Comparative Liver Transcriptome Analysis on Kunming mice with Streptozotocin and Natural Food Induced Type 2 Diabetes Mellitus

YANJUN WU
Guizhou University Of Traditional Chinese Medicine
XIANGXING ZHU (ZHU_XIANGXING@126.COM)
Foshan University Medical College https://orcid.org/0000-0002-5265-2620
Arome Solomon Odiba
Guangxi University
Zisheng LIN
Foshan University Medical College
JIANCONG WEN
Foshan University Medical College
DAOYUAN GONG
Foshan University Medical College
JING LIANG
Guangxi University
Shuguang WU
Guizhou University Of Traditional Chinese Medicine
Ganqiu LAN
Guangxi University

Research Article

Keywords: Type 2 diabetes mellitus (T2DM), Streptozotocin (STZ), Transcriptome analysis, Kunming mice, Animal model

Posted Date: July 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-673596/v1

Abstract

Background: Streptozotocin is a classic drug used to induce diabetic animal models.

Objective: The aim of this study is to investigate the liver transcriptome of diabetic Kunming mice induced by streptozotocin (STZ) or Non-STZ.

Methods: Forty male mice were randomly assigned into 4 groups, namely Control (Ctr, standard diet), mHH (high fat and high carbohydrate diet), mHS (high fat and high carbohydrate diet for 4 weeks followed by 60 mg/kg STZ for 3 consecutive days) and mSH (60 mg/kg STZ for 3 consecutive days and then fed with high fat and high carbohydrate diet for 12 weeks). All mice injected with STZ were identified as diabetes despite the sequential feeding of high fat and high carbohydrate diet.

Results: Only 7 of 13 mice in mHH group met the diagnostic criteria for diabetes. The FBG of mHH, mHS, mSH and Ctrl groups were 13.27 ± 1.14, 15.01 ± 2.59, 15.95 ± 4.38 and 6.28 ± 0.33 mmol/L at 12th week, respectively. Compared with mHH group, the transcriptions of 85 genes were elevated in liver of mHS mice while 21 genes were down-regulated, and 97 genes were shown to be up-regulated in mSH group while 35 genes were decreased. Total 43 co-expressed genes were identified in mHSvsmHH and mSHvsmHH groups. GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) analyses showed that there were 2 corporate GO terms and 2 KEGG pathways significantly annotated in STZ-treated groups. Both the GO term and pathway were related to the metabolism mediated by p53.

Conclusion: High fat and high carbohydrate diet combined with low dose of STZ can effectively induce diabetic models in Kunming mice despite the abnormal expressions of genes in the liver. The differentially expressed genes were related to the metabolism mediated by p53.

Introduction

Diabetes mellitus (DM) is a metabolic diseases characterized by hyperglycemia and caused by a combination of environmental and genetic factors[1]. At present, a large number of animal models, which was induced by high fat and high carbohydrate diet, drug or transgenic technology, are used in the field of T2DM research[2,3]. However, the high cost and strict technical requirements have hinder the wide use of transgenic induction technology. On the contrary, the induction of diabetes in models by diet and drug is commonly accepted by scientific researchers because of the strong stability, low cost and typical symptoms of diabetes[4,5]. At present, the most commonly used chemical agents for inducing diabetes in animal models include STZ and four alloxan. Compare to four alloxan, STZ can specifically destroy pancreatic cells and exhibit less toxicity[6]. Studies have shown that, in the various induction strategies, the combination of high fat and high carbohydrate diet with low dose of STZ is an ideal method for establishment of T2DM with its short modeling time and stable clinical symptoms of diabetes[7,8].

Streptozotocin is isolated from Streptomycetes achromogenes synthetic and is widely used to induce both T1DM and T2DM animal models[9]. Streptozotocin features important biological characteristics as

demonstrated by its antibiotic and toxicity to β-cell, as well as the oncogenic effects[10]. Due to the specific toxicity associated with pancreatic β-cells, STZ is currently accepted as an investigational drug for inducing diabetic animal models. The application of STZ to induce diabetes models include simple STZ injection (a high dose injection or multiple low dose injection), high fat and high carbohydrate diet combined with STZ injection or nicotinamide combined with STZ injection [11–13]. However, A large number of studies have shown that a high dose of STZ injection (> 65 mg/kg) can lead to massive pancreatic islet beta cells damage. Multiple low doses of STZ injections (40–55 mg/kg) can destroy part of islet beta cells through the immune mechanism, leading to the defective insulin secretion, and result in diabetes[14,15].

Kunming mice are the most widely used experimental mouse strain in pharmacology and genetically related studies in China, which are derived from Swiss mice[16,17]. Kunming mice exhibit many advantages such as large and frequent litters, high disease resistanceas well as rapid growth rates. The diabetic model of Kunming mice induced by STZ has been widely used in the study of diabetes, such as diabetic nephropathy, retinopathy, and in the development of antidiabetic drugs[18,19]. However, the effect of STZ-induced diabetetic animal model in liver gene expressions is unknown. There is also dearth of information in the relationship between human diabetes and STZ. In a previous study, we have compared the liver transcriptome of T2DM in Guangxi Bama Mini-pig induced by STZ or Non-STZ[20]. The purpose of this study is to compare the characteristics of different indcued methods of T2DM animal models, and uncover the alterations at the transcription level of liver genes caused by STZ in Kunming mice.

Materials And Methods

Materials

Forty (40) male Kunming mice (30 ± 3 g) aged 6 weeks were obtained from the Laboratory Animal Center of Guangxi Youjiang Medical University for Nationalities. STZ was purchased from Sigma. Ultra cold storage freezer was purchased from Thermo fisher. Trizol was purchased from Takara, Japan. Roche blood glucose meter and test strip were purchased from by Roche, Germany.

Breeding methods of laboratory animals and experimental groups

Total of 40 Kunming mice were used for the study. Briefly, the mice were randomly assigned into 4 groups, namely, Ctr (n = 7), mHH (n = 13), mHS (n = 10), and mSH (n = 10) groups. The experimental period was 12 weeks. Body weight (BW) and fasting blood glucose (FBG) were measured per week. The Ctr and mHH group were fed with standard diet and high fat and high carbohydrate diet for 12 weeks, respectively. Mice in mHS group were fed with high fat and high carbohydrate diet for 4 weeks followed by 60 mg/kg STZ injection for three days in a row, and then continued with high fat and high carbohydrate diet until 12 weeks. Similarly, the mSH group was injected with 60 mg/kg STZ for three consecutive days and then fed with high fat and high carbohydrate diet for 12 weeks. Food and water

were administered ad libitum. The STZ was dissolved in sodium citrate buffer (0.1 mol/L, pH 4.4–4.5) at a concentration of 150 mmol/L and immediately intraperitoneally injected by sterile syringe filter[21]. The Ctr mice were given equal amounts of sodium citrate buffer according to the weight. All mice were maintained under specific pathogen-free (SPF) conditions according to the People's Republic of China Laboratory Animal Regulations, and the study was approved by the Experimental animal Ethics Committee of Guizhou University of Traditional Chinese Medicine.

The standard diet was obtained from Jiangsu Xietong Pharmaceutical Bioengineering Company, containing 50% nitrogen free extract, 20% crude protein, 10% crude fat, 8% crude ash, 5% crude fiber, 1.8% calcium, 1.2% phosphorous, 1.32% lysine, 0.78% methionine and cysteine. The high fat and high carbohydrate diet was standard formula with 30% sucrose and 10% fat. Over the experimental period the body weight of the mice were measured and statistically compared per week.

Determination of FBG and IGTT

Before the determination of blood glucose, mice were fasted 12 hours and weighed. Weekly Fasting blood glucose (FBG) levels in mmol/L were determined in blood of the tail vein using Roche automatic glucometer. Mice were considered diabetic when FGB level records above 11.1mmol/L [18]. For the glucose tolerance tests, mice were fasted for 8 hours and blood glucose measured at indicated time point (3, 10, 30, 60, 90 and 120 min) following an intraperitoneal injection of glucose (2 mg/g body weight)[22,23].

RNA isolation

At the 12th week of the experiment, all the mice were humanely sacrificed after fasting for 12 hours and weighed. Liver RNA was extracted by Trizol cleavage. The degradation and contamination of RNA was observed on 1% agarose gels. Nano Photometer® spectrophotometer (IMPLEN, CA, USA) was used to check the purity. Five diabetic mice in each group were used for data analysis as biological replicates.

Transcriptome Sequencing

Three microgram (μg) RNA from each sample was used as the original input material. According to the manufacturer's recommendations, the NEB Next® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) was used to generate sequencing libraries with an index code added to each sample. All the sequencing tasks were in order with Illumina Hiseq platform and the paired-end reads (125 bp/150 bp) were generated. Top Hat v2.0.12 was used to aligne the clean reads from the reference genome[24]. The reads numbers which mapped to each gene were count by HTSeq v0.6.1 and thereafter. FPKunming was calculated based on the length and reads count of each gene[25]. Differential genes (DEGs) were identified using the DESeq R package (1.18.0) analysis. The P- value was adjusted by the Benjamini and Hochberg method. Gene was considered as significantly different expression when the corrected P-value of 0.005 and log2 (Fold change) of 1[26]. Functional analysis of differentially expressed genes was performed by GO[27] and Kyoto KEGG[28]. GO terms and KEGG pathways which was enriched by differentially expressed genes were considered significantly when the corrected *P*-value less than 0.05.

Validation of differentially expressed genes by qRT-PCR

The different expressions of genes in mHS vs mHH and mSH vs mHH groups mice were confirmed by qRT-PCR. The quantitative variation was evaluated using $2^{-\Delta\Delta Ct}$ method adjust with β-actin as internal control. Specific primers (Table 1) and SYBR Green real-time PCR master mix were used to amplify cDNA. qRT-PCR was performed in five biological replicates and each sample was conducted in triplicates.

Table 1
Specific primers for qRT-PCR

Gene	Sequences (5′→3′)	Tm (°C)
Zmat3	F: AGCTCAGAGTCACTCATTCTCG	60
	R: GGAGCGGGCATTGAAGTAAGG	
Sesn2	F: AGTGTTCTTACCTGGTGGGTTC	60
	R: GATGCGCCAGTAACTTGTTGAC	
Cdkn1a	F: AGTACTTCCTCTGCCCTGCTG	60
	R: AATCTGTCAGGCTGGTCTGC	
Mdm2	F: ATGTGTTTGGAGTCCCGAGTTTC	60
	R: ATCCTTCAGATCACTCCCACC	
Ccng1	F: ACTGCTACACCAGCTGAACAC	60
	R: AGCTAGGGAAAATGTCTCCGTG	
Cyp4a10	F: GGGAAGCAAGGCCTACTTAAC	60
	R: AGAGCAAACCATACCCAATCC	
β-actin	F: GAGACCTTCAACACCCCAGC	60
	R: CCACAGGATTCCATACCCAA	

Results

Weight comparison

The body weights were shown in Fig. 1. The result indicated that the body weight (g) of all the 4 groups of mice increased with time. Group mHH gained much more weights compared with the STZ-treated groups.

FBG and IGTT

The result showed that FBG levels of STZ-treated mice were higher than non-STZ treated mice (Fig. 2a). Three weeks following STZ injection, FBG rose rapidly in the mSH group and peaked at 8th week. The decline of FBG can be observed and the level is higher than mHS and mHH group until the 12th week. Glucose tolerance test at the 12th week showed that, the glucose disappearance rate of the diabetic groups had reduced compared to the Ctr group, (Fig. 2b).

Quality of RNA

RNA concentration of liver samples in mHH, mHS and mSH groups were all aboved 300 ng/μL and total quantifiations were over 20 μg. The ratio of 260 to 280 nm was 1.8–2.1 (Supplementary Table S1).

Recognition of differentially expressed genes

To identify clusters with functional enrichments, hierarchical clustering was performed based on gene expression patterns (Fig. 3). Gene expressions showed small differences between the two STZ-treated groups. When compared to mHH, 106 DEGs were identified in mHS group (Supplementary Table S2), and 132 DEGs were identified in mSH (Supplementary Table S3). Forty-three co-expressed genes were determined in mHS vs mHH and mSH vs mHH groups (Fig. 4).

Functional analysis of differentially expressed genes using GO and KEGG

GO is an international standard classification system for all species which defines and describes the functions of genes and proteins and can be updated with the latest research. GO is divided into three parts: cellular component; molecular function and biological process. All genes can be assigned into these three main categories. GO analysis of the DEGs were shown in Fig. 5 and Fig. 6. Seven and 16 significantly enriched GO terms in mHSvsmHH and mSHvsmHH animals were identified, respectively. A GO term is considered to be significantly enriched if the corrected p-value is belowed 0.05 (Supplementary Table S4 and Supplementary Table S5). Among these significantly GO terms, “signal transduction by p53 class mediator” (GO:0072331) and “intrinsic apoptotic signaling pathway by p53 class mediator” (GO:0072332) were enriched in mHSvsmHH and mSHvsmHH synchronously. There were 5 up-regulated co-expressive genes in the 2 GO terms, including E3 ubiquitin-protein ligase (Mdm2), Cyclin-dependent kinase inhibitor 1 (Cdkn1a), Apoptosis-enhancing nuclease (Aen), Ectodysplasin A2 receptor (Eda2r) and Pleckstrin homology-like domain family A member 3 (Phlda3).

In the animal models, different genes coordinate with each other to exert biological functions, and the most important biochemical metabolic and signal transduction pathways of differentially expressed genes can be determined through pathway significant enrichments. KEGG is a systematic analysis of gene functions and genome information database, which helps researchers to investigate genes and expression information as a whole network. KEGG analysis showed that “p53 signaling pathway” (mmu04115) and “Retinol metabolism” (mmu00830) were significantly enriched in mHSvsmHH and mSHvsmHH synchronously (Table 2 and Table 3). There were 6 up-regulated co-expressive genes in the 2

KEGG pathways, including E3 ubiquitin-protein ligase (Mdm2), Cyclin-dependent kinase inhibitor 1 (Cdkn1a), zinc finger matrin type 3 (Zmat3), Sestrin-2(Sesn2), Cyclin-G1(Ccng1) and Cytochrome P450 4A10 (Cyp4a10). Cdkn1a and Mdm2 gene were involved in the regulation of p53 in GO and KEGG as up-regulated co-expressive genes.

Table 2
The significantly enriched pathway terms in mHSvsmHH

Term	KEGG ID	Corrected *P*-Value	Input number	Input names
p53 signaling pathway	mmu04115	0.002015	6	*Gtse1, Zmat3, Sesn2, Cdkn1a, Mdm2, Ccng1*
Bladder cancer	mmu05219	0.019351	4	*Cdh1, Cdkn1a, Src, Mdm2*
Retinol metabolism	mmu00830	0.026008	5	*Cyp4a10, Cyp4a31, Rdh9, Ugt2b37, Cyp2c55*

Table 3
The significantly enriched pathway terms in mSHvsmHH

Term	KEGG ID	Corrected *P*-Value	Input number	Input names
p53 signaling pathway	mmu04115	0.00051745	7	*Bax, Zmat3, Sesn2, Cdkn1a, Mdm2, Ccng1, Adgrb1*
Retinol metabolism	mmu00830	0.012533914	6	*Cyp4a10, Cyp4a12b, Cyp4a12a, Ugt2b35, Aox1, Cyp3a44*

Verification of the gene expressions using qRT-PCR

To validate the expression of differentially expressed genes in the transcriptome analysis, the 6 of 43 co-expressive genes (Cdkn1a, Mdm2, Ccng1, Sesn2, Zmat3, Cyp4a10) which were annotated into the GO terms and KEGG pathway of p53 were evaluated by qRT-PCR. The expression patterns of Cdkn1a, Mdm2, Ccng1, Sesn2, Zmat3, Cyp4a10 in the qRT-PCR analysis were consistent with the RNA-Seq analysis (Fig. 7).

Discussion

A reasonable animal model of T2DM is vital for diabetic research. The T2DM induced by a high-fat diet was reported first time in 1947[29], and a large number of scholars have utilized this animal model in diabetes research. Despite the high-fat diet method is significantly suitable for human T2DM disease studies, it requires long induction period and the cost is ineffecitve. To overcome this challenge, a new strategy that combines high fat diet with STZ to induced T2DM in animal models has been adopted and widely used by researchers today. This synergistic effect is based on the concept that, high fat diet induces insulin resistance and the treatment with STZ can destroy islet beta cells, resulting in hyperglycemia. This method shortens the experimental times and improves the efficiency of the

model[30,31]. The application of STZ is so common in animal diabetic models, and previous reports indicate that the type and characteristics of the induced diabetes vary with different doses of STZ and animal species[32–35].

To avoid Type 1 diabetes caused by high dose of STZ, the 60 mg/kg STZ was administered for three consecutive days in the STZ-treated group. All the mice fed with high-fat and high-carbohydrate diet gained more weight while STZ-treated mice had a higher FBG. This is consistent with a report published by Reed of STZ and high fat diet diabetes model in rats[36]. In the present study, two groups of mice were treated with STZ at different time points. Fasting blood glucose of the mice injected with STZ first followed by high fat and high carbohydrate diet (mSH) increased rapidly from 3rd week and started to decline from the 8th week. We speculated that this might be related to the small weight of the mouse when STZ was injected. The development of pancreas in the mouse was in the early stage of birth and the cells were relatively sensitive to STZ. Similarly, a study by Gilbert showed that the combination of high fat diet and three 40 mg/kg STZ injections induced a T2DM model with a dynamic change of FBG in mouse[37].

Streptozotocin caused 43 differentially co-expressive genes in mHS vs mHH and mSH vs mHH groups. Functional classifications of differentially expressed genes using GO and KEGG analysis showed that, compared with no-STZ treatment, STZ caused p53 metabolic abnormalities whether in the GO terms or KEGG pathway. This confirmed the previous studies of the correlation between STZ and tumor genesis[38–41].

Among the differentially co-expressive genes, Cdkn1a, Mdm2 were annotated into the GO terms and KEGG pathway of p53. The p53 tumor suppressor factor was upregulated by DNA repair, cell cycle arrest, anti-angiogenesis, apoptosis and autophagy in response to cell stress[42]. Progression of the cell cycle can be controlled by the genes of the p53 pathway (ARF-Mdm2-TP53- Cdkn1a) when DNA is damaged[43]. This pathway supports the idea that STZ can damage cellular DNA. The Cdkn1a gene is necessary for efficient p53-dependent cell cycle arrest in response to DNA damage and metabolic perturbation, and is therefore a ‘bona fide’ effector of p53[44,45]. The p53 protein binds to a specific site in the Mdm2 gene promoter and activates its transcription. As a negative feedback mechanism, Mdm2 protein can bind to p53 by blocking its ability to activate Mdm2 gene transcription[46]. Consequenently, Mdm2 protein negatively regulates the p53 tumor suppressor protein[47]. In this study, we confirmed the correlation between STZ treatment and p53 metabolism on gene levels for the first time. This provides a reference for the rational application of STZ in diabetic animal models.

Conclusions

High fat and high carbohydrate diet combined with low dose of STZ can effectively induce diabetic models in Kunming mice despite the abnormal expressions of genes in the liver. The differentially expressed genes were related to the metabolism mediated by p53.

Declarations

AUTHOR CONTRIBUTIONS

G.Q.L. and Y.J.W. conceived and designed the experiments; Y.J.W., X.X.Z. and L.J. performed the experiments and analyzed the data; A.S.O., Z.S.L., J.C.W. and D.Y.G. participated in experiment assistant. G.Q.L., Y.J.W., X.X.Z. and S.G.W. drafted the manuscript. All authors participated in discussions of the results and reviewed the manuscript.

Funding Statement

This work was supported by the Science and technology funds of the chairman of the Autonomous Region (No. 16449-10), the Science and Technology Major Special Project of Guangxi (No. Guike-AA17292002), the Guangdong Basic and Applied Basic Research Fund (2019A1515110280), the Foshan Science and Technology Innovation Project (1920001001203) and the Guangdong Science and Technology Innovation Strategy Fund (The Special Fund for “Climbing Plan”; pdjh2020a0616).

Data Availability

The underlying data supporting the results are available from the corresponding authors upon reasonable request.

Supplementary Materials

Supplementary Tables S1, S2 and S3 are provided in the Supplementary Materials File.

CONFLICTS OF INTEREST

The main original data of this study are in the supplemental. No conflicts of interest, financial or otherwise, are declared by the authors.

References

1. Kahn SE, Hull RL, Utzschneider Kunming (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444(7121):840–846
2. Wolf E, Braun-Reichhart C, Streckel E, Renner S (2014) Genetically engineered pig models for diabetes research. Transgenic Res 23(1):27–38
3. Kavanagh K, Flynn DM, Nelson C, Zhang L, Wagner JD (2011) Characterization and validation of a streptozotocin-induced diabetes model in the vervet monkey. J Pharmacol Toxicol Methods 63(3):296–303
4. Xi S, Yin W, Wang Z et al (2004) A minipig model of high-fat/high-sucrose diet-induced diabetes and atherosclerosis. Int J Exp Pathol 85(4):223–231

5. Liu Y, Wang Z, Yin W et al (2007) Severe insulin resistance and moderate glomerulosclerosis in a minipig model induced by high-fat/ high-sucrose/ high-cholesterol diet. Exp Anim 56(1):11–20
6. Emonnot L, Cohen R, Lo M (2007) Neonatal streptozotocin-induced glucose intolerance: different consequences in Lyon normotensive and hypertensive rats. J Hypertens 25(2):429–438
7. Ma H, You GP, Zhang XP et al (2014) A novel role of globular adiponectin in treatment with HFD/STZ induced T2DM combined with NAFLD rats. ScientificWorldJournal 2014:230835
8. Gerrity RG, Natarajan R, Nadler JL, Kimsey T (2001) Diabetes-induced accelerated atherosclerosis in swine. Diabetes 50(7):1654–1665
9. Szkudelski T (2001) The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 50(6):537–546
10. Volk BW, Wellmann KF, Brancato P (1974) Fine structure of rat islet cell tumors induced by streptozotocin and nicotinamide. Diabetologia 10(1):37–44
11. Podell BK, Ackart DF, Richardson MA et al (2017) A model of type 2 diabetes in the guinea pig using sequential diet-induced glucose intolerance and streptozotocin treatment. Dis Model Mech 10(2):151–162
12. Kim JH, Lee DE, Choi SH et al (2014) Diabetic characteristics and alveolar bone loss in streptozotocin- and streptozotocin-nicotinamide-treated rats with periodontitis. J Periodontal Res 49(6):792–800
13. Prathaban S, Gnanaprakasam V (2000) Streptozotocin induced diabetes mellitus in dogs. Indian Veterinary Journal 77(3):219–222
14. Ganda OP, Rossini AA, Like AA (1976) Studies on streptozotocin diabetes. Diabetes 25(7):595–603
15. Katsumata K, Katsumata KJ, Katsumata Y (1992) Protective effect of diltiazem hydrochloride on the occurrence of alloxan- or streptozotocin-induced diabetes in rats. Horm Metab Res 24(11):508–510
16. Nie YW, Zhang P, Zhang J et al (2016) Isolation and characterization of white and brown adipocytes in Kunming mice. Genet Mol Res 15(1):15017355
17. Yu S, Yan X, Liu H et al (2014) Improved establishment of embryonic stem (ES) cell lines from the Chinese Kunming mice by hybridization with 129 mice. Int J Mol Sci 15(3):3389–3402
18. Xie H, Huang L, Li Y, Zhang H, Liu H (2016) Endoplasmic reticulum stress and renal lesion in mice with combination of high-fat diet and streptozotocin-induced diabetes. Acta Cir Bras 31(3):150–155
19. Wu F, Jin Z, Jin J (2013) Hypoglycemic effects of glabridin, a polyphenolic flavonoid from licorice, in an animal model of diabetes mellitus. Mol Med Rep 7(4):1278–1282
20. Wu Y, Zhang L, Liang J et al (2018) Comparative analysis on liver transcriptome profiles of different methods to establish type 2 diabetes mellitus models in Guangxi Bama mini-pig. Gene 673:194–200
21. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P (2005) Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res 52(4):313–320

22. Kawashima Y, Chen J, Sun H et al (2009) Apolipoprotein E deficiency abrogates insulin resistance in a mouse model of type 2 diabetes mellitus. Diabetologia 52(7):1434–1441
23. Nagata M, Suzuki W, Iizuka S et al (2006) Type 2 diabetes mellitus in obese mouse model induced by monosodium glutamate. Exp Anim 55(2):109–115
24. Trapnell C, Pachter L, Salzberg SL (2009) TopHat: discovering splice junctions with RNA-SEq. Bioinformatics 25(9):1105–1111
25. Trapnell C, Williams BA, Pertea G et al (2010) Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol 28(5):511–515
26. Anders S, Huber W (2010) Differential expression analysis for sequence count data. Genome Biol 11(10):R106
27. Young MD, Wakefield MJ, Smyth GK, Oshlack A (2010) Gene ontology analysis for RNA-seq: accounting for selection bias. Genome Biol 11(2):R14
28. Mao X, Cai T, Olyarchuk JG, Wei L (2005) Automated genome annotation and pathway identification using the KEGG Orthology (KO) as a controlled vocabulary. Bioinformatics 21(19):3787–3793
29. Houssay BA, Martinez C (1947) Experimental Diabetes and Diet. Science 105(2734):548–549
30. Graham ML, Janecek JL, Kittredge JA, Hering BJ, Schuurman HJ (2011) The streptozotocin-induced diabetic nude mouse model: differences between animals from different sources. Comp Med 61(4):356–360
31. Skovso S (2014) Modeling type 2 diabetes in rats using high fat diet and streptozotocin. J Diabetes Investig 5(4):349–358
32. RAKIETEN N, RAKIETEN ML, NADKARNI MR (1963) Studies on the diabetogenic action of streptozotocin (NSC-37917). Cancer Chemother Rep 29:91–98
33. Brosky G, Logothetopoulos J (1969) Streptozotocin diabetes in the mouse and guinea pig. Diabetes 18(9):606–611
34. Junod A, Lambert AE, Orci L et al (1967) Studies of the diabetogenic action of streptozotocin. Proc Soc Exp Biol Med 126(1):201–205
35. Rerup CC (1970) Drugs producing diabetes through damage of the insulin secreting cells. Pharmacol Rev 22(4):485–518
36. Reed MJ, Meszaros K, Entes LJ et al (2000) A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. Metabolism 49(11):1390–1394
37. Gilbert ER, Fu Z, Liu D (2011) Development of a nongenetic mouse model of type 2 diabetes. Exp Diabetes Res 2011:416254
38. Goto Y, Nagasawa H, Iguchi T (1995) Streptozotocin-induced diabetes accelerates mammary tumorigenesis in shn and sln mice. Oncol Rep 2(1):37–40
39. Gruys ME, Back TC, Subleski J et al (2001) Induction of transplantable mouse renal cell cancers by streptozotocin: in vivo growth, metastases, and angiogenic phenotype. Cancer Res 61(16):6255–

6263

40. Fienhold MA, Kazakoff K, Pour PM (1997) The effect of streptozotocin and a high-fat diet on BOP-induced tumors in the pancreas and in the submandibular gland of hamsters bearing transplants of homologous islets. Cancer Lett 117(2):155–160
41. Dombrowski F, Bannasch P, Pfeifer U (1997) Hepatocellular neoplasms induced by low-number pancreatic islet transplants in streptozotocin diabetic rats. Am J Pathol 150(3):1071–1087
42. Levine AJ (1997) p53, the cellular gatekeeper for growth and division. Cell 88(3):323–331
43. Kim MK, Jeon BN, Koh DI et al (2013) Regulation of the cyclin-dependent kinase inhibitor 1A gene (CDKN1A) by the repressor BOZF1 through inhibition of p53 acetylation and transcription factor Sp1 binding. J Biol Chem 288(10):7053–7064
44. El-Deiry WS, Tokino T, Velculescu VE et al (1993) WAF1, a potential mediator of p53 tumor suppression. Cell 75(4):817–825
45. El-Deiry WS, Harper JW, O'Connor PM et al (1994) WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis. Cancer Res 54(5):1169–1174
46. Chua HW, Ng D, Choo S et al (2010) Effect of MDM2 SNP309 and p53 codon 72 polymorphisms on lung cancer risk and survival among non-smoking Chinese women in Singapore. Bmc Cancer 10(1):88–88
47. Momand J, Zambetti GP, Olson DC, George D, Levine AJ (1992) The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. Cell 69(7):1237–1245

Figures

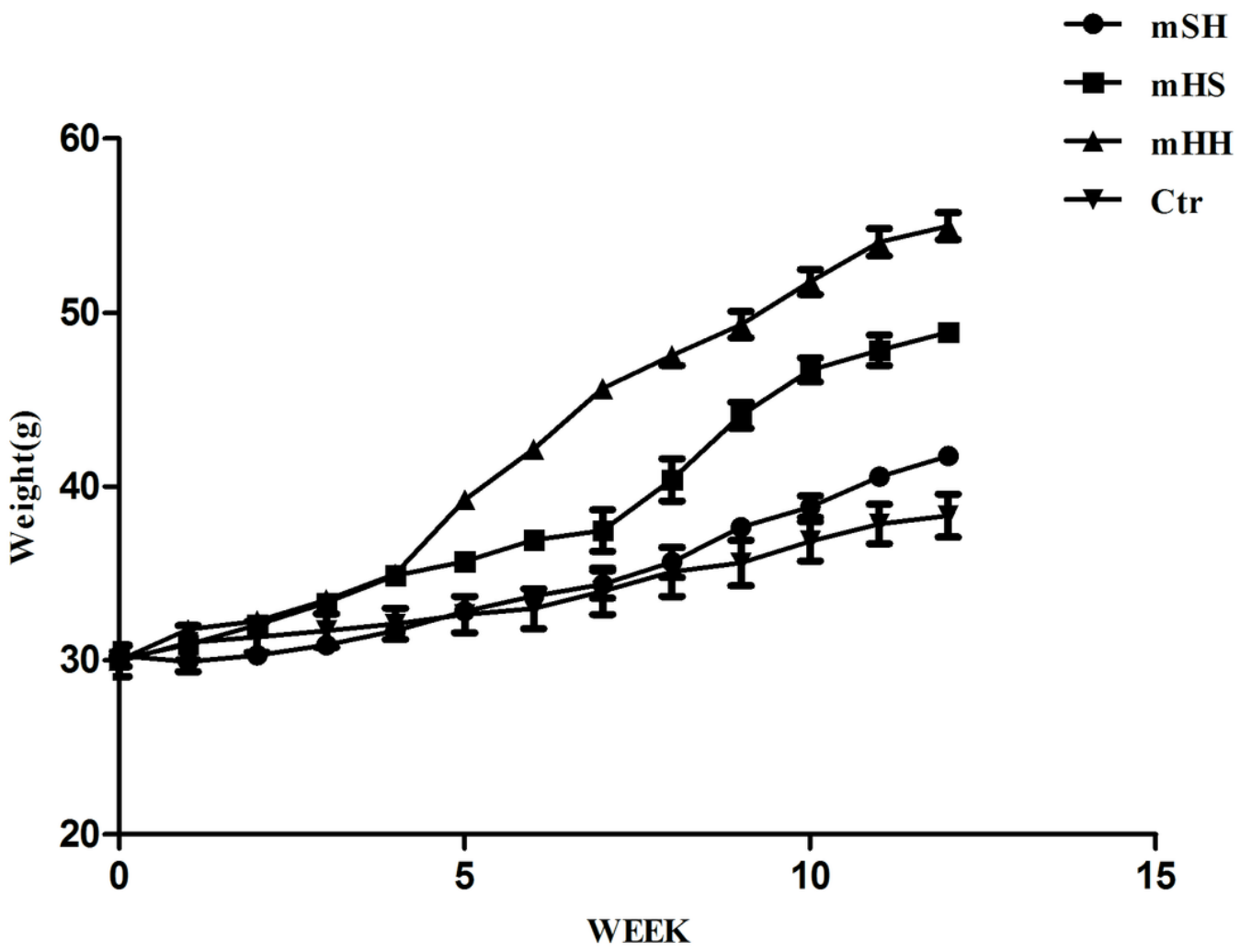

Figure 1

Increase in Kunming mice body weights among different groups across time.

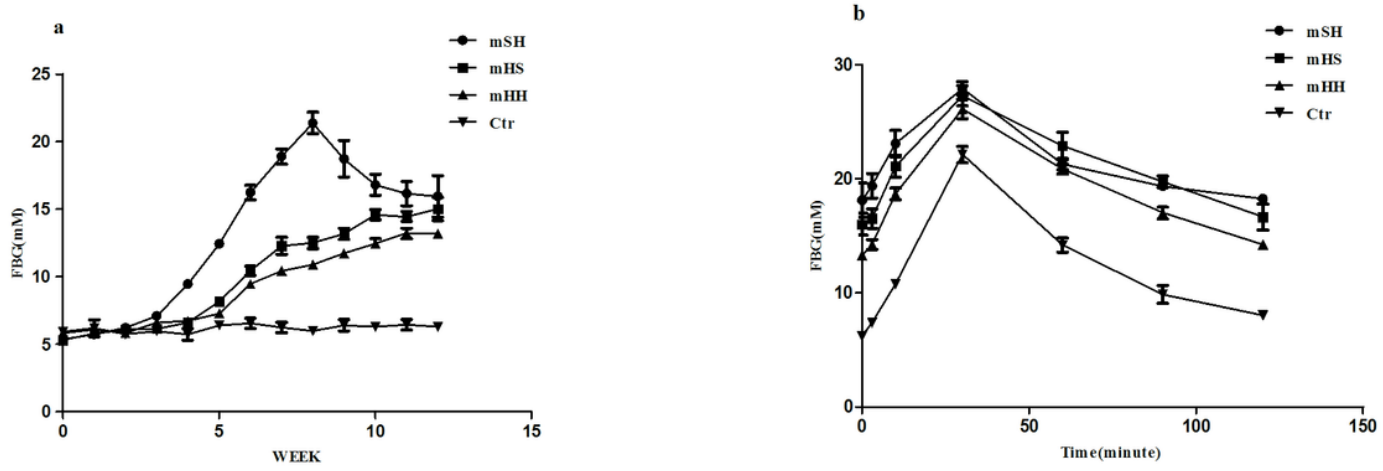

Figure 2

Changes in FBG and IGTT levels. FBG of mSH group rapidly increased from 3rd week and declined from the 8th week, despite the levels in the STZ-treated groups were higher than non-STZ groups. IGTT indicated that glucose disappearance rate of diabetic groups reduced across time.

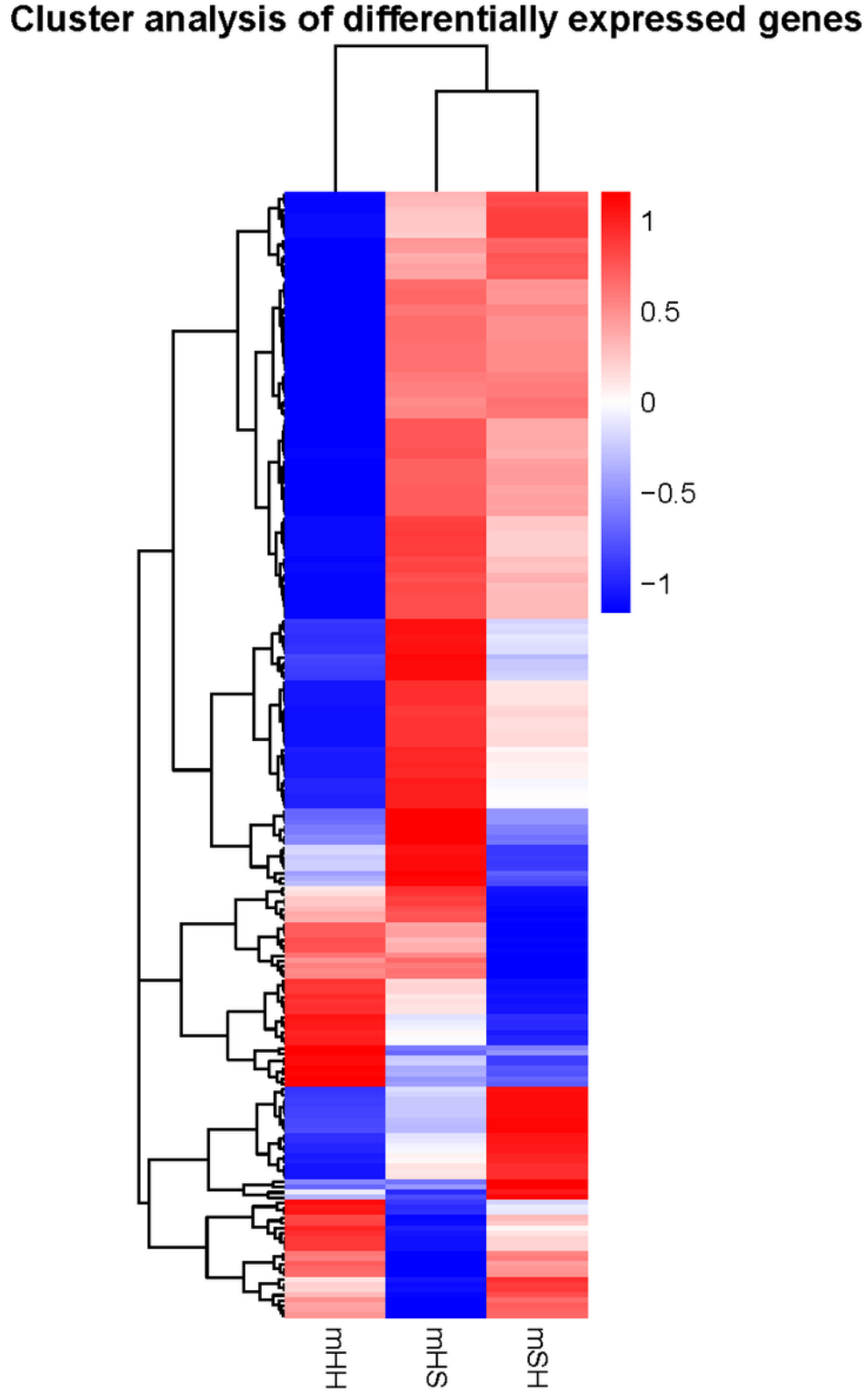

Figure 3

Cluster analysis of differentially expressed genes. Different colors represent various levels of expressions. Red denotes up-regulated transcription and blue color denotes down-regulation.

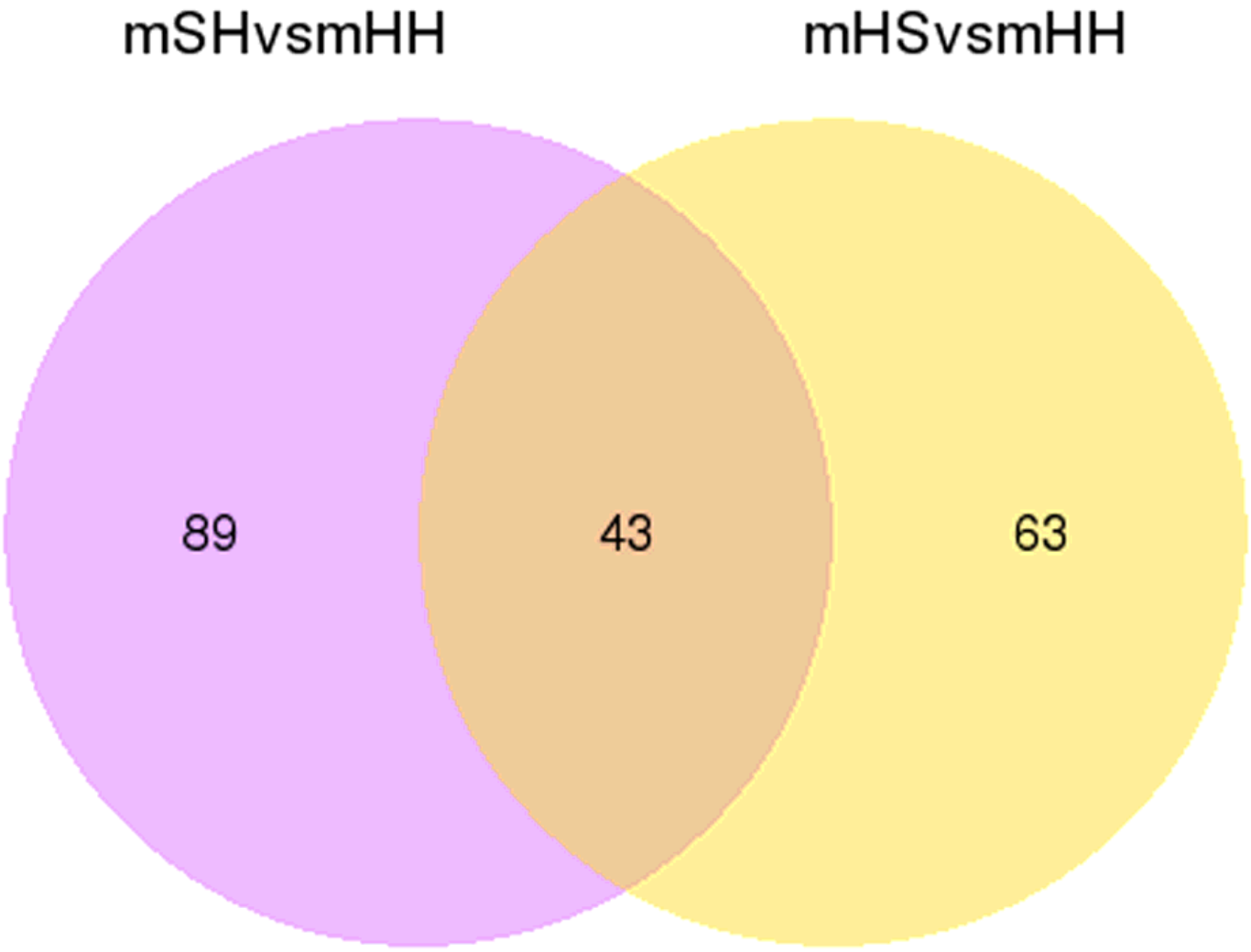

Figure 4

Differentially expressed genes in mHS vs mHH and mSH vs mHH groups.

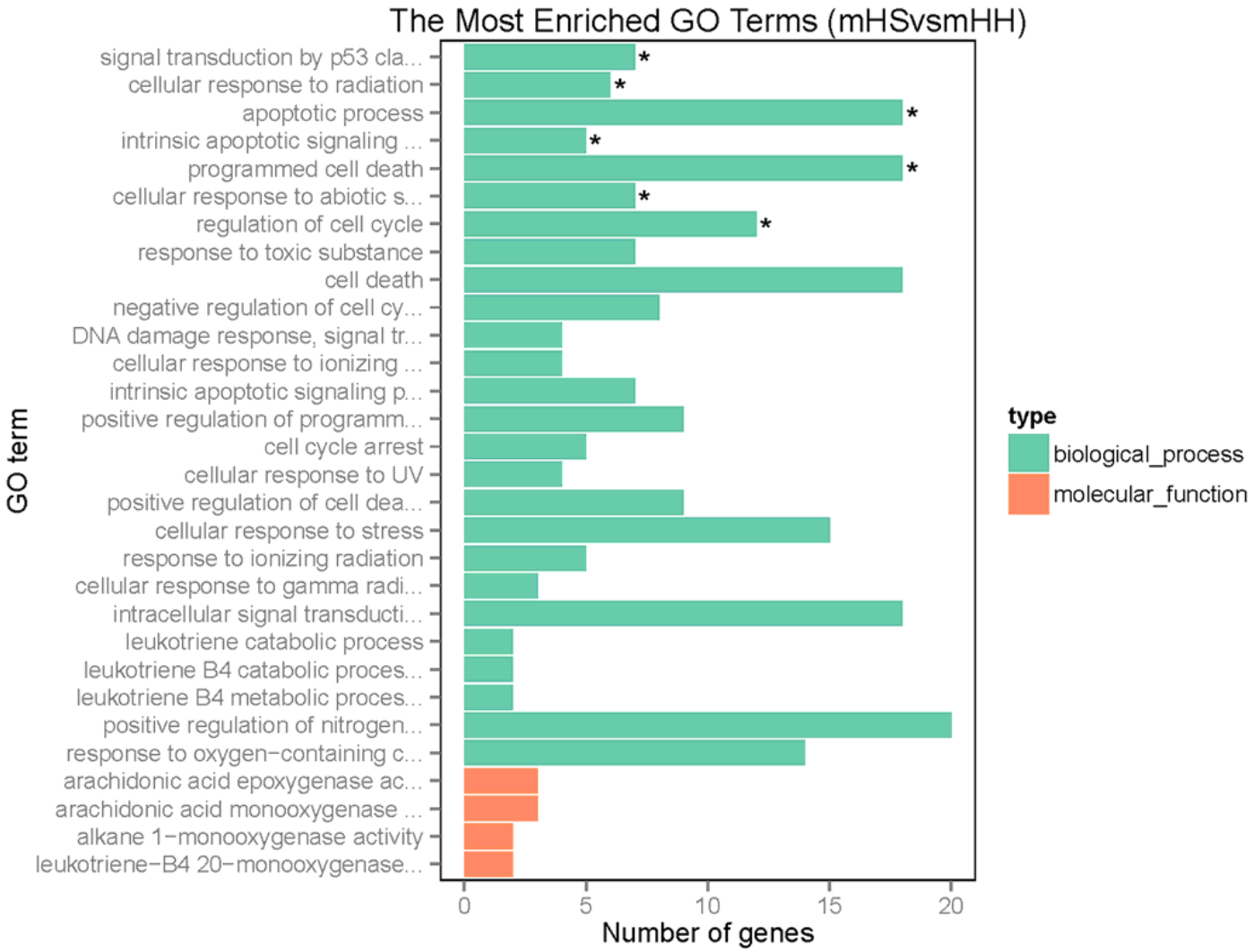

Figure 5

GO classifications of DEGs in mHS vs mHH groups. *indicates significantly enriched GO terms with corrected P-value < 0.05.

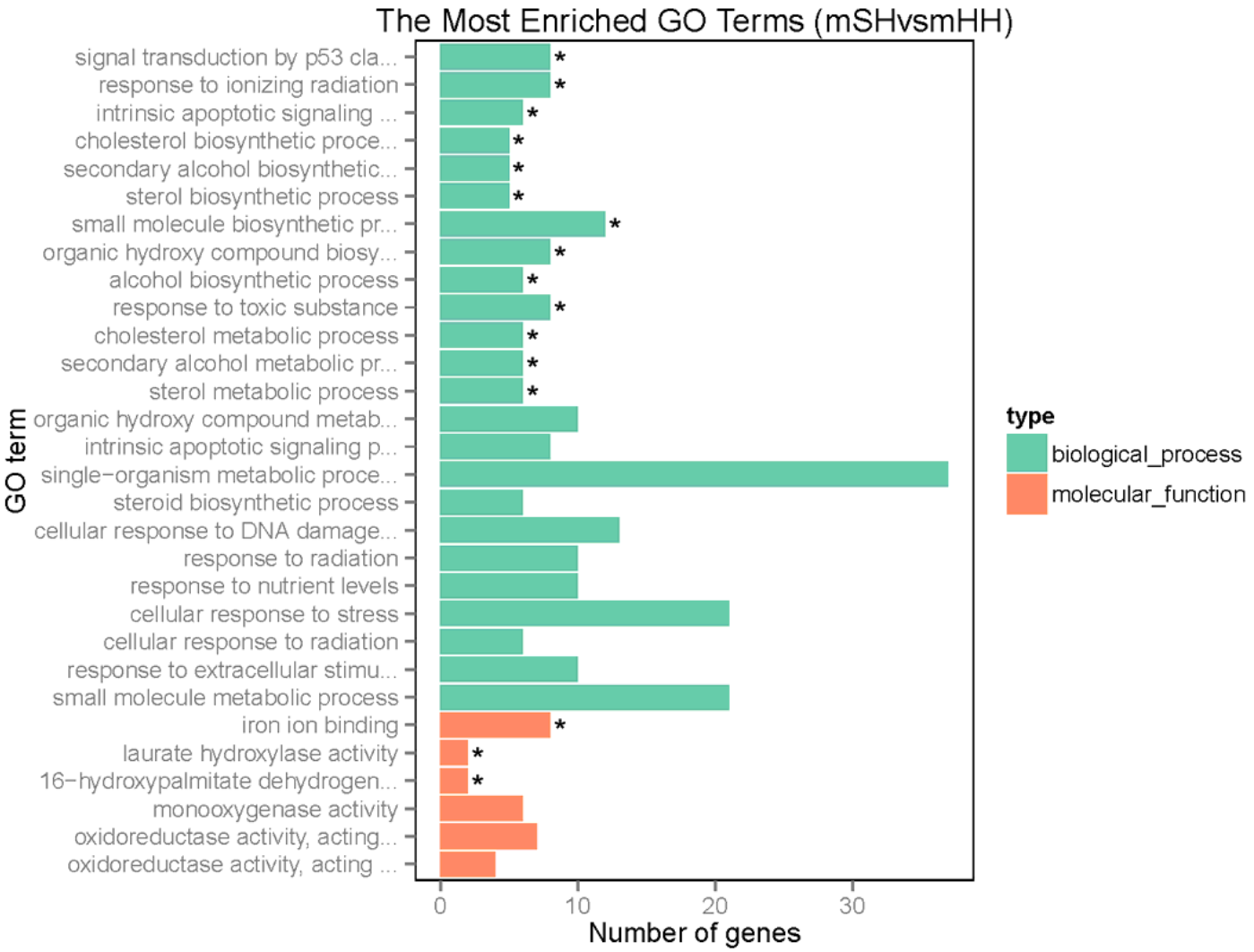

Figure 6

GO classifications of DEGs in mSHvsmHH. * indicates significantly enriched GO terms with corrected P-value < 0.05.

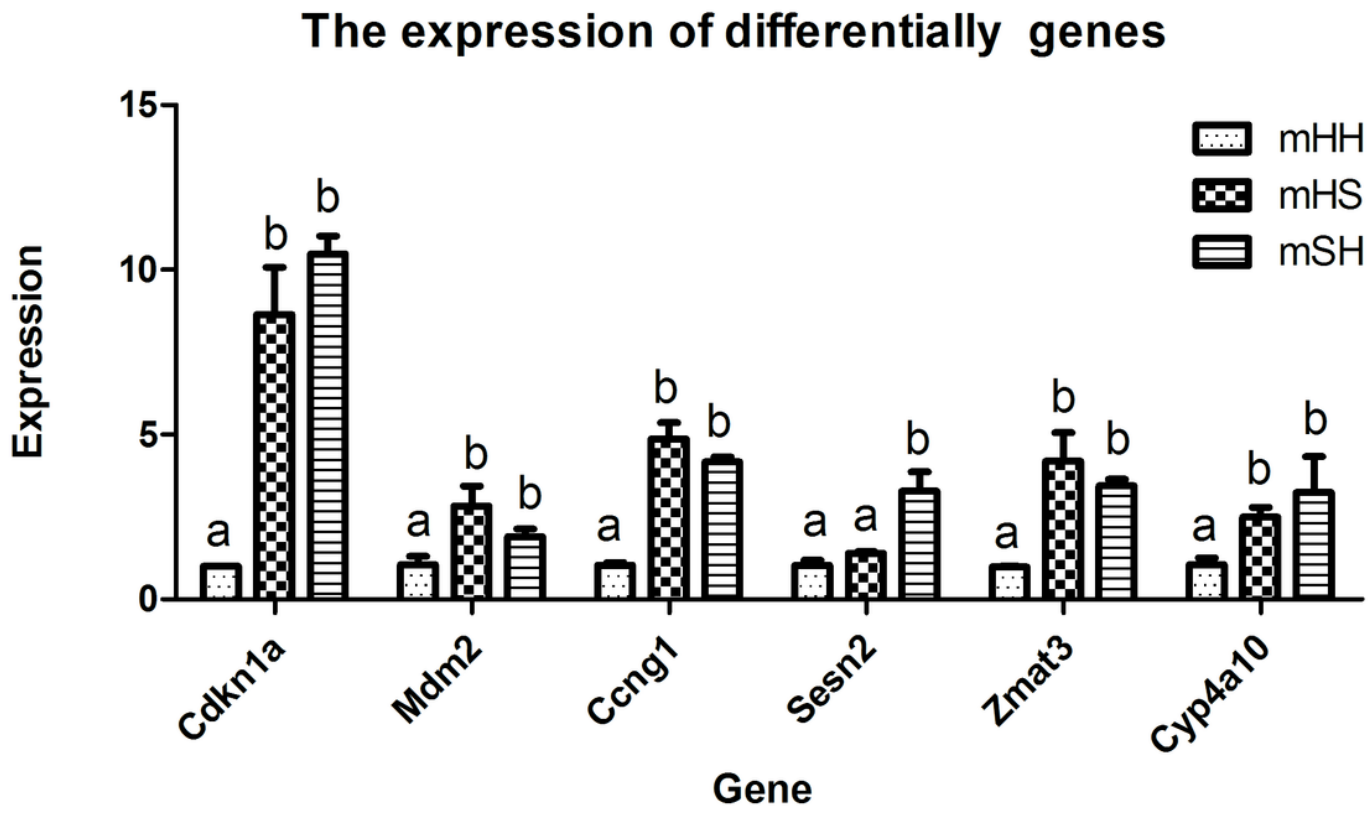

Figure 7

Validation of differentially expressed genes by qRT-PCR. The relative expressions were normalized to mouse β-actin gene as internal control.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementary.docx