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Dossier

Grape seed procyanidin B2 inhibits adipogenesis of 3T3-L1 cells by targeting peroxisome proliferator-activated receptor γ with miR-483-5p involved mechanism

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ABSTRACT

Procyanidins have lipolysis effect on adipose metabolism, but the underlying mechanism is not fully understood. The aim of present study was to examine the effect of grape seed procyanidin B2 (GSP) on the adipogenic differentiation of 3T3-L1 preadipocyte cell line and investigate the underlying mechanism. The results showed that GSP treatment significantly reduced the intracellular lipid accumulation in induced 3T3-L1 cells by targeting miR-483-5p as well as peroxisome proliferator-activated receptor γ (PPARγ). In addition, our results revealed that overexpression of miR-483-5p increased adipogenic differentiation, while inhibition of miR-483-5p reduced the lipid accumulation by suppressing the adipogenic differentiation. Moreover, overexpression of miR-483-5p could reverse GSP's inhibition of adipocyte differentiation as well as increase the level of PPARγ. These results demonstrate that GSP inhibits adipogenesis by targeting PPARγ and suggest this effect might be mediated by miR-483-5p.
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1. Introduction

Obesity is one of the most common global metabolic disorders. The data from WHO demonstrate that more than 1.3 billion adults worldwide are overweight (body mass index from 25 to 30 kg/m^2) and a further 600 million are obese (body mass index ≥30 kg/m^2) [1]. Specifically, the rapid economic development in China since 1980 and the change of living style has resulted in an increased prevalence of overweight and obesity not only in adults but also in children [2–5]. Obesity has been conclusively linked to dyslipidemia, diabetes mellitus, hypertension, some cancers, and osteoarthritis [6,7]. Since obesity is caused by both adipogenesis and adipocyte hypertrophy, inhibition of adipogenesis has been regarded as an effective strategy for preventing obesity. 3T3-L1 preadipocyte cell line is one of the best characterized in vitro systems for unraveling the molecular mechanism during adipogenesis [8].

Procyanidins have been studied heavily due to their benefits for cancer, cardiovascular disease, type 2 diabetes, and ulcerative colitis [9–15]. With the limited number of literatures reported so far, the role of procyanidins on adipogenesis, especially its underlying mechanism, is not fully understood [16–21].

MicroRNAs are small noncoding RNAs that associate with the RNA-induced silencing complex (RISC) and bind target mRNAs with partial complementarity. Some microRNAs have been identified as inducers of adipogenesis, including miR-148a, miR-125a-3p and miR-483-5p [22,23]. The miRWalk Database identified the down regulation of procyanidins on miR-483's expression in pancreatic islets [24].

This present study was designed to investigate the putative effect of GSP on the adipogenesis of 3T3-L1 cells and unravel the underlying mechanism by finding some mediated candidate.

2. Material and methods

2.1. Reagents and materials

This study protocol was approved by the Institutional Review Boards of Zhejiang Provincial People's Hospital, Hangzhou. GSP, (CAT No. 29106-49-8, Lot No. HP104375, with purity ≥98% analyzed using high performance liquid chromatography (HPLC) and LC–MS), was purchased from Hangzhou Neway Chemical Co., Ltd., Hangzhou, China. The miR mimics, mimic-NC, cholesterol-conjugated 2′-*O*-methyl-modified mimics, agomir-NC, antagomir,

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and antagomir-NC were purchased from RiboBio (RiboBio, Guangzhou, China). The antibodies of PPARγ and β-actin were purchased from Cell Signaling Technology (Danvers, MA). Lipofectamine RNAiMAX was purchased from Invitrogen (Carlsbad, CA, USA).

2.2. Cell cultures, treatment and transfection

3T3-L1 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM; GIBCO-BRL, Grand Island, NY, USA) containing 10% calf serum (CS; GIOCO-BRL) and antibiotics (100 U/mL penicillin and 100 ug/mL streptomycin; GIBCO-BRL). We seeded 5×10^5 cells in each well in six-well plate. To induce adipocytic differentiation, two days after reaching 100% confluence (Day 0), the cells were exposed to culture medium containing 0.5 mM 3-isobutyl- methylxanthine (IBMX; Sigma), 1 uM dexamethasone (Sigma) and 1 ug/ml insulin (Bovine; Sigma) (MDI hormonal cocktail) for 2 days (Day 2). The medium was replaced with a fresh complete medium containing insulin with 10% FBS and the cells were incubated for another 2 days (Day 4). Thereafter, until the cells were fully differentiated, medium with 10% FBS were changed every other day.

We used various concentration of GSP (dissolved in deionized water) (10–200 ug/ml) for screening the optimal concentration for 3T3-L1 cells. Based on its effect on adipogenesis, 150 ug/ml GSP was chosen as the optimum concentration in the following experiment. 3T3-L1 cells were treated with 150 ug/ml GSP for 48 h at day 0. Cells were fed with complete medium without antibiotics 24 h before transfection. miR-483-5p mimics were transfected when the cells were approximately 60–70% confluence and cultured for 72 h before harvesting. Cells were transfected with either agomir or antagomir of miR-483-5p 24 h before they reached 100% confluence. After the treatment and transfection for 72 h, the medium was replaced by the corresponding medium with hormonal cocktail.

2.3. Glycerol-3-phosphate dehydrogenase (G3PDH) assay

On day 8 of differentiation, culture medium was removed. 3T3-L1 adipocytes were rinsed twice with DPBS and lysed in enzyme extraction buffer. An equal volume of dilution buffer supplemented with β-mercaptoethanol was added and the substrate solution was added into each 96-well plate. After incubated at 30 °C, 25 ul of diluted sample was added and the absorbance was read at 340 nm at 30 °C with a spectrophotometer (Fluostar Optima; BMG Labtech, Offenburg, Germany).

2.4. RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from cells using Trizol (Invitrogen) according to the manufacturer's instructions. cDNA synthesis was carried out with a Reverse Transcription Kit (Life Technologies, Carlsbad, CA, USA). Briefly, 1 ug of RNA was used for reverse transcription into cDNA. Real-time quantitative RT-PCR analysis was performed using the LightCycler (Roche) and SYBR RT-PCR Kits (Takara). The relative expression level of mRNA was normalized to the internal control β-actin while the relative expression level of miRNA was normalized to U6 in each sample using the previous described method [25]. The sequence of primers are as followings: mPPARγ sense (5′-TGTCGGTTTCAGAAGTGCCTTG-3′), antisense (5′-TTC— AGCTGGTCGATATCACTGGAG-3′); mC/EBPα sense (5′-GTTAGCCATGTGGT- AGGAGACA-3′), antisense (5′-GTTAGC-CATGTGGTAGGAGACA-3′); mC/EBPβ sense (5′-ACGACTTCCTCTCC-GACCTCT-3′), antisense (5′-ACG- ACTTCCTCTCCGACCTCT-3′); mLPL sense (5′-GGGAGTTTGGCTCCAGAGT- TT-3′), antisense (5′-TGTGTCTTCAGGGGTCCTTAG-3′); maP2 sense (5′-AGCATCA-TAACCCTAGATGGCG-3′), antisense (5′-CATAACACATTCCA- CCAC-CAGC-3′) and mβ-actin sense (5′-GCATTGTTACCAACTGGGAC-3′), antisense (5′-CATCACAATGCCTGTGGTAC-3′).

2.5. Oil red O staining and quantitation

Cells were washed twice in DPBS solution, fixed in 4% formaldehyde for 30 min, and washed three times with water. Then, cells were stained with Oil red O (Sigma) for 15 min. Following three washes in water, the lipid droplets were observed and photographed under a microscope (TE2000-E; Nikon, Japan). Oil Red O positive areas were quantified as previously described [26].

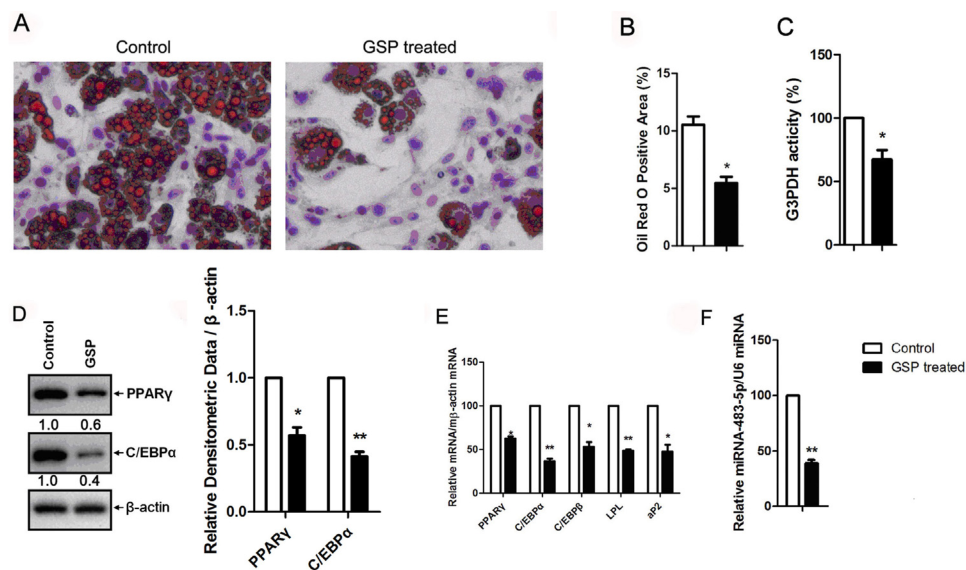

Fig. 1. Inhibition of adipogenesis, downregulation of PPARγ and differentiation makers and miR-483-5p by GSP. (A) Oil Red O staining was performed 6 days after induction of differentiation with MDI hormonal cocktail. (B) Quantitation of Oil Red O staining. (C) G3PDH activity assay. (D) Western Blotting of indicated proteins and its densitometry and statistical analysis result. (E) The expression level of adipogenic markers was measured by qRT-PCR. (F) qRT-PCR analysis was performed to determine the miR-483-5p level. All results represent mean ± SD (n = 3). *P < 0.05, **P < 0.01 compared with control group, respectively.

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