GH receptor(GHR)

Ⅰ. INTRODUCTION

The important function of GH is body weight gain, skeletal growth and nitrogen retention was concern with Insulin-like growth factor (IGF-I) in each tissue (Daughaday et al., 1972). The GH gene is involved in body weight gain and metabolic action in whole body while Growth Hormone Receptor (GHR) express liver, adipose tissue, small intestine, heart, skeletal muscle, brain and testis *in vivo* (Mathews et al., 1989; Frick et al., 1990). The GHR is a member of the hematopoietin receptor gene family and located on chromosome 5 and has 10 exons over a distance of 87 kb. Several molecular weight forms of GHR have been observed for complexes with GH ranging from 122,000 to 300,000 Daltons for different species. The receptor is probably 100,000 Daltons bound with 22,000 GH molecules. The GHR is in the transducing active form as a homodimer. The membrane-bound hGH then complexes with a second hGHR to form the

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receptor homodimer, which initiates signal transduction (Cosman et al., 1990).

The presence of large numbers of receptors in the endosomal compartment probably reflects rapid receptor synthesis, since the half-life of the GHR is varying short (Baxter, 1985). In rat intestine, heart, skeletal muscle, brain, and testis, GHR mRNA has been identified (Busiguina et al., 2000; Burton et al., 1992; Tiong et al., 1991). The biological action of GHR signaling following binding of GH seems to be very complex. Identification of which pathways elicit which responses, how the different signaling cascades intersect and further characterization of the biological role of GHR signaling in different tissues promise to be fascinating challenges (Christin et al., 1996).

We have previously produced transgenic mice containing GHR gene fused with metallothionein (MT) promoter (Kim & Jin, 2001). The phenotypic effects of GHR expression in transgenic mice on growth was analyzed in this study.

Ⅱ. MATERIALS AND METHODS

1. Vector and Production of Transgenic Mice

The MT locus control region (MT-LCR) expression vector 2999 (pMT5′3′) was inserted cDNA of GHR into the unique Nru I site of vector (Fig. 1). Transgenic mice were generated by the microinjection of DNA into zygote pronucleus (Kim & Jin, 2001). 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.

2. DNA Analysis

Tail DNA was extracted from newborn mice. A piece of 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 ethanol and diluted in $T_{10}E_1$ buffer (10mM Tris-HCl/ 1mM EDTA).

Primers for PCR of the inserted DT-A gene are following:

forward primer 5′ -GACGTCCAATCACGTGTCGA-3′
Reverse primer 5′ -TTATTAGGACAAGGCTGGT-3′

PCR was carried out in 20 μl reaction volume containing 100 ng/μl of DNA solution, 2mM of the dNTP, 10 pM of the primers and the 1 unit of *Taq* polymerase (Promega Corp.). 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.

3. RNA Isolation and RT-PCR

Adult (4~8 weeks old) male/female mice were sacrifices by CO_2 saturation and various tissues were kept in liquid nitrogen. Total RNA was isolated from these frozen tissues using TRI REAGENT (molecular research center, INC). RT-PCR reaction was carried out 20 μl reaction volume (containing 100 mM of $MgSO_4$, 4 μl of 5 x

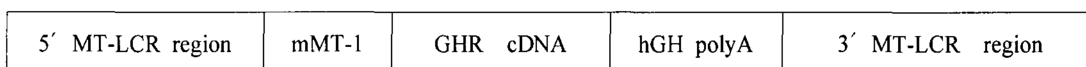

Fig. 1. Representative scheme of MT-GHR transgene.

buffer, 2.5 mM of dNTP, 7 μl of ddH_2O, 2.5 units of Reverse Transcriptase, 10 pmol of Poly A primer) and 3 attomole of RNA sample. The mixture was incubated at 42 ℃ for 40 min and boiled at 100 ℃ for 3 min. Immediately mixture was kept on ice and added 100 mM of $MgSO_4$, 4 μl of 5 x buffer, 2.5 mM of dNTP, 57 μl of ddH_2O, 2.5 units of Reverse Transcriptase, 10 pmol of upstream primer, 10 pmol of downstream primer and 2 mM of DNA polymerase (Access RT-PCR system, Promega Corp.). Primer GHR sequences were 5′-GACGTCCAATCACGTGTCGA-3′ and reverse primer (for Poly A) was used for PCR amplification. 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, DNA was separated in 2 % agarose gel electrophoresis.

4. Statistical Analysis

Comparison of values between subgroups was done by ANOVA test and Duncans multiple range tests.

Ⅲ. RESULTS AND DISCUSSION

1. Transgenic Mice and Transmission

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. The copy number of transgene was estimated 4 to 5 copies by Southern blotting analysis (Data not shown). GHR gene was stably transmitted to next generation. In Table 1, transmission rate of transgenic founder mice with GHR genes to F_1 progeny were 33~50 %. Line 1 may be mosaic. However, transmission rate of transgene to F_2 progeny was all most 40~50 % that means GHR gene is integrated in the mouse chromosomes.

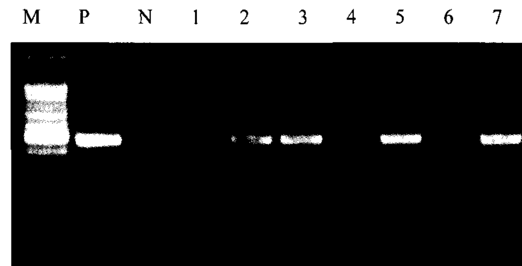

Fig. 2. PCR screening of transgenic mice with GHR gene.
M: 100-bp ladder marker, P: positive control, N: negative control, Lane 2, 3, 5 and 7: transgenic mice with transgene, Lane 1, 4 and 6: non-transgenic mice without transgene.

Transgenic mice that are mosaic in transgene occur frequently. Mosaicism in the transgenic mice can be detected as infrequent transmission of the transgene to 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,

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

Line	Sex of founders	No. F_1 transgenics/ progeny (%)	No. F_2 transgenics/ progeny (%)
1	Male	3/ 9 (33)	13/26 (50)
2	Male	5/10 (50)	11/24 (46)
3	Female	9/20 (45)	9/21 (43)

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., 1983). 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. Analysis of 262 transgenic mouse pedigrees suggests that about 30 % of the mice produced by microinjection of plasmids into pronuclei are mosaic in the germline. This implies that in these lines integration of the foreign DNA occurred after the first round of chromosomal DNA replication (Burdon et al., 1992). In general, the foreign DNA is stably transmitted for many generations with no evidence of rearrangement.

2. Expression Analysis of mRNA by RT-PCR

RT-PCR approaches have been developed for the quentitation of mRNA. mRNAs are ideal targets for cloning and *in vitro* manipulation by the genetic engineering because they represent only the protein-encoding regions of a gene. RT-PCR was performed to detect expression of GHR genes in transgenic mice. Primers used for this study was specific for GHR transgenics and designed to discriminate endogenous GHR mRNA by use reverse primer of human GH 3′ polyA signal which was contained in GHR transgenics. Transgene was successfully expressed in tissues of transgenic mice. Fig. 3 showed mRNA expression of transgene using RT-PCR with GHR transgenic mice in liver and detection size of cDNA was 550 bp. The band intensity of transgenic mouse line 1 (lane 1, 2) was lower than line 2 (lane 3, 4). The mRNA expression of GHR gene in transgenic mice with GHR was not different between sex. And transgnen expression was detected in varios tissues including

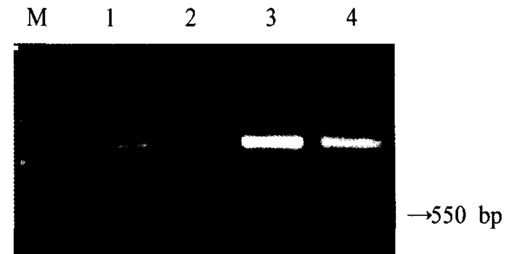

Fig. 3. RT-PCR analysis of mRNA expression of transgenic mice with GHR gene in liver. M : 100 bp ladder marker, Lane 1: transgenic line 1 female, Lane 2 : transgenic line 1 male mice, Lane 3 : transgenic lien 2 female mice with IGF-IR/GHR genes, Lane 4: transgenic line 2 male.

liver, kidney, muscle, and heart in transgenic mice. MT promoter used in this experiment can direct the expression of transgene basically at many organs for all stage of development (Palmiter et al., 1983).

3. Growth Performance in Transgenic Mice

The body weight of transgenic and mice was checked every 3 days after birth. The growth rate of transgenic male mice was higher than that of female. For all developmental stages, body weight of transgenic mice was more rapidly higher than non-transgenic female mice. In Table 2 and 3, body weights of transgenic mice were compared to those of non-transgenic mice at 4, 10 and 14 weeks after birth. The body weight of transgenic mice was higher compared with that of non-transgenic control mice regardless of sex ($P<0.05$). Difference of body weights between transgenic and non-transgenic mice was tended to increase with aging. Overall, about 10 to 15 % of bodyweight in GHR transgenic mice was increased compared to non-transgenic mice. All transgenic mice were healthy and fertile without any pathological defects.

Transgenic mice produced by human (Palmiter et al., 1982), rat (Palmiter et al., 1983) or bovine GH (McGrane et al., 1988) showed accelerated growth

Table 2. Comparison of body weights between transgenic and non-transgenic female mice

Line	4 week	10 week	14 week
Control	14.51 ± 0.56^{a}	22.32 ± 0.55^{a}	25.51 ± 0.50^{a}
GHR-TG mice	17.91 ± 0.46^{b}	26.11 ± 0.32^{b}	28.70 ± 0.31^{b}

^{ab} Different superscripts within column denote significant differences ($P < 0.05$).
Unit: g±se, se : standard error.

Table 3. Comparison of body weights between transgenic and non-transgenic male mice

Line	4 week	10 week	14 week
Control	18.11 ± 0.53^{a}	25.97 ± 0.45^{a}	28.53 ± 0.50^{a}
GHR-TG mice	19.41 ± 0.40^{b}	28.47 ± 0.47^{b}	33.47 ± 0.50^{b}

^{ab} Different superscripts within column denote significant differences ($P < 0.05$).
Unit : g±se, se : standard error.

rates and adult body weights reaching 100 % of the body weight of their normal littermates. However, overexpression of growth hormone (GH) gene in transgenic mice was associated with various degrees of impairment of female reproductive functions (Cecim et al., 1995). Those mice had diverse pathological pattern, for instance, premature aging, infertility, libido etc. In female transgenic, GH acting directly influenced sexual maturation, ovarian cycle, ovulation, fertility, and lactation. Hammer et al. (1985, 1986), initiated studies on large animals by microinjecting the fusion gene, MT-GH, into the pronuclei of eggs from superovulated rabbits, sheep and pigs. They reported integration of the gene in all three species and expression of the gene in transgenic animals. GH exerted definite biological effects in transgenic animals as evidenced by significantly depressed backfat measurements and moderate elevation of body weight. However, those transgenic animals with GH gene had suffered serious pathological disorders. Many important problems remain to be resolved in understanding of complex molecular mechanisms of growth. The results of this study indicated that the transgenic application to improved farm animals basically can be pursued using receptor genes with minimizing deleterious effects.

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