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Maternal high-fat diet affects Msi/Notch/Hes signaling in neural stem cells of offspring mice☆,☆☆

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Abstract

Numerous research have begun to reveal the importance of maternal nutrition in offspring brain development. Particularly, the maternal obesity or exposure to high-fat diet has been strongly suggested to exert irreversible impact on the structure and function of offspring's brain. However, it remains obscure about whether neonatal neural stem cells (NSCs) in offspring's brain are susceptible to maternal exposure to high-fat diet. Here we focused on the alternation in the Notch signaling in NSCs derived from neonatal mice, which had been given birth by female mice with a high-fat diet and found that, in fact, the high-fat diet administration imposed effects on not only maternal mice, indicated by the accumulation of viscera fat as well as the increase in body weight and serum total cholesterol, but also NSCs in the offspring's brain, where significant increase was observed in the expression of genes, either downstream of Notch signaling or regulating this pathway, which have been shown essential for the maturation of NSCs. Therefore, our data provided the first evidence for the potential effect of maternal exposure to the high-fat diet on the Notch signaling pathway in offspring's NSCs, indicating this altered signaling response might contribute to a profound change in offspring's brains as a result of maternal high-fat diet prior to and during gestation.

Keywords: Maternal high-fat diet; Neural stem cell; Notch signaling pathway

1. Introduction

Obesity has become a major public health issue in the world. Tremendous efforts have been dedicated into define the effects of obesity or overweight on childhood and adulthood health. However, it is until recently that the effect of diet disorders through generations began to attract researchers' attention. The mothers with obesity and overweight are at increased risk in having babies with neural tube defects [1]. Niculescu and colleagues have found that maternal obesity induces developmental delay of the fetal hippocampus in an experiment animal model [2]. Another in vivo experiment showed that the mice fed with high-fat diet (HFD) generated fewer new neurons and retained more old neurons [3]. These studies indicated that maternal high-fat diet or obesity might be an important trigger to alter brain development in the offspring. Fetal exposure to high-fat intrauterine environment can activate pre-inflammatory pathways which could impact on substrate metabolism and mitochondrial function, so as to stem cell fate. However, the cellular and molecular mechanisms underlying brain development and neurogenesis in the context of HFD-induced maternal obesity are less clear.

Neurogenesis is a extremely delicate process, where neural stem cells (NSCs), which have the ability to self-renew and differentiate to neurons and glia in an orchestra of infra- and extra- regulation factors. Therefore, any impact which has been placed on NSCs is highly likely spread over the whole brain development. Previous studies has proposed Notch signaling pathway as a major regulating system in NSCs. Notch signaling, including its downstream molecules Hes1/5, is required to maintain a reservoir of undifferentiated NSCs and preserve neural stem cell proliferation [4,5]. Additionally, the Musashi (Msi) family has been found to activate Notch signaling via the translational repression of NUMB [6,7].

In this study, we applied a "western-style" high-fat diet prior to and during gestation to female C57/6 J mice to develop a maternal

Abbreviations: NSCs, neural stem cells; HFD, high-fat diet; TC, total cholesterol; TG, triglyceride.

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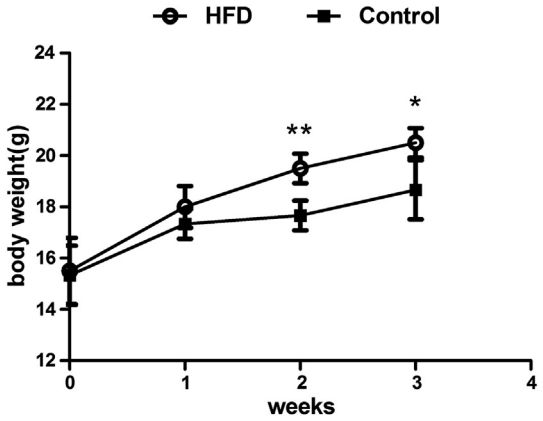

Fig. 1. Effect of HFD on body weights of maternal mice. From the second week of feeding period, the mice in HFD group tended to gain more weight than the control group ($P<.01$). And at the third week, there was still a significant difference in the body weight between the two groups ($P<.05$). The data are presented as mean±standard deviation.

obesity or over-weight model, which was confirmed by the accumulation of viscera fat as well as the increases in body weight and total serum cholesterol level in these mice. Then, we monitored the expression of Notch signaling targeting genes and the genes involved in regulating Notch signaling pathway in NSCs from the neonatal offspring. Our data showed that the mRNA and protein levels of Msi1 and Notch1 were significantly elevated in these NSCs. Combining our data with results from other work, we here raise a possibility that maternal HFD activates Notch signaling in offspring NSCs, thereby affects the final maturation of neurons from NSCs.

2. Materials and methods

2.1. Animals and diets

All protocols and procedures regarding to animal experiments in this study were approved and authorized by the Tianjin Medical University Animal Ethics Committee. Pregnant C57BL/6 J mice were purchased from Beijing HFK Bio-Technology (Beijing, China) and were fed with basal diet and water until their offspring reached 5 weeks in a room with controlled temperature (22–24°C), humidity and an inverse alternating light and dark cycle (12:12-hour light:dark cycle). Then, seven pairs of female littermate mice were randomly divided into HFD group and control group according to matched-pair design. The control group was fed with the basal diets (10% calories from fat, 19% calories from protein and 71% calories from carbohydrate) and the HFD group was fed with high-fat diet (35% calories from fat, 16% calories from protein and 49% calories from carbohydrate). Both diets were purchased from Beijing HFK Bio-Technology. After 3 weeks of the experimental diets, the mice from two groups were made to mate with male mice fed with basal diet. All female mice were maintained on the respective experimental diet during gestation. Blood and adipose tissues were collected when the maternal mice were humanely euthanized after giving birth to the baby mice. The neonatal mice were euthanized for primary culture of neural stem cells.

2.2. Body weight, length and adipose tissue weight

Body weights of all maternal mice in experiments were measured weekly. Body weight, length and adipose tissues were assessed after CO_2 asphyxiation sacrifice.

2.3. Neural stem cells primary culture

Brains of the neonatal mice were removed from the skull and eliminated cerebellum and vascular tunica covering the cerebrum. Then, the cerebrum was transferred into phosphate-buffered saline (PBS) to wash off the blood and transferred into DMEM/F12 (Dulbecco's modified eagle medium/nutrient mixture F-12) medium (Invitrogen, USA) and was cut into small pieces and dissociated by incubation with 0.25% parenzyme and 0.02% EDTA, filtered through cell strainer (76 μm), centrifuged (800 rpm for 5 min) and the supernatant removed. After the cells were re-suspended in the DMEM/F12 medium supplemented with 2% B27 supplement, 20 ng/ml epidermal growth factor (EGF), 20 ng/ml basic fibroblast growth factor (bFGF), 2 mmol/L L-glutamine, at a density of 1×10^6 cells/ml. Cells were grown under standard conditions at 37°C and 5% CO_2. The medium was changed every 2 days. After 6-day culturing, the neural stem cells were collected.

2.4. Serum total cholesterol, triglyceride and glucose concentrations

Blood was collected by cardiac vessel bleeding from anesthetized maternal mice. The blood was allowed to clot at room temperature for 30 min and subsequently was separated by centrifugation at 3,000×*g* for 5 min at 4°C. Serum triglyceride, cholesterol and glucose concentrations were determined using the enzymatic assay kits (Beijing BHKT Clinical Reagent Co., Ltd), as specified by the manufacturers.

2.5. Immunocytochemistry

For fluorescent staining, cells were fixed in 4% paraformaldehyde for 20 min at room temperature. After rinsing with PBS and permeabilized with 0.1% Triton X-100 in PBS for 15 min at room temperature. After blocking with 10% goat serum in PBS for 1 h, cells were incubated with the primary antibodies [anti-Sox2, 1:100, Abcam; anti β-tubulin, 1:30, Abcam; anti-glial fibrillary acidic protein (GFAP), 1:100, Millipore] overnight at 4°C. After repeated rinses in PBS, appropriate secondary antibodies (FITC, 1:100 and TRITC, 1:100, Zhongshan Goldbridge Biotechnology, Beijing, China) were applied as previous described [8]. Immunostained cells were visualized by indirect fluorescence under the fluorescent microscope (Olympus, Tokyo, Japan).

2.6. Real-time polymerase chain reaction (PCR) analysis

Neural stem cells were harvested and 1 ml TRIzol was added per sample mixing uniformly. RNA was extracted following standard protocol provided by TRIzol Reagent manual (Invitrogen). The upper and lower primers used were 5′-gatggcctcaatgggtacaag-3′ and 5′-tcgttgttgttgatgtcacagt-3′ for Notch1, 5′-ttcatcggaggactcagttgg-3′ and 5′-catcaccagacactct- ttcacc-3′ for Msi1, 5′-cgagcagtttggcaaggtaga-3′ and 5′-cagactttctccacaacgtctt-3′ for Msi2, 5′-tcaacacgacaccggacaaac-3′ and 5′-atgccgggagctatctttctt-3′ for Hes1 and 5′-agtcccaaggaga- aaaaccga-3′ and 5′-gctgtgtttcaggtagctgac-3′ for Hes5, respectively. One microgram of total RNA was used for cDNA synthesis, with 1 μl oligo(dT)18 primer, 5 μl reaction buffer 4 μl, RiboLock RNase Inhibitor (20 U/μl) 1 μl, 10 mmol/L dNTP Mix 2 μl and RevertAid M-MuLV Reverse Transcriptase (200 U/μl) 1 μl,

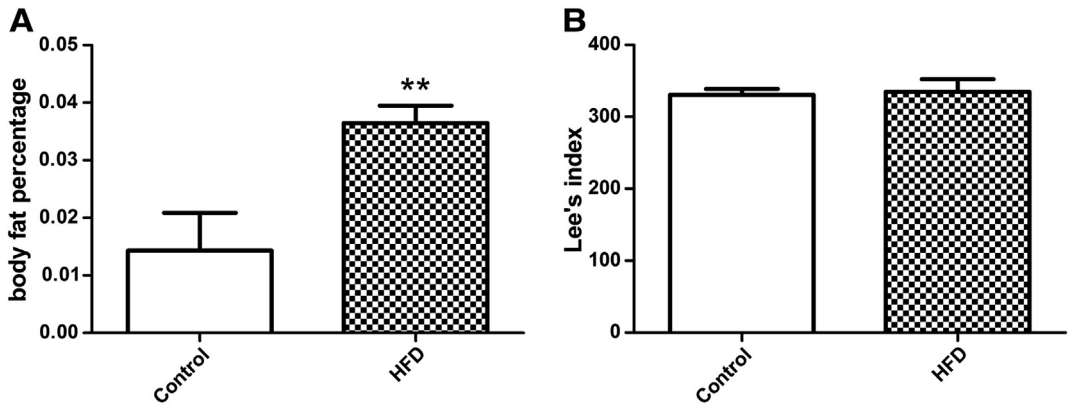

Fig. 2. The body fat percentage and Lee's index of HFD and control groups. (A) Body fat percentage of the HFD group was higher than the control group ($P<.01$). (B) Lee's index of the HFD group showed no significant difference compared to the control group ($P>.05$). Significance is denoted by asterisk (*, ** and *** represent $P<.05$, $P<.01$ and $P<.001$, respectively). All data are presented as mean±standard deviation.

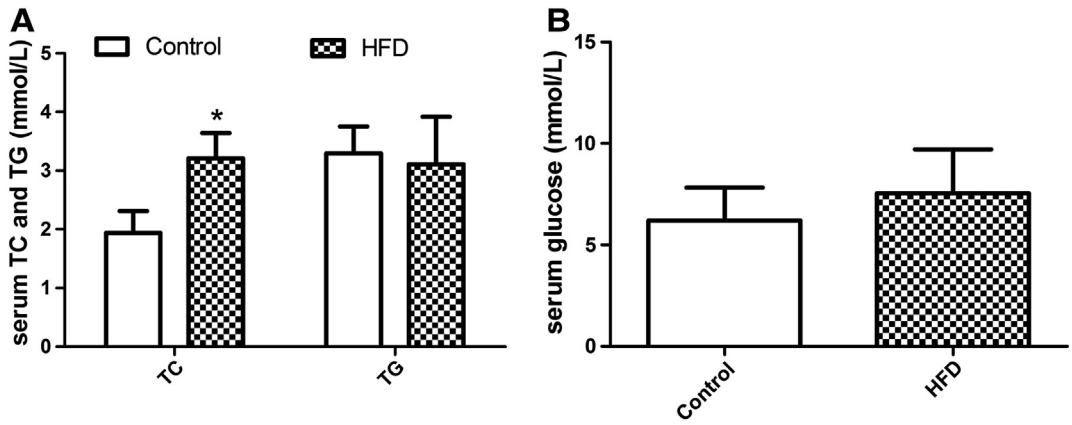

Fig. 3. The assessment of serum TC, TG and glucose of maternal mice fed with either HFD or control diet. (A) Serum TC level of maternal HFD group is higher than control group ($P<.05$), but TG showed no significant difference ($P>.05$). (B) Serum glucose measurement showed no significant difference between maternal HFD and control groups ($P>.05$).

mixed gently, centrifuged then incubated at 42°C for 1 hour and heat at 70°C for 5 min. Expression of genes was quantified with real-time PCR, utilizing LightCycler 480 SYBR Green I Master kit. Reactions were carried out on Roche 480 system. GAPDH was used for normalization of real-time PCR results.

2.7. Western immunoblotting

Equal amounts of cell protein lysates were electrophoresed on 12% SDS-polyacrylamide gel electrophoresis gel and electrophoretically transferred onto a polyvinylidene fluoride membrane. Membranes were blocked in Tris-buffered saline with 5% milk and 0.1% Tween. The blots were probed with primary antibodies overnight and revealed with horseradish peroxidase-conjugated secondary antibodies. Primary antibodies used are as follows: Hes1 (D6P2U) Rabbit mAb (#11988, Cell Signaling Technology), Anti-Musashi 1 (ab52865, Abcam) and Notch1 (D6F11) XP Rabbit mAb (#4380, Cell Signaling Technology).

2.8. Statistical analysis

Data were expressed as mean±standard deviation. Paired t test was used to determine the differences between high-fat diet and control groups by SPSS 13.0. Statistical significance for all analyses was set at $P<.05$, and *, ** and *** represent $P<.05$, $P<.01$ and $P<.001$, respectively.

3. Results

3.1. Effects of high-fat diet on maternal mice body weight

The female littermate mice (5 week age) were randomly assigned into either HFD ($n=7$) or normal diet group ($n=7$), with body weights being noted once a week until mating. The collected data are presented in Fig. 1, showing that, upon two weeks of the experimental diet feeding, the body weight gain in the HFD group was higher than the control group ($P<.05$).

3.2. HFD induced the mice gaining more adipose tissue

At the end of the study, the maternal mice were euthanatized and their body weight, body length and adipose tissue weight (adipose tissue weight was calculated as the sum of perimetrial, mesenteric and retroperitoneal adipose tissue weights) were assessed to calculate the body fat percentage (the total weight of fat divided by total weight) and the Lee's index [$\sqrt[3]{}$body weight (g)×1000/naso-anal length (cm)], which are presented in Fig. 2. The body fat percentage of the HFD group was significantly increased than the control group ($P<.05$), but Lee's index showed no difference ($P>.05$).

3.3. Effect of HFD on serum TC, TG and glucose

Blood samples were collected at necropsy to determine serum total cholesterol, glucose and triglyceride. In comparison with the control group, the HFD group had a higher level of the total cholesterol ($P<.05$), whereas there were no significant differences in triglyceride and glucose between the two groups ($P>.05$) (Fig. 3).

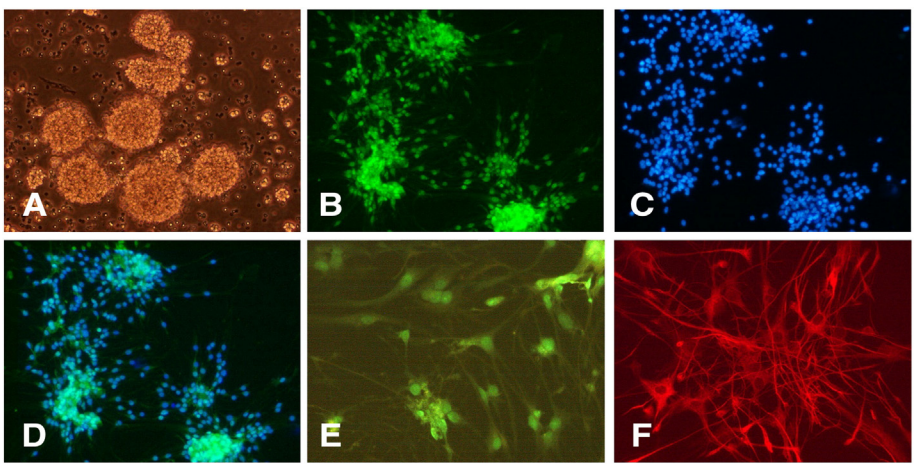

Fig. 4. Identification of NSCs. (A) Photomicrographs of NSC neurospheres (100×). (B) Immunofluorescent staining with Sox2 antibody (green) (100×). (C) Cells were counterstained for DAPI (blue) (100×). (D) Merged picture for C and D. (E) Cells were counterstained for β-tubulin-III (green, 200×); (F) Cells were counterstained for GFAP (red, 200×).

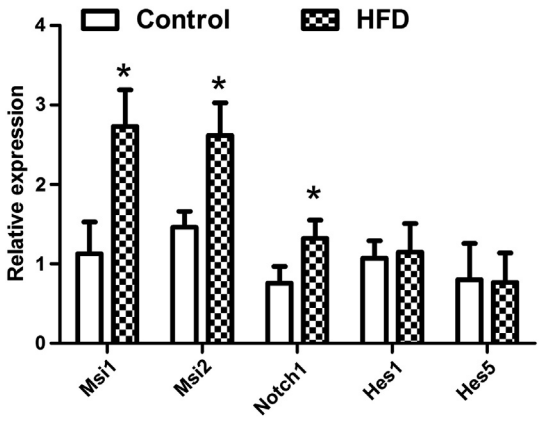

Fig. 5. The mRNA levels of Msi1, Msi2 and Notch1 are higher in the HFD group ($P<.05$). But the expressions of Hes1 and Hes5 show no significant difference ($P>.05$). Significance is denoted by asterisk. All data are presented as mean±standard deviation.

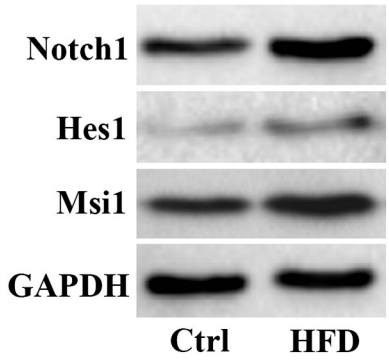

Fig. 6. The protein levels of Msi1, Notch1 and Hes1.

3.4. Neural stem cell identification

Brains from the neonatal offspring mice were isolated, dissociated and cultured in serum-free medium composed of DMEM/F12 with bFGF and EGF. After one week of culture, NSCs were aggregated as neurospheres (Fig. 4A). Sox2 is essential to maintain self-renewal of undifferentiated stem cells and widely used to identify multipotential neural stem/progenitor cells in vitro. Immunofluorescence staining showed neurosphere reacted positives with anti-Sox2 antibody (Fig. 4B–D). When kept in the serum-free medium with bFGF and EGF, NSCs continued to proliferate for several weeks. To differentiate NSCs, 5% fetal bovine serum was added to the medium without bFGF and EGF. Subpopulations of NSCs exhibited immunoreactivity to β-tubulin-III and GFAP (Fig. 4E, F), the commonly used markers for neurons and astrocytes, respectively. These results indicated that the isolated cells were NSCs which could self-renew and differentiate into neurons and astrocytes.

3.5. Effect of maternal HFD on Notch signaling in offspring NSCs

The data of real-time PCR and western blot showed that the expression of Msi1, Msi2 and Notch1 were significantly higher in offspring's NSCs of the HFD group than the control group (Fig. 5 and Fig. 6). Because of this observation, the expression of Hes1 were determined and it showed that Hes1 protein increased in the HFD group (Fig. 6), while no differences were found in Hes1 and Hes5 mRNA levels.

4. Discussion

The C57BL/6 J mouse has been used extensively to study high-fat diet induced obese. However, few studies have investigated the impact of maternal high-fat diet on offspring NSCs in this animal model. In our study, the female C57/6 J mice were fed with a "western-style" high-fat diet for three weeks before mating and during gestation. And then, serum lipid profile in maternal mice and the expression of Notch signaling in neonatal offspring mice were determined. Our data showed that HFD induced accumulation of viscera fat and higher body weight significantly, whereas Lee's index showed no difference between HFD and control groups. This suggests the obesity developed in this model remained at an early stage. Our results also demonstrated that HFD resulted in increases of serum total cholesterol, but triglyceride and glucose remained on the level the same as the control, which implied that the short period of HFD had insufficiently led to significant lipid and glucose dysregulation. Also, it is possible that female mice tend to be more tolerant to the metabolic disturbance.

It is becoming more and more intriguing to verify whether the maternal HFD affects neurogenesis in offspring, as there is a growing body of evidence for supporting this notion [1–3,9–11]. More importantly, fetal exposure to excess blood lipids has proved to be able to activate pre-inflammatory reaction and, hence, affect the metabolism and mitochondrial function in stem cell. It has been rarely reported on the molecular linkage between maternal HFD and neural stem cells. In NSCs, Notch signaling pathway plays a critical role in maintaining neural stem cell proliferation; also Notch induces the expression of Hes1 and Hes5, which repress proneural gene expression and thereby inhibit neuronal differentiation [7,12,13]. There are other important series of proteins involved in NSCs fate named Musashi (Msi) family including Msi1 and Msi2, co-expressing in neural stem cells [14,15]. In mammals, the function of Msi is to activate Notch signaling through the translational repression of NUMB [6,7,14,15]. This study showed that Notch1, Msi1 and Hes1 are up-regulated in the NSCs of maternal HFD offspring. Unexpectedly, the mRNA levels of Hes1 and Hes5 in offspring NSCs showed no significant difference between maternal HFD and control groups. This possibly can be interpreted by the observation, that is, Hes1 expression dynamically oscillates in neural progenitors because sustained expression of Hes1 represses the expression of Notch ligands [13]. Thus, our data implied that maternal HFD activated Notch signaling in offspring NSCs, thereby affecting the final maturation of neurons from NSCs.

References

[1] Stothard KJ, Tennant PW, Bell R, Rankin J. Maternal overweight and obesity and the risk of congenital anomalies: a systematic review and meta-analysis. JAMA 2009;301:636–50.
[2] Niculescu MD, Lupu DS. High fat diet-induced maternal obesity alters fetal hippocampal development. Int J Dev Neurosci 2009;27:627–33.
[3] McNay DE, Briancon N, Kokoeva MV, Maratos-Flier E, Flier JS. Remodeling of the arcuate nucleus energy-balance circuit is inhibited in obese mice. J Clin Invest 2012;122:142–52.
[4] Bergstrom T, Forsberg-Nilsson K. Neural stem cells: brain building blocks and beyond. Ups J Med Sci 2012;117:132–42.
[5] Faigle R, Song H. Signaling mechanisms regulating adult neural stem cells and neurogenesis. Biochim Biophys Acta 1830;2013:2435–48.
[6] Wang XY, Yin Y, Yuan H, Sakamaki T, Okano H, Glazer RI. Musashi1 modulates mammary progenitor cell expansion through proliferin-mediated activation of the Wnt and Notch pathways. Mol Cell Biol 2008;28:3589–99.
[7] Nishimoto Y, Okano H. New insight into cancer therapeutics: induction of differentiation by regulating the Musashi/Numb/Notch pathway. Cell Res 2010;20:1083–5.
[8] Li W, Yu M, Luo S, Liu H, Gao Y, Wilson JX, et al. DNA methyltransferase mediates dose-dependent stimulation of neural stem cell proliferation by folate. J Nutr Biochem 2013;24:1295–301.

[9] Heerwagen MJ, Miller MR, Barbour LA, Friedman JE. Maternal obesity and fetal metabolic programming: a fertile epigenetic soil. Am J Physiol Regul Integr Comp Physiol 2010;299:R711–22.
[10] Chang GQ, Gaysinskaya V, Karatayev O, Leibowitz SF. Maternal high-fat diet and fetal programming: increased proliferation of hypothalamic peptide-producing neurons that increase risk for overeating and obesity. J Neurosci 2008;28: 12107–19.
[11] Namavar MR, Raminfard S, Jahromi ZV, Azari H. Effects of high-fat diet on the numerical density and number of neuronal cells and the volume of the mouse hypothalamus: a stereological study. Anat Cell Biol 2012;45: 178–84.
[12] Imayoshi I, Sakamoto M, Yamaguchi M, Mori K, Kageyama R. Essential roles of Notch signaling in maintenance of neural stem cells in developing and adult brains. J Neurosci 2010;30:3489–98.
[13] Shimojo H, Ohtsuka T, Kageyama R. Oscillations in notch signaling regulate maintenance of neural progenitors. Neuron 2008;58:52–64.
[14] Okano H, Imai T, Okabe M. Musashi: a translational regulator of cell fate. J Cell Sci 2002;115:1355–9.
[15] Sakakibara S, Nakamura Y, Yoshida T, Shibata S, Koike M, Takano H, et al. RNA-binding protein Musashi family: roles for CNS stem cells and a subpopulation of ependymal cells revealed by targeted disruption and antisense ablation. Proc Natl Acad Sci U S A 2002;99:15194–9.