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Short communication

Repressive effects of oat extracts on intracellular lipid-droplet formation in adipocytes and a three-dimensional subcutaneous adipose tissue model

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ABSTRACT

We assessed the repression of lipid-droplet formation in mouse mesenchymal stromal preadipocytes OP9 by specified oat extracts (Hatomugi, *Coix lacryma-jobi* var. *ma-yuen*) named "SPH" which were proteolytically and glucosyl-transferredly prepared from finely-milled oat whole-grain. Stimulation of OP9 preadipocytes with insulin-containing serum-replacement promoted differentiation to adipocytes, concurrently with an increase in the intracellular lipid droplets by 51.5%, which were repressed by SPH-bulk or SPH-water-extract at 840 ppm, to 33.5% or 46.9%, respectively, but not by SPH-ethanol-extract at the same dose, showing the hydrophilic property of the anti-adipogenetic ingredients. The intracellular lipid droplets were scanty for intact preadipocytes, small-sized but abundant for the SPH-unadministered adipocytes, and large-sized but few for SPH-bulk-administered adipocytes being coexistent with many lipid-droplet-lacking viable cells, suggesting "the all-or-none rule" for lipid-droplet generation in cell-to-cell. Hydrogen-peroxide-induced cell death in human epidermal keratinocytes HaCaT was prevented by SPH-bulk at 100 or 150 ppm by 5.6–8.1%, being consistent with higher viabilities of SPH-bulk-administered OP9 cells, together with repressions of both cell shrinkage and cell detachment from the culture substratum. In three-dimensional subcutaneous adipose tissue models reconstructed with HaCaT-keratinocytes and OP9-preadipocytes, lipid droplets were accumulated in dermal OP9-cell-parts, and repressed to 43.5% by SPH-bulk at 840 ppm concurrently with marked diminishment of huge aggregates of lipid droplets. Thus SPH-bulk suppresses adipogenesis-associated lipid-droplet accumulation during differentiation of OP9 preadipocytes together with lowered cytotoxicity to either HaCaT keratinocytes or the preadipocytes.

1. Introduction

For therapies of obesity-associated metabolic syndromes, physiological conditions including oxidative stress are considered as a crucial cause of adipogenesis [1,2]. Recent investigations have shown that reactive oxygen species (ROS) is increased during adipocyte differentiation, whereas antioxidant enzyme system inhibits it [3,4]. Oat extracts can act as adjuvant therapeutics for obesity, body fat, lipids, and lipoproteins along with the liver metabolic regulation and lipid clearance in vivo [5]. Accordingly, we have examined whether antioxidants inhibit intracellular adipogenesis [6–9]. It is found that the oat extracts have an antioxidant activity [10]. Based on these findings, we hypothesized that the redox state control by oat extracts on adipocytes was a potentially effective strategy against lipid accumulation preceded by adipogenic differentiation. Bone marrow-derived mouse stromal cells OP9 can propose a useful model for adipogenesis due to rapid differentiation into adipocytes [11]. In the present study, we examined the anti-adipogenic activity of the specified oat extract SPH in OP9 cells or three-dimensional (3D) subcutaneous adipose tissue models.

2. Materials and methods

2.1. Preparation of the oat extract [Super Hatomugi (oat): SPH]

The SPH was a processed food provided by HABA Laboratories Inc. (Tokyo), which was prepared by the procedures of (a)–(d): (a) whole-grain of oat (Hatomugi, *Coix lacryma-jobi* var. *ma-yuen*) was finely-milled to make effective utilization of polyphenols and thereafter (b) facilitated for the absorption of proteins by hydrolytic degradation to free amino acids by 98%, then (c) enzyme-treated to convert carbohydrates to isomaltooligosaccharides for intestinal regulation and low-

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calorie, (d) subsequently condensed, pasteurized and dried, which was designated as SPH-bulk powder. The SPH-bulk powder was extracted with water or ethanol and applied in this study as SPH-water extract or SPH-ethanol extract.

2.2. Cell culture

Mouse mesenchymal stromal pluripotent cells OP9 [12] were obtained from Riken BioResource Center (RCB1124; Ibaraki, Japan). OP9 preadipocytes were cultured at a low cell density to adopt a spindly morphology and poorly differentiate into adipocytes without any treatments. Human skin epidermal keratinocytes HaCaT were kindly provided by Prof. Norbert E. Fusening [13].

2.3. In vitro reconstruction of a 3D-subcutaneous adipose tissue model and application of SPH on it

We developed a 3D-subcutaneous adipose tissue model to serve as a living subcutaneous adipose tissue substitute in experiments [9]. OP9 cells of 4.0×10^5 cells/well were mixed with collagen type I (Cellmatrix type I-A, Nitta Gelatin, Inc., Osaka, Japan) and added in a cell culture insert set in 24-well cell culture plates, and incubated at 37 °C in 95% humidified air and 5% CO_2 for 5 days in a state submerged in Dulbecco's modified Eagle's medium (DMEM; Nissui Pharmaceutical Co. Ltd.) supplemented with 10% FBS. A suspension of HaCaT keratinocytes of 3.5×10^5 cells/well was added to the surface of the collagen-preadipocyte layer and cultured in a state submerged in DMEM/Ham's F12 (1:1, Nissui Pharmaceutical Co. Ltd.) medium supplemented with 5% FBS and 15% KnockOut™ serum replacement containing 1.7 μM of insulin (SR; Life Technologies Japan, Ltd., Tokyo). On the next day, the spent medium was replaced by DMEM/Ham's F12 (1:1) medium supplemented with 1% FBS and 20% SR. The SPH-bulk powder was dissolved in the medium and administered in the medium and on surface of a 3D-subcutaneous adipose tissue model according to the procedure of adipogenic differentiation [14]. After 4 days, the surface of the skin equivalent was exposed to air to promote keratinocyte differentiation and stratum-corneum formation, and cultured for 14 days in DMEM/Ham's F12 (1:1) medium supplemented with 20% SR and SPH-bulk powder. The medium was replaced every second day. The cryosections (5 μm in thickness) of a 3D-subcutaneous adipose tissue model were stained with Oil Red O and hematoxylin dyes separately, and then observed using a Hoffman modulation contrast microscope (ECLIPSE Ti, Nikon Corp., Tokyo). The amounts of lipid droplets in the dermis were assessed semi-quantitatively as Oil Red O-stained densities for three microphotographic areas using an ImageJ software (National Institute of Health, Bethesda, MD, USA).

2.4. Application of the SPH to OP9 cells

OP9 preadipocytes were stimulated to differentiate to adipocytes by serum replacement (SR) containing insulin of 1.7 μM as a final concentration, and Oil Red O staining was employed to determine the amount of intracellular lipid droplets [8,11,15,16]. OP9 cells of 2.0×10^4 cells/well were seeded in 24-well cell culture plates (Becton, Dickinson and Co., BD Falcon™, NJ, USA) and incubated at 37 °C in 95% humidified air and 5% CO_2 for 24 h. Then, the spent medium was replaced by alpha-Modified Eagle Minimum Essential Medium (α-MEM; Nissui Pharmaceutical Co. Ltd., Tokyo) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Biological Industries Ltd., Kibbutz Beit-Haemek, Israel) and 2 mM L-glutamine (Wako Pure Chemical Industries, Ltd.,

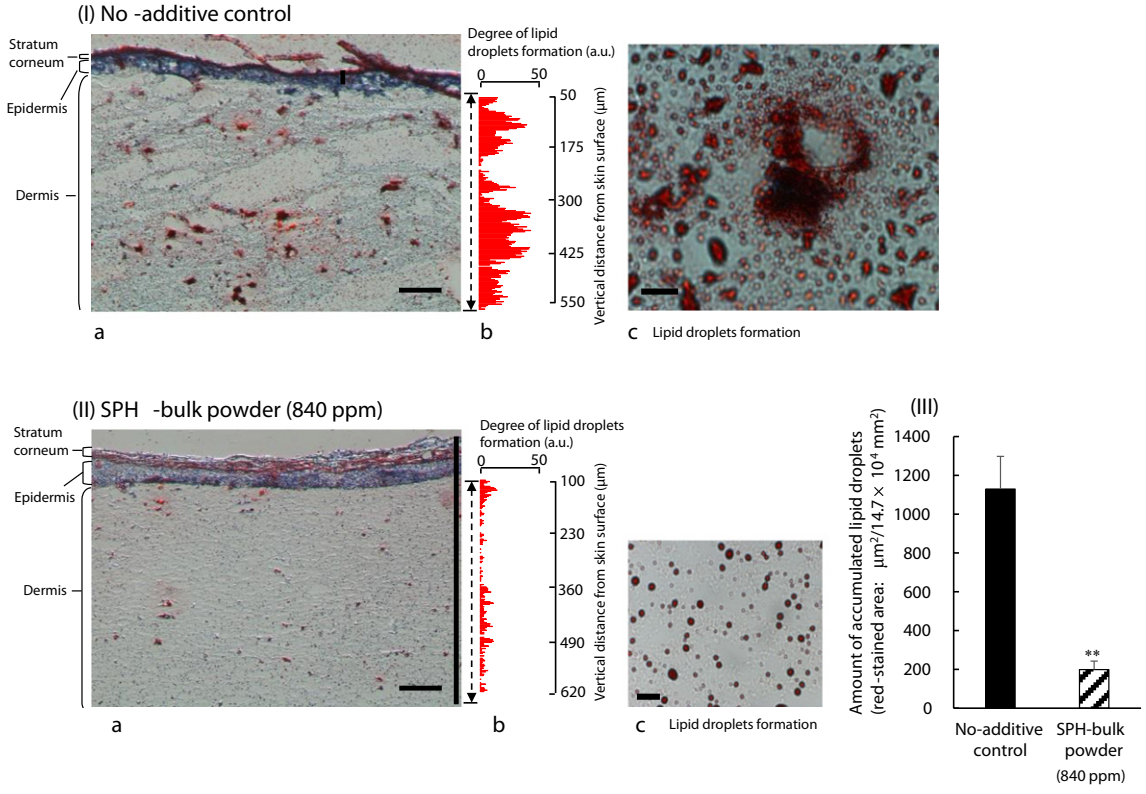

Fig. 1. Inhibitory effects of oat extracts 'Super Hatomugi (SPH)' on intracellular lipid droplet accumulation in a three-dimensional (3D) subcutaneous adipose tissue model as assessed by a), c) Hoffman modulation contrast microscopy. The 3D-subcutaneous adipose tissue models, consisting of HaCaT keratinocytes in stratum corneum and epidermis, and OP9 preadipocytes in dermis, were stained separately with Oil Red O and hematoxylin dyes. The typical area of each test was shown as line profiles of b) vertical section, and (III) amounts of accumulated lipid droplets were assessed semi-quantitatively as red-stained densities for five microphotographic areas in the cell-differentiated (II) and no-additive control (I) or the SPH-administered cells using an ImageJ software. a) Scale bars = 100 μm, magnification: ×100. c): Scale bars = 10 μm, magnification: ×1000. (III) Mean ± SD, n = 3, $^{**}p < 0.01$ (vs. no additive control, unpaired Student's *t*-test).