論文の内容の要旨

東京大学大学院農学生命科学研究科水圏生物科学専攻
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論文題目　Studies on growth hormone as a regulator of lipid metabolism in torafugu *Takifugu rubripes*
(トラフグ脂質代謝制御因子としての成長ホルモンの機能解析)

Lipids are essential for maintaining energy metabolism in animals. Excess energy intake results in lipid deposition in specific tissues, such as adipose tissue, liver, and skeletal muscle. Fish is known to be categorized into two groups mainly depending on the lipid contents in their muscle; one is “fatty fish”, containing high ratio of lipids in their muscle, and the other is “lean fish”, hardly accumulating lipids in their muscle but predominantly in their liver. The fish belonging to the latter group usually do not have mature adipose tissues, suggesting that their lipid metabolism rely on the function of liver. Since the lipid contents of fish body affect its taste and the property of final products, most previous studies on fish lipid metabolism have been conducted on the former group. However, cross-species comparison between fatty and lean groups may also help us to understand it from a broad perspective. For these reasons, this study focused on the mechanisms of maintaining lipid metabolism in lean fish.

Torafugu (Japanese pufferfish) *Takifugu rubripes* is the typical lean fish species. It marginally accumulates lipid in its skeletal muscle (approximately 1%); meanwhile, substantial lipids are deposited in its liver (approximately 65%). Previous studies revealed that the lipid content in torafugu muscle is not altered with the lipid content of the diet, and that the gene expression level of lipoprotein lipase (LPL), an enzyme responsible for lipid uptake, is significantly low in muscle compared to that in liver. These results suggest that lipid metabolism in torafugu muscle is repressed and therefore its liver would play an important role for maintaining lipid metabolism in torafugu whole body.

Growth hormone (GH) is a peptide hormone produced and released from the pituitary gland and other sites in vertebrates. This hormone has pluripotent functions in regulating growth, osmoregulation, immunity, and energy metabolism. Many previous studies indicate that GH regulates the lipid mobilization in teleost species. However, almost all studies were performed using fatty fish species as mentioned before. Because of the popular and important function of GH in aquaculture industry, enhancing the growth performance of cultured fish, it is meaningful to increase the knowledge about the molecular functions of GH on lipid metabolism in lean fish for understanding overall ability of this industrially important hormone in fish.

In Chapter 1, the molecular mechanisms of torafugu GH on torafugu liver and muscle metabolism were investigated using *ex vivo* system. Torafugu GH cDNA was cloned and its open reading frame sequence was inserted into pQE-30 Xa bacterial vector. Using bacterial protein expression system, recombinant torafugu GH (rtGH) was produced. Tissue slices from liver and muscle of torafugu were cultured in L15 medium and treated with rtGH (5 ng/mL at a final concentration) for expected periods of time. Transcriptome analysis with oligo-microarray indicated that the gene regulations of rtGH were tissue-specific. The activation of GH signaling molecules was also investigated by Western blot analysis using specific antibodies for signal transducer and activator of transcription (STAT), protein kinase B (Akt), and extracellular signal-regulated kinase (ERK) to detect their phosphorylation ratio. As for liver slices, both Akt and ERK were significantly activated after 60 min stimulation with rtGH. On the other hand, in muscle slices, all of these signaling molecules tested in this study did not alter their activation during 60 min stimulation with rtGH. These results suggest that the pathway of rtGH signaling is also tissue-specific. Quantitative real-time PCR analysis for lipid metabolism related genes revealed that rtGH down-regulated the gene expressions of LPL and fatty acid synthase (FAS) after 1 and 12 hours of rtGH addition, respectively, and up-regulated those of carnitine palmitoyltransferase 1 (CPT1), an enzyme essential in the beta-oxidation of fatty acids, after 24 hours of rtGH addition in liver slices. These results indicate that rtGH repress the lipid influx and lipogenesis followed by promoting lipid consumption in liver. As for muscle slices, only the gene expressions of FAS were down-regulated after 12 hours of rtGH addition, suggesting the suppression effect of *de novo* fatty acid synthesis in muscle. Overall, these experiments indicate that rtGH has a function for regulating lipid metabolism in tissue-dependent manners.

The action of GH is initiated by its binding to the specific receptors, growth hormone receptors (GHRs). In Chapter 2, cDNA cloning of torafugu growth hormone receptor isoforms was performed to understanding their molecular characteristics. The full-length cDNAs of two GHR isoforms, GHR1 and 2 were cloned and identified as so by phylogenetic analysis together with their counterparts from other fish. The comparison of the deduced amino acid sequences of each GHR isoforms showed that torafugu GHR2 lacked two extracellular cysteine and five intracellular tyrosine residues, suggesting that these GHR isoforms have different functions in GH/GHR signaling. The tissue distributions of the torafugu GHRs were investigated by quantitative real-time PCR using gill, heart, skin, fast muscle, slow muscle, liver, and intestine. As a result, all of these tested tissues were found to express both torafugu GHRs. The transcript abundance of torafugu GHR1 and GHR2 tended to be high in fast muscle, slow muscle, and liver. In mammal and other teleost fish, the highest relative mRNA levels of GHR have been found in the liver. In torafugu, notably, the significantly highest mRNA level of torafugu GHR1 was observed in fast muscle. Taking the results of Chapter 1 into account, these GHR distributions imply that the lipogenesis is strongly repressed in torafugu muscle by GH signaling.

The stored lipids mainly consist of triacylglycerol (TAG). TAGs are neutral lipids provided

by biosynthesis and/or transported by lipoproteins, solubilized complexes of TAG and apolipoproteins, produced in liver and intestine by using stored and dietary-derived TAGs, respectively. The TAG distribution among tissues and serum lipoproteins would provide us the information about lipid mobilization capacity. In Chapter 3, the difference of such distribution between torafugu and red seabream *Pagrus major*, a typical fatty fish species, were compared. As expected, torafugu accumulated TAG predominantly in liver and hardly did in muscle, whereas red seabream did in liver, muscle, and adipose tissue. However, the TAG contents in their sera were not so different from each other. Blue-native polyacrylamide gel electrophoresis followed by Oil red O staining also revealed that there was no significant difference in lipid distribution among lipoproteins. Further analysis using high-performance liquid chromatography demonstrated that TAG amount of a fraction containing very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) was significantly higher in torafugu than red seabream (39.4 ± 15.6 vs. 24.7 ± 12.3 mg/100 mL, respectively), whereas those in the high-density lipoprotein fraction were similar (54.0 ± 23.1 vs. 51.4 ± 14.0 mg/100 mL, respectively). Since VLDL and LDL are thought to be major TAG transporters in fish, these results suggest that the low TAG content in torafugu muscle is not derived from the little TAG secretion from liver and intestine, but probably from low TAG incorporation into the muscle cell. This is also supported by the further experiment using ex vivo system showing that the gene expression of 14 kDa apolipoprotein (Apo-14), a specific apolipoprotein in fish species, was up-regulated by high concentration of rtGH (200 ng/mL) in liver slices. This result implies that torafugu is capable of producing the lipoproteins in its liver in response to GH stimulation.

In conclusion, the present study elucidated the molecular mechanisms of GH to maintain lipid metabolism in torafugu, possibly with controlling the lipid contents in liver and muscle. The knowledge obtained in this study will help us to understand the overall function and importance of GH in teleost fish species.

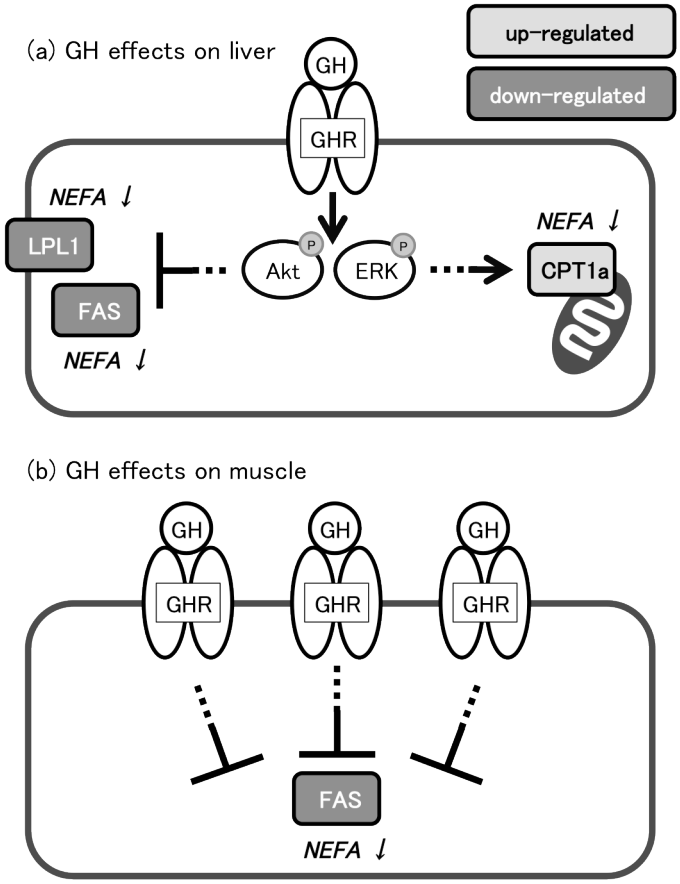

Figure 1. Schematic models of GH regulation of lipid metabolism in torafugu (a) liver and (b) muscle. Non-esterified fatty acid (NEFA) is an important source of fatty acids for TAG synthesis. The decrease of NEFAs would lead to the reduction of TAG contents in each tissue.