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Fucoxanthin attenuates fatty acid-induced lipid accumulation in FL83B hepatocytes through regulated Sirt1/AMPK signaling pathway

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ABSTRACT

The fucoxanthin, isolated from brown algae, was reported to have multiple biological functions to anti-inflammation, anti-tumor, and ameliorated obesity in mice. In this study we investigated whether fucoxanthin could inhibit lipids accumulation in FL83B hepatocytes. FL83B cells were induced as fatty liver cell model by 0.5 mM oleic acid for 48 h, and treated with various concentration of fucoxanthin for 24 h. The results demonstrated that fucoxanthin significantly suppressed lipid accumulation and decreased lipid peroxidation in hepatocytes. Fucoxanthin could decrease lipogenesis-related transcription factor expression, including sterol regulatory element-binding proteins 1c and peroxisome proliferator-activated receptor γ. It also reduced fatty acid synthase expression and increased adipose triglyceride lipase and the phosphorylation of hormone-sensitive lipase production for lipolysis. Furthermore, fucoxanthin significantly increased phosphorylation of AMP-activated protein kinase (AMPK), and decreased activity of acetyl-CoA carboxylase for regulating fatty acid synthesis. The results suggest that fucoxanthin is an effective marine nature compound for increasing lipolysis and inhibiting lipogenesis in oleic acid induced fatty liver cells through promoted Sirt1/AMPK pathway.

1. Introduction

Obesity is currently a global health problem, and excessive lipid accumulation could cause more chronic diseases such as hyperlipidemia, coronary heart diseases, type 2 diabetes and non-alcoholic fatty liver disease (NAFLD) [1]. Hepatic steatosis is an initial characteristic and pivotal step in the development of NAFLD [2]. Excessive triglyceride in hepatocytes did not only interfere with energy metabolism, but also cause inflammation, oxidative damage, insulin resistance, even developed as liver fibrosis, and liver cancer [1]. In recent years, studies found that excessive lipid accumulation and fatty acid metabolic abnormalities was an important factor to induce obesity and NAFLD, and metabolic disorders of lipid was also closely associated with insulin resistance and metabolic syndrome [3]. Hence, blocking excessive lipid accumulation of hepatocytes is most effective way for improved progression from NAFLD to nonalcoholic steatohepatitis.

Triglycerides are composed of glycerol and fatty acids, and studies demonstrated that fatty acid and triglyceride synthesis contained more multiple and complex pathways [4]. Transcriptional regulation of hepatic lipogenesis including sterol regulatory element-binding protein 1c (SREBP-1c), CCAAT/enhancer-binding protein (C/EBP) and peroxisome proliferator-activated receptor

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(PPAR) can activate gene expression of fatty acid synthesis for exacerbated lipid accumulation in hepatocytes [5]. Free fatty acid and glucose could induce those transcription factors for lipogenic genes expression for involving multiple various phosphatases and kinases to regulate lipogenesis [6]. Additionally, in hepatocytes, if excess triglycerides were catalysed by lipolytic enzymes to accelerate the decomposition of lipid oil droplets, this metabolic characteristic would improve the development of NAFLD [1]. Therefore, blocking the transcription factor of lipid synthesis and promoting the enzyme expression for triglyceride decomposition will have the opportunity to prevent and attenuate hepatic steatosis and NAFLD.

AMP-activated protein kinase (AMPK) was an important key sensor to regulate cellular energy status, and AMPK activation also regulated lipid and carbohydrate metabolism of hepatocytes [7]. Numerous studies demonstrated that AMPK activation suppressed acetyl-CoA carboxylase (ACC) for blocked the effect of fatty acid synthase (FAS) and decreased lipid accumulation on hepatocytes [8]. AMPK activation also increased the activation of fatty acid β-oxidation to decompose fatty acids for reducing the incidence of hepatic steatosis in NAFLD patients [9].

Fucoxanthin is an orange red color carotenoid pigment, can be isolated from marine diatoms (Bacillariophyta) and brown seaweeds (Phaeophyceae) [3]. In recent years, many studies demonstrated that fucoxanthin had antioxidant, anti-inflammatory and anti-tumor effects [10]. Fucoxanthin also was found that could decrease lipid accumulation in 3T3-L1 adipocyte cells by increasing the phosphorylation of AMPK and ACC [11]. However, it is not clear whether fucoxanthin regulates lipid metabolism in hepatocytes. In this study, we would investigate whether fucoxanthin regulated lipogenesis, lipolysis, and AMPK pathway in oleic acid-induced FL83B hepatocytes.

2. Materials and methods

2.1. Chemical reagent

Fucoxanthin (purity ≥95% by HPLC) was used for this study, and purchased from Sigma-Aldrich (St. Louis, MO, USA). Fucoxanthin dissolved in DMSO at stock concentrations of 100 mM, and the final concentration of DMSO in culture medium was ≤0.1% in all experiments. 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR, an AMPK activator) and compound C (AMPK inhibitor) were also purchased from Sigma-Aldrich.

2.2. Cell culture and induced fatty liver cells

The FL83B cell line obtained from the Bioresource Collection and Research Center (BCRC, Taiwan). FL83B cells were grown in F12K medium supplemented with 10% foetal bovine serum (FBS)(Biological Industries, Haemek, Israel) and 100 mg/L penicillin and streptomycin at 37 °C in a humidified atmosphere of 5% CO2. Cells induced lipid accumulation in hepatocytes using 0.5 mM oleic acid (OA) for 48 h, then 3–100 μM fucoxanthin were treated for 24 h for all experiment analysis.

2.3. Cell viability assay

FL83B cells seeded on 96 well culture plate, and treated with various concentrations of fucoxanthin for 24 h, then the plate added 5 mg/ml MTT solution (Sigma) for 4 h as previously described [12]. Washed the plate and added isopropanol to dissolve purple formazan crystals, and detected absorbance value by spectrophotometer (Multiskan FC, Thermo, Waltham, MA, USA) at 570 nm.

2.4. Hepatic lipid accumulation and lipoperoxidation

FL83B cells incubated with 0.5 mM oleic acid for 48 h, then fucoxanthin treated with cells for 24 h. Cells washed and fixed with 10% formalin, then cells stained with BODIPY 493/503 (Invitrogen, Carlsbad, CA, USA) for evaluated lipid accumulation. Furthermore, cells stained with BODIPY 581/591C11 (Invitrogen) for evaluated hepatic lipoperoxidation. DAPI stained nuclei and observed the results using fluorescence microscopy (Olympus, Tokyo, Japan).

2.5. Oil red O staining

FL83B cells seeded on 6 well culture plate, and incubated with 0.5 mM oleic acid for 48 h, then fucoxanthin treated with cells for 24 h. Cells washed and fixed with formalin and oil droplets stained using Oil Red O solution, as previously described [13]. The hepatocyte oil droplets observed using microscopy (Olympus). Next, culture plates treated with isopropanol to measure lipid accumulation by microplate reader (Multiskan FC, Thermo Fisher Scientific), and recorded absorbance values at 490 nm.

2.6. Malondialdehyde (MDA) activity assay

FL83B cells were seeded on 6 well culture plate, and incubated with 0.5 mM oleic acid for 48 h, then fucoxanthin treated with cells for 24 h. Next, cells were lysed and MDA activity was determined as previously described [14]. In brief, we used a lipid peroxidation (MDA) assay kit (Sigma) to evaluate MDA activity, according to the manufacturer's instructions. MDA activities were detected by a multi-mode microplate reader (BioTek SynergyHT, Bedfordshire, United Kingdom).

2.7. Western blot analysis

Equal amounts of protein were separated by 8–10% sodium dodecyl sulfate–polyacrylamide gels, and transferred into polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). The membranes were washed and blocked with 5% FBS in TBST buffer (150 mM NaCl, 10 mM Tris-HCl pH 8.0, 0.1% Tween 20) for 1 h, and then the membranes were incubated specific primary antibodies overnight at 4 °C. In this experiment used primary antibodies including: pACC-1, ACC-1, PPAR-α, PPAR-γ, phosphorylated hormone-sensitive lipase (pHSL), HSL, and adipose triglyceride lipase (ATGL) (Epitomics, Burlingame, CA, USA); pAMPKα, AMPK, FAS (Santa Cruz, CA, USA); sirtuin 1 (Sirt1) (Millipore); SREBP-1c, carnitine palmitoyltransferase 1 (CPT-1), and CPT-2 (Cell Signaling Technology, MA, USA), and β-actin (Sigma). Next, the membranes were washed and incubated with secondary antibodies, and specific protein signals were presented with Luminol/Enhancer solution (Millipore) and protein expressions were detected using the BioSpectrum 600 system (UVP, Upland, CA, USA).

2.8. Statistical analysis

Statistical method used one-way ANOVA and Dunnett's post-hoc test. The results were presented as mean ± standard deviation, and *P* values less than 0.05 were statistically significant.

3. Results

3.1. Cell viability and cytotoxicity of fucoxanthin in FL83B cells

The cytotoxicity of fucoxanthin in FL83B cells were determined by MTT method, and fucoxanthin had no significant effect on cell viability at concentrations ≤100 μM (Fig. 1A). Therefore,

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