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Desialylation of gangliosides regulates triglyceride accumulation in fish liver

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Introduction

Lipid is important energy resource for fish. The majority of lipid uptake by the feeding is immediately consumed as energy and the excess amount of lipids are stored as triglyceride (TG) in the fish body. The major sites of lipid accumulation in fish are the liver, skeletal muscles, and adipose tissues [1]. It is known that amount of storage lipid is changed depending on the nutritional status and seasons. Also, regulation of muscle lipid content is an important concern in aquaculture fish in terms of fish meat quality. While lipid-uptake genes were identified by some research [2], few studies have examined detailed mechanism of lipid accumulation in fish so far.

In this study, we focused on sialidase Neu3 which is ganglioside specific sialidase. Sialidase Neu3 is localized at cell membrane and ganglioside is its good substrate. Recently, Yoshizumi et al. [3] reported that overexpression of human *NEU3* in mouse was increased TG level of the liver and suggested that desialylation of ganglioside was affected in TG accumulation. In this study, the influence of desialylation of ganglioside on lipid metabolism was examined with fish hepatocyte.

Materials and methods

Animals and cell culture
Commercial medaka (*Oryzias latipes*) was used in this study. Fish were fed commercial diet twice a day under 14/10 h light and dark cycle at 25°C.

Hepa-T1 cells were maintained in E-RDF medium containing 10% FBS without CO_2 supplementation at 28°C. Empty pcDNA3.1 or pcDNA3.1-Neu3a-HA expression plasmid were transfected into Hepa-T1 cells using X-treme Gene HP DNA (Roche, Swiss) according to manufacturer's instructions. Transfectants were selected by G418 to establish stable transfectants. Cellular ganglioside contents were analyzed by TLC using orcinol-H_2SO_4.

Analysis of gene expression
mRNA levels of medaka *neu3a* and lipid accumulation related genes in the liver of fasting medaka were analyzed by real-time PCR (Lightcycler nano, Roche). Fasting medaka liver were collected at 0, 3, 7, 10 day. Total RNA was extracted using Sepasol-RNA I Super G (nacalai tesque, Japan), followed by the cDNA synthesis using ReverTra Ace qPCR RT Master Mix with gDNA Remover (TOYOBO, Japan). For real-time PCR, the sequence-specific primers were used [4,5]. For the correction of the difference of RNA quality and quantity between samples, *actb* was used as internal reference. The PCR reaction was carried out using KOD SYBR qPCR Mix (TOYOBO) with amplification program involving a 2 min denaturation step, followed by 45 cycles at 98°C for 10 s, 65°C for 10 s and 68°C for 30 s. Transcription start site (TSS) of *neu3a* was determined by 5´ RACE and upstream sequence of TSS was analyzed by Ensembl genome browser (http://www.ensembl.org/index.html). Transcription factor binding sites were predicted by TFBIND (http://tfbind.hgc.jp/).

Oil Red O staining
Oil Red O staining was performed to observe lipid droplets. *neu3a*-stable transfectant cells were serum-starved for 16 h. Cells were treated with 1 mM oleic acid for 9 h. The cultured cells were washed with PBS and fixed with 4% PFA/PBS for 10 min. Fixed cells were washed and stained for 30 min with a filtered solution of Oil Red O (WALDECK GmbH & Co KG, Germany). After washing cells, preparations were mounted onto glass slides and examined by sectioning fluorescence microscopy system (Apotome, Carl-zeiss, Germany).

Results

Alterations of gene expressions under the deficient energy conditions
To examine whether ganglioside desialylation was

related to lipid metabolism, the liver of fasting medaka was analyzed by real-time PCR. *neu3a* expression was up-regulated until day 7. *lpl1*, relate to uptake lipid in blood, was markedly increased in day 7. Expression of *pparg* was increased in day 3 and then gradually decreased. These results suggested that desialylation of ganglioside could be related to lipid accumulation.

To confirm whether Neu3a was involved in lipid metabolism, analysis of transcription factor binding site in 5´ upstream of *neu3a* was carried out. Upstream of TSS had a *pparg*-related transcription factor binding sites related to lipid metabolism.

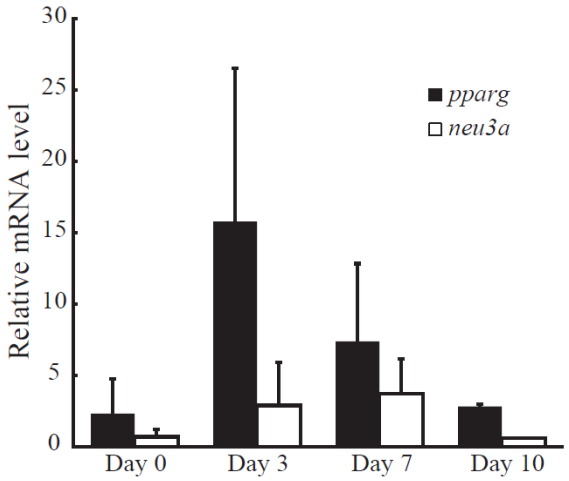

Fig. 1. Relative mRNA levels of lipid metabolism-related genes. Medaka was maintained under fasting during 10 days. *neu3a* expression was estimated by real-time PCR. *actb* isoform expression in medaka was used as a internal reference.

Observation of lipid droplets induced by oleic acid

To investigate whether ganglioside desialylation affected lipid metabolism, *neu3a*-stable transfectant cells were treated with oleic acid and its lipid droplet formation were assessed. Bigger size of lipid droplets were observed in *neu3a*-stable transfectant cells compared with vector. This result suggested that desialylation of ganglioside affected lipid accumulation.

Alteration of gangliosides in neu3a-stable transfectant cells

To elucidate which ganglioside was involved in this phenomenon, glycolipids composition were analyzed by TLC. *neu3a*-stable transfectant cells showed increase of LacCer and decrease of ganglioside GM3, suggesting that LacCer or GM3 played important role in the lipid accumulation.

Discussion

In fish, excess amount of lipid is accumulated in the liver, muscle and adipose tissue. Some studies reported lipid uptake related gene, while regulatory system of lipid accumulation in fish remained unclear. In this study, we examined regulation of lipid accumulation in fish liver focusing on to ganglioside desialylation.

neu3a mRNA expression in the fasting medaka liver was analyzed by real-time PCR. *neu3a* gene expression was up-regulated until day 7 under the deficient energy conditions, accompanied with *pparg* and *lpl1* expression. Furthermore, analysis of 5´ upstream of *neu3a* TSS was predicted *pparg*-related transcription factor binding site. It is a possibility that *pparg* could regulate *neu3a* mRNA expression.

To examine whether ganglioside desialylation was related to lipid uptake, *neu3a*-stable cells were exposed to oleic acid. Bigger size of lipid droplets were observed in *neu3a*-stable transfectant cells compared with vector. Neu1 sialidase interacts with perilipin 1, lipid droplet-associated protein, inducing lipolysis inhibition [6]. So, it is a possibility that Neu3a affects perilipin and regulates lipid droplet size. Then, glycolipids composition was analyzed by TLC. *neu3a*-stable transfectant showed increase of LacCer and decrease of GM3. Wada et al. [7] reported that ganglioside GM3 regulated cell surface receptor structure. Therefore, it is a possibility that medaka Neu3a would affect structure of cell surface fatty acid transporter mediated desialylation of ganglioside and regulate lipid accumulation.

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