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Hippocampal Genetic Knockdown of PPARδ Causes Depression-Like Behaviors and Neurogenesis Suppression

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ABSTRACT

Background: Although depression is the leading cause of disability worldwide, its pathophysiology is poorly understood. Our previous study showed that hippocampal peroxisome proliferator-activated receptor δ (PPARδ) overexpression displays antidepressive effect and enhances hippocampal neurogenesis during chronic stress. Herein, we further extended our curiosity to investigate whether downregulating PPARδ could cause depressive-like behaviors through downregulation of neurogenesis.

Methods: Stereotaxic injection of lentiviral vector, expressing short hairpin RNA complementary to the coding exon of PPARδ, was done into the bilateral dentate gyri of the hippocampus, and the depression-like behaviors were observed in mice. Additionally, hippocampal neurogenesis, brain-derived neurotrophic factor and cAMP response element-binding protein were measured both in vivo and in vitro.

Results: Hippocampal PPARδ knockdown caused depressive-like behaviors and significantly decreased neurogenesis, neuronal differentiation, levels of mature brain-derived neurotrophic factor and phosphorylated cAMP response element-binding protein in the hippocampus. In vitro study further confirmed that PPARδ knockdown could inhibit proliferation and differentiation of neural stem cells. Furthermore, these effects were mimicked by repeated systemic administration of a PPARδ antagonist, GSK0660 (1 or 3 mg/kg i.p. for 21 d).

Conclusions: These findings suggest that downregulation of hippocampal PPARδ is associated with depressive behaviors in mice through an inhibitory effect on cAMP response element-binding protein/brain-derived neurotrophic factor-mediated adult neurogenesis in the hippocampus, providing new insights into the pathogenesis of depression.

Keywords: depression, PPARδ, hippocampus, neurogenesis, BDNF
Introduction
Depression is a common disorder worldwide, associated with an increased risk of suicide, impaired social skills, and social withdrawal (Rosenström and Jokela, 2017). Although many advances have been made in understanding the neurobiolgy of this complex disorder, the pathophysiological mechanisms are still unclear. Accumulating studies have supported a strong association between adult hippocampal neurogenesis, the formation of new neurons in the dentate gyrus (DG) of the adult brain, and depression (Serafini et al., 2014; Schoenfeld and Cameron, 2015). People with depression often display decreased hippocampal neurogenesis that results in hippocampal atrophy (Small et al., 2011; Fotuhi et al., 2012). Stress suppresses hippocampal neurogenesis, which can be reversed by antidepressant treatments (Dranovsky and Hen, 2006; Li et al., 2009; Boldrini et al., 2012; Schoenfeld and Gould, 2012). Inhibiting hippocampal neurogenesis blocks some behavior-modulatory effects of antidepressants (Santarelli et al., 2003), which suggests that neurogenesis might be critical for antidepressant action.

It is well known that neurotrophins serve as important regulators of depression. Brain-derived neurotrophic factor (BDNF) is the most extensively studied neurotrophin, which is upregulated in the hippocampus by antidepressant treatment and is sufficient to produce antidepressant-like effects (Wang et al., 2008; Taliaz et al., 2010; Son et al., 2012). BDNF mediates its effects by activating several intracellular pathways, such as the mitogen-activated protein kinases and/or extracellular-regulated kinase cascade (Peng et al., 2008; Xiao et al., 2011), thus leading to an increase in cAMP-response element binding protein (CREB) and promoting B-cell lymphoma-2 (Bcl-2) synthesis. Moreover, CREB is able to modify BDNF and Bcl-2 transcriptions. An increase in the BDNF/CREB/Bcl-2 regulatory pathways underlies the molecular basis for the improvement of neurogenesis, synaptic plasticity, memory, and mood (Li et al., 2009; Mariga et al., 2017).

Peroxisome proliferator-activated receptor δ (PPARδ, aka PPARδ1) is one of the 3 known PPARs (the others are PPARα and PPARγ), which are part of the nuclear receptor superfamily of transcription factors. PPARδ is a critical regulator of diverse biological processes, including maintenance of lipid and glucose homeostasis, inflammation, cell proliferation, and differentiation (Feige et al., 2006; Straus and Glass, 2007; Yu et al., 2014). Interestingly, in addition to the peripheral organs, PPARδ is also expressed throughout the brain, with particularly high levels in the hippocampus, entorhinal cortex, and hypothalamus (Woods et al., 2003; Hiqashiyama et al., 2007). Neuronal expression of this subtype is relatively higher compared with that of PPARα and PPARγ (Lemberger et al., 1996). To date, the neuroprotective benefits of PPARδ agonists have been reported in several experimental models of stroke (Arsenijevic et al., 2006; Pialat et al., 2007), Alzheimer’s disease (Kalinin et al., 2009), Parkinson’s disease (Martin et al., 2013; Das et al., 2014), autoimmune encephalomyelitis (Polak et al., 2005), and spinal cord injury (Paterniti et al., 2010). Our previous study has found that chronic stress, a known risk factor for depression, could decrease the expression of PPARδ in the hippocampus, and overexpression of hippocampal PPARδ could produce antidepressant-like effects, as observed in the chronic mild stress and learned helplessness paradigms (fi et al., 2015). Herein, we further extended our curiosity to investigate the effects of hippocampal PPARδ downregulation on mood-related behaviors and neurogenesis in vivo or in vitro.

Materials and Methods
Animals
Male ICR mice (18–22 g, 6–8 weeks) (Yangzhou University Medical Center, Yangzhou, China) were housed under controlled temperature, humidity, and lighting (22°C ± 2°C, 55% ± 5%, and a 12-h light/dark cycle with lights on at 7:00 AM), with food and water freely available unless otherwise noted. All experiments were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Laevis and Tropica1s, 1996) and approved by the Animal Care and Use Committee of China Pharmaceutical University.

Lentivirus Generation
Lentiviral miRNA-mediated knockdown (Brummelkamp et al., 2002; Yu et al., 2002; Stegmeier et al., 2005) was used to silence the PPARδ gene. We generated lentiviral vector constructs expressing short hairpin RNA (shRNA) complementary to the coding exon of mice PPARδ tagged with a fused enhanced green fluorescent protein (EGFP) and named it as LV-PPARδ-shRNA-EGFP. We also generated a lentiviral vector expressing EGFP alone (LV-EGFP). The sequence for the PPARδ shRNA (shRNA-mir hairpin structure) was 5'-tcgACGAGGTGAGAAGCTCGAGACTCTCTCTCCGACGGTTTCGAGCTGTATTTCCGAGAAGTTG-3'. In both PPARδ shRNA and normal control sequences, the middle 6 nucleotide (underlined) were hairpin loops. The coding sequence of PPARδ shRNA was amplified by polymerase chain reaction (PCR). The primer sequences were as follows: 5'-GCCGCCGTTAATTTTGCTAT-3' (forward) and 5'-GAGCAGATCTCGGTGGGTTG-3' (reverse). The PCR fragments and the GV118 vector (U6-MCS-UBi-EGFP) plasmid were digested with Age I and ligated with Age I to produce GV118-PPARδ-shRNA-EGFP. The plasmid was used to transform DH5α Escherichia coli for identification. For recovery of recombinant...
lentivirus-PPARδ-shRNA-EGFP (LV-PPARδ-shRNA-EGFP), HEK293 cells were co-transfected with 20 μg of the GV118 plasmid with a CDNA encoding PPARδ-shRNA and 15 μg pHelper 1.0 and 10 μg pHelper 2.0 plasmid to generate the recombinant lentivirus (LV), and LV-PPARδ-shRNA-EGFP. After 48 hours, the supernatant was harvested from HEK293 cells. The virus amplification was repeated thrice and the supernatant was filtered through a 0.45-μm filter. After resuspension, serially diluted LV was used to transfect HEK293 cells. Seven days later, labeled HEK293 cells were counted to calculate the viral titer (8 × 10^5 TU/mL). All of the lentiviral vectors contained the EGFP as a reporter to track δ-shRNA and 15a cDNA encoding PPARδ.

**Animal Surgery and LV Microinjection**

Mice were anesthetized with chloral hydrate (350 mg/kg, i.p.) and placed on a stereotaxic device. A 30-gauge infusion cannula was inserted into the dorsal/ventral DG (dorsal: 1.5 mm posterior to bregma, 1.0 mm lateral to the midline, and 1.7 mm below dura; ventral: 3.0 mm posterior to bregma, 2.0 mm lateral to the midline, and 1.9 mm below dura) with 2 injection sites on each side (Kheirbek et al., 2013). LV (2 × 10^5 TU/μL, 2 μL/side) containing PPARδ-shRNA with or without the EGFP was infused (0.2 μL/min) using a micro-injection pump (CMA402 Siringo Pump, Dakumar Machinery). Injectors were left intact for 5 minutes in place after completing the injection to ensure complete diffusion from the syringe tip. Behavioral tests and immunostaining assays were performed on the 3rd week after the LV transfection or after repeated systemic administration of the PPARδ antagonist GSK0660 (1 or 3 mg/kg i.p. once daily) for 21 days.

**Behavioral Tests**

Open field test (OFT), tail suspension test (TST), forced swimming test (FST), novelty-suppressed feeding test (NSFT), and elevated plus maze test (EPMT) were performed as described previously (Guo et al., 2012). Detailed descriptions of these tests can be found in the supplemental Methods and Materials.

**mRNA and Protein Analysis**

The descriptions of reverse transcription-PCR (RT-PCR) for mRNA analysis and western blot (WB) can be found in the supplemental Methods and Materials.

**Immunostaining**

For analyzing hippocampal neurogenesis and neural differentiation, the mice received 4 injections of 5-Bromo-2’-Deoxyuridine (BrdU; 50 mg/kg i.p. every 2 hours) on the 3rd week after LV injection. The mice were anesthetized with chloral hydrate (350 mg/kg, i.p.) after the last BrdU administration and transcardially perfused (0.1 M phosphate buffered saline followed by 4% paraformaldehyde). After fixation, the brains were sectioned into 40 μm sections and dehydrated with 30% sucrose over 2 days. Serial sections (35 μm) were cut throughout the hippocampus using an oscillating tissue slicer and preserved in normal saline. Then the sections were incubated with rat polyclonal antibody anti-BrdU (1:40, Abcam), rabbit polyclonal antibody anti-NeuN (1:200, Millipore), and rabbit polyclonal antibody anti-GFAP (1:200, Millipore) under 4°C overnight. We used the following secondary antibodies: cyanin 3 (1:500, Beyotime Biotechnology), Alexa Fluor 350 (1:500, Beyotime Biotechnology), DyLight 405 (1:100, BioWorld Biotechnology), and Alexa Fluor 647 (1:500, Beyotime Biotechnology). Fluorescent signals were detected using a fluorescence microscope (Olympus DF72). The cells were counted as described previously (Jedynak et al., 2014) by another blinded experimenter. Briefly, every 9th section was kept for BrdU immunohistochemistry. The cells in the DG were counted through a 40× objective lens in each section and multiplied by 10, regarded as the total quantity of labeled cells. The quantification was carried out using Image-Pro Plus software. The percentage of differentiated cells was calculated as the number of marker-positive cells divided by the total number of cells.

**In Vitro Assays for Proliferation and Differentiation of Adult Neural Stem Cells in Vitro**

Adult neural stem cells (NSCs) from the DG of 8- to 9-week-old female mice were dissected and cultured as reported previously (Guo et al., 2012). The NSCs proliferation was assessed by 3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) and cell counting kit (CCK-8, Beyotime Biotechnology) as well as BrdU incorporation observation. The NSC differentiation was determined using neuronal marker neuronal nuclear antigen (NeuN) or astrocytic marker glial fibrillary acidic protein (GFAP) antibodies respectively. DAPI+ cells were used to count the total number of cells. Images were analyzed by Image-Pro Plus software. The proportion of cells positive for specific markers was calculated to the total number. The detailed descriptions were provided in our previous publication (Ji et al., 2015).

**Data and Statistical Analyses**

Data shown are expressed as mean ± SEM. All data were analyzed by a 1-way ANOVA followed by a Dunnett’s post hoc analysis for multiple comparisons. All analyses were carried out using SPSS v20.0. P < .05 was considered as significant difference between the groups.

**RESULTS**

**Hippocampal PPARδ Knockdown Causes Depressive-Like Behaviors**

To assess the effect of hippocampal PPARδ knockdown on mood-related behaviors in mice, we generated LV encoding shRNA designed to target and downregulate PPARδ expression (Figure 1A). In vivo validation was confirmed by observation of EGFP+ cells (Figure 1B). RT-PCR and WB quantifications revealed that hippocampal PPARδ levels were significantly decreased on the 7th day after infection with LV-PPARδ-shRNA-EGFP (RT-PCR: P < .01; WB: P < .01; Figure 1C). In vitro validation revealed that the PPARδ protein level in the NSCs infected with LV-PPARδ-shRNA-EGFP was significantly less than that of noninfected cells or cells infected with the LV-EGFP (F2,33 = 25.89; P < .01; Figure 1D). These results indicate that the LV-PPARδ-shRNA-EGFP is effective and can be used to knockdown PPARδ in the hippocampus or NSCs.

We then investigated whether knockdown of hippocampal PPARδ could affect mood-related behaviors. Three weeks after LV injection, we performed behavioral tests and analyzed the total distance traveled and line crossings in the OFT to detect locomotor activity. One-way ANOVA revealed that hippocampus-specific knockdown of PPARδ did not affect locomotor activity (distance: F2,33 = 0.082, P > .05; line crossings: F2,33 = 0.203, P > .05; Figure 2A). The data of the FST and TST showed that hippocampus-specific knockdown of PPARδ significantly increased the immobility time (FST: F2,33 = 6.708, P < .05; TST: F2,33 = 4.094, P < .05; Figure 2B). In addition,
because of the frequent overlapping of symptoms of depression and anxiety in human beings (Xin et al., 2015), we examined anxiety in these mice using the NSFT and EPMT. In the NSFT, hippocampus-specific knockdown of PPARδ significantly increased the latency to feed in the novel environment ($F_{(2,33)} = 5.474, P < .05$; Figure 2C) but did not alter the home cage food consumption index ($F_{(2,33)} = 0.070, P > .05$), suggesting that the PPARδ knockdown-induced changes in the latency to feed in a novel environment cannot be explained by possible changes in appetite. However, in the EPMT, neither the time spent in the open arms ($F_{(2,33)} = 0.672, P > .05$; Figure 2D) nor the entries into the open arms ($F_{(2,33)} = 0.620, P > .05$; Figure 2D) was significantly affected by hippocampus-specific knockdown of PPARδ.
To further verify the role of PPARδ in the pathogenesis of depression, we next investigated the effect of GSK0660, a selective PPARδ antagonist that can penetrate the blood-brain barrier (Savage et al., 2015), on the depressive behaviors in mice. GSK0660 treatment increased the immobility time in the FST (F2,27 = 5.339, P < .05; supplemental Figure 1A) and the TST (F2,27 = 3.850, P < .05; supplemental Figure 1A). In the NSFT, GSK0660 treatment markedly increased the latency to feed (F2,27 = 7.103, P < .05; supplemental Figure 1B) without changing mice home cage consumption index (F2,27 = 0.012, P > .05; supplemental Figure 1B). The OPT showed that GSK0660 treatment did not affect the locomotor activity (distance: F2,27 = 0.691, P > .05; supplemental Figure 1C). In addition, neither the time spent in the open arms (F2,27 = 1.308, P > .05; supplemental Figure 1D) nor the entries into the open arms (F2,27 = 1.753, P > .05; supplemental Figure 1D) was significantly affected by GSK0660 treatment in the EPMT.

Collectively, we found that downregulation of hippocampal PPARδ through specific gene knockdown or using a selective PPARδ antagonist induced depressive behaviors in mice.

**Hippocampal PPARδ Knockdown Decreases Neurogenesis and Neuronal Differentiation**

Next, we were curious about the regulatory role of PPARδ on neurogenesis and neuronal differentiation that are suppressed in depression. BrdU+ cells in the DG were examined on the 16th day after the first BrdU injection in mice. Mice injected with the LV-PPARδ-shRNA-EGFP displayed a significant decrease in the number of BrdU-labeled cells in the DG (F2,15 = 8.436, P < .01; Figure 3A–B) compared with the mice injected with the LV-EGFP.

To examine the phenotype of BrdU+ cells in the DG, double labeling for BrdU and NeuN, a neuronal marker, or GFAP, an astrocyte marker, was performed after BrdU injection. The results indicated that hippocampus-specific knockdown of PPARδ decreased the percentage of NeuN+/BrdU+ cells (F2,15 = 4.882, P < .05; Figure 3C–D), but did not affect the percentage of GFAP+/BrdU+ cells (F2,15 = 0.353, P > .05; Figure 3E–F). In addition, GSK0660 treatment also showed significant decrease in the number of the BrdU-labeled cells in the DG (F2,15 = 8.743, P < .05; supplemental Figure 2A).

![Figure 3](image-url)

Figure 3. Hippocampus-specific knockdown of peroxisome proliferator-activated receptor δ (PPARδ) decreased hippocampal neurogenesis and neuronal differentiation in mice. (A) Representative micrographs and (B) quantification of 5-Bromo-2’-Deoxyuridine (BrdU)-labeled cells (red) in the dentate gyrus (DG) of the mice. (C) Representative micrographs of cells double-labeled for BrdU (red, left) and the neuronal marker NeuN (green, middle). (D) Percentages of neurons labeled by BrdU in the DG of the mice injected with LV-EGFP or LV-PPARδ-shRNA-EGFP. (E) Representative micrographs of cells double-labeled for BrdU (red, left) and the astrocyte marker glial fibrillary acidic protein (GFAP) (green, middle). (F) Percentages of glial cells labeled by BrdU in the DG of the mice injected with LV-EGFP or LV-PPARδ-shRNA-EGFP. Data shown are mean ± SEM; n = 6. *P < .05, **P < .01 vs control.
**PPARδ Knockdown Inhibits Proliferation and Differentiation of NSCs**

To further confirm the in vivo results, we observed the effect of PPARδ knockdown or blockade on the proliferation and differentiation of NSCs in vitro. In a floating culture medium, the NSCs from mouse hippocampus showed neurosphere formation with obvious nestin expression (Figure 4A). CCK-8 and MTT reduction assays revealed that cell proliferation was significantly decreased in the NSCs transfected with LV-PPARδ-shRNA-EGFP (MTT: F2,15 = 5.199, P < .05; CCK-8: F2,15 = 7.570, P < .01; Figure 4B). GSK0660 also produced similar effects with LV (MTT: F2,15 = 12.20, P < .01; CCK-8: F2,15 = 5.604, P < .01; supplemental Figure 2C). BrdU incorporation experiment showed a significant decrease of the BrdU+ cells in the monolayer-cultured NSCs treated with LV-PPARδ-shRNA-EGFP (F2,15 = 5.011, P < .05; Figure 4C–D) or GSK0660 (F2,15 = 4.776, P < .05; supplemental Figure 2D–E).

We also investigated the effect of PPARδ knockdown or blockade on cell differentiation in the cultured NSCs. The results showed that PPARδ knockdown significantly reduced the percentage of NeuN+/total cells (F2,15 = 4.098, P < .05; Figure 4E–F). Similarly, GSK0660 treatment (0.1 or 10 μM) substantially decreased the percentage of NeuN+/total cells (F2,15 = 6.174, P < .05; supplemental Figure 2F–G). Neither PPARδ knockdown nor blockade changed the percentage of GFAP+/total cells (LV-PPARδ knockdown: F2,15 = 0.016, P > .05; Figure 4G–H; GSK0660: F2,15 = 0.622, P > .05; supplemental Figure 2H). These results indicate that downregulating PPARδ inhibits NSCs differentiated into neurons in vitro.

**PPARδ Knockdown Decreases mBDNF Generation and CREB Phosphorylation**

It is well known that BDNF-CREB signaling plays a crucial role in neurogenesis (Mariga et al., 2017). As shown in Figure 5, the mice with LV-PPARδ-shRNA-EGFP treatment displayed a significant decrease in hippocampal mBDNF, but not in pro-BDNF (mBDNF: F2,15 = 14.42, P < .01; Figure 5A). Assay for pCREB using an antibody directed against pCREB (Ser133) showed much lower phosphorylation of CREB at Ser133 in the hippocampus of mice treated with the LV-PPARδ-shRNA-EGFP (F2,15 = 7.775, P < .05; Figure 5B). Decreases of mBDNF and pCREB were also observed in the in vitro NSCs after treatment with LV-PPARδ-shRNA-EGFP (proliferation: mBDNF: F2,15 = 13.45, P < .01; Figure 5C; pCREB: F2,15 = 5.760, P < .05; Figure 5D). In addition, decreases of mBDNF and pCREB were found in the mice (mBDNF: F2,15 = 9.496, P < .05; pCREB: F2,15 = 12.72, P < .05; supplemental Figure 3A) or NSCs treated with GSK0660 (proliferation: mBDNF: F2,15 = 15.81, P < .05; supplemental Figure 3B; pCREB: F2,15 = 6.729, P < .05; supplemental Figure 3C) or NSCs treated with GSK0660 (proliferation: mBDNF: F2,15 = 15.81, P < .05; supplemental Figure 3B; pCREB: F2,15 = 6.729, P < .05; supplemental Figure 3C). These results suggest that PPARδ downregulation inhibits BDNF-CREB signaling, which is involved in hippocampal neurogenesis.

**DISCUSSION**

The present study showed that downregulating hippocampal PPARδ by intra-hippocampal microinfusion of LV, expressing shRNA complementary to the coding exon of PPARδ, or by repeated systemic administration of PPARδ antagonist GSK0660 induced depressive-like behaviors in mice. These treatments also resulted in a reduction of hippocampal neurogenesis and neuronal differentiation as well as decreases in mBDNF and pCREB, both in vivo and in vitro. PPARδ is expressed throughout the brain, with prominent localization in mouse hippocampus, entorhinal cortex, and hypothalamus, but lower levels in the corpus callosum and caudate putamen (Woods et al., 2003; Hiqashiyama et al., 2007). The expression patterns of PPARδ support the idea that this receptor has important constitutive roles in these brain subregions. While there are no significant differences between PPAR subtypes distribution in stress-related brain subregions (i.e., prefrontal cortex, paraventricular nucleus of hypothalamus) (Moreno et al., 2004), PPARδ shows a relatively high neuronal expression compared with the other PPAR subtypes (Lemberger et al., 1996). Notably, PPARδ plays an important role in modulating the activities of the other 2 PPAR subtypes (Shi et al., 2002). Our previous study showed that acute or chronic stress downregulated hippocampal PPARδ expression and induced depressive-like phenotype in mice, whereas hippocampal PPARδ overexpression reversed such a phenomenon (Ji et al., 2015). Therefore, in the present study, we extended our curiosity to find the effects of PPARδ downregulation in mouse hippocampus. We found that hippocampal PPARδ downregulation induced several behavioral impairments associated with depression, including increased immobility time in the TST and FST and latency to feed in the NSF test. Moreover, findings from EPMT indicated that knockdown of hippocampal PPARδ had the potential to induce anxiety-like behaviors. Moreover, these effects were mimicked by repeated administration with the selective PPARδ antagonist. All data indicated that PPARδ could be a key molecule in the hippocampus that might have potential regulatory roles in the pathophysiology of depression.

It is well known that hippocampal volume is decreased in people with recurrent depression relative to age- and sex-matched controls (Videbech and Ravkilde, 2004; Geerlings and Gerritsen, 2017). Moreover, the hippocampus is very susceptible to stress and contains high levels of glucocorticoid receptors and glutamate. The optimal function of the hippocampus is critical for modulation of the hypothalamus-pituitary-adrenal axis, and its dysregulation is observed in almost one-half of all depressed patients (Sapolsky, 2000). Therefore, the hippocampus is one of the most commonly studied brain regions in depression. In the past decade, researchers have established that the hippocampus is one of the few brain regions in the healthy mammalian brain where neurogenesis occurs throughout adult life (Kempermann et al., 2004), and it plays central roles in the formation of memory and emotional processes (Egan et al., 2003; Drevets et al., 2008). Adult hippocampal neurogenesis is known to contribute to the behavioral modulatory effects of antidepressant treatments (Surget et al., 2011; Snyder et al., 2011). Recent studies showed that neurogenesis-related changes specific to a dorsal/ventral subregion are associated with observed behavioral phenotypes, and dorsal hippocampus is associated with cognitive functions, while the ventral hippocampus is related with stress, and emotion (Fanselow and Dong, 2010; O’Leary and Cryan, 2014). However, it is difficult to induce changes in PPARδ at some specific subregion by microinfusion of LV into the hippocampus because of its infectious diffusion. Less BrdU+ cells in dorsal and ventral sub-region were observed after downregulation or antagonism of hippocampal PPARδ in the present study, and on the contrary, more BrdU+ cells in the subregions were displayed after its upregulation or activation (Ji et al., 2015). Furthermore, the in vitro study showed that PPARδ...
Figure 4. Knockdown of peroxisome proliferator-activated receptor δ (PPARδ) inhibited proliferation and differentiation of NSCs. (A) Neural stem cells (NSCs) from mice hippocampus expressed nestin (red), a protein marker for the NSCs. (B) Cell proliferation was determined by 3-(4, 5-dimethythiazole-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) and cell counting kit (CCK-8) assays. (C) Representatives of 5-Bromo-2’-Deoxyuridine (BrdU)-labeled cells of the NSCs. (D) Statistical graph showed declined BrdU+ cells in monolayer-cultured NSCs. (E) Representatives of immunofluorescence for neuronal marker, NeuN (red). (F) Percentages of neurons labeled by NeuN in the NSCs. (G) Representatives of immunofluorescence for astrocytic marker glial fibrillary acidic protein (GFAP) (red). (H) Percentages of glial cells labeled by GFAP in the NSCs. Data are shown as mean ± SEM; n = 6. *P < .05, **P < .01 vs control.
downregulation inhibited the proliferation of NSCs as well as their differentiation into neurons. These in vivo and in vitro data strongly suggest that PPARδ plays a crucial role in neurogenesis and makes a plausible explanation that PPARδ regulates both depression and memory.

BDNF, like other neurotrophins, is synthetized as a pro-BDNF that is proteolytically processed into mBDNF by intracellular and/or extracellular proteases (Seidah et al., 1996). It is a key signaling molecule involved in a wide range of central functions such as the maintenance of neuronal plasticity, learning, memory, neurogenesis, and mood control (Malcangio and Lessmann, 2003; Duman and Monteggia, 2006; Castren, 2014; Lu et al., 2014; Hempstead, 2015). Over the last decade, several studies have consistently highlighted BDNF as a key player...
in antidepressant action, and it serves as a transducer, acting as the link between the antidepressant drugs and the neuroplastic changes that result in the improvement of the depressive symptoms (Hempstead, 2015; Björnholm and Monteggia, 2016). CREB was described as one of the components downstream of the signaling pathways of BDNF in response to stress (Finkbeiner, 2000). Some stressful stimuli can induce the phosphorylation of CREB at serine-133 site by means of an intracellular signal transduction pathway (Lessmann et al., 1994; Otten et al., 2000). Phosphorylation of CREB subsequently results in the transcriptional regulation of c-fos, c-jun, and bcl-2, which play important roles in the processes of regeneration, survival, and neuronal repair (Marmigere et al., 2001; Arthur-Farraj et al., 2012; Harris et al., 2013; Li et al., 2013). Interestingly, our work showed that PPARδ downregulation decreased levels of CREB phosphorylation (serine-133) and mBDNF, while PPARδ upregulation increased their levels. All such evidence indicates that the role of PPARδ in depression is involved in BDNF-CREB signaling. Further elucidation of the specific mechanism will enable us to better understand what is required to trigger antidepressant effects in hope of developing better treatment options.

Taken together, the present study provides a persuasive demonstration for the role of hippocampal PPARδ in depression and further reinforces the interesting finding that hippocampal PPARδ downregulation by genetic manipulation or a pharmacological blockade displays depressive-like effects through BDNF/CREB-associated adult neurogenesis in the hippocampus. Overall, this study strongly supports the idea that the hippocampal PPARδ is critically involved in mood regulation and its dysfunction underlies the manifestation of depressive-like behaviors. Hopefully, hippocampal PPARδ could be a novel and promising target for developing new drugs for the treatment of depressive disorders.

**Supplementary Materials**

Supplementary data are available at International Journal of Neuropsychopharmacology (IJNPPY) online.

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**Interest Statement**

None.

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