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Microarray Analysis of MicroRNA Expression Profiles in Newborn and Adult Rats Hippocampus

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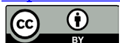

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Abstract

MicroRNAs (miRNAs) are a functional small non-coding RNA and play essential roles in gene regulation indevelopment, differentiation and proliferation. In order to investigate the miRNAs function in Lewis rat hippocampal development, in this study, newborn and adult hippocampi were deliberately selected to analyze the miRNA expression profiles by microarray. Microarray analyses identified 22 differentially expressed miRNAs (>1.5 fold, intersection of two sets). Of these, 12 were down-regulated and 10 were up-regulated during hippocampus development. DAVID Functional Annotation Cluster (FAC) analysis of the 317 predicted target genes of down-regulated miRNAs revealed confident enrichment scores for cell adhesion and neuron development etc., indicating the functional significance and importance of these miRNAs during hippocampal development. Bioinformatic analyses of the differentially expressed miRNAs have identified a number of miRNAs with putative involvement in the hippocampus developing process. This study lays a solid foundation for further studies to clarify the important regulation function of miRNAs in brain tissue.

Keywords

MicroRNA, Microarray, Hippocampus, Development

1. Introduction

The microRNAs (miRNAs) are a group of small non-coding RNAs that are sin-

gle stranded chains consisting of 19 - 25 nucleotides (~22 nucleotides) and transcribed by RNA polymerase II or III in the nucleus [1]. More and more studies showed that miRNAs play an important role in gene regulations by binding to the 3'-untranslated regions (3'-UTRs) of target messenger RNAs (mRNAs), resulting in post-transcriptional silencing by translational repression, mRNA degradation, or a combination of the two [2] [3].

A unique feature of these miRNAs is their ability to bind and regulate many genes, and in some cases multiple miRNAs target similar families of genes [4] [5], thereby enhancing their regulation ability. And emerging evidence indicates that miRNAs are actively involved in regulating gene expression patterns in the adult brain [6] [7].

miRNA microarray technology is an efficient method to generate miRNA expression profiles. These microarray data can be used to extract information regarding the regulatory pathways initiated by miRNAs, especially regulation due to development, by integrating the mRNA expression profiles of predicted miRNA target genes. Suchan approach has been applied to study the functional linkage between miRNAs and physiological or pathological processes [8] [9].

The microarray technology has facilitated large-scale studies on miRNAs expression changes during brain development [9] [10] [11]. Recent estimates put the number of human miRNAs at 1100 or more, composing complex regulatory networks that influence the expression of as many as two thirds of all genes [12]. Such a large family of genes could explain some of the difficulties that neurobiologists generally have encountered in their efforts to link individual miRNA genes to mental disorders, because they are single molecular entities that dictate the expression of fundamental regulatory pathways, miRNAs represent potential drug targets of unprecedented power.

Previous studies mainly focused on miRNA expression in human, mouse and rat central nervous system (CNS) or brain different regions. Lewis rat is a general animal model for biological research, in order to investigate the miRNAs expression profiles in Lewis rat hippocampus in detail, unlike the previous reports, newborn and adult hippocampi were deliberately selected to analyze the miRNA expression profiles by microarray.

Furthermore, the functional information of these hippocampus specific miRNAs and the related regulatory networks were investigated, and the target genes of the differentially expressed miRNAs predicted by three public datasets (Mirbase, Miranda, and Mirdb) were studied in detail by function and pathway enrichment analysis. Thus, a global view of developmental Lewis rat hippocampus specific miRNA expression profiles and their target maps was developed in this study.

2. Methods

2.1. Animals

Adult male (10 months old) and pregnant female (in gestation days 14 - 16)

Lewis rats were obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The adult animals were acclimated for 5 - 7 days under standard conditions and the pregnant females were kept for generation of newborns. Seven 10 months old and seven newborn (postnatal day two, P2) Lewis rats were killed and the brains were quickly removed, two of them were used for chip assay, the remaining five were used for quantitative real time PCR (qRT-PCR) verification in each group. Fresh hippocampi were then dissected from the brain on a chilled glass plate on ice according to the procedures described in reference [13]. The hippocampi were sent to microarray detection or stored at −70˚C until the day of assay. All animal experiments in this study were approved by Medical Ethics Committee of Yangtze University, and carried out in accordance with the guiding principles for the care and use of laboratory animals published by the U.S. National Institutes of Health (NIH Publication No. 85 - 23, revised 1996) and the ARRIVE guidelines.

2.2. MiRNA Microarray

Microarray analysis was performed by Kangcheng Bio-tech Inc. (Shanghai, China). Briefly, total RNA was harvested using Trizol (Invitrogen, CA, USA) and miRNeasy mini kit (Qiagen, CA, USA) according to manufacturer's instructions. After RNA quantity measurement with the NanoDrop 1000, the samples were labeled using the miRCURYTMHy3TM/Hy5^{TM} Power labeling kit and hybridized on the miRCURYTMLNA Array (v.16.0, Exiqon, Skelstedet, Vedbaek, Denmark). After hybridization, scanning was performed with the Axon GenePix 4000B microarray scanner (Molecular Devices, PA, USA). The raw intensity of the image was read with GenePix pro V6.0 (Molecular Devices) and the intensity of green signal was calculated after background subtraction. Four replicated spots of each probe on the same slide were averaged. Median Normalization Method was used to obtain 'Normalized Data': Normalized Data = (Foreground - Background)/median, where the median was the 50% quantile of miRNA intensity, which was larger than 50 in all samples after background correction.

2.3. Quantitative RT-PCR Validation

Total RNA was extracted using Trizol reagent (Invitrogen). cDNA was synthesized using the PrimeScript RT Reagent Kit (GeneCopoeia, MD, USA). A total of five differentially expressed miRNAs were selected based on their function and involvement in pathways and processes important to Lewis rat development. Detection of the mature form of miRNAs was performed using Quantitect SYBR Green PCR Kit (GeneCopoeia) and quantitative RT-PCR Primer Sets (GeneCopoeia) with the U6 small nuclear RNA as an internal control.

2.4. Bioinformatics Analysis of Normalized Microarray Data

The 10 months old vs newborn 1.5 fold change up and down regulated miRNAs were selected from miRNAs expression profiling data (Intersection of two sets of

chip results). Three types of miRNA target prediction software, ENCORI (http://starbase.sysu.edu.cn/) and Mirdb (http://mirdb.org/miRDB/) were used to predict the target genes of the selected miRNAs. The intersection of these three datasets was used as the prediction results of the target gene of the selected miRNAs.

2.5. Clustering and Principal Component Analysis

The hierarchical clustering method [14] was used to classify different group patterns. Principal component analysis (PCA) [15] was used to produce a two-dimensional graph of the distances between different groups.

Gene Ontology (GO) classification systems were used to assign putative function to each clone by the way of biological process, molecular function and cellular components. The Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7b [16] was used to determine pathways and processes of major biological significance and importance through the Functional Annotation Cluster (FAC) tool based on the GO annotation function.

2.6. DAVID Functional Annotation Cluster Analysis

DAVID FAC analysis was conducted on two independent normalized gene lists containing the target genes (Intersection of 3 or more miRNAs) of 1.5-fold up-regulated normalized miRNAs and 1.5-fold down-regulated normalized miRNAs (3 or more). High stringency ease score parameters were selected, to indicate confident enrichment scores of functional significance and importance of the given pathways and processes investigated. The GO system in DAVID was utilized to identify enriched biological themes in both gene lists.

2.7. Mapping and Visual Pathway Analysis

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway tools were used to visually map cluster of the target genes involved in common pathways and processes for both pathway-specific and molecular overview purposes. KEGG pathway tools were utilized through DAVID online tools. Since visual mapping was the primary objective, all target genes (Intersection of 3 or more miRNAs) of normalized miRNAs that were differentially expressed by 1.5-fold were considered for the KEGG pathway analysis. Heat map analyses were also conducted through DAVID to produce a matrix of enriched GO terms with common target genes (Intersection of 3 or more miRNAs) of normalized miRNAs that were 1.5-fold or more up and down-regulated. The green and black shading on the heat map matrix indicates a positive and negative correlation between the enriched GO term and the given target gene, respectively.

3. Results

3.1. All Differentially Expressed MiRNAs

The 6th generation of miRNA array (Exiqon) contains about 680 capture probes,

covering rat microRNAs annotated in miRBase 16.0. 10 months old and newborn Lewis rats hippocampi were used for miRNAs microarray analysis. 10 months old vs newborn 1.5 fold change up and down regulated miRNAs (Intersection of two sets of chip results) were chosen for further study (**Table 1** and **Table 2**). In total, two sets replicate microarray hybridizations were performed and analysis of the results identified 22 differentially expressed miRNAs (>1.5 fold, intersection of two sets). Of these, 10 were up-regulated and 12 down-regulated during hippocampus development. The intersections of three or more selected miRNAs' predicted target genes were analyzed using the FAC tool contained in DAVID [16]. DAVID FAC analysis of the 317 predicted target genes of down-regulated miRNAs (>1.5-fold) produced a total of 63 enriched functional clusters under high stringency conditions (Enrichment Score > 1). The enrichment score gives an indication of the biological significance of the gene groups being analyzed, from which the top 8 were considered in our study, they are cell adhesion, response to hormone stimulus, regulation of neuron development, regulation of cell growth, neutral lipid metabolic process, regulation of cell motion, regulation of apoptosis and neuron projection (**Figure 1**). DAVID FAC analysis of the 311 predicted target genes of up-regulated miRNAs (>1.5-fold) produced a total of 26 enriched functional clusters under high stringency conditions (Enrichment Score > 1). The top 8 were shown in **Figure 2**. Considering the negative regulation is the main characteristic of miRNA function, target genes of down-regulated miRNAs (>1.5-fold) were chosen for deeper analysis.

Table 1. The miRNAs of 1.5 fold and above down-regulated in hippoccampus of 10 months old VS newborn rats (intersection of 10 m_1 VS $P2_1$ and 10 m_2 VS $P2_2$).

Name	10 m_1	$P2_1$	10 $m_1/P2_1$	10 m_2	$P2_2$	10 $m_2/P2_2$
miR-100	0.0775	0.9293	0.0834	0.1835	0.4524	0.4055
miR-135a	1.6155	4.7287	0.3416	0.5144	0.8333	0.6173
miR-145	0.0226	0.3160	0.0716	0.2230	0.3762	0.5928
miR-181b	0.2569	0.8638	0.2974	0.3777	0.8190	0.4611
miR-181c	0.0420	0.5010	0.0838	0.1331	0.4286	0.3106
miR-20b-5p	0.0355	0.1622	0.2192	0.2050	0.3381	0.6064
miR-21*	0.0695	0.1133	0.6131	0.3345	0.6571	0.5091
miR-341	0.1228	0.9865	0.1245	2.5324	4.3143	0.5870
miR-466d	0.0145	0.3368	0.0432	0.5108	1.0571	0.4832
miR-500	0.0194	0.3378	0.0574	0.2338	0.4000	0.5845
miR-664	0.0290	0.1154	0.2520	0.9604	2.7048	0.3551
miR-665	0.0388	0.0759	0.5109	0.2410	0.4667	0.5164

(10 m: 10 months old; P2: postnatal day two).

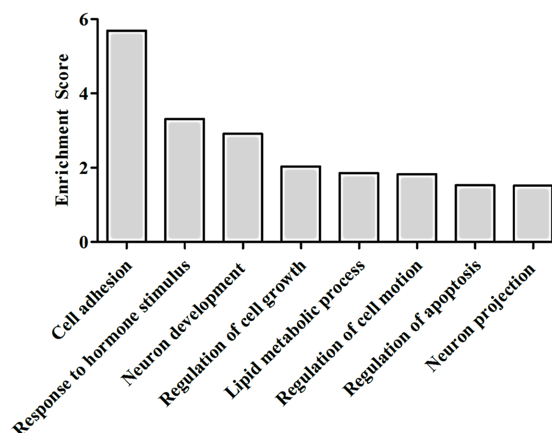

Figure 1. DAVID FAC analysis of target genes of 10 months old VS newborn down-regulated miRNAs (>1.5 fold). Major FACs for down-regulated miRNAs. Significance is determined by corresponding enrichment scores.

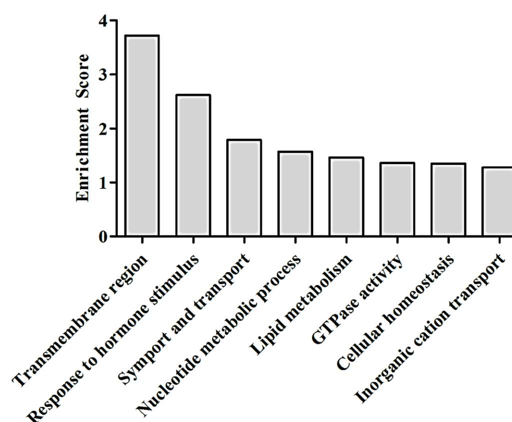

Figure 2. DAVID FAC analysis of target genes of 10 months old VS newborn up-regulated miRNAs (>1.5 fold). Major FACs for up-regulated miRNAs. Significance is determined by corresponding enrichment scores.

Table 2. The miRNAs of 1.5 fold and above up-regulated in hippoccampus of 10 months old VS newborn rats (intersection of 10 m_1 VS $P2_1$ and 10 m_2 VS $P2_2$).

Name	10 m_1	$P2_1$	10 $m_1/P2_1$	10 m_2	$P2_2$	10 $m_2/P2_2$
miR-101b	1.3360	0.7495	1.7826	1.4281	0.7905	1.8066
miR-128	3.7609	1.9220	1.9567	1.4604	0.7762	1.8815
miR-137	1.7447	1.0000	1.7447	0.5288	0.1857	2.8473
miR-139-5p	0.9790	0.1871	5.2322	2.4173	1.6000	1.5108
miR-222	0.8271	0.4938	1.6752	9.8058	2.2286	4.4000
miR-24	4.5186	1.0790	4.1878	1.8381	0.7619	2.4125
miR-27b	1.9790	0.3773	5.2446	0.6367	0.3905	1.6305
miR-329*	0.6478	0.1538	4.2108	1.8561	1.0000	1.8561
miR-675	0.5299	0.0738	7.1796	1.9784	0.9857	2.0071
miR-9	27.2051	15.6112	1.7426	4.9065	1.3857	3.5408

(10 m: 10 months old; P2: postnatal day two).

3.2. Validation of Microarray Data by qRT-PCR

In order to validate microarray results, qRT-PCR were performed to determine the expression levels of five miRNAs selected from the list of miRNAs differentially expressed across newborn and 10 months old rats hippocampus. The qRT-PCR results are correlated with the microarray expression data (Figure 3, Table 1 and Table 2).

3.2.1. Cell Adhesion

Cell adhesion showed the highest enrichment score in the FAC analysis with the target genes of down-regulated miRNAs (>1.5-fold) during the development (Figure 1). DAVID two dimensional figure analysis shows 43 genes are correlated with cell adhesion, biological adhesion, etc. of GO classifications (Figure 4(a)). Among them, neuronal cell adhesion molecular (NCAM) is a kind of glycoprotein, it can mediate interactions between cells and cells, or cells and extracellular matrix, it also play a certain role on recognition and transfer in cells, tumor infiltration and growth, neural regeneration, transmembrane signal transmission, learning and memory and so on. Transforming growth factor-βII (TGF-βII) binding with TβRII, the signal could transmitted by SMAD signaling pathway. These important cytokines are closely related to the cell adhesion, growth and development, and they are all the predicted target genes of significant reduced miRNAs during the process of growth and development from newborn to adult hippocampus.

3.2.2. Regulation of Neuron Growth and Development

FAC analysis identified neuron development and regulation of cell growth as significant biological processes during hippocampus development (Figure 1). DAVID heat map analyses identified 23 genes that functionally clustered into common GO terms related to neuron differentiation, regulation of cell development,

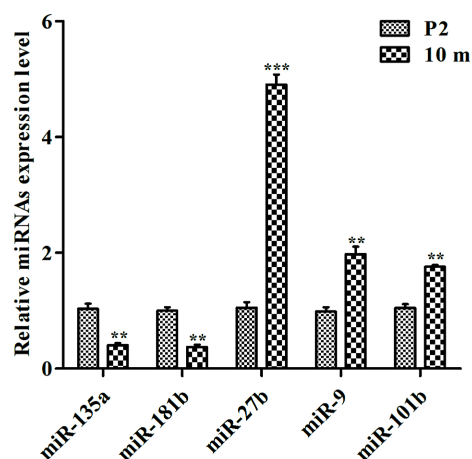

Figure 3. Validation of microarray data. A total of five miRNAs in our microarray experiments were selected and their relative expression determined using qRT-PCR. The bars represent relative levels (mean ± SEM, n = 5) of miRNAs normalized by U6 small nuclear RNA (**P < 0.01, ***P < 0.001) (10 m: 10 months old; P2: postnatal day two).

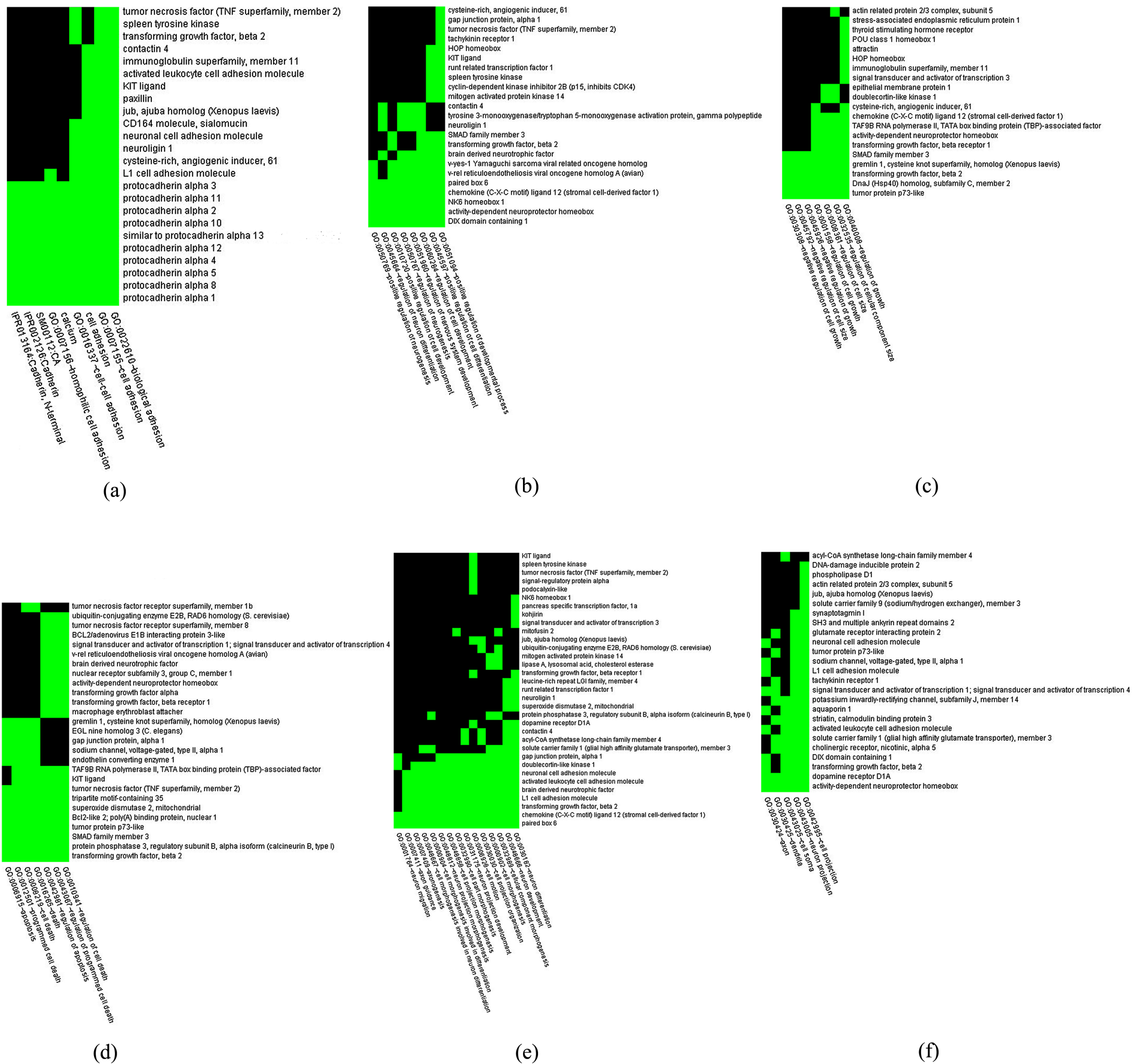

Figure 4. DAVID 2D view analysis of biologically significant FACs containing target genes of 10 months old VS newborn down-regulated miRNAs (>1.5 fold). (a) Cell adhesion. (b) Regulation of neuron development. (c) Regulation of cell growth. (d) Apoptosis. (e) Regulation of cell motion. (f) Neuron projection. Green and black shading indicates positive and unconfirmed correlation of annotated gene and functional GO terms, respectively.

regulation of nervous system development and regulation of neuron development (Figure 4(b)). 20 genes that functionally clustered into common GO terms related to regulation of growth, regulation of cell size and negative regulation of cell growth (Figure 4(c)). These include brain derived neurotrophic factor (BDNF), TGF-βII, transforming growth factor-β receptor 1 (TGF-β R1), neuroligin 1, SMAD family member 3 (SMAD3) and tumor necrosis factor (TNF) etc. The results suggest, these significantly reduced miRNAs are negative regulation factors of hippocampus normal growth, and are inhibited in the process of growth and development.

3.2.3. Regulation of Apoptosis

FAC analysis identified regulation of apoptosis as important biological processes during hippocampus development (Figure 1). DAVID heat map analyses identified 27 genes that functionally clustered into common GO terms related to regulation of apoptosis, regulation of cell death and programmed cell death. B-cell translocation gene 2 (Bcl2), BDNF, TGF-βII, TβRI, TGF-α, SMAD 3, and TNF are among them (Figure 4(d)).

3.2.4. Regulation of Neuron Motion and Projection

FAC analysis also showed regulation of cell motion and neuron projection are significant clusters during hippocampus development (Figure 1). DAVID heat map analysis revealed a total of 33 genes that functionally cluster with GO terms related to neuron differentiation, cell motion, cell morphogenesis and axonogenesis (Figure 4(e)). A total of 25 genes that functionally cluster with GO terms related to neuron projection, dentrite and axon (Figure 4(f)). These include dopamine receptor D1A, TGF-βII, TβRI, SMAD 3 and NCAM.

3.2.5. Response to Endogenous Stimulus and Lipid Metabolism

DAVID FAC analysis indicated that response to endogenous stimulus and lipid metabolism are active in both newborn and adult hippocampus (Figure 1 and Figure 2). The enrichment scores for response to endogenous stimulus and lipid metabolism were comparable between both FAC analyses (Figure 1 and Figure 2). DAVID heat map analysis identified 41 target genes of down-regulated miRNAs (Figure 5(a)) and 35 target genes of up-regulated miRNAs (not shown)

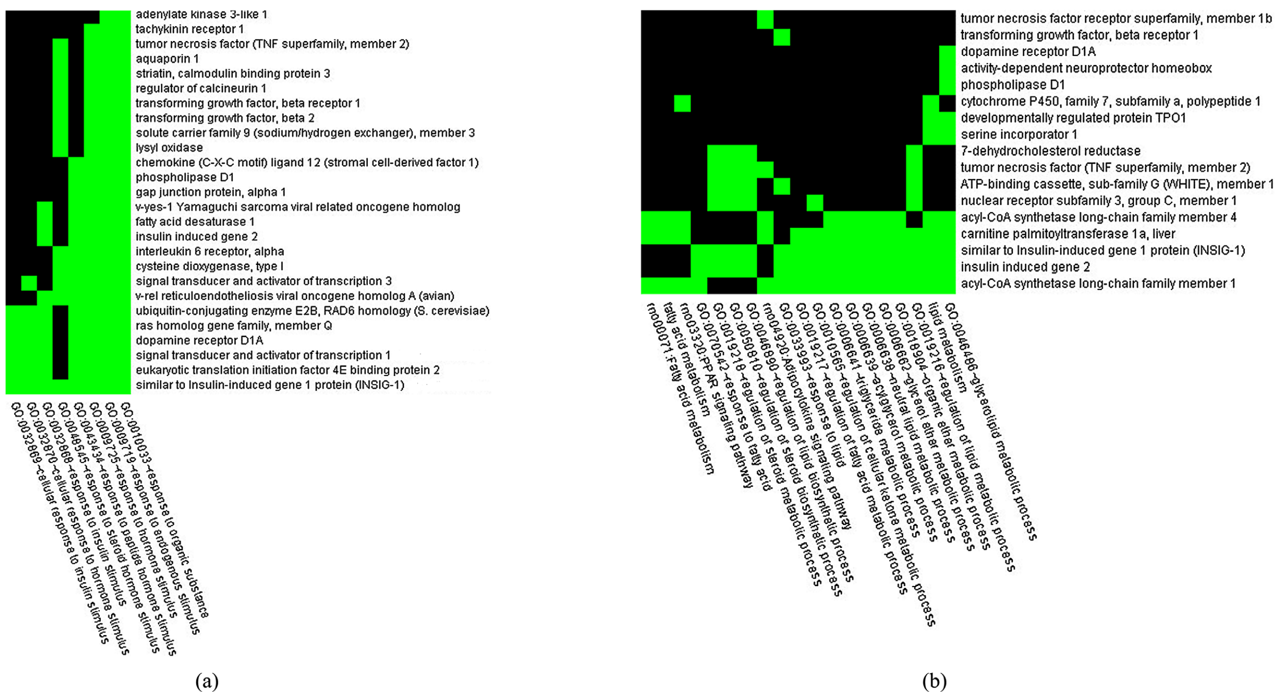

Figure 5. DAVID 2D view analysis of important FACs containing target genes of 10 months old VS newborn up and down-regulated miRNAs (>1.5 fold). (a) Response to endogenousstimulus. (b) Neutral lipid metabolic process. Green and black shading indicates positive and unconfirmed correlation of annotated gene and functional GO terms, respectively.

involved in response to endogenous stimulus, and 20 target genes of down-regulated miRNAs (Figure 5(b)) and 17 target genes of up-regulated miRNAs (not shown) involved in lipid metabolism. These include dopamine receptor D1A, TGF-βII, TβRI, ras gene, TNF and acyl-CoA synthetase etc. These results illustrated that endogenous stimulus and lipid metabolism are both important for newborn and adult hippocampus, and significant cytokines of endogenous stimulus and lipid metabolism could be modulated by miRNAs. The differentially expressed miRNAs might regulate the expression of target genes, and involve in endogenous stimulus and lipid metabolism from early differentiation to synaptogenesis and maturation, and then, they exert an important influence in the molecular networks of nervous system.

3.3. Results of KEGG Pathway Analysis

The important GO terms of FAC analysis were chose for KEGG pathway analysis. Among them, neurotrophin signaling pathway, which widely exists in brain tissue, and plays an important physiological role in all kinds of neural cells growth, differentiation, maintenance and regeneration (Figure 6(a)); TGF-β signaling pathway, which can regulate multiple cellular functions, including cell growth, adhesion, cell transfer, differentiation and apoptosis, and plays an important role in regulating immune inflammation, wound healing, immune steady and tolerance (Figure 6(b)); cell apoptosis signaling pathway, which is closely related to the hippocampal growth and aging (Figure 6(c)); dopamine synaptic pathway, which participates in the control of the movement in central nervous system, and plays an important role in regulating brain development, learning and memory function (Figure 6(d)). In the KEGG pathway maps, red colour was used to mark the predicted target genes of miRNAs and differentially expressed miRNAs (Figure 6, Table 3). If further studies confirm these differentially

Table 3. List of miRNAs from Mirbase, Miranda and Mirdb with predicted target mRNAs KEGG ID, description and KEGGpathway.

KEGG ID	Description	KEGG Pathway	miRNAs
K04355	Bdnf, brain derived neurophic factor	Neurotrophin signaling pathway	miR-27b, miR-181b/c, miR-20-5p, miR-145, miR-128;
K07828	Rhoq, ras homolog family member Q	Neurotrophin signaling pathway	miR-9, miR-101b, miR-139-5p;
K04500	Smad3, SMAD family member 3	TGF-beta signaling pathway	miR-9, miR-145, miR-135a, miR-101b, miR-20-5p;
K13376	Tgfb2, transforming growth factor, beta 2	TGF-beta signaling pathway	miR-27b, miR-135a, miR-20-5p;
K04674	Tgfbr1, transforming growth factor, beta receptor 1	TGF-beta signaling pathway	miR-139-5p, miR-27b, miR-9, miR-135a, miR-101b, miR-128;
K02161	Bcl2, B-cell CLL/lymphoma 2	Apoptosis	miR-9, miR-181b/c, miR-135a;
K03156	Tnf, tumor necrosis factor	Apoptosis	miR-181b/c, miR-27b, miR-145, miR-24;
K04144	Drd1, dopamine receptor D1A	Dopaminergic synapse	miR-181b/c, miR-20-5p, miR-24.

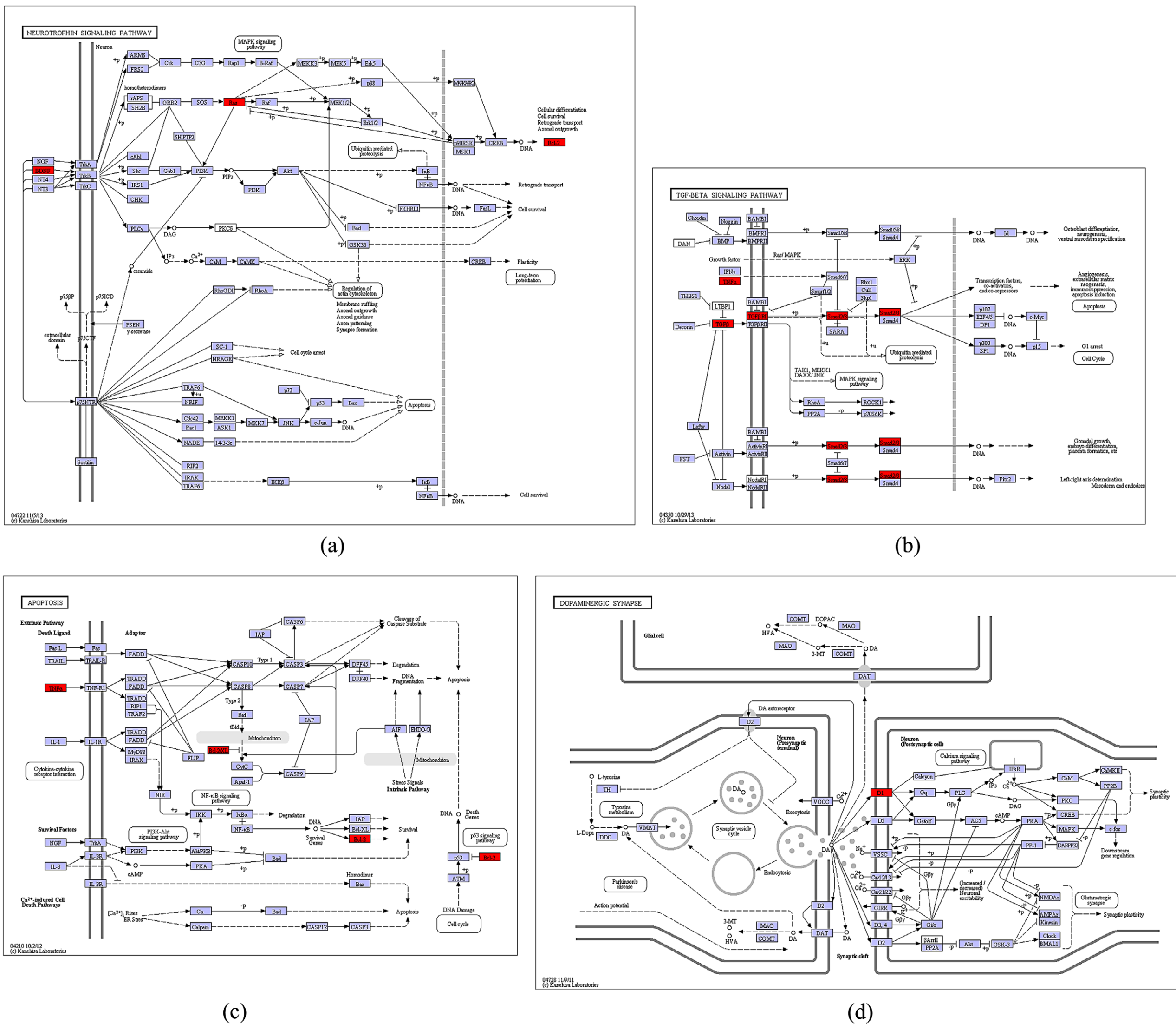

Figure 6. KEGG pathway map analyses of significant GO terms correlated with target genesof differentially expressed miRNAs. (a) Neurotrophin signaling pathway. (b) TGF-β signaling pathway. (c) Apoptosis. (d) Dopamine synaptic pathway.

expressed miRNAs (Table 3) could combine with mRNA 3'UTR sequence of predicted target genes and inhibited the expression of target genes, that suggests these miRNAs are involved in regulation of the above mentioned important signaling pathways by inhibiting the expression of the important cytokines, and attend in regulated hippocampal growth, development and maintenance, and then regulate learning and memory function.

4. Discussion

The hippocampus is an essential part of the archaeo cortex. In mammals, it is three-layer structure located on the medial surface of the temporal lobe in the back of each cerebral hemisphere. The name hippocampus is derived from Greek and means sea horse, which it resembles in shape [17]. The hippocampus is an important part of the limbic system, which plays a vital role in the learn-

ing/memory, spatial navigation, emotional behavior and regulation of the neuroendocrine stress axis processes [17] [18].

In order to comprehensive understanding of miRNAs expression differences and regulation functions in hippocampal growth and development, newborn and 10 months old Lewis rats were chosen, two sets and a total of four hippocampal samples were sent to miRNAs microarray detection. 10 months old vs newborn 1.5 fold change up and down regulated miRNAs (Intersection of two sets of chip results) were chosen for further study (**Table 1** and **Table 2**). Through bioinformatic analysis, these predicted target genes are distributed in neurotrophic factor signaling pathway, transforming growth factor signal pathway, apoptosis pathway, dopamine synaptic pathway, and many of them are the significant cytokines of these signaling pathways.

Neurotrophic factors, especially the BDNF can regulate the development and function of the CNS. The neurotrophin BDNF has been shown to modulate the development and function of synapses in the nervous system [19]. BDNF is important in modulating dentate gyrus neurogenesis [20] and in synaptogenesis [21]. BDNF has been implicated in activity dependent synaptic plasticity and network remodeling [22]. Moreover, it is able to regulate the extent of adult hippocampal neurogenesis [23], presumably via its specific Trk Breceptors [24]. Proliferating neural progenitor cells in the dentate gyrus have been demonstrated to express TrkB receptors [25], suggesting a direct influence of BDNF on neurogenesis. It is well known that BDNF binding to TrkB receptors evokes several intracellular signaling pathways, including MAP/ERK pathway and the activation of CREB [26]. BDNF is known to regulate dendritic development in particular, and it has been shown to induce primary dendrite formation in developing neurons via PI3-K and MAPK pathway activation [27]. Through bioinformation analysis, here from newborn to adult development process, miR-27b, miR-181b/c, miR-20-5p, miR-145, miR-128 may bind with BDNF mRNA to participate in the regulation of neurotrophin signaling pathway (**Table 3**, **Figure 6(a)**).

TGF-β signaling pathway, which can regulate multiple cellular functions, including cell growth, adhesion, cell transfer, differentiation and apoptosis, and plays an important role in regulating immune inflammation, wound healing, immune steady and tolerance [28]. TGF-β signaling pathway was precision regulated in different levels, and dysfunction is closely related to the occurrence of a variety of diseases. Smad3 protein was the key molecule in TGF-β signal transduction, it play an important role in maintaining the normal function of cells [29]. Through bioinformation analysis, here from newborn to adult development process, miR-27b, miR-135a, miR-20-5p may bound with TGF-β II mRNA, miR-139-5p, miR-27b, miR-9, miR-135a, miR-101b, miR-128 may bind with TGF-βR I mRNA, miR-9, miR-145, miR-135a, miR-101b, miR-20-5p may bind with SMAD3 mRNA to participate in the regulation of TGF-β signaling pathway (**Table 3**, **Figure 6(b)**).

FAC analysis identified apoptosis as an important biological process during hippocampus development (**Figure 1**). DAVID heat map analyses identified Bcl2, TNF, TGF-β, SMAD 3, BDNF etc. functionally clustered into common GO terms related to apoptosis and other associated processes (**Figure 4(d)**). Apoptosis suits itself into antagonistic pleiotropy theory and as it has deleterious effects in aging neurons while it is needed during development where it removes neurons that don't integrate into the growing neuronal network [30]. However, after the development of the neuronal network into the mature nervous system, the fight of neurons for survival and healthy existence with plasticity becomes anti-apoptotic [31]. In the mitochondrial pathway of apoptosis, intracellular signals result in releasing cytochrome c from mitochondria, which binds to the adaptor protein APAF-1 (apoptotic protease-activating factor-1), which further leads to the activation of caspase 9 and subsequently caspase 3 [32].

Bcl2 as a pro-survival gene is a member of the anti-apoptotic group, which binds to and inhibits the action of multi-domain pro-apoptotic proteins like Bax [33]. Moreover, the up-regulation of Bcl2 expression leads to down-regulation of Bax, and interferes with the release of cytochrome c from the mitochondrial intermembrane space into the cytosol, where it associates with caspase 9 and Apaf1 to form the apoptosome complex, thus effecting apoptosis [34]. The Bcl2 protein resides in the outer mitochondrial wall, and acts as an anti-apoptotic factor by controlling mitochondrial permeability, thus regulating apoptosis [35]. TNF-α is secreted by non-neural cells in the brain, including activated astrocytes and microglial cells [36]. TNF-α has been reported to play important roles in neuronal functions such as neuron apoptosis, microglia activation, synaptic transmission, and synaptic plasticity [37].

Previous evidence of miRNA impact on cell survival mechanisms has been shown with miR-15, miR-16, miR-34a and miR-181a-1* targeting Bcl2 mRNA and inducing apoptosis [38] [39] [40]. Accordingly, these differentially expressed miRNAs could targeting their predicted targets, such as Bcl2, TNF, BDNF etc. and joined in regulation of apoptotic death in neuronal cells. Through bioinformation analysis, here from newborn to adult development process, miR-9, miR-181b/c, miR-135a may bind with Bcl2 mRNA, miR-181b/c, miR-27b, miR-145, miR-24 may bind with TNF mRNA to participate in the regulation of neural cells apoptosis (**Table 3**, **Figure 6(c)**).

Dopamine is the mainly catecholamine neurotransmitter in mammalian brain, It controls the motion, cognition, emotion, positive reinforcement, feeding and endocrine regulation and many other functions [41]. Dopamine receptors can be divided into D1 and D2 receptors they are both expressed in hippocampus. Dopamine play an important role for motion control, and dopamine levels were positively correlated with cognitive function, and negatively correlated with age [42]. Through bioinformation analysis, here from newborn to adult development process, miR-181b/c, miR-20-5p, miR-24 may bind with D1 mRNA to participate in the regulation of dopamine synaptic pathway (**Table 3**, **Figure 6(d)**).

In conclusion, here from newborn to adult hippocampal development process, significantly differentially expressed miRNAs may bind with mRNA of their predicted target genes to participate in the regulation of neurotrophic factor signaling pathway, transforming growth factor signal pathway, apoptosis pathway, dopamine synaptic pathway, and then regulate the hippocampal growth and development, aging and degradation, learning and memory functions. This study lays a solid foundation for further studies to clarify the important regulation function of miRNAs in brain tissue.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

Bcl2, B-cell translocation gene 2; BDNF, brain derived neurotrophic factor; CNS, central nervous system; DAVID, database for annotation, visualization, and integrated discovery; FAC, functional annotation cluster; GO, gene ontology; KEGG, kyoto encyclopedia of genes and genomes; miRNA, microRNA; NCAM, neuronal cell adhesion molecular; P2, postnatal day two; TGF-βII, transforming growth factor-βII; TGF-β R1, transforming growth factor-β receptor 1; TNF, tumor necrosis factor; UTR, untranslated region.