（学位第３号様式）

学 位 論 文 要 旨

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題　目	Physiological functions of fish hepatic glycosphingolipids (魚類肝臓におけるスフィンゴ糖脂質の生理機能解析)

Glycosphingolipids (GSLs) are mainly expressed on cell surface. In mammals, GSLs are involved in various physiological roles. Although GSLs express in fish tissues, the physiological roles of fish GSLs remain unclear. Here, the present study focused on the effect of fish hepatic GSL in metabolism and bacterial infection.

Fish liver play various physiological roles such as lipid metabolism and detoxification. Fish store triglyceride (TG) in the liver and adipose tissue, and TG is consumed as an energy source upon metabolic demand. Alteration of GSL composition affect lipid metabolism in mammalian liver, while it is unclear whether similar mechanism exist in fish liver. First, this study aimed to clarify whether the alteration of ganglioside composition affects lipid metabolism of fish hepatocyte. To elucidate how lipid metabolism is associated with fasting in medaka liver, the biological parameters and *neu3a* expression were estimated. As a result, *neu3a* level was significantly up-regulated in the liver accompanied by the decrease of TG content. Next, to determine the role of Neu3a in hepatic lipid metabolism, Neu3a stable transfectants were generated using fish liver Hepa-T1 cells. Oleic acid exposure to Neu3a cells resulted in the reduction of TG and increase of free fatty acid and diacylglycerol in comparison with mock cells. Furthermore, lipase activities induced by oleic acid treatment were higher in Neu3a cells than in mock. To examine which gangliosides were related to these events, ganglioside composition of Neu3a cells were analyzed by thin layer chromatography (TLC). Neu3a cells showed accumulation of lactosylceramide (LacCer). In addition, exposure of LacCer toward Hepa-T1 cells resulted in an increase of lipase activity. These results suggest that Neu3a up-regulation in medaka under fasting condition promotes hepatic TG degradation for energy production via LacCer accumulation.

The present study has also revealed that LacCer was involved in *Edwardsiella tarda* infection. Previous studies indicate that *E. tarda* invade to host cell via membrane microdomain, but the mechanisms have been unclear. First, the present study examined whether the GSL composition was involved in the *E. tarda* infection. Intraperitoneal injection of *E. tarda* reduced medaka hepatic glucosylceramide (GlcCer) levels accompanied by the decrease of GlcCer and GM3 synthase mRNA levels. These results suggested that host GSL may be involved in *E. tarda* infection. Next, the significance of GSL in *E. tarda* infection was examined using fish cultured cells, DIT29 with high amount of LacCer and GlcCer and GAKS with low amount of these GSLs. Disruption of the membrane microdomain affected the susceptible of DIT29 cells to *E. tarda*, suggesting that the involvement of microdomain LacCer and GlcCer in the infection. In addition, incubation with the GSL synthase inhibitor suppressed *E. tarda* infection in DIT29 cells, and Neu3-overexpressing GAKS cells, which accumulated LacCer, elevated the infection. Furthermore, incubating *E. tarda* with LacCer, but not GlcCer, suppressed subsequent cell invasion in DIT29 cells. Thus, LacCer may be a positive regulator of *E. tarda* infection.

These results indicate that LacCer is an important regulator of lipid metabolism and bacterial infection in fish liver.