Unveil the transcriptome alteration of POMC neuron in diet-induced obesity

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High-Fat-Diet (HFD), Diet-Induced Obesity (DIO), POMC neuron, Neuron homeostasis, pRb phosphorylation
Abstract

Background: Loss of neuron homeostasis in the Arcuate nucleus (ARC) is suggested to be responsible for the development diet-induced-obesity (DIO). We previously reported that loss of Rb1 gene compromised the homeostasis of anorexigenic POMC neurons in ARC and induced obesity in mice.

Method: To shed light on how DIO develops, we propose to analyze the transcriptomic alteration of POMC neurons in mice following high fat diet (HFD) feeding. We isolated the POMC neurons from established DIO mice and performed transcriptomic profiling on them by RNA-seq.

Results: A total of 1,066 genes (628 up-regulated and 438 down-regulated) were identified as differentially expressed genes (DEGs). Pathway enrichment analysis with these DEGs further revealed that ‘cell cycle’, ‘apoptosis’, ‘chemokine signalling’ and ‘sphingolipid metabolism’ pathways were correlated with the development of DIO. Moreover, we validated that the pRb protein, key regulator of ‘cell cycle pathway’, was inactivated by phosphorylation in POMC neurons with HFD feeding. Importantly, reversal of deregulated cell cycle by stereotaxic delivering of the unphosphorylated pRbΔP in ARC significantly meliorated the DIO. Together, our study provides insights into the mechanisms related to the loss of homeostasis of POMC neurons in DIO, and suggests pRb phosphorylation as a potential intervention target to treat DIO.

Conclusion: The Arcuate nucleus is the material basis that controlled energy balance and glucose metabolism, which is vulnerable to high-fat-diet (HFD) in diet-induced-obesity (DIO). In this study, we conducted transcriptomic profiling in anorexigenic POMC neurons of ARC with HFD to disclose the underlying mechanisms related with the homeostasis maintenance and the development of DIO. Importantly, we suggest that DIO could be prevented of treated by reversal of the deregulated cell cycle in POMC neurons through targeting pRb phosphorylation. Keywords: High-Fat-Diet (HFD); Diet-Induced Obesity (DIO); POMC neuron; Neuron homeostasis; pRb phosphorylation

Full-text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Tables
| Group | Sample | Raw Reads Number | Raw Bases Number | Clean Reads Number | Clean Bases Number | Clean Base (%) |
|-------|--------|------------------|------------------|--------------------|--------------------|----------------|
| Nor1  | 42,570,070 | 6,385,510,500 | 41,267,938 | 6,162,876,844 | 94.73 |
| RD    | Nor2  | 53,454,716 | 8,018,207,400 | 51,704,866 | 7,737,845,536 | 92.62 |
| Nor3  | 43,364,668 | 6,504,700,200 | 42,183,346 | 6,273,289,498 | 94.40 |
| Obe1  | 45,023,084 | 6,753,462,600 | 43,880,392 | 6,550,472,008 | 94.33 |
| HFD   | Obe2  | 43,741,698 | 6,561,254,700 | 42,722,324 | 6,387,524,011 | 94.72 |
| Obe3  | 47,826,996 | 7,174,049,400 | 46,655,612 | 6,982,206,476 | 92.92 |

Table 2 The top enrichment terms in GSEA analysis
| Term name                                           | NES | P-val |
|----------------------------------------------------|-----|-------|
| Sphingolipid Metabolism                           | 1   | 0.05  |
| Chemokine Signaling Pathway                       | 1   | 0.01  |
| Cytokine-Cytokine Receptor Interaction            | 0   |       |
| B Cell Receptor Signaling Pathway                 | 0.96 | 0.01  |
| Cell Adhesion Molecules Cams                      | 0.91 | 0.03  |
| Allograft Rejection                               | 0.82 | 0.09  |
| Amino Sugar and Nucleotide Sugar Metabolism       | 0   | 0.1   |
| Apoptosis                                          | 0   |       |
| Adipocytokine Signaling Pathway                   | 0   |       |
| Glycerolipid Metabolism                           | 0   |       |
| Long Term Potentiation                            | 1   | 0.09  |
| Bladder Cancer                                    | 0.68 | 0.09  |
| Amyotrophic Lateral Sclerosis Als                 | 0.61 | 0.08  |
| Galactose Metabolism                              | 0.46 |       |
| Adherens Junction                                 | 0.45 | 0.09  |
| Oocyte Meiosis                                    | 0.39 | 0.1   |
| Cell Cycle                                        | 0.1 | 0.05  |
Figures

A

Food intake (kcal/day)

 RD  HFD

Days

B

Body weight (g)

 RD  HFD

Weeks

C

Body weight gain (%)

 RD  HFD

Weeks

D

RD

HFD

E

Abdominal white fat (g)

 RD  HFD

F

Hypothalamic neurons of Pomc-cre-;Rosa<sup>tdTomato</sup> mice

SSC-A

FSC-A

APC-Cy7-A

PE(561)-A

P1 41.7%

P2 100.0%

P3 0.0%

G

Hypothalamic neurons of Pomc-cre+;Rosa<sup>tdTomato</sup> mice

SSC-A

FSC-A

APC-Cy7-A

PE(561)-A

P1 45.0%

P2 95.4%

P3 3.21%

H

Relative abundance

non-sorted cells (P1)

sorted tdTomato+ cells (P3)

POMC
tdTomato

***
The construct of DIO model of Pomc-Cre; ROSA-tdTomato mice and the cell sorting of hypothalamic POMC neurons. (A) Average (± SEM) 24 h caloric intake (kcal) of the regular diet (RD) group and the high-fat diet (HFD) group mice. (B) Body weight of the RD group and the HFD group mice. (C) Body weight gain of the RD group and the HFD group mice. (D) Abdominal white fat of the RD group and the HFD group mice. (E) The quantification of abdominal white fat of the RD group and the HFD group mice. The flow cytometric sorting of hypothalamic neurons from Pomccre-; ROSAtdTomato mice (F) and Pomc-Cre+; ROSA-tdTomato mice (G). (H) The enrichment of Pomc and tdTomato genes in sorted tdTomato+ cells (P3 of Fig.1G) as compared to non-sorted cells (P1 in Fig.1G)
The analysis results of differentially expressed genes. (A) Venn diagram for DEG between HFD group and RD group (Upregulated in HFD: blue, Upregulated in RD: green). Reported DEGs required q-value < 0.05, abs[log2(fc)] > 1. (B) The volcano map of differentially expressed genes. NA means no significant change between HFD and RD. (C) The clustering map of differentially expressed genes. HFD indicated high fat diet group, and RD indicated regular diet group.
Figure 3

Functional analysis of differentially expressed gene (DEG) in POMC neurons of HFD group compared to RD group. (A) GO functional classification of DEGs according to the enriched gene numbers. (corrected P value < 0.05). Top enriched GO terms of 17 cellular components, 25 molecular functions and 12 biological processes were shown. (B-D) The significant pathway enrichment in GO cellular components, biological process, and molecular functions pathways according to the Bonferroni corrected False Discovery Rate (FDR) and gene counts. (E) The significant KEGG pathway enrichment. Gene counts and p-value corrected by Bonferroni are shown.
Figure 4

Pathway correlation analysis and RT-qPCR validation of differentially expressed genes (DEGs) of POMC neurons in HFD group compared to RD group. (A-D) The GSEA plots showing the enrichment of indicated gene set categories of identified DEGs. NES: normalized enrichment score. Positive NES indicates enrichment of gene signatures in HFD group, negative NES indicates enrichment of gene signatures in RD group. (E-H) The expression heatmaps and clustering of identified DEGs, classified belonging to indicated KEGG pathways. Red signal denotes higher abundance, while blue signal denotes lower
abundance. (I-L) The RT-qPCR validation of selected DEGs belonging to related KEGG pathways as in plots E-H. "**, ***" and "****" indicated $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. The representative KEGG pathways were shown: pathway of cell cycle genes and E2F targets (A, E, I), pathway of apoptosis genes (B, F, J), chemokine signal pathway (C, G, K), pathway of sphingolipid metabolism (D, H, L).
HFD induced phosphorylation of pRb in POMC neurons and stereotaxic injection of unphosphorylated pRb in ARC meliorated the HFD induced DIO. (A) ARC sections from Pomc-Cre; RosaR(GFP) mice in RD and HFD groups were stained with pRb(S608p). Direct GFP was photographed in green. (B) Phosphorylation sites of pRb protein and the schematic maps of the lenti- GFP and lenti-pRbΔP plasmids. (C) Body weights and body weight gains of the HFD fed mice injected with lenti-GFP or lenti-pRbΔP lentivirus in ARC (n=5 for each group). (D) Abdominal white fat content of HFD fed mice injected with lenti-GFP or lenti-pRbΔP lentivirus in ARC (n=5 for each group). (E) Predicted schematic model of HFD induced POMC neuron dysregulation.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.
Supplimentary file 1_gene_list.xlsx
Supplementary figure2.jpg
Supplementary Figure 1.jpeg
Supplementary file 2_Biological function analysis.xls
Supplementary Figure 4.jpg
Supplementary file 3_specific genes primer list.xls
Supplementary Figure 3.jpg