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DNA methylation and histone deacetylation regulating insulin sensitivity due to chronic cold exposure

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ABSTRACT

In this study, we investigated the causal relationship between chronic cold exposure and insulin resistance and the mechanisms of how DNA methylation and histone deacetylation regulate cold-reduced insulin resistance. 46 adult male mice from postnatal day 90–180 were randomly assigned to control group and cold-exposure group. Mice in cold-exposure group were placed at temperature from -1 to 4 °C for 30 days to mimic chronic cold environment. Then, fasting blood glucose, blood insulin level and insulin resistance index were measured with enzymatic methods. Immunofluorescent labeling was carried out to visualize the insulin receptor substrate 2 (IRS2), Obese receptor (Ob-R, a leptin receptor), voltage-dependent anion channel protein 1 (VDAC1), cytochrome C (cytC), 5-methylcytosine (5-mC) positive cells in hippocampal CA1 area. Furthermore, the expressions of some proteins mentioned above were detected with Western blot. The results showed: ① Chronic cold exposure could reduce the insulin resistance index ($P < 0.01$) and increase the number of IRS2 positive cells and Ob-R positive cells in hippocampus ($P < 0.01$). ② The expressions of mitochondrial energy-relative proteins, VDAC1 and cytC, were higher in cold-exposure group than in control group with both immunohistochemical staining and Western blot ($P < 0.01$). ③ Chronic cold exposure increased DNA methylation and histone deacetylation in the pyramidal cells of CA1 area and led to an increase in the expression of histone deacetylase 1 (HDAC1) and DNA methylation relative enzymes ($P < 0.01$). In conclusion, chronic cold exposure can improve insulin sensitivity, with the involvement of DNA methylation, histone deacetylation and the regulation of mitochondrial energy metabolism. These epigenetic modifications probably form the basic mechanism of cold-reduced insulin resistance.

1. Introduction

Chronic cold exposure could be the root-cause for some diseases, such as skin freezing and some respiratory and cardiovascular diseases [9]. Therefore, it is of great importance to understand the risks of cold exposure for people's health. Certainly, mitochondrial energy metabolism is involved in body's response to chronic cold exposure [4], and energy production has a very close relationship with glucose metabolism, which is regulated through leptin signaling pathway and insulin pathway [36]. When animal is exposed to cold, there is an increase in the secretion of adrenal corticosteroids and thyroid hormone. These hormones will accelerate cellular energy metabolism, glycogenolysis and gluconeogenesis [2]. In addition, some proteins of glucose metabolism, such as glucagon, epinephrine, norepinephrine and growth hormone, will increase in body as well [6]. Cold-induced gluconeogenesis is

Abbreviations: CpG, cytosine-phosphate-guanine; cytC, cytochrome C; Dnmt1, DNA methyltransferase 1; DNMT3a, DNA methyltransferase 3a; FPG, fasting plasma glucose; FINS, fasting serum insulin; HDAC1, histone deacetylase 1; HOMA-IR, Homeostasis model assessment for insulin resistance; HRP, horseradish peroxidase; IRS2, insulin receptor substrate 2; JAK2, Janus kinase 2; JAK2-STAT3, JAK2-signal transducer and activator of transcription 3; 5-mC, 5-methylcytosine; MAPK, mitogen-activated protein kinase; MBPs, Methyl binding protein; MBD2, methyl CpG binding domain 2; Ob-R, Obese receptor; OD, Optical density; STZ, streptozotocin; VDAC1, voltage-dependent anion channel protein 1.

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most likely due to newly-released adrenergline interacting with norepinephrine and its receptor [46], which is conducive to energy production. No doubt, the energy production needs proper mitochondrial functions to maintain normal body temperature. Studies have showed that some mitochondrial relative proteins, such as voltage-dependent anion channel protein 1 (VDAC1) and cytochrome C (cytC), participate in energy metabolism [48,55], since they can increase the uptake of glucose and cause changes in blood glucose level [1]. Insulin resistance is an important consideration for diabetes, and high insulin resistance usually is a risk factor of type II diabetes [41]. Insulin resistance is also used to evaluate glucose and energy metabolisms, since blood glucose can be regulated by insulin [34]. For instance, high insulin resistance usually leads to high blood glucose due to decreased glucose utilization. A study showed that cold exposure could improve glucose turnover and insulin sensitivity in rats, and IRS (insulin receptor substrate)-1, -2, ERK (extracellular signal-related kinase), serine (Akt, protein kinase B) phosphorylation in insulin signaling cascade probably were involved in the processes [25].

DNA methylation and histone deacetylation are two types of key epigenetic modifications in eukaryotic cells. Heavy metal exposure, smoking, alcohol consumption and chronic cold exposure can alter DNA methylation and histone deacetylation [13,38]. However, the relationship between cold exposure and insulin resistance should be interesting issue and needs further elucidation [7], moreover, how epigenetic modifications regulate cold reduced-insulin resistance is not certain. In this study, with chronic cold exposure animal model, we worked to further reveal how cold stress affects the insulin resistance, and the regulations of DNA methylation and histone deacetylation to energy metabolism and insulin resistance were investigated as well. Our study will make valuable contribution to further knowledge in cryomedicine.

2. Materials and methods

2.1. Animal and grouping

All experiments were carried out in accordance with the institutional guidelines of Henan University for animal welfare. Adult male C57BL/6J mice (25–30 g) at postnatal day 90–180 (P90-180) were housed with a 12 h light: 12 h dark cycle at 20–25 °C. Mouse cages were prepared for animal housing with sawdust litter, standard food and adequate clean water. The mice were randomly divided into control group and cold exposure group (treatment group), with more than 20 cases (survival mice) in each group. To decrease the mortality from sudden exposure at −1–4 °C, pre-exposure at 10 °C was carried out for treatment group. After pre-exposure for 1 week, the treatment animals were cold-exposed at −1–4 °C for 30 days according to previous studies [15,25]. During cold exposure, one cage was prepared for each experimental mouse in order to avoid mutual contact for warmth. The animals' behaviors and mortality were recorded. The control animals were housed under standard conditions at 20–25 °C. Finally, both control and treatment animals were harvested, and anesthetized with sodium pentobarbital (20 mg/kg, i.p.). Then the mice were perfused transcardially with 4% paraformaldehyde (pH 7.2). After brains were removed from the skull, immersion fixation was carried out at 4 °C for 1–2 days. Total 46 adult male mice were used in the study.

2.2. Insulin resistance index analysis

The mice in both control group and treatment group were fasted for 12 h. After anesthesia, the eyeballs of mice were removed, and 1000 μl blood was collected, then, blood plasma was centrifuged at 3000 rpm for 20 min. With chemical assay, the concentrations of blood glucose (fasting plasma glucose, FPG) and blood insulin (fasting serum insulin, FINS) were tested with insulin ELISA kit. According to protocol, blood serum sample, various reagents and horseradish peroxidase (HRP) conjugated antibody were added successively. After washing thoroughly, 3, 3′, 5, 5′-Tetramethylbenzidine (TMB) was added to visualize for colorimetry. Optical density (OD) value was then measured at 450 nm wavelength with microplate reader. Finally, insulin was calculated according to the standard curve from standards sample. Moreover, with the parameters, such as FPG and FINS concentration, insulin resistance index (Homeostasis model assessment for insulin resistance, HOMA-IR) was calculated according to the formula, HOMA-IR = FINS × FPG/22.5. In the study, 8 cases were used in either control group or treatment group.

2.3. Immunohistochemistry

Coronal sections of hippocampus were cut and rinsed with 0.01 mol/L PB. Then nonspecific antigens were blocked with 10% normal goat serum (with 0.3% Triton X-100, 1% BSA in 0.01 M PB) for 30 min. The slices were incubated with primary antibodies at 4 °C overnight. After rinsing three times, the slices were incubated with secondary antibodies for 3 h at room temperature. The following was the primary antibodies used in this experiment: ① the antibodies for insulin metabolism proteins: rabbit polyclonal anti-Obese receptor (Ob-R) antibody (1:500, Santa Cruz, SC1834); rabbit monoclonal anti-IRS2 antibody (1:200, Abcam, ABl34101) ② the antibodies for mitochondrial proteins: rabbit polyclonal anti-VDAC1 antibody (1:500, Abcam, AB15895); mouse monoclonal anti-cytC antibody (1:300, Invitrogen, 456100); ③ the antibodies for epigenetic modifications: rabbit polyclonal anti-DNA methyltransferase 3a (Dnmt3a) antibody (1:500, Abcam, AB4897); rabbit polyclonal anti-DNA methyltransferase 1 (Dnmt1) antibody (1:200, Abcam, AB19905); mouse monoclonal anti-5-methylcytosine (5-mC) antibody (1:200, Abcam, AB10805); rabbit polyclonal anti-histone deacetylase 1 (HDAC1) antibody (1:800, Abcam, AB53091); rabbit monoclonal anti-methyl CpG binding domain 2 (MBD2) antibody (1:250, Abcam, AB109260). Corresponding second antibodies: donkey anti-mouse IgG Alexa Fluor 568 (1:600, Invitrogen, A10037); donkey anti-rabbit IgG Alexa Fluor 488 (1:500, Invitrogen, A21206). Sections were coverslipped with medium (65% glycerol in 0.01 M PB + 1:1000 DAPI for counterstaining). After that, cells were imaged with an epifluorescent microscope (BX61; Olympus) under rhodamine, fluorescein isothiocyanate (FITC) or ultraviolet excitation. High quality sections were photographed with an Olympus laser confocal microscope (FV1000; Olympus).

2.4. Western blot assay

To further verify the results from immunocytochemistry, the expressions of Ob-R, IRS2, VDAC1, cytC, DNMT3a, Dnmt1, HDAC1 and MBD2 in hippocampus tissues were investigated using Western blot technique. Mice were sacrificed with cervical dislocation. The brains were quickly stripped and hippocampal tissue was isolated. The proteins were extracted with the cytoplasmic protein extraction kit (Beyotime Institute of Biotechnology, P0027). With BCA assay, total protein concentrations were measured according to standard curve. Then, they were subjected to electrophoresis and transferred to membranes. The primary antibodies, such as rabbit polyclonal anti-Ob-R antibody (1:1000, Santa Cruz, SC1834); rabbit monoclonal anti-IRS2 antibody (1:1000, Abcam, ABl34101); rabbit polyclonal anti-VDAC1 antibody (1:2000, Abcam, AB15895); mouse monoclonal anti-cytC antibody (1:2000, Invitrogen, 456100); rabbit polyclonal anti-Dnmt3a antibody (1:2000, Abcam, AB4897); rabbit polyclonal anti-Dnmt1 antibody (1:1000, Abcam, AB19905);