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GSK126 alleviates the obesity phenotype by promoting the differentiation of thermogenic beige adipocytes in diet-induced obese mice

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ABSTRACT

A close relationship between epigenetic regulation and obesity has been demonstrated in several recent studies. Histone methyltransferase enhancer of Zeste homolog 2 (Ezh2), which mainly catalyzes trimethylation of histone H3K27 to form H3K27me3 was found to be required for the differentiation of white and brown adipocytes in vitro. Here, we investigated the effects of the Ezh2-specific inhibitor GSK126 in a mouse model of obesity induced by a high-fat diet (HFD). We found that GSK126 treatment reduced body fat, improved glucose tolerance, increased lipolysis and improved cold tolerance in mice by promoting the differentiation of thermogenic beige adipocytes. Moreover, we discovered that GSK126 inhibited the differentiation of white adipocytes, and the decrease of Ezh2 enzymatic activity and H3K27me3 also changed the morphology of brown adipocytes but did not alter the expression of thermogenic genes in these cells. Our results indicated that GSK126 was a novel chemical inducer of beige adipocytes and may be a potential therapeutic agent for the management of obesity. Furthermore, they also prompted that Ezh2 and H3K27me3 play different roles in the differentiation of the white, brown, and beige adipocytes in vivo.

1. Introduction

The incidence of obesity, which is related to excessive energy intake without adequate energy expenditure, has been increasing rapidly both in developed and developing countries [1]. Because obesity is associated with insulin resistance, cardiovascular disease, and cancer [2–4], it is necessary to improve our understanding of the pathogenesis and treatment strategies, besides diet and exercise, for this condition.

In mammals, there are three types of adipocytes: white adipocytes, classical brown adipocytes [5], and beige adipocytes [6]. White adipocytes have a single large lipid droplet with triglycerides (TGs) inside and store excess energy. In contrast, brown adipocytes contain multiple small lipid droplets and mitochondria expressing uncoupling protein 1 (Ucp1), which diminishes the proton gradient and uncouples oxidative phosphorylation during ATP synthesis [7], leading to energy dissipation by consumption TGs and/or glucose [8,9]. Beige adipocytes located within white adipose tissue (WAT) have similar morphology and function with classical brown adipocytes and can be induced by cold exposure and some chemical reagents; this phenomenon is called white fat browning [9]. Classical brown adipocytes and beige adipocytes can both relieve obesity and glucose intolerance in rodent models and humans by producing heat. Notably, so-called brown adipocytes found in adult

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humans are mainly composed of beige adipocytes [10]; thus, beige adipocytes have become an attractive therapeutic target for counteracting obesity and type 2 diabetes [9], and their differentiation and inducing reagents have become a major focus of researchers in this field.

Recently, researchers have found that some epigenetic factors contribute to the differentiation of adipocytes; for example, enhancer of Zeste homolog 2 (Ezh2), the enzymatic subunit of polycomb repressive complex 2, mainly catalyzes trimethylation of histone H3K27 to form H3K27me3 [11,12], was found to be required for the differentiation of both white and brown adipocytes in vitro [13,14]. In a study by Ferrari and colleagues, a chemical inhibitor of histone deacetylases, MS-275, was used to attenuate diet-induced obesity by white fat browning in mice [15]. However, it is unclear whether biochemical inhibitors of Ezh2 could modulate obesity.

Therefore, in this study, we evaluated the effects of GSK126, a potent, highly selective small-molecule inhibitor of Ezh2 methyltransferase activity, which decreases global H3K27me3 levels [16], in obese mice fed a high-fat diet (HFD). Our results provided important insights into the mechanisms of beige adipocytes differentiation in mice and revealed the potential therapeutic effects of GSK126 in diet-induced obesity.

2. Materials and methods

2.1. Animal care and treatment

C57BL/6J male mice were housed in a pathogen-free facility at the The Air Force Medical University with a 12-h light/dark cycle and free access to standard irradiated rodent chow (5% energy from fat; XieTong Organism, China) until reaching 6 weeks of age. The diet was then changed to an HFD (60% energy from fat; D12492i; Research Diets, USA). After 18 weeks, mice were randomized into two groups (n = 9 per group), matched for body weights (Fig. 1A). Mice were treated with the same volume of vehicle (20% Captisol; HY-17031; MedChemExpress, USA; Control group) or GSK126 (50 mg/kg; S7061; Selleck Chemicals, USA; GSK126 group) intraperitoneally once a day for 10 days. During the treatment, food intake was measured every day, and body weights were measured every other day. Fecal lipids were collected and tested as previously described [17]. After 10 days of treatment, for fasting blood glucose and glucose tolerance tests, mice were fasted for 16 h, and blood from tail vein was tested before and at 30, 60, 90, and 120 min after intraperitoneal injection of glucose (1.0 g/kg). For cold challenge experiments, rectal temperatures were measured before and every hour after housing in a 4 °C environment with an electronic thermometer for 5 h. During the cold challenge, mice had free access to food and water. After anesthetizing animals, blood from the heart was collected to prepare plasma for enzyme-linked immunosorbent assays (ELISAs), and tissues were collected for morphology and molecular biology experiments. All experiments were approved by the Air Force Medical University Animal Ethics Committee, China.

2.2. Histological analysis

Epididymal, mesenteric, subcutaneous white adipose tissues and interscapular brown adipose tissues were fixed, dehydrated, embedded in paraffin, sliced (3-μm-thick sections), and stained with hematoxylin and eosin (HE). Images were taken at 200 × or 400 × magnification.

2.3. Immunohistochemistry

Sections of WAT and BAT were deparaffinized, and antigen retrieval was performed with a pressure cooker in citric acid-sodium citrate buffer for 2 min. Endogenous peroxidase activity was blocked with 3% H_2O_2 for 15 min. Antigen blocking was performed with 5% goat serum. Anti-Ucp1 antibodies (1:300; ab10983; Abcam, USA) were applied overnight at 4 °C, and sections were then incubated with biotinylated secondary antibodies (SP9001; ZSGB-BIO, China). Histochemical reactions were performed using diaminobenzidine.

2.4. Western blotting and ELISAs

Total proteins were extracted using RIPA buffer containing a protease inhibitor cocktail according to the manufacturer's instructions, and proteins were then separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis on 8% or 15% gels and transferred to polyvinylidene difluoride membranes. Membranes were then blocked with 5% nonfat dry milk in 1 × phosphate-buffered saline with Tween20. The following primary antibodies were used: anti-H3K27me3 (1:1000; ab192985; Abcam), anti-Ucp1 (1:1000; ab10983; Abcam), and anti-β-tubulin (1:1000; KM9003T; SUNGENE BIOTECH, China). Horseradish peroxidase-conjugated secondary antibodies (1:5000; ZDR-5306, ZDR-5307; ZSGB-BIO) were used for detection with chemiluminescence reagents (WBKLS0100; Millipore, USA).

ELISAs was carried out strictly according to the manufacturer's instructions (Mouse Total Cholesterol ELISA Kit: ml037202; Mouse TG ELISA Kit: ml0377871; Mlbio, China).

2.5. Real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNA was isolated from tissues using RNAiso Plus (9108; Takara, Japan). cDNA synthesis was performed with PrimeScript RT Master Mix (RR036A; Takara). Real-time qPCR was performed using TB Green Premix Ex Taq II (RR820A; Takara) on a CFX96 Real-Time PCR System (C1000 Touch Thermal Cycler; Bio-Rad, USA). Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method and was normalized to the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The PCR program was as follows: initial denaturation at 95 °C for 5 s, followed by 45 cycles of denaturation at 95 °C for 5 s and annealing at 60 °C for 30 s. Primers were designed and synthesized by Takara Company (Table 1).

2.6. Statistical analysis

Data are presented as means ± standard deviations (SDs). Values were analyzed by two-tailed independent-sample Student's t tests or paired t tests using SPSS16.0 or GraphPad Prism 5.0. Differences with P values of less than 0.05 were considered statistically significant.

3. Results

3.1. Treatment with GSK126 alleviated the obese phenotype and improved glucose tolerance in mice

After 18 weeks of feeding the HFD, mice were randomized into two groups and treated with vehicle or GSK126 once a day for 10 days. Starting from the sixth day of treatment, mice in the GSK126 group showed significantly decreased body weights compared with those before treatment (Fig. 1D; $p < 0.05$). On day 10, mice in the GSK126 group showed lower body weights than those in the control group (Fig. 1D; $p < 0.05$), without significant differences in food intake nor in fecal lipid excretion (Fig. 1B and C; $p > 0.05$), indicating that these mice showed similar energy absorption.

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