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Effects of insulin, triiodothyronine and fat soluble vitamins on adipocyte differentiation and LPL gene expression in the stromal-vascular cells of red sea bream, *Pagrus major*

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Abstract

Various kinds of hormones including insulin, triiodothyronine (T_3) and fat-soluble vitamins have been proposed as mediators of adipocyte differentiation in mammals. To investigate the factors which are responsible for fish adipocyte differentiation, we developed a serum-free culture system of stromal-vascular cells of red sea bream adipose tissue and examined the effects of bovine insulin, T_3, and fat-soluble vitamins (all-*trans* retinoic acid, retinyl acetate and 1,25-dihydroxyvitamin D_3) on the differentiation-linked expression of the lipoprotein lipase (LPL) gene. As assessed by the increase in LPL gene expression after 3 day cultivation, like in mammalian adipocytes, insulin enhanced the adipocyte differentiation in a concentration-dependent manner. During 2 week cultivation, bovine insulin promoted lipid accumulation in differentiating adipocytes concentration-dependently until the terminal differentiation. These results indicate that the differentiation of fish adipocytes is inducible by insulin alone. T_3 alone had no effect but enhanced the differentiation-linked LPL gene expression in the presence of insulin. Fat-soluble vitamins, unlike in mammalian adipocytes, did not show any significant effects. The method developed in this study should be of interest for the characterization of factors involved in fish adipocyte differentiation.

Keywords: Adipocyte; Stromal-vascular cells; Differentiation; Lipoprotein lipase (LPL); Teleost; Red sea bream; *Pagrus major*

1. Introduction

Adipocyte differentiation is an important factor for fat accumulation in the body. Adipocytes are derived from fibroblast-like preadipocytes and grow in size by accumulation of lipids in the cytoplasms in association with the terminal differentiation (Hausman et al., 1980). In the early stage of adipocyte differentiation, preceding the terminal differentiation, many adipocyte-characteristic genes are sequentially activated and play established roles in promoting the differentiation process (Gaskins et al., 1989; Ntambi and Kim, 2000). Lipoprotein lipase (LPL) gene is one of such genes and represents an early marker of adipocyte differentiation (Dani et al., 1990). LPL gene encodes a lypolytic enzyme responsible for lipid uptake in adipocytes (Nilsson-Ehle et al., 1980) and the gene expression and enzymatic activity are utilized to assess the degree of adipocyte differentiation in vivo and in vitro (Vu et al., 1996; McNeel et al., 2000; Ding and Mersmann, 2001).

Adipocyte differentiation is mediated positively or negatively by various kinds of hormones (Gregoire et al., 1998; Boone et al., 2000). For example, insulin is required for adipocyte differentiation in mammals and birds (Dani et al., 1986; Suryawan et al., 1997; Ramsay and Rosebrough, 2003). Triiodothyronine (T_3) also stimulates adipocyte differentiation in the preadipocyte cell line Ob17 (Gharbi-Chihi et al., 1981). Furthermore, it is well known that fat-soluble vitamins, especially metabolites of vitamin A and D, modulate the adipocyte differentiation in cultured cells (Kawada et al., 1996). All-*trans* retinoic acid (RA) and 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) inhibit the adipocyte differentiation in cultured cells at a superphysiological concentration (Sato and Hiragun, 1988; Kawada et al., 1990; Suryawan and Hu, 1997). Very low concentration (1 pM–10 nM) of RA stimulates the adipocyte differentiation (Safonova et al., 1994). Moreover, Vu et al. (1996) have

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reported the stimulatory effects of $1,25(OH)_2D_3$ on the differentiation-linked LPL gene expression in 3T3-L1.

The adipocyte is a major site of lipid deposition in fish. The tissue distribution and number of adipocytes in fish body are critical factors in determining the body lipid content and the flesh quality of cultured species (Yamada and Nakamura, 1964; Yamada, 1981; Shindo et al., 1986; Zhou et al., 1996). An in vitro method for studying the proliferation and differentiation of Atlantic salmon preadipocytes has been developed (Vegusdal et al., 2004). Salmon preadipocytes proliferate in vitro as characterized by the expression of proliferating cell nuclear antigen (PCNA) (Vegusdal et al., 2004). And the supplementation of exogenous lipids to the culture medium was shown to induce salmon preadipocyte differentiation as characterized by lipid accumulation in the cytoplasm and the appearance of adipocyte characteristic proteins including PPARγ, C/EBP and leptin (Vegusdal et al., 2004). These findings have provided us with information on the molecular mechanisms of fish adipocyte proliferation and differentiation. However, knowledge about the extrinsic regulation of fish adipocyte differentiation is still limited.

For the eventual control of adiposity in cultured species, the factors which modulate the adipocyte differentiation in fish need to be identified. In this study, to investigate the factors which may be responsible for fish adipocyte differentiation, we developed a primary culture system of adipose stromal-vascular cells of a marine teleost red sea bream (*Pagrus major*) and tested the effects of insulin, T_3, and fat-soluble vitamins on adipocyte differentiation in vitro. A preliminary step for defining the factors responsible for regulation of fish adipocyte differentiation is the development of a defined cell culture system. In the present study, we employed a serum-free culture system to investigate the hormonal effects on the adipocyte differentiation under strictly controlled conditions. The lipoprotein lipase (LPL) gene expression level was used as an early marker of the adipocyte differentiation and the lipid accumulation was used as a marker of the terminal differentiation process.

2. Materials and methods

2.1. Experimental fish

Juveniles of red sea bream (*Pagrus major*) were purchased from a local hatchery station (Nissin Marine Tech, Aichi, Japan) and maintained in our institute (National Research Institute of Aquaculture, Minamiise, Mie, Japan). After growing to 800–1200 g in body mass, the fish were used.

2.2. Culture media

The plating medium consisted of the DME/F12 (1:1) medium (Invitrogen Corp., Carlsbad, CA, USA) supplemented with 65 mM

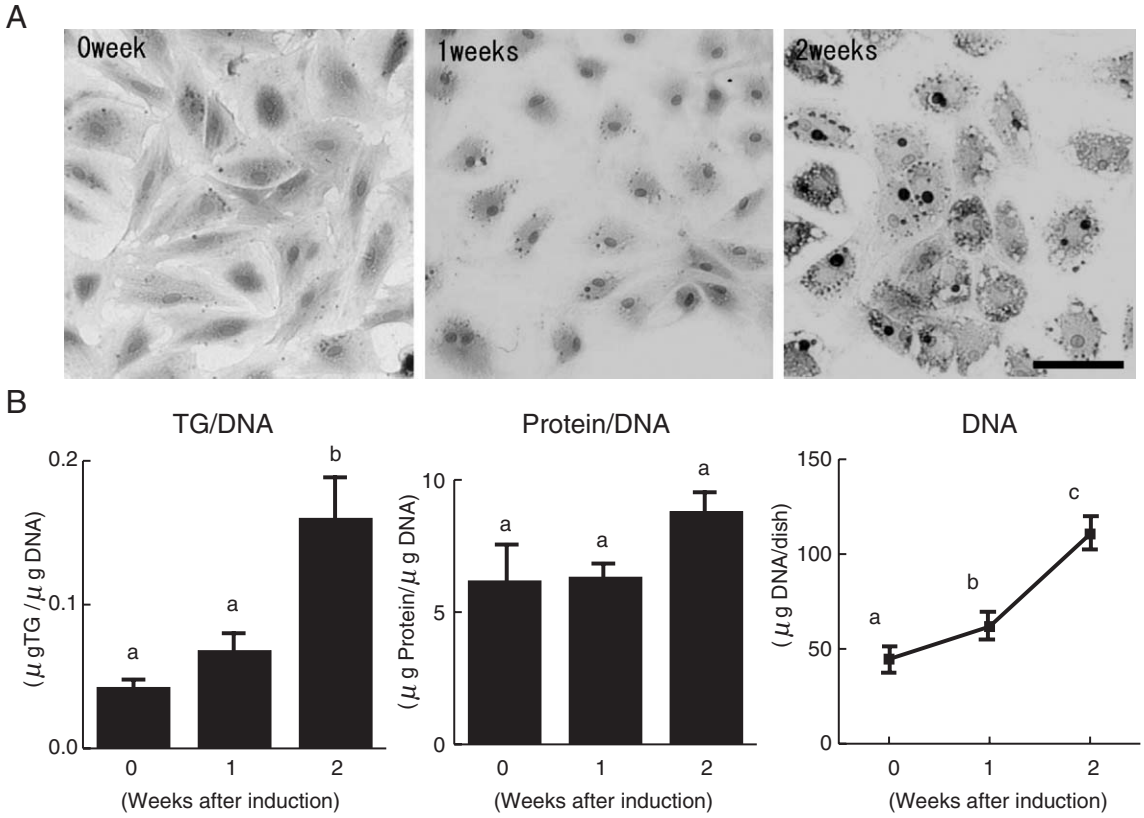

Fig. 1. The lipid accumulation in the cytoplasm (A) and changes in the amounts of triglyceride, protein and DNA (B) during the terminal differentiation of red sea bream SV cells. The red sea bream SV cells were cultured for 2 weeks in the FA(−) induction medium containing 5 μg/mL bovine insulin. (A) The cells were fixed in 10% formaldehyde and stained with Sudan black B and Nuclear fast red. The bar indicates 50 μm. (B) The experiment was repeated six times with isolated cell cultures derived from six different fish. Means (mean±SEM) not sharing a common superscript are significantly different ($P<0.05$).