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Genome-wide transcriptional changes in type 2 diabetic mice supplemented with lotus seed resistant starch

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ABSTRACT

Resistant starch has been studied extensively for its hypoglycemic activity, while its underlying molecular mechanism is not fully understood. In this study, we investigated the hypoglycemic effect of different doses of lotus seed resistant starch (LSRS) supplementation on type 2 diabetic mice and elucidated the molecular basis of its hypoglycemic effect. LSRS supplementation significantly reduced blood glucose level by 16.0%–33.6%, recovered serum insulin level by 25.0%–39.0% and improved lipid metabolism disorder in the diabetic mice. The genome-wide expression patterns in pancreatic tissue were analyzed, and 511 differentially expressed genes (DEGs) were identified. The analysis results of gene ontology (GO) functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways indicated that the protective effect of LSRS supplementation was most likely driven by modulating expression levels of various key factors involved in insulin secretion, insulin signal transmission, cell apoptosis, antioxidant activity and p53 signaling pathways.

1. Introduction

Globally, the incidence of diabetes mellitus (DM) has increased more than 13-fold since the early 1980s, and has become one of the most concerned public health challenges and economic burden of the twenty-first century (Fernandes et al., 2016). DM occurs due to insufficient secretion or inefficient processing of insulin and thus is broadly classified into type 1 (T1DM) and type 2 DM (T2DM), with the latter accounting for around 90% of DM (Lysy, Corritore, & Sokal, 2016). Insulin resistance (IR) and pancreatic β-cell dysfunction are the important factors characterized in T2DM, which lead to persistent hyperglycemia in the T2DM population (Kokil, Veedu, Ramm, Prins, & Parekh, 2015). Although existing treatments, such as oral hypoglycemic drugs and insulin, could alleviate some of the complications of T2DM, the effective control of this chronic disease at the population level remains unsolved.

Dietary and lifestyle modifications may delay and manage the onset of T2DM (O'Brien et al., 2017; Parillo & Riccardi, 2004). Moreover, the use of healthy natural products with certain antidiabetic properties can be an alternative approach for the preferable long-term strategy for T2DM treatment with minimum or no side-effects (Ramachandran, Xiao, & Xu, 2017; Zhao, et al., 2018; Zhao, et al., 2018). Among these natural products, resistant starch (RS), which escapes digestion in the small intestine of healthy individuals and is completely or partially fermented in the colon (Englyst, Kingman, & Cummings, 1992), has been studied extensively for its weight control and hypoglycemic activity (Bodinham et al., 2014; Wang et al., 2015; Zhou, Wang, Ren, Wang, & Blanchard, 2015).

In recent years, a significant body of research has been devoted to the preparation of RS from various starch crops (Lertwanawatana, Frazier, & Niranjan, 2015; Morales-Medina, Del Mar Munio, Guadix, & Guadix, 2014; Xia, Li, & Gao, 2016), easily replacing regular starch by RS in various food products to decrease the glycemic index (GI) and enhance the health functions, especially for control of diabetes (Ahmed & Urooj, 2015; Menon, Padmaja, & Sajeev, 2015). Lotus (*Nelumbo nucifera* Gaertn.) seeds with high content of amylose starch (~40%, w/w) are a valuable source for RS production (Zeng et al., 2015; Zhang et al., 2015; Zhang, Zeng, Wang, Zeng, & Zheng, 2014). Previously, lotus seed resistant starch (LSRS) was prepared by autoclaving, ultrasonic-autoclaving and microwave-moisture methods, and their structural characteristics and physicochemical properties were extensively studied (Zeng et al., 2015). Unlike soluble fibers with viscous properties within

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the gastrointestinal tract, RS is one of insoluble fibers and thus have no effect on glucose absorption (Robertson, Bickerton, Dennis, Vidal, & Frayn, 2005).

Growing evidence has shown that RS supplementation offers benefit to human health by manipulating gut microbiota, improving glucose and lipid metabolism and reducing insulin resistance (Harazaki, Inoue, Imai, Mochizuki, & Goda, 2014; Hu et al., 2016; Nichenametla et al., 2014). T2DM is linked to metabolic abnormalities within the liver and pancreas. Recent reports demonstrated that the diet containing high level of RS could prevent or manage diabetes in experimental animals by manipulating the expression of hepatic genes involved in lipid and glucose metabolism (Choi, Gwon, Ahn, Jung, & Ha, 2013; Zhou et al., 2015). Some other studies investigated the potential of RS in modulating pancreatic function by improving the glucose intolerance and insulin resistance, which contribute to its hypoglycemic effect (Robertson et al., 2005). T2DM is a result of chronic insulin resistance and pancreatic β-cell dysfunction, which result in poorly regulated blood glucose level (Wang, Zhao, Yang, Wang, & Kuang, 2016). The possible molecular mechanism of β-cell dysfunction related to T2DM mainly focuses on the oxidative stress, endoplasmic reticulum stress and glucolipotoxicity (Prentki & Nolan, 2006). However, there is limited information about how LSRS supplementation regulates the global gene expression in the pancreatic tissue in type 2 diabetic mice.

RNA-seq is a new efficient and comprehensive method for whole transcriptome sequencing, and is mainly applied for quantitative gene expression analyses of various biological processes in a tissue or cell (Li et al., 2016; Liu, Lian, Liu, Zhou, & Yang, 2016). RNA-seq provides a cost-effective way to achieve large amounts of transcriptome data with accurate quantization and higher repeatability. Therefore, RNA-seq has been used as a potential tool to provide useful and detailed information on the molecular mechanisms, unknown biological pathways and networks of a certain disease, and may contribute to the identification of novel treatments (Tonne et al., 2013).

The present study evaluated the effect of the LSRS supplementation on type 2 diabetic mice, and genome-wide analyses were performed in pancreatic tissues of experimental animals with and without LSRS treatment using RNA-seq method.

2. Materials and methods

2.1. Materials and reagents

The dried lotus (*Nelumobo nucifera* Gaertn.) seeds were purchased from local market, ground and sieved. The resulting lotus seed powder was used to prepare lotus seed resistant starch (LSRS) using the ultrasonic-autoclaving method as previously reported (Zeng et al., 2015).

Streptozotocin (STZ) was purchased from Sigma (St. Louis, USA). All other chemical reagents used in this study were of analytical grade.

2.2. Animals and diets

A total of 30 healthy male Kunming mice weighting 25.0 ± 2.0 g were obtained from Shanghai laboratory animal center (Shanghai, China), and housed at a controlled temperature (23 ± 2 °C) and humidity (60 ± 10%) under a 12/12 h light-dark cycle. During the experiment, the mice were free to access to diet and water. Type 2 diabetic mice were induced by high-fat diet (HFD) and streptozotocin (STZ) injection as previously described (Zheng et al., 2016). After one week of adaptive feeding, the mice were fed with high-fat diet (basal diet 63.6%, cholesterol 1.2%, sodium salt 0.2%, egg yolk powder 10%, sucrose 15%, and lard 10%). After 4 weeks, the mice were randomly divided into 5 groups: NC (normal control group, basal diet); DC (diabetic model control, basal diet); RS5 (diabetic mice, basal diet containing 5% of LSRS); RS10 (diabetic mice, basal diet containing 10% of LSRS) and RS15 (diabetic mice, basal diet containing 15% of LSRS). Except for the mice in the NC group, the other mice received intravenous injection of fresh STZ solution (40 mg/kg body weight). The fasting blood glucose levels were measured by an One-Touch Ultra blood glucose meter (LifeScan, Milpitas, CA, USA) after 7 days. The mice with blood glucose level ≥12.0 mmol/L were considered diabetic and used for further experiments (Lomas-Soria et al., 2015). All procedures involving the experimental animals in this study were in accordance with the Guidelines for the Care and Use of Laboratory Animals, and approved by the Animal Care Review Committee, Fujian Agriculture and Forest University, China.

2.3. Biochemical analysis

During the experiment, the fasting blood glucose levels were monitored and recorded weekly. The initial and final body weights of the mice were measured and recorded. At the end of experiment, blood from eyeball was collected, and the serum was immediately separated from part of blood sample by centrifugation (3000 rpm, 4 °C, 10 min). Serum insulin levels were measured using an insulin ELISA kit (Xinyu Biotechnology Co., Shanghai, China), and blood lipids composition, including the levels of high-density lipoprotein-cholesterol (HDL-c), total cholesterol (TC) and triglyceride (TG) were measured with corresponding kits (Jiancheng Bioengineering Institute, NanJing, China).

2.4. RNA isolation, library construction and sequencing

To survey the genome-wide transcriptional changes in the pancreatic tissues of type 2 diabetic mice supplemented with LSRS, three pancreatic tissue samples were obtained from the mice in DC group (used as control) (CK-1, CK-2 and CK-3) to compare with another three samples from RS10 group (RS-1, RS-2 and RS-3). After the mice were sacrificed, the pancreatic tissues were immediately collected and frozen in liquid nitrogen, and stored at −80 °C before homogenizing for total RNA extraction. Total RNA was extracted and purified from the frozen pancreatic tissues, and then quantitated and subjected to quality inspection as previously described (Li et al., 2016). Afterwards, six RNA samples were packed in dry ice and delivered to Gene Denovo Biotechnology Co. (Guangzhou, China) for further library construction and sequencing on an Illumina HiSeq 2500 platform.

2.5. Raw data processing and alignment analysis

To obtain high-quality clean reads, raw reads containing adapters or more than 10% of unknown nucleotides and low quality reads containing more than 50% of low quality bases were removed from the raw data. Meanwhile, short reads alignment tool Bowtie2 was used for mapping reads to ribosome RNA (rRNA) database, and the rRNA mapped reads were removed (Langmead & Salzberg, 2012). The clean reads were mapped to the reference genome (Ensembl release 80 GRCm38) using TopHat2 (version 2.0.3.12) with default parameters (Dobin & Gingeras, 2013). The reconstruction of transcripts was carried out with software Cufflinks (Trapnell et al., 2012).

2.6. Identification of DEGs

Gene abundances were quantified using the software RSEM (Li & Dewey, 2011), and normalized by using the FPKM (Fragments Per Kilobase of transcript per Million mapped reads) method. Subsequently, the differentially expressed genes across samples were identified by using the edgeR package (http://www.r-project.org/). Genes were identified with absolute value of log2 (fold change) ≥1 and a false discovery rate (FDR) < 0.05 in a comparison as significant DEGs. DEGs were then subjected to enrichment analysis of gene ontology (GO) functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.