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Docosahexaenoic acid inhibits insulin-induced activation of sterol regulatory-element binding protein 1 and cyclooxygenase-2 expression through upregulation of SIRT1 in human colon epithelial cells

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ABSTRACT

Multiple lines of compelling evidence from clinical and population-based studies support that hyperinsulinemia often accompanying obesity-associated insulin insensitivity promotes colon carcinogenesis. Insulin can acetylate, thereby activating sterol regulator element-binding protein 1 (SREBP-1), a prime transcription factor responsible for expression of genes involved in lipogenesis. Moreover, SREBP-1 upregulates cyclooxygenase-2 (COX-2), a key player in inflammatory signaling. Docosahexaenoic acid (DHA), a representative omega-3 polyunsaturated fatty acid, has been known to negatively regulate SREBP-1, but the underlying molecular mechanism is not fully clarified yet. This prompted us to investigate whether DHA could inhibit insulin-induced activation of SREBP-1 and COX-2 expression in the context of its potential protective effect on obesity-induced inflammation and carcinogenesis. SIRT1, a NAD^+-dependent histone/non-histone protein deacetylase, has been reported to inhibit intracellular signaling mediated by SREBP-1 through deacetylation of this transcription factor. We found that DHA induced SIRT1 expression in CCD841CoN human colon epithelial cells. DHA abrogated insulin-induced acetylation as well as expression of SREBP-1 and COX-2 upregulation. Insulin-induced stimulation of CCD841CoN cell migration was also inhbited by DHA. These effects mediated by DHA were attenuated by pharmacologic inhibition of SIRT1. Hyperinsulinemia or insulin resistance is considered to be associated with obesity-associated inflammation. Genetically obese (*ob/ob*) mice showed higher colonic expression levels of both SREBP-1 and COX-2 than did normal lean mice. Likewise, expression of SREBP-1 and COX-2 was elevated in human colon tumor specimens compared with surrounding normal tissues. In conclusion, DHA may protect against obesity-associated inflammation and colon carcinogenesis by suppressing insulin-induced activation of SREBP-1 and expression of COX-2 through up-regulation of SIRT1.

1. Introduction

Hyperinsulinemia is a well-known risk factor for obesity-induced colon carcinogenesis [1]. Obese subjects often exhibit hyperinsulinemia as a consequence of insulin resistance [2,3]. It has been reported that the insulin receptor is overexpressed in various types of cancer cells, implying that obesity-associated hyperinsulinemia can triger the oncogenic signal transduction

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[4]. In line with this notion, insulin directly promotes growth of aberrant crypt foci as well as tumors in the colon of rats [5,6]. Thus, hyperinsulinemia is likely to link obesity and colon cancer.

In obesity-induced hyperinsulinemia, insulin activates sterol regulatory element-binding protein 1 (SREBP-1), a prime transcription factor responsible for expression of genes involved in lipogenesis [7,8]. SREBP-1 upregulated under conditions of overnutrition linked to obesity enhances lipogenesis [9]. The expression level of SREBP-1 is also elevated in cetain types of tumors [10,11]. In addition, SREBP-1 has been reported to upregulate cyclooxygenase-2 (COX-2), a key enzyme implicated in the inflammation-associated carcinogenesis, through direct binding to the promoter of *COX-2* gene [12]. These findings suggest that SREBP-1 can coordinately mediate insulin-induced proinflammatory and oncogenic events assoiated with obesity.

Several molecular mechanisms have been proposed to explain insulin-induced activation of SREBP-1. First, insulin increases mRNA and protein levels of SREBP-1. Second, insulin stimulates nuclear accumulation of SREBP-1 [13]. Insulin also enhances the binding affinity of SREBP-1 for its sterol response element (SRE) motif, resulting in potentiation of SREBP-1 transcriptional activity [14]. Notably, insulin-induced acetylation of SREBP-1 is crucial for its transcriptional activity as well as stabilization [15].

Docosahexaenoic acid (DHA) is a representative omega-3 polyunsaturated fatty acid abundant in fish oil [16]. DHA is well known for its anti-obesity and anti-carcinogenic effects [17,18]. It has been reported that DHA improves insulin resistance and suppresses insulin-induced inflammatory responses through inhibition of NF-κB signaling [19,20]. Moreover, DHA can attenuate insulin-induced activation of SREBP-1, thereby repressing transcription of lipogenic genes [21].

SIRT1 has been reported to deacetylate and consequently inhibit insulin-induced activation of SREBP-1 [22]. Here, we report that SIRT1 could mediate the inhibitory effect of DHA on insulin-induced activation of SREBP-1 signaling in human colon epithelial cells and their migration via deacetylation of SREBP-1.

2. Materials and methods

2.1. Reagents

DHA was purchased from Cayman Chemical Co. (MI, USA). Sirtinol, nicotinamide and insulin were obtained from Sigma–Aldrich (MO, USA).

2.2. Animals

Male C57BL/6J and *ob/ob* mice (5 weeks of age) were purchased from Central Laboratory Animal Inc. (Seoul, South Korea). All the animals were maintained at the Animal Facility of Seoul National University according to the Institutional Animal Care Guidelines. Animal experimental procedures were approved by the Institutional Animal Care and Use Committee at Seoul National University.

2.3. Human colon tissue samples

All the specimens were obtained from Gil Medical Center (Incheon, South Korea). The samples included 6 pairs of colon tumor and surrounding tissues for Western blot analysis. All investigations were conducted in accordance with the ethical guidelines of the Helsinki Declaration (1898).

2.4. Cell culture

CCD841CoN cells were maintained routinely in modified Eagle's medium (MEM) supplemented with 10% fetal bovine serum and a 100 ng/ml antibiotics mixture at 37 °C in a humidified atmosphere of 5% CO_2/95% air. The cells were plated at an appropriate density according to each experimental scale.

2.5. Western blot analysis

Lysates from cells or tissues were separated by running through 6–10% SDS-PAGE gel and transferred to the PVDF membranes. Antibodies included SIRT1 (Abcam, Cambridge, UK), Nrf2 (Santa Cruz Biotechnology Inc., CA, USA), actin (Sigma–Aldrich), lamin B1 (Invitrogen, CA, USA), α-tubulin (Santa Cruz Biotechnology Inc.), COX-2 (Thermo Fischer Scientific, CA, USA), and SREBP-1 (Santa Cruz Biotechnology, Inc.). The membranes were probed with horseradish peroxidase-conjugated secondary antibodies (Invitrogen).

2.6. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from CCD841CoN cells by using TRIzol® reagent (Invitrogen), and RT-PCR was conducted according to standard procedures. The primer sequences were as follows: *SIRT1* 5′-TCA GTG TCA TGG TTC CTT TGC-3′ (forward) and 5′-AAT CTG CTC CTT TGC CAC TCT-3′ (reverse); *actin* 5′-CCC CAG GCA CCA GGG CGT GAT-3′ (forward) and 5′-GGT CAT CTT CTC GCG GTT GGC CTT GGG GT-3′ (reverse).

2.7. Immunoprecipitation

Cellular proteins (500 μg) were subjected to immunoprecipitation by shaking with the SREBP-1 antibody at 4 °C for 12 h, followed by the addition of protein G-agarose bead suspension and additional shaking for 2 h at the same condition. After centrifugation at 10,000 × *g* for 1 min, immunopricipitated beads were collected by discarding the supernatant and washing with cell lysis buffer. After final washing, the immmunoprecipitate was resuspended in 40 μl of 2× electrophoresis sample buffer and boiled for 5 min. Supernatant from each sample was collected after centrifugation and loaded on SDS-PAGE.

2.8. Electrophoretic mobility shift assay (EMSA)

EMSA was performed by using a DNA–protein binding detection kit (Gibco®, Invitrogen) according to the manufacturer's protocol. The double stranded DNA probe containing the SIRT1 gene antioxidant response element (ARE) sequence 5′-GGA GGC GAA GTC ATT TCC TT-3′ was labeled with [γ-^{32}P]ATP by using T4 polynucleotide kinase (Elpis Biotech. Inc., Seoul, South Korea). Nuclear extracts (10 μg) were incubated with 0.1 mg/ml sonicated salmon sperm DNA and binding buffer [10 mM Tris–HCl (pH 7.5), 100 mM NaCl, 1 mM dithiothreitol (DTT), 1 mM EDTA and 4% (v/v) glycerol] on ice. To determine the sequence specificity of the Nrf2-*SIRT1* ARE binding, the excess amount of the cold probe was added. After 20-min incubation at room temperature, 2 μl of 0.1% bromophenol blue was added, and the samples were electrophoresed through 6% non-denaturing polyacrylamide gel at 130 V in a cold room. Finally, the gel was dried and exposed to an X-ray film.

2.9. Immunocytochemistry

Cells grown in 4-well chamber slides were fixed in 95% methanol/5% acetic acid solution for 5 min at −20 °C. After a rinse with PBS, the cells were blocked for 1 h and incubated with an antibody against Nrf2 or SIRT1 overnight. After an additional incubation with FITC-conjugated secondary antibody, the cells were further stained with propidium iodide (PI, Molecular Probes®, Invtrogen) for the nucleus, and analyzed under a confocal microscope.