韓國家畜繁殖學會誌 24(4) : 419~428(2000)
Korean J. Animal Reprod.

Expression of Antisense Mouse *Obese* Gene in Transgenic Mice

Kwon, B. S., K. H. Hong, J. W. Jahng[1], H. T. Lee and K. S. Chung[†]
College of Agriculture, Animal and Life Science, Konkuk University

ABSTRACT

Leptin, the product of *obese* (*ob*) gene, is an adipocyte-derived satiety factor that plays a major role in the regulation of food intake, energy homeostasis, body weight, reproductive physiology and neuropeptide secretion. The present study was designed to generate transgenic mice expressing antisense mouse *ob* (m*ob*) gene. Total RNA was extracted from the adipose tissues of mouse, then reverse transcription was performed. The 303 and 635 bp fragments of anti Ⅰ and Ⅱ cDNAs were amplified from m*ob* cDNAs by PCR. The two m*ob* cDNAs were reversely ligated into between adipose tissue specific aP2 promoter and SV40 poly(A) site. Transgenic mice carrying two different kinds of antisense m*ob* transgenes were generated by DNA microinjection into pronucleus. Total 14 transgenic mice were born, and the 4 and 5 founder lines of the transgenic mice with anti Ⅰ and Ⅱ transgenes were respectively established. Antisense mRNA expression was detected in transgenic F_1 mice by RT-PCR analysis. This result suggests that the transgenic mice expressing antisense m*ob* mRNA may be useful as an animal disease model to be obesity caused by decreased amount of leptin secretion.
(Key words : Leptin, Transgenic, Antisense, aP2 promoter, Obesity)

Ⅰ. INTRODUCTION

Obesity is an increased body fat mass resulted from unblance between energy intake and energy expenditure in body composition (Bray and York, 1971; Weigle et al., 1994). The increased body weight (obesity), defined as a serious health problem, is associated with important psychological and medical morbidities, the latter including hypertension, hyper-lipidaemia, and Type Ⅱ or non-insulin-dependent diabetes mellitus (Friedman and Leibel, 1992). Recently, the pathogenesis of obesity was investigated at the part of molecular mechanism (Zhang et al., 1994).

Obese (*ob*) gene was firstly cloned and sequenced by Friedman and his colleagues (Zhang et al., 1994). Leptin, the product of ob gene, is an adipocyte-derived satiety factor. It plays a major role in the regulation of food intake, energy homeostasis, body weight (Campfield et al., 1995; Pelleymounter et al., 1995; Halaas et al., 1995; Stephens et al., 1995) and reproductive physiology (Clarke and Henry, 1999). Leptin acts as a negative-feedback in brain, and many neuropeptides relating to ingestive behavior, such as neuropeptide Y (NPY), agouti-related peptide (AGRP), cocaine- and amphetamin-regulated transcript (CART), and

[†] Corresponding author : E-mail : cks123@kkucc.konkuk.ac.kr
[1] 연세대학교 의과대학 (Dept. of Medicine, Yonsei University)

proopiomelanocortin (POMC), are regulated by leptin levels in the central nervous system (CNS) (Stephens et al., 1995; Ahima et al., 1999; Ebihara et al., 1999; Thornton et al., 1997; Kristensen et al., 1998). Releases of hypothalamic NPY and AGRP, potent orexigenic peptides, were reduced in leptin-treated *ob/ob* mice (Stephens et al., 1995; Ebihara et al., 1999). Hypothalamic CART and POMC expression, potent anorexigenic peptides, was increased in leptin-treated *ob/ob* mice (Kristensen et al., 1998; Shwartz et al., 1997; Thornton et al., 1997). These results indicate that leptin elevates the anorectic effects in the brain. However, the CNS mechanism regulated by leptin is not yet perfectly known.

Obese ob/ob mice with a mutation in the *ob* gene are diabetic, infertile, and exhibit the reduced activity, metabolism and body temperature (Bray and York, 1971; Herberg and Coleman, 1977; Coleman, 1978). Intraperitoneal or intracerebroventricular administration of recombinant leptin reduced food intake, body weight, percent body fat, and serum concentration of glucose and insulin (Campfield et al., 1995; Pelleymounter et al., 1995; Halaas et al., 1995; Stephens et al., 1995) and repaired reproductive function by promoting growth of the reproductive organs and increasing secretion of gonadotropins (Chehab et al., 1996; Buttle et al., 1997) in *ob/ob* mice. In addition, transgenic mice overexperssing leptin showed decreased body weight, increased the thermogenesis and repaired fertility in transgenic *ob/ob* mice (Ioffe et al., 1998), and increased the glucose metabolism and insulin sensitivity in transgenic skinny mice (Ogawa et al., 1999). The pathgenesis of obesity caused by leptin deficiency or resistance has been studied in many laboratories (Friedman and Halaas, 1998; Schwartz et al., 2000). However, the mechanism which results in obesity is still largely unclear. Until now, although there are many reports relating to over-administration or ablation of leptin, the reduced level of blood leptin in animal models have been little investigated.

Antisense transgenesis provides a methodology for ablating or decreasing gene expression in targeted tissues through the use of tissue-specific or controllable promoters (Erickson, 1999). Antisense RNA can bind in a highly specific manner to complementary endogenous RNA and then, block the ability of the endogenous RNA to be processed or translated (Izant and Weintraub, 1985; Chang and Stoltzfus, 1987; Yokoyama and Imanoto, 1987; Inouye, 1988). Thus, this antisense method using homologous recombination has been used to regulate specific gene expression in transgenic mouse.

In the present study, to elucidate the physiological and neural modulation caused by the low level of leptin *in vivo*, transgenic mice expressing antisense mouse *ob* (m*ob*) mRNA were generated by microinjection of recombinant antisense m*ob* gene into pronucleus. Furthermore, we examined the expression levels of antisense m*ob* genes by RT-PCR assay.

Ⅱ. MATERIALS AND METHODS

1. Cloning of the Mouse *Obese* Gene

Total RNA was extracted from mouse adipose tissues by using Trizol (Gibco, NY, USA). Isolated total RNA was reversely transcribed to synthesize the first strand cDNA by using AMV Reverse Transcriptase First-strand cDNA Synthesis kit (Life Sciences, FL, USA). All primers were designed according to the published m*ob* cDNA sequence (accession no. MMU18812), and synthesized in Bioneer Co., Korea. Primer sequences for each cDNA are as follow: anti Ⅰ, forward primer 5'-CAGCTGCAAGGTGCAAGAAGAA-3'; reverse primer 5'-TGGTGAGGACCTGTTGATAG-3'; pro-

duct size, 303 bp; anti Ⅱ, forward primer 5'-CAGCTGCAAGGTGCAAGAAGAA-3'; reverse primer 5'-GTGCACATGGCTCTCTTCTC-3'; product size, 635 bp. Thermal cycling profiles for amplifying each cDNA were as follow: denaturation at 94℃ for 30 sec, annealing at 60℃ for 30 sec, and extension at 72℃ for 40 sec for 35 cycles. Then, the PCR products were ligated into pCR®2.1-TOPO vectors (Invitrogen, CA, USA).

2. Construction of an Adipocyte Specific Expression Vector

The reversely orientated m*ob* cDNAs in the vector were identified by using restriction enzyme analysis. The reversely orientated TOPO-m*ob* was digested with Kpn Ⅰ and Not Ⅰ. The digested m*ob* was ligated with pEGFP-N1 vector (Clontech, CA, USA) digested with Kpn Ⅰ and Not Ⅰ. The reversely orientated pEGFP-m*ob*, in order to express antisense m*ob* mRNA in adipocyte, was digested with Xho Ⅰ and Kpn Ⅰ. Adipocyte P2 (aP2) promoter, a fat cell specific promoter, was ligated with pEGFP-m*ob* digested with Xho Ⅰ and Kpn Ⅰ. The aP2 promoter was kindly provided from Dr. Moitra (National Cancer Institute, USA). The aP2-m*ob*-poly(A) gene for microinjection was prepared according to the standard recombinant DNA procedure (Sambrook et al., 1989). The recombinant pEGFP-m*ob* containing aP2 promoter was digested with Xho Ⅰ and Nae Ⅰ for the microinjection, and the 8.7 and 9 kb fragments were dissolved respectively as a 10 μg/ml of concentration in 10 mM Tris (pH 7.4) and 1 mM EDTA buffer.

3. Generation of Transgenic Mice

Mice of B6D2F_1 (C57BL/6J × DBA/2J)F_1 and ICR strains were purchased from Daehanbiolink Co., Korea and kept under the regulated temperature (24～26℃) and humidity (40～60%) through the experiments. Mice were maintained on 12 h light and 12 h dark (light on at 09:00 h). Approximately 1 to 2 pl containing about 10 μg/ml of DNA solution was injected into the pronucleus of the one-cell embryos of B6D2F_1 hybrids. Survived embryos were incubated in M16 medium overnight in a 5% CO_2 incubator at 37℃ for growth to the two-cell stage. Then, the live eggs (1～2 cell stage) were transferred to the oviduct of pseudopregnant ICR mice by methods of Hogan et al. (1994).

4. Screening of Transgenic Mice

At three week age, tail biopsy was performed by clipping 0.5 cm from each pup at the time of weaning. Each genomic DNA was extracted from the tail samples by phenol extraction methods (Hogan et al., 1994). Then, PCR was performed by using primers (forward 5'-CTGCCAGAGTCTGGTCCATC- 3'; reverse 5'-AACACTCAACCCTATCTCGG-3'; product size, 697 bp). Thermal cycling profiles for identifying transgenic mice were as follow: denaturation at 94℃ for 30 sec, annealing at 65℃ for 30 sec, and extension step at 72℃ for 40 sec for 25 cycles.

For detection of transgene copy number, PCR assay was performed with the same of thermal cycling profiles except 22 cycles as described in the screening of transgenic mice. To analyze the concentration and purity of the genomic DNAs extracted from transgenic mice, the ratio of A_{260}/A_{280} was measured with a spectrophotometer (Beckmann, CA, USA). The transgene copy number was determined according to the density of amplified bands by densitometry (Vilber Lourmat, France).

5. Expression of Antisense *Obese* Gene

To investigate the expression of antisense m*ob* gene, peritoneal adipose tissues were removed from transgenic and non-transgenic mice at 11:00 h. The

removed tissues were rapidly submerged in LN_2 and stored at −70℃ deep freezer until used. Total RNA was isolated from frozen tissue by using Trizol (Gibco, NY, USA) and then reverse transcription was performed by using AMV Reverse Transcriptase First-strand cDNA Synthesis kit (Life Sciences, FL, USA). PCR was performed by using primers (forward 5'-CTAGTAACGGCCGCCAGT-GT-3'; reverse 5'-CAGCTGCAAGGTGCAAGAAG -3'; product size, 343 bp in case of anti Ⅰ, 675 bp in case of anti Ⅱ). Thermal cycling profiles for estimating antisense *mob* gene expression were as follow: denaturation at 94℃ for 30 sec, annealing at 55℃ for 30 sec, and extension step at 72℃ for 60 sec for 35 cycles.

Ⅲ. RESULTS

1. Construction of Microinjection DNA

Total RNA was isolated from adipose tissues and the two fragments (303 and 635 bp) of *mob* cDNAs were cloned by RT-PCR (Fig. 1). In order to produce reversely orientated *mob* cDNAs, they were inserted into pCR®2.1-TOPO vectors and then, the reversely orientated *mob* cDNAs were successfully confirmed and isolated by enzyme digestion. To confirm the exact sequence of cloned *mob* cDNAs, the PCR products were sequenced in GENE Co., Korea. As a result of sequencing, the sequences of *mob* cDNAs was correct (data not shown). Plasmid

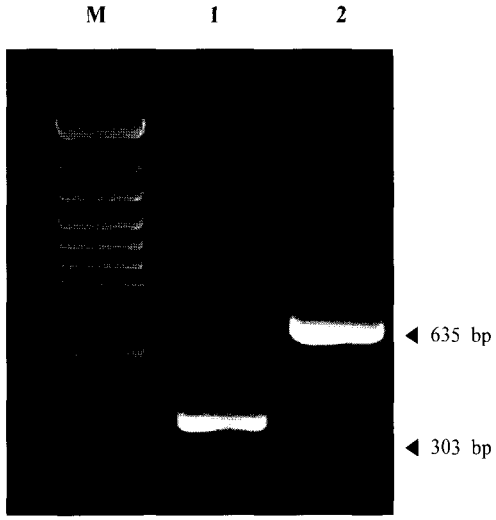

Fig. 1. PCR amplifications of *mob* cDNAs from adpose tissues. Lane M: 100 bp ladder, lane 1: The 303 bp of PCR product with anti Ⅰ primers, lane 2: The 635 bp of PCR product with anti Ⅱ primers.

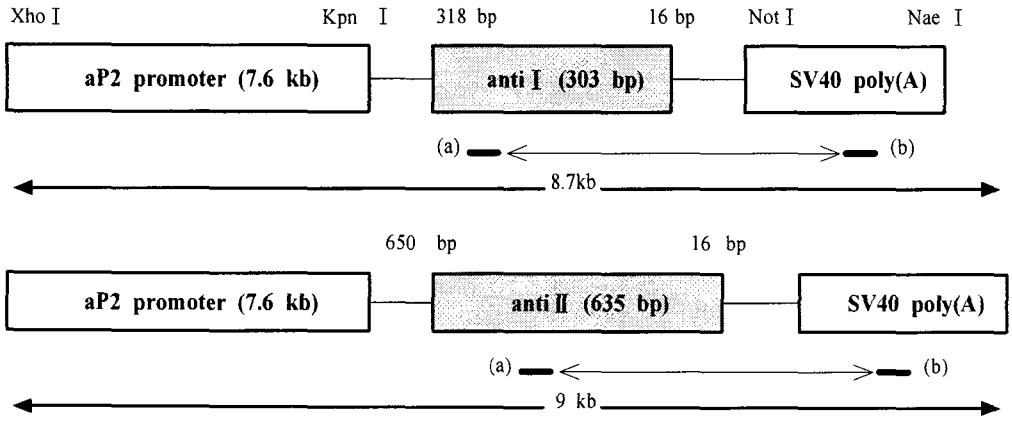

Fig. 2. Schematics for the aP2 promoter/antisense *mob* fusion gene constructs. (a), (b) : PCR primers for transgene screening.

EGFP-N1 vectors with SV40 poly (A) were ligated with the reversely orientated m*ob* cDNAs. To induce the expression of antisense m*ob* mRNA in adipocyte, they were ligated with the 7.6 kb aP2 promoter leading to specific expression of transgene in adipose tissues. For microinjection into pronucleus, the recombinant vectors were digested with Xho Ⅰ and Nae Ⅰ. As a result of enzyme reaction, two microinjection DNAs of 8.7 and 9 kb long were constructed as shown in Fig. 2.

2. Generation and Screening of Transgenic Mice

The anti Ⅰ and Ⅱ transgenes were microinjected into pronuclei of total 779 fertilized mouse oocytes and among them, 597 embryos were survived at the one-cell stage in the day or developed to the two-cell stage in the next morning (Table 1). These survived embryos at the one- or two-cell stage were transferred to the oviduct of pseudopregnant foster mother. After 19~20 days, 59 live pups were delivered. At 3 weeks later, genomic DNAs were isolated from the tails of pups and PCR was performed with specific primers. As a result of PCR, 7 of transgenic mice with anti Ⅰ gene were generated, and 7 of transgenic mice with anti Ⅱ gene were generated. However, 5 pups among detected transgenic mice were dead within 3 days after birth because of poor care of them by their mothers. The transgene copy number which detected by PCR assay was shown in Fig. 3. In

Table 1. Production of transgenic mice from embryos microinjected with anti Ⅰ and Ⅱ transgenes

Injected genes	No. of embryos injected	No. of embryos transferred	No. of offsprings	No. of transgenic mice	% of transgenic mice from offsprings
anti Ⅰ	520	411	32	7(3)	22
anti Ⅱ	259	186	27	7(2)	26
Total	779	597	59	14(5)	24

( ) : Number of dead offsprings after birth.
anti Ⅰ, Ⅱ : See Fig. 2.

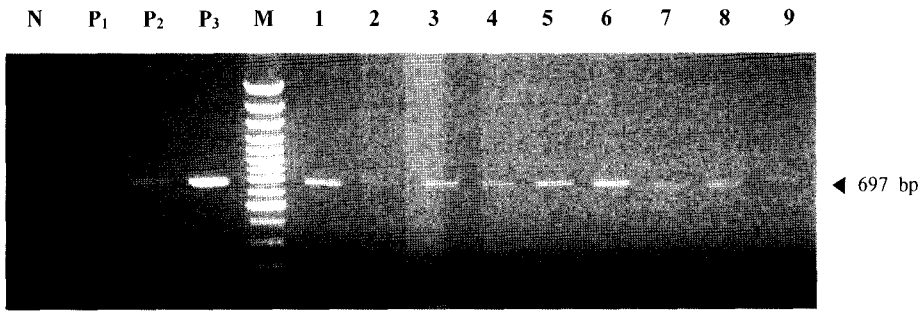

Fig. 3. Detection of transgene copy number by PCR assay. Lane N: non-transgenic mouse. Lane P_1-P_3: negative genomic DNAs mixed with microinjected genes show 1, 10 and 100 copies respectively. Lane M: 100 bp ladder. Lane 1~5: transgenic mice with anti Ⅰ gene. Lane 6~9: transgenic mice with anti Ⅱ gene. The bands formed in each lane show transgene copy number according to the density.

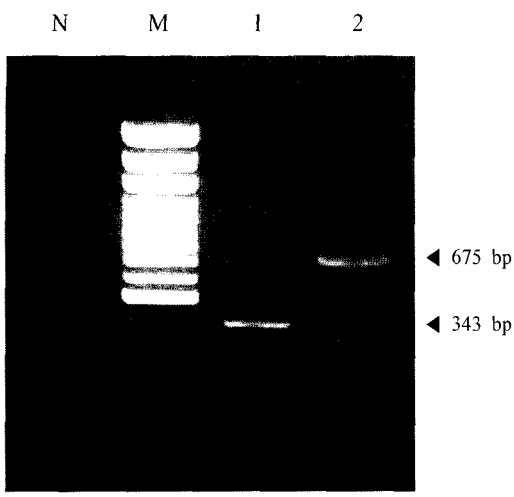

Fig. 4. RT-PCR analysis of the expressions of antisense *mob* genes. Lane N: non-transgenic mouse. Lane M: 100 bp ladder. Lane 1: The 343 bp of PCR product from transgenic mouse with anti Ⅰ gene. Lane 2: The 675 bp of PCR product from transgenic mouse with anti Ⅱ gene.

densitometry analysis (data not shown), the copy number of transgene is approximately as follow: 5 copies in lane 2, 7 and 9; 10 copies in lane 3, 4 and 8; 20 copies in lane 5; 30 copies in lane 6; 40 copies in lane 1.

3. Expression of Antisense *Obese* Gene

RT-PCR was perfomed with the adipose tissues from transgenic and non-transgenic mice. As a result of RT-PCR (Fig. 4), expressions of antisense *mob* mRNAs were detected in transgenic mice but not in non-transgenic mice. In the transgenic mice with anti Ⅰ and Ⅱ genes, 343 and 675 bp bands were respectively amplified by expressions of antisense mRNAs.

Ⅳ. DISCUSSION

The transgenic technology of the mouse genome by transfer of exgenous DNA provides powerful approaches for evaluating gene expression and function *in vivo* system. A lot of reports have been documented in researches to elucidate the mechanism of human disease and to investigate physiological characterization by the gene transfer technology. Now, the transfer of foreign genetic materials by pronuclear microinjection technology (Gordon et al., 1980) is a popular method designed to generate animal with an altered genotype.

There are many of reports available in antisense transgenesis to reduce the expression of some proteins. Type Ⅱ glucocorticoid receptor and $G\alpha_{i2}$, were expressed at the low level of 30 and 5%, respectively, in transgenic mice expressing the antisense mRNA of their genes (Pepin et al., 1992; Moxham et al., 1993). In the nucleus of antisense transgenesis, binding of the antisense RNA to the transcribed RNA presumably blocks processing and transport out of the nucleus (Erickson, 1999) or the hybrid RNA strands, once formed, are rapidly degraded by ribonucleases (Pepin et al., 1992). In order to suppress leptin expression *in vivo*, therefore, we adopted the use of a construct to express specific antisense *ob* mRNA instead of gene disruption.

Excluding some exprimental results, the general trend is that the larger length of an antisense construct is the more effective as an inhibitor (Erickson, 1999). Mouse *ob* gene, including three exons (Brousse et al., 1996), encodes a 4.5 kb mRNA with a highly conserved 167 amino-acid open reading frame (Zhang et al., 1994). In this study, the antisense *mob* cDNAs of two different lengths of 303 and 635 bp were successfully cloned from mouse adipose tissues. The antisense *mob* cDNA of 303 bp long includes the exon 2 with a starting ATG codon and the antisense *mob* cDNA of 635 bp long partially includes exon 2 and 3 with 5` and 3` untranslated regions. Since leptin is

secreted from adipocytes (Zhang et al., 1994; Masuzaki et al., 1995), we used aP2 promoter, which already verified by other researches (Hunt et al., 1986; Moitra et al., 1998), to induce the adipocyte-specific expression of antisense *mob* mRNA.

There are three general ways in which alterations of the leptin regulatory loop could lead to obesity. One is a failure to produce leptin, another is inappropriately low leptin secretion for a given fat mass, the third is a obesity which results from relative or absolute insensitivity to leptin at its site of action (Friedman and Halaas, 1998). The result of RT-PCR, the expression of antisense *mob* genes was detected from adpose tissues of transgenic mice and thus, it would be result in low leptin secretion in the transgenic mice.

The gene for mouse leptin receptors was firstly cloned from choroid plexus (Tartaglia et al., 1995) and shown to be expressed in arcuate nucleus (Arc), paraventricular nucleus and other hyperthalamic areas (Fei et al., 1997; Hakansson et al., 1998). NPY and AGRP are co-localized in Arc neurons (Broberger et al., 1998; Hahn et al., 1998) and CART and POMC are co-localized in Arc neurons (Elias et al., 1998). In the Arc, the secretion of these peptides regulating the ingestive behavior is regulated by leptin levels (Stephens et al., 1995; Ahima et al., 1999; Ebihara et al., 1999; Thornton et al., 1997; Kristensen et al., 1998). Therefore, in our transgenic mice with antisense *mob* genes, the secretion of NPY, AGRP, CART and POMC might be modulated by central and peripheral effects of low leptin level. Administration of leptin restored the decreased levels of testosterone and LH caused by fasting to nearly 40% of control mice and blocked the delay of estrus cycle caused by fasting (Ahima et al., 1996). In the transgenic mice, reproductive physiology might be affected by modulated leptin levels. However, the more detail analysis of the factors will be carried out to reach our assumption.

In the present study, transgenic mice carrying antisense *mob* transgene were generated by ligating the adipose tissue specific aP2 promoter to a reverse *mob* gene with a SV40 poly(A) site. Further studies are required to examine the level of plasma leptin and to investigate the physiological and neural modulation caused by the decreased amount of leptin in the transgenic mice.

V. REFERENCES

1. Ahima, R.S., Prabakaran, D., Mantzoros, C., Qu, D., Lowell, B., Flier, E. M. and Flier, J. S. 1996. Role of leptin in the neuroendocrine response to fasting. Nature 382:250-252.
2. Ahima, R. S., Kelly, J., Elmquist, J. K. and Flier, J. S. 1999. Distinct physiologic and neuronal responses to decreased leptin and mild hyperleptinemia. Endocrinology 140:4923-4931.
3. Bray, G. A. and York, D. A. 1971. Genetically transmitted obesity in rodents. Physiol. Rev. 51: 598-646.
4. Broberger, C., Johansen, J., Johansson, C., Schalling, M. and Hokfelt, T. 1998. The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. Proc. Natl. Acad. Sci. USA. 95:15043-15048.
5. Brousse, F. C., Shan, B. and Chen, J. L. 1996. Identification of the promoter of the mouse *obese* gene. Proc. Natl. Acad. Sci. USA. 93: 4096-4101.
6. Buttle N. F., Hopkinson, J. M. and Nicolson, M. A. 1997. Leptin in human reproduction: Serum leptin levels in pregnant and lactating women. J. Cli. Endocrinol. Metab. 82:585-589.
7. Campfield, L. A., Smith, F. J., Guisez, Y., Devos, R. and Burn, P. 1995. Recombinant

mouse OB protein: Evidence for a peripheral signal linking adiposity and central neural networks. Scince 269:546-549.

8. Chang, L. J. and Stoltzfus, C. M. 1987. Inhibition of rous sarcoma virus replication by antisense RNA. J. Virol. 61:921-924.
9. Chehab, F. E., Lim, M. E. and Lu, R. H. 1996. Correction of sterility defect in homozygous *obese* female mice by treatment with the human recombinant leptin. Nat. Genet. 12:318-320.
10. Clarke, I. J. and Henry, B. A. 1999. Leptin and reproduction. Rev. Reprod. 4:48-55.
11. Coleman, D. L. 1978. *Obese* and Diabetes: Two mutant genes causing diabetes-obesity syndroms in mice. Diabetologia 14:141-148.
12. Ebihara, K., Ogawa, Y., Katsuura, G., Numata, Y., Masuzaki, H., Satoh, N., Tamaki, M., Yoshioka, T., Hayase, M., Matsuoka, N., Aizawa-Abe, M., Yoshimasa, Y. and Nakao, K. 1999. Involvement of agouti-related protein, an endogenous antagonist of hypothalamic melanocortin receptor, in leptin action. Diabetes 48: 2028-2033.
13. Elias, C. F., Lee, C., Kelly, J., Aschkenasi, C., Ahima, R. S., Couceyro, P. R., Kuhar, M. J., Saper, C. B. and Elmquist, J. K. 1998. Leptin activates hypothalamic CART neurons projecting to the spinal cord. Neuron 21:1375-1385.
14. Erickson, R. P. 1999. Antisense transgenics in animals. Methods Enzymol. 18:304-310.
15. Fei, H., Okano, H. J., Li, C., Lee, G. H., Zhao, C., Darnell, R. and Friedman, J. M. 1997. Anatomic localization of alternatively spliced leptin receptors (*ob*-R) in mouse brain and other tissues. Proc. Natl. Acad. Sci. USA. 94: 7001-7005.
16. Friedman, J. M. and Halaas, J. L. 1998. Leptin and the regulation of body weight in mammals. Nature 395:763-770.
17. Friedman, J. M. and Leibel, R. L. 1992. Tackling a weighty problem. Cell 69:217-220.
18. Gordon, J. W., Scangos, G. A., Plotkin, D. J., Barbosa, J. A. and Ruddle, F. H. 1980. Genetic transformation of mouse embryos by microinjection of purified DNA. Proc. Natl. Acad. Sci. USA. 77:7380-7384.
19. Hahn, T. M., Breininger, J. F., Baskin, D. G. and Schwartz, M. W. 1998. Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. Nat. Neurosci. 4:271-272.
20. Hakansson, M. L., Brown, H., Ghilardi, N., Skoda, R. C. and Meister, B. 1998. Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. J. Neurosci. 18:559-572.
21. Halaas, J. L., Gajiwala, K. S., Maffei, M., Cohen, S. L., Chait, B. T., Rabinowitz, D., Lallone, R. L., Burley, S. K. and Friedman, J. M. 1995. Weight-reducing effects of the plasma protein encoded by the *obese* gene. Science 269:543-546.
22. Herberg, L. and Coleman, D. L. 1977. Laboratory animals exhibiting obesity and diabetes syndromes. Metabolism 26:59-99.
23. Hogan, B., Constantini, F., and Lacy, E. 1994. Manipulating the Mouse Embryos: A Laboratory Manual, 2nd ed., Cold Spring Habor Laboratory Press, New York, USA., pp:170-181, 293-298.
24. Hunt, C. R., Ro, J. H. S., Dobson, D. E., Min, H. Y. and Spiegelman, B. M. 1986. Adipocyte P2 gene: Developmental expression and homology of 5'-flanking sequences among fat cell -specific genes. Proc. Natl. Acad. Sci. USA. 83:3786-3790.
25. Inouye, M. 1988. Antisense RNA: its functions and applications in gene regulation - a review. Gene 72:25-34.
26. Ioffe, E., Moon, B., Connolly, E. and Fried-

man, J. M. 1998. Abnormal regulation of the leptin gene in the pathogenesis of obesity. Proc. Natl. Acad. Sci. USA. 95:11852-11857.

27. Izant, J. G. and Weintraub, H. 1985. Constitutive and conditional suppression of exogenous and endogenous genes by anti-sense RNA. Science 229:347-352.
28. Kristensen, P., Judge, M. E., Thim, L., Ribel, U., Christjansen, K. N., Wulff B. S., Clausen, J. T., Jensen, P. B., Madsen, O. D., Vrang, N., Larsen, P. J. and Hastrup, S. 1998. Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature 393:72-76.
29. Moitra, J., Mason, M. M., Olive, M., Krylov, D., Gavrilova, O., Samuels, B. M., Feigenbaum, L., Lee, E., Aoyama, T., Eckhaus, M., Reitman, M. L. and Vinson, C. 1998. Life without white fat: a transgenic mouse. Genes Dev. 12:3168-3181.
30. Moxham, C. M., Hod Y. and Malbon, C. C. 1993. Induction of $G\alpha_{i2}$ - Specific antisense RNA *in vivo* inhibits neonatal growth. Science 260:991-995.
31. Msuzaki, H., Ogawa, Y., Isse, N., Satoh, N., Okazaki, T., Shigemoto, M., Mori, K., Tamura, N., Hosoda, K., Yoshimasa, Y., Jingami, H., Kawada, T. and Nakao, K. 1995. Human *obese* gene expression: Adipocyte-specific expression and Regional differences in the adipose tissue. Diabetes 44:855-858.
32. Ogawa, Y., Masuzaki, H., Hosoda, K., Aizawa-Abe, M., Suga, J., Suda, M., Ebihara, K., Iwai, H., Matsuoka, N., Satoh, N., Odaka, H., Kasuga, H., Fujisawa, Y., Inoue, G., Nishimura, H., Yoshimasa, Y. and Nakao, K. 1999. Increased glucose metabolism and insulin sensitivity in transgenic skinny mice overexpressing leptin. Diabetes 48:1822-1829.
33. Pelleymounter, M. A., Cullen, M. J., Baker, M. B., Hecht, R., Winters, D., Boone, T. and Collins, F. 1995. Effects of the *obese* gene product on body weight regulation in *ob/ob* mice. Science 269:540-543.
34. Pepin, M. C., Pothier, F. and Barden, N. 1992. Impaired type Ⅱ glucocorticoid-receptor function in mice bearing antisense RNA transgene. Nature 355:725-728.
35. Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, New York, USA., pp: 1.21-1.110.
36. Schwartz, M. W., Woods, S. C., Porte Jr, D., Seeley, R. J. and Baskin, D. G. 2000. Central nervous system control of food intake. Nature 404:661-671.
37. Shwartz M. W., Seeley, R. J., Woods, S. C., Weigle, W. D., Campfield, L. A., Burn, P. and Baskin, D. G. 1997. Leptin increases hypothalamic proopomelanocortin mRNA expression in the rostral arcuate nucleus. Diabetes 46: 2119-2123.
38. Stephens, T. W., Basinski, M., Bristow, P. K., Bue-Vallesket, J. M., Burgett, S. G., Craft, L., Hale, J., Hoffmann, J., Hsiung, H. M., Kriauciunas, A., Mackellar, W., Rosteck Jr, P. R., Schoner, B., Smith, D., Tinsley, F. C., Zhang, X. Y. and Heiman, M. 1995. The role of neuropeptide Y in the antiobesity action of the *obese* gene product. Nature 377:530-532.
39. Tartaglia, L. A., Dembski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., Richards, G. J., Campfield, L. A., Clark, F. T., Deeds, J., Muir, C., Sanker, S., Moriarty, A., Moore, K. J., Smutko, J. S., Mays, G. G., Woolf, E. A., Monroe, C. A. and Tepper, R. I. 1995. Identification and expression cloning of a leptin receptor, OB-R. Cell 83:1263-1271.
40. Thornton, J. E., Cheung, C. C., Clifton, D. K. and Steiner, R. A. 1997. Regulation of hypothalamic poopiomelanocortin mRNA by leptin

in *ob/ob* mice. Endocrinology 138:5063-5066.
41. Weigle, D. S. 1994. Appetite and the regulation of body composition. FASEB. J. 8:302-310.
42. Yokoyama, K. and Imanoto, F. 1987. Transcriptional control of the endogenous MYC protooncogene by antisense RNA. Proc. Natl. Acad. Sci. USA. 84:7363-7367.
43. Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. and Friedman, J.M. 1994. Positional cloning of the mouse *obese* gene and its human homologue. Nature 372:425-432.

요 약

형질전환 생쥐에서 Antisense 비만유전자의 발현

권범섭 · 홍권호 · 장정원[1] · 이훈택 · 정길생†
건국대학교 농축산생명과학대학
[1]연세대학교 의과대학

렙틴은 지방세포의 비만유전자에서 분비되는 포식인자로써 음식섭취, 에너지대사, 체중, 번식생리 및 신경호르몬 분비를 조절하는 역할을 한다. 본 연구는 antisense 비만유전자를 발현하는 형질전환 생쥐를 생산하기 위하여 실시하였다. 먼저 렙틴을 분비하는 지방세포에서 RNA를 추출한 후 역전사 PCR을 실시하여 303 bp의 anti Ⅰ과 635 bp의 anti Ⅱ cDNA들을 합성하였다. 이러한 cDNA들을 지방세포 특이적 발현 프로모터인 aP2 프로모터와 SV40 poly(A) 사이에 역방향으로 결합하여 미세주입용 유전자를 구축하였다. 생쥐의 수정란전핵에 antisense 비만유전자를 미세 주입하여 14마리의 형질전환 생쥐를 생산하였으며, anti Ⅰ을 지닌 4마리의 형질전환 생쥐와 anti Ⅱ를 지닌 5마리의 형질전환 생쥐 계통을 확립하였다. 그리고 형질전환 생쥐의 지방세포를 추출하여 RT-PCR을 실시한 결과 antisense 비만유전자 mRNA 발현을 확인하였다. 따라서, 본 연구에서 생산된 형질전환생쥐는 생체 렙틴저하에 의해 비만을 일으키는 질환모델동물로써의 사용가능성을 나타내었다.

(접수일자 : 2000. 11. 3. / 채택일자 : 2000. 11. 20.)