Development of ghrelin transgenic mice for elucidation of clinical implication of ghrelin

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Abstract. To elucidate the clinical implication of ghrelin, we have been trying to generate variable models of transgenic (Tg) mice overexpressing ghrelin. We generated Tg mice overexpressing des-acyl ghrelin in a wide variety of tissues under the control of β-actin promoter. While plasma des-acyl ghrelin level in the Tg mice was 44-fold greater than that of control mice, there was no differences in the plasma ghrelin level between des-acyl ghrelin Tg and the control mice. The des-acyl ghrelin Tg mice exhibited the lower body weight and the shorter body length due to modulation of GH-IGF-1 axis. We tried to generate Tg mice expressing a ghrelin analog, which possessed ghrelin-like activity (Trp3-ghrelin Tg mice). The plasma Trp3-ghrelin concentration in Trp3-ghrelin Tg mice was approximately 85-fold higher than plasma ghrelin (acylated ghrelin) concentration seen in the control mice. Because Trp3-ghrelin is approximately 24-fold less potent than ghrelin, the plasma Trp3-ghrelin concentration in Trp3-ghrelin Tg mice was calculated to have approximately 3.5-fold biological activity greater than that of ghrelin (acylated ghrelin) in the control mice. Trp3-ghrelin Tg mice did not show any phenotypes except for reduced insulin sensitivity in 1-year old. After the identification of ghrelin O-acyltransferase (GOAT), we generated doubly Tg mice overexpressing both mouse des-acyl ghrelin and mouse GOAT in the liver by cross-mating the two kinds of Tg mice. The plasma ghrelin concentration of doubly Tg mice was approximately 2-fold higher than that of the control mice. No apparent phenotypic changes in body weight and food intake were observed in doubly Tg mice. Further studies are ongoing in our laboratory to generate Tg mice with the increased plasma ghrelin level to a greater extent. The better understanding of physiological and pathophysiological significance of ghrelin from experiments using an excellent animal model may provide a new therapeutic approach for human diseases.

Key words: Ghrelin, Transgenic mice

GHRELIN, an endogenous ligand for the growth hormone (GH) secretagogue receptor (GHS-R), is a 28-amino acid peptide with unique acylation modification, which is essential for its biological action [1]. Ghrelin is mainly produced in the stomach, and is also expressed in the hypothalamus [1]. The human plasma ghrelin level is increased during fasting and is rapidly decreased after feeding [2]. Peripheral administration of ghrelin not only induces GH secretion [3] but also increases food intake [4]. These results indicate that ghrelin/GHS-R signaling is implicated in both GH release and energy homeostasis. Furthermore, using more sensitive PCR techniques, expression of GHS-R has been identified in a wide range of organs, such as anterior pituitary, hypothalamus, stomach, small intestine, pancreas, ventricular myocardium, aorta, adipose tissue, and lymphocytes [1, 5]. The wide distribution of ghrelin and its receptor suggests that ghrelin has a wide range of physiological roles and that ghrelin is a potential drug candidate for the treatment of various diseases.

In order to elucidate the clinical implication of ghrelin and explore the therapeutic potential of ghrelin for...
human diseases, we have generated variable kinds of ghrelin-overexpressing transgenic (Tg) mice and investigated their phenotypes. In this review, we introduce the genetically engineered mouse models with modified ghrelin systems, and discuss the clinical implication of ghrelin.

**Des-acyl ghrelin Tg mice**

We generated Tg mice bearing the preproghrelin gene under the control of chicken β-actin promoter [6]. These mice overexpressed des-acyl ghrelin, which lacked the n-octanoyl modification at Ser⁴ of the ghrelin molecule, in plasma and a wide variety of tissues. Plasma total ghrelin level in the Tg mice was approximately 44-fold higher than that in the control mice (control vs. Tg: 1,105 ± 94.4 and 48,570 ± 9,291.5 fmoL/mL, p < 0.01). No difference in plasma ghrelin level was detected between the Tg and the control mice (control vs. Tg: 83.7 ± 11.9 and 86.3 ± 21.1 fmoL/mL). The Tg mice were significantly lighter in body weight and the shorter in body length than the control mice (body weight, control vs. Tg: 23.2 ± 0.5 and 16.6 ± 0.6 g, p < 0.01, body length, control vs. Tg: 9.2 ± 0.3 and 7.7 ± 0.3 cm, p < 0.01). Although the absolute amount of daily food intake was reduced in the Tg mice, the amount per body weight was not significantly different between the Tg and the control mice (control vs. Tg: 149.1 ± 7.6 and 155.2 ± 5.9 mg/g/day). There were tendencies for decline in serum GH level in the Tg mice compared with the control mice, although the differences between them were not significant (control vs. Tg: 5.5 ± 1.9 and 2.3 ± 0.9 ng/mL). Serum IGF-1 level in the Tg mice was significantly reduced compared with that in the control mice (control vs. Tg: 522 ± 23.6 and 364.1 ± 25.6 ng/mL, p < 0.01). These results indicate that small phenotype of des-acyl ghrelin Tg mice is attributed to altered GH-IGF-1 axis, not to poor nutritional condition.

Other groups have generated variable models of Tg mice by overexpressing preproghrelin using different promotors [7-9]. Almost all these animals produced des-acyl ghrelin and did not result in increased plasma ghrelin level. They did not exhibit increased body weight or food intake compared with control mice. A possible explanation for the absence of distinct phenotype in des-acyl ghrelin Tg mice is that plasma ghrelin levels of Tg mice were not increased.

**Tg mice overexpressing a ghrelin analog (Trp⁴-ghrelin Tg mice)**

We generated Tg mice overexpressing a ghrelin analog possessing ghrelin-like activity without Ser⁴ acylation. Among ghrelin analogs with replacement of the octanoylated Ser at the third position with other amino acids, Trp⁴-ghrelin most strongly showed ghrelin-like activity [10] and could be synthesized in vivo. Thus, we generated mice overexpressing Trp⁴-ghrelin by using human serum-amyloid-P (hSAP) promoter [11]. While plasma Trp⁴-ghrelin concentration in Trp⁴-ghrelin Tg mice was 3,438 ± 571.6 fmoL/mL, which was approximately 85-fold higher than plasma ghrelin (acylated ghrelin) concentration seen in the control mice, plasma ghrelin concentration did not differ between genotypes (control vs. Tg: 36.6 ± 4.4 and 40.5 ± 10.2 fmoL/mL). Because Trp⁴-ghrelin is approximately 24-fold less potent in biological activity than ghrelin [10], plasma Trp⁴-ghrelin concentration in Trp⁴-ghrelin Tg mice was calculated to have approximately 3.5-fold greater biological activity than that of ghrelin (acylated ghrelin) in the control mice. The average food intake of Trp⁴-ghrelin Tg mice did not differ from that of control mice. There were also no differences between Trp⁴-ghrelin Tg and control mice in anthropometric parameters including body weight, body length, total body fat percentage, and lean body mass. One-year-old Trp⁴-ghrelin Tg mice exhibited slightly impaired glucose tolerance and reduced insulin sensitivity, indicating that activation of ghrelin for a long-term may worsen insulin sensitivity.

**Tg mice overexpressing both mouse des-acyl ghrelin and GOAT (doubly Tg mice)**

After the identification of ghrelin O-acyltransferase (GOAT) in 2008, we generated Tg mice designed to express both mouse ghrelin and mouse GOAT genes in the liver under the control of the hSAP promoter (doubly Tg mice) by interbreeding of male heterozygous des-acyl ghrelin Tg mice and female heterozygous GOAT Tg mice. Plasma ghrelin level in doubly Tg mice was approximately 2-fold higher than that in the control mice under the control diet. There were no differences between doubly Tg and the control mice in food intake and body weight in 10-week-old. We thought that plasma ghrelin level in doubly Tg mice
was not elevated sufficiently to increase body weight or food intake.

Further investigations including feeding doubly Tg mice with medium-chain triglyceride (MCT) diet are ongoing in our laboratory, because ingested MCT diet is known to serve as a source of fatty acids in the acyl modification of ghrelin [12].

**Conclusion and future prospects**

We have been generating various kinds of ghrelin Tg mice by variable methods. These mice did not exhibit elevated plasma ghrelin level sufficient to increase body weight or food intake. It is challenging to generate ghrelin gain-of-activity models.

Previously, we have generated mice overexpressing transgenic form of leptin to assess the clinical implications of leptin [13]. We have also demonstrated that the transgenic overexpression of leptin reverses the metabolic abnormalities in a mouse model of lipodystrophy by crossbreeding with leptin transgenic mice [14]. Based on these findings, we have conducted a translational research of leptin and demonstrated the efficacy and safety of leptin replacement therapy in patients with lipodystrophy [15]. These lessons from animal models and rare human disease can provide us with a better understanding and possible novel treatment for common human disease.

Eating abnormalities are associated with various diseases, including obesity, diabetes, cachexia, and anorexia nervosa. A better understanding of the ghrelin from experiments using an excellent animal model may provide a new therapeutic approach for these diseases. Ongoing efforts are being made to generate Tg mice overexpressing ghrelin for evaluating the long-term pathophysiological and/or pharmacological effects of ghrelin.

**References**


