



# Peroxisome biogenesis factor 5 controlled Histone deacetylase 6 and Sirtuin1 expression and modulated mitochondrial biogenesis in rat dorsal hippocampus

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## ABSTRACT

**Introduction:** Mitochondria and peroxisomes are tightly connected organelles that cooperate in lipid oxidation and maintenance of redox homeostasis. However, the peroxisome's role in the modulation of the mitochondrial regulatory factors has remained unanswered. SIRT1- PGC-1 $\alpha$  interaction as a pivotal pathway in energy expenditure leads to mitochondrial biogenesis. Histone deacetylase (HDAC)6 and HDAC10 also regulate mitochondrial dynamics. Mitochondrial dysfunction is a cause and/or consequence of aging and neurodegenerative disorders.

**Methods:** In this study, to disturb importing proteins into the peroxisomes, PEX5 was down-regulated in the dorsal hippocampus by lentivirus-mediated shRNA. The impact of PEX5 reduction on peroxisomes was explored by assessment of catalase activity, a regular peroxisome matrix enzyme, and PMP70 and PEX14 expression. Then, mitochondrial biogenesis factors, PGC-1 $\alpha$ , and mitochondrial transcription factor A (TFAM) were measured by quantitative polymerase chain reaction and mitochondrial-related HDACs, SIRT1, SIRT3, HDAC6 and HDAC10, by western blotting. Besides, spatial learning and memory were assessed using the Morris water maze task.

**Results:** Our results revealed a significant reduction of HDAC6 and SIRT1, alongside with decrease in mitochondrial biogenesis factors PGC-1 $\alpha$  and TFAM, and no alteration in HDAC10 and SIRT3. Despite all observed changes, memory performance displayed no detectable alteration in the experimental groups. These data suggest the role of peroxisomes in modulating mitochondrial dynamics via regulation of HDAC6 and SIRT1 expression.

**Conclusion:** Peroxisome dysfunctions may occur upstream to mitochondrial failure and can be considered as a potential therapeutic target for aging and age-related disorders.

## Keywords:

Peroxisome  
PEX5  
Mitochondrial biogenesis factors  
Histone deacetylases  
Dorsal hippocampus

## Introduction

Impairment of mitochondrial function with aging has been associated with the advent and progress of neuro-

degenerative disorders (Grimm and Eckert, 2017; Sun et al., 2016). Increased oxidative stress is considered both a cause and a result of mitochondrial dysfunction

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(Wang et al., 2020). However, recent evidence suggests peroxisomes as an upstream signal for the mitochondrial response to oxidative stress (Jo et al., 2020). The peroxisome-mitochondria connection is the well-known interplay of the two organelles in lipid metabolism, antiviral-defense, preserving redox state and common molecules in organelle division (Schrader et al., 2015).

Peroxisomes are the eukaryotic organelles participating in diverse metabolic functions, such as lipid oxidation, reactive oxygen species (ROS) homeostasis and synthesis of bile acids and ether phospholipids. They are crucial for the proper development and performance of the brain. Peroxisomes are linked to several human age-related disorders, for instance, neurodegenerative diseases (Islinger et al., 2018). Peroxisome function decrease with aging and their metabolite alterations have been reported in neurodegenerative diseases (Cipolla and Lodhi, 2017). Moreover, postmortem brain studies disclosed that changes in lipid components due to abnormal peroxisome lipid metabolism occurred in Alzheimer's patients (Kou et al., 2011). It is also indicated that amyloid beta generation elevates in the rat brain following inhibition of peroxisome lipid oxidation (Shi et al., 2012).

Peroxisins (PEXs) are the families of proteins required for peroxisome biogenesis. PEX5 is a cytosolic receptor that recognizes and imports matrix proteins into the peroxisomes (Otera et al., 1998). PEX5 defects are one of the mutations that occurred in the neonatal adrenoleukodystrophy, Zellweger syndrome and infantile Refsum disease (IRD) with common neurological deficits (Argyriou et al., 2016). It has been shown that PEX5 abundance declines during aging. Moreover, its inactivation creates peroxisomes with abnormal structure and function (Baes et al., 1997; Liu et al., 2012).

Sirtuin1/ peroxisome proliferator-activated receptor gamma [PPAR $\gamma$ ] coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) pathway plays a pivotal role in energy homeostasis and metabolic adaptation. In response to energy-demanding situations, SIRT1, the NAD<sup>+</sup>- dependent histone deacetylase, can activate PGC-1 $\alpha$  through deacetylation (Rodgers et al., 2005). PGC-1 $\alpha$  acts as the main co-transcription factor for the peroxisome and mitochondrial activity regulation (Fransen et al., 2017). By inducing the mitochondrial transcription factor A (TFAM), it also controls mitochondrial DNA transcription for genes such as subunit 2 of cytochrome c oxidase and other oxidative phosphory-

lation proteins (Kaufman et al., 2007). Dysregulation of expression or activity of brain SIRT1 has a destructive impact on mitochondrial function, metabolic homeostasis and neurological function (Xu et al., 2018). Maintenance of the mitochondrial function by SIRT1 induces a protective effect against axon degeneration of the X-linked adrenoleukodystrophy (X-ALD) mouse model (Morató et al., 2015). SIRT3, a downstream target for PGC-1 $\alpha$ , exerts its effect by mitochondrial biogenesis and the suppression of cellular ROS (Kong et al., 2010).

HDAC6 and HDAC10 the subclass IIb of histone deacetylases (HDACs), are the most frequent cytosolic HDACs with non-histone proteins as preferred substrates. They play a significant role in mitochondrial dynamics (Lee et al., 2010; Oehme et al., 2013). It has been shown that down-regulation of HDAC6 can decrease the metabolic activity of mitochondria (Kamemura et al., 2012). Increasing activity of HDACs in the peroxisome disorder of IRD has been reported to induce cell death in the *in vitro* condition (Nagai, 2015). HDAC inhibitors have also exhibited beneficial effects in restoring peroxisome function in IRD and X-ALD models (Singh et al., 2011). Several studies provide evidence on the importance of SIRT1 in cognitive function, synaptic plasticity and learning and memory in health and disease (Ng et al., 2015). Besides, it has been reported that HDAC6 has a role in stabilizing synaptic strength in *Drosophila* that is important for learning at the behavioral level (Perry et al., 2017). Given that the dorsal and ventral hippocampus have distinct extrinsic and intrinsic connections, they are responsible for different functions. In other words, the ventral part is involved in emotional processing while the dorsal one is responsible for cognitive functions, including spatial learning and memory (Lee et al., 2017).

This study was designed to determine the effect of PEX5 down-regulation in the dorsal hippocampus (dHIP) on the expression of HDAC-related mitochondrial factors. To test how PEX5 reduction may change the peroxisomes, we measured PMP70, PEX14 and catalase activity. Then, we explored the expression of SIRT1, SIRT3, HDAC6, HDAC10, PGC-1 $\alpha$  and TFAM as mitochondrial regulatory factors. In the next step, we performed Morris water maze (MWM) task to check out whether peroxisome alteration and/or the subsequent alteration in cellular signaling could be manifested at the behavioral level.

## Material and Methods

### Animals

Animals used in the present study were provided by the Pasteur Institute breeding colony (Tehran, Iran). Adult male Wistar rats weighed 200-250g were housed four in each cage and cared for according to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80-23, revised 1996). The animal room was equipped with standard 12h light/dark and 22–25°C temperature. Water and chow were available for each cage. All experiments received ethical approval from the research and ethics committee of the Neuroscience Research Center of Shahid Beheshti University of Medical Sciences under permit number IR.SBMU.MSP.REC.1396.168.

### Construction of shRNA-expressing lentivirus

The short hairpin RNA (shRNA) sequence-targeting rat PEX5 was designed and synthesized as follows<sup>1</sup>:

Top strand: gatccGGGAGTAGGCACTGAAGATGAGTTGttggCAACTCATCTTCAGTGCCTACTCCtttttg

Bottom strand: aattcaaaaaGGGAGTAGGCACTGAAGATGAGTTGccaaCAACTCATCTTCAGTGCTACTCCCg

The oligonucleotide fragments were annealed and cloned into the BamHI/EcoRI sites of pGreenPuro plasmid (System Biosciences, CA), which express shRNA sequence under the control of H1 and green fluorescent protein (GFP) under the regulation of cytomegalovirus promoter. The cloned construct was verified by restriction enzyme digestion and sequencing. In order to produce viral particles, the shRNA cloned pGreenPuro vector was co-transfected with the Virapower packaging mix (Invitrogen, USA, #k497000) into the lentiX-293T cells (Clontech, USA, #632180). The same protocol was used for the insert-minus pGreenPuro vector as control. To pellet down viral particles, the growth medium was filtered through a 0.45µm PVDF filter (Jet biofilm, China) 48h after transfection and ultra-centrifuged at 50×10<sup>3</sup> g for 3h. The pellet dissolved in sterile phosphate buffer saline (PBS). Lentiviral particles at a titer of approximately 10<sup>9</sup> TU/ml were used for *in vivo* experiments.

### Stereotaxic injections

Intraperitoneal injection of a mixture of ketamine (80mg/kg) and xylazine (10mg/kg) was performed before the animals were placed on a stereotaxic apparatus (Stoelting, USA). Based on the Paxinos and Watson atlas (2006) virus was injected in the following coordinates in the dentate gyrus: (AP:3, ML:±1.3, DV:4.3), (AP:4, ML: ±2.4, DV: 3.3) and (AP:5, ML:±3.4, DV:3.7). In two experimental groups, viral particles were injected at a volume of 2µl per site through a 30-gauge needle attached to a 10µl Hamilton syringe by a polyethylene tube (flow rate of 0.5µl/1min). The shPEX5 group received lentivirus carrying shPEX5 expressing pGreenPuro vector. Control animals received the same volume of lentiviral particles containing the insert-minus vector. On the 21st day, the injected animals were subjected to behavioral tests (n=8) or decapitated to remove brain tissues for immunohistochemistry (n=3), quantitative polymerase chain reaction (qPCR, n=3) and western blotting (n=3).

### Immunohistochemistry (IHC) staining

Deep anesthetized rats were subjected to transcardial perfusion with ice-cold PBS following 4% paraformaldehyde (PFA). After overnight post-fixation in PFA, the removed brains were stored at 4°C in 30% sucrose solution for 48h. Frozen-embedded brains were sliced into 10µm coronal sections by a cryostat apparatus (Scilab, UK) to obtain hippocampal slices in the anterior-posterior axis. IHC staining was started by section incubation in acetone for 20min, followed by incubation in 3% hydrogen peroxide for 15min after washing in PBST (PBS+ 0.1% tween 20). Permeabilization and blocking were performed by incubation in 1% Triton X-100 for 10min and 10% normal goat serum in PBST for about 1h, respectively. The final step was the overnight treatment of the sections with anti-GFP antibody (ab290) diluted in PBST with 5% normal goat serum at 4°C, followed by 1h incubation in HRP-linked secondary antibody (EnVision™+ Dual Link System-HRP, Dako Denmark A/S, Glostrup, Denmark). Immunoreactivities were visualized with 3, 3'- Diaminobenzidine tetrahydrochloride (DAB) solution. To capture images by the light microscope (Nikon), we used hematoxylin staining for nuclear counterstaining. Stereotaxic PBS injected rats were used as the control for staining experiments.

1. The loop and cloning sequences are in lowercase.

*Analysis of mRNA expression by q-PCR*

Total RNA was extracted from hippocampal tissues using the YZol Pure RNA (YektaTajhizAzma, Tehran, Iran, #YT9063) based on the manufacturer’s instructions. The extracted mRNA purity and concentration were estimated by NanoDrop 2000 (Thermo Scientific, USA). Total RNA (2µg) was used for the cDNA synthesis according to the manufacturer’s instructions of the cDNA synthesis Kit (YektaTajhizAzma, Tehran, Iran). The qPCR was carried out by the SYBR Green qPCR Master Mix (2X) (Amplicon-Denmark) and ABI StepOne instrument (Applied Biosystems, USA). The relative mRNA levels were estimated using β-actin genes as an internal control and calculated as 2<sup>-ΔΔCt</sup>. The primers used in this study are listed in Table 1.

*Analysis of protein expression by Western blot*

On the 21st day after virus injection, hippocampal tissues were extracted after decapitation of animals. Then, the dorsal and ventral portions of the hippocampus were separated. Homogenate of hippocampal tissue was prepared in cold lysis buffer containing 50mM Tris-HCl (PH=8), 10mM NaCl, 0.1%Triton X-100, 0.25% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 1mM sodium orthovanadate, 50mM sodium fluoride and 1% complete protease inhibitor cocktail. The supernatant of proteins was collected after homogenate centrifugation at 13000g for 10min. Protein concentration was determined based on the method of Bradford (Bradford 1976). Separated proteins on 10% SDS–PAGE acrylamide gel were then electrotransferred to PVDF membranes (Millipore, USA). The following antibodies were used in immunoblotting: rabbit monoclonal antibody against PEX5 (1:1000, cell signaling), rabbit polyclonal antibody against PMP70 (1:1000, Abcam), rabbit polyclonal anti-PEX14 antibody (1:1000, Abxexa), rabbit antibodies against HDAC6, HDAC10, SIRT1 and SIRT3 (1:1000, cell signaling) and secondary HRP conjugated anti-rabbit (1:3000, cell signaling).

The chemiluminescence signal was detected by the ECL kit (GE Healthcare, Italy) and then analyzed by Image J software (1.4.3.67 version). Data normalization was done by β-actin (1:1000, cell signaling) as an internal control.

*Enzymatic assay for catalase activity*

Catalase activity was assayed according to the method of Beers and Sizer (1952). The reaction started by adding 100mM H<sub>2</sub>O<sub>2</sub> to the reaction mixture containing 50mM phosphate buffer, pH 7.0 and 60µg protein. We measured changes in absorbance at 240nm for 15min. The rate of H<sub>2</sub>O<sub>2</sub> decomposition was expressed as µmol/min/mg protein.

*Assessment of learning and memory by MWM*

Spatial learning and memory were assessed using the MWM task in the injected rats 21 days after virus injection (Khatami et al., 2018). The water maze utilized in the study was a circular swimming pool (150cm in diameter, 60cm high) filling with water up to 45cm. Four points as north, south, east and west on the rim pool divided it into the four quadrants. Several spatial cues were surrounding the pool in the experiment room. During the training trials, the platform was hidden 2cm under the water in the target quadrant and the animals were trained in four consecutive trials with a 10-min inter-trial interval. Then, the animals were released from a random point into the pool and allowed to find the platform in 60s. The time in which the rat reached the platform was considered escape latency. Memory assessment was in the probe test without any platform. The test was started from the opposite point of the target quadrant and continued for 60s. The time spent in all quadrants and the number of times each rat crossed the platform were recorded in 60s. Animal locomotion activity was recorded by a CCD camera (Panasonic Inc., Japan) and analyzed using the Ethovision (version XT7) video tracking software.

**TABLE 1:** Primer sequences used in qPCR

Gene	Forward primer (5'-3')	Reverse primer(5'-3')
PEX5	AGAGTTGGAGGAGATGGCAA	TGGTCACGCAAGGGATTCTC
TFAM	AGAGTTGTCATTGGGATTGG	CATTCAGTGGGCAGAAGTC
PGC1-α	GTGCAGCCAAGACTCTGTATGG	GTCCAGGTCATTACATCAAGTTC
β-actin	TCTATCCTGGCCTCACTGTC	AACGCAGCTCAGTAACAGTCC

Statistical analysis

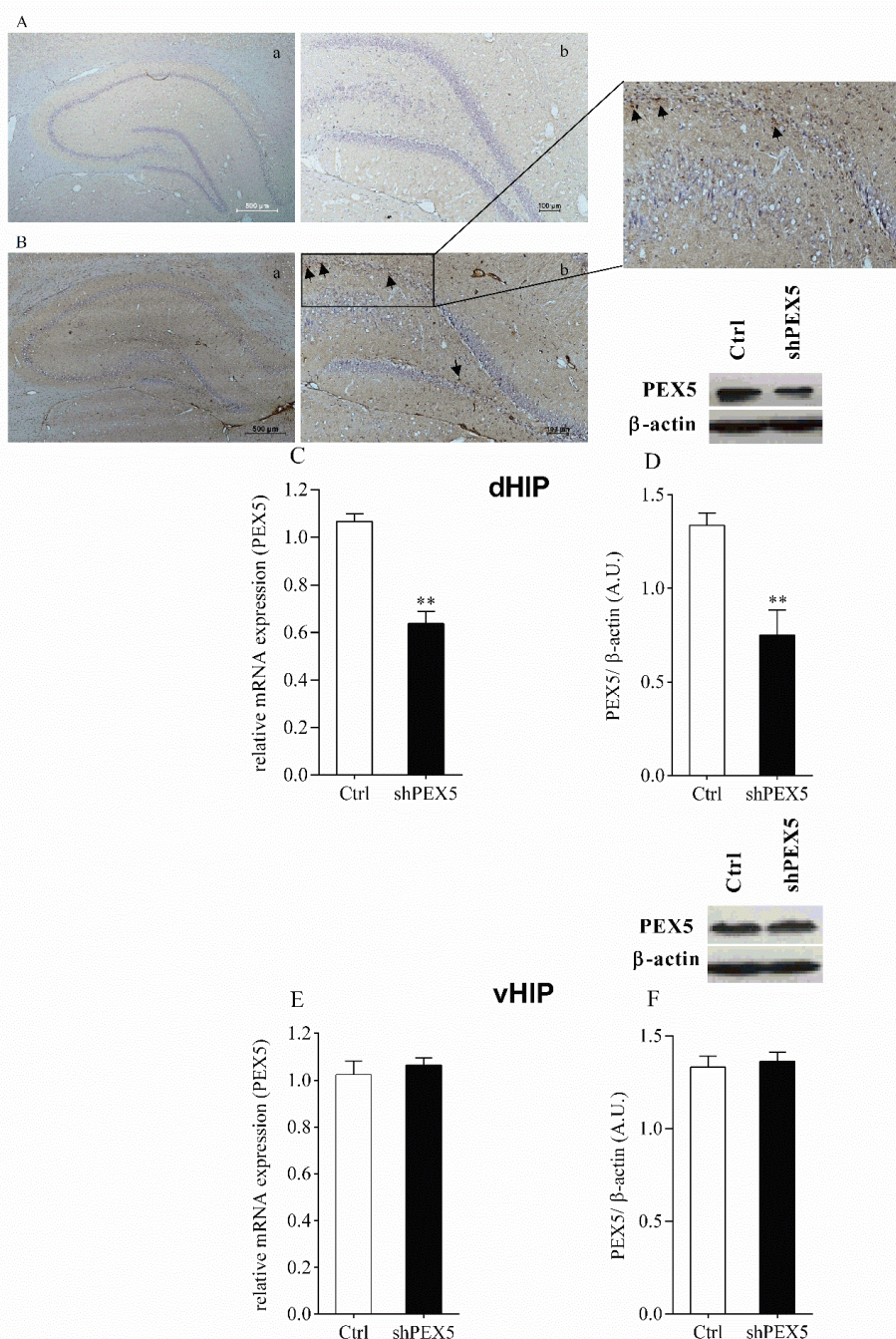
GraphPad Prism (6.07) was used for statistical analysis. For comparison between two groups, data were analyzed using Student's t-test. The escape latency in the training test and time in quadrants in the probe test were analyzed by repeated-measure two-way ANOVA and one-way ANOVA, respectively. All data were expressed as mean±SEM and statistical significance was set at  $P<0.05$ .

Results

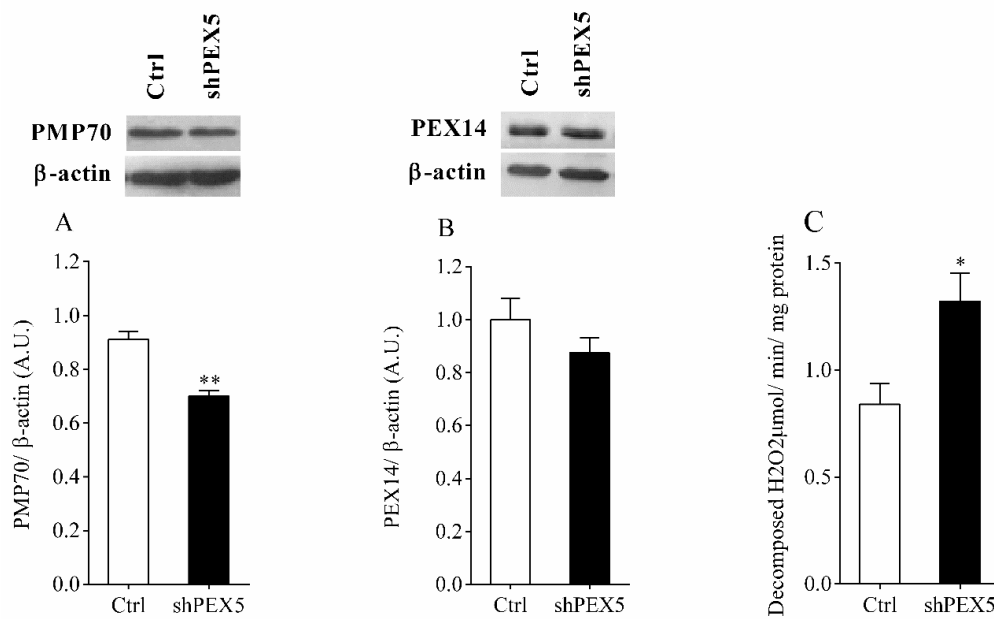
shRNA against PEX5 reduced mRNA and protein con-

tent of PEX5 in the dHIP

The GFP expression was detected using IHC staining in the granular layer of the dentate gyrus on the 21st-day post-injection in PBS-injected and shPEX5 groups (Figures 1A and B). The efficacy of the lentiviral construct in down-regulating PEX5 was assessed by western blotting and qPCR. As it is shown in Figures 1C and D, the mRNA ( $P<0.01$ ) and protein content ( $P<0.01$ ) of PEX5 in the dHIP were decreased significantly by the construct. However, in the ventral hippocampus (vHIP), no significant alteration was detected in neither mRNA nor protein contents (Figures. 1E and F).



**FIGURE 1.** Efficacy of lentivirus carrying shRNA against PEX5. GFP immunoreactivity was detected in the dentate gyrus of the hippocampus in the PBS-injected (A) and the shPEX5 groups (B), respectively. Scale bar: a) 500µm, b) 100µm; Quantification of PEX5 mRNA levels extracted from dHIP of the control and shPEX5 groups (C). Representative and quantification of PEX5 protein level from dHIP of the two comparing groups (D). Quantification of PEX5 mRNA level from the vHIP of control and shPEX5 (E). Representative and quantification of PEX5 protein level from vHIP of the two mentioned groups (F). Data are reported as the mean±SEM; \* $P<0.05$ , \*\* $P<0.01$  versus the control group (n=3 per each group).



**FIGURE 2.** Effect of PEX5 down-regulation on peroxisome biogenesis markers and catalase activity. Representative and quantification of PMP70 (A), PEX14 (B) levels and enzymatic activity of catalase (C) in the dHIP of control and ShPEX5 groups. Data are reported as the mean±SEM; \* $P<0.05$ , \*\* $P<0.01$  versus control group (n=3 per each group).

*PEX5 down-regulation declined the peroxisome biogenesis marker PMP70 and enhanced catalase activity without alteration in PEX14*

Since PEX5 has a regulatory role in peroxisome biogenesis, we checked out peroxisome biogenesis markers. As shown in Figures 2A and B, there was a significant decrease in the protein level of the peroxisome membrane protein PMP70 ( $P<0.01$ ) with no detectable change in expression of PEX14 in the dHIP of the shPEX5 group. Because PEX5 is important in catalase import to the matrix, we measured enzyme activity in both groups. Data analysis represented in Figure 2C shows a significant increase in catalase activity in the shPEX5 group compared to the control group ( $P<0.05$ ).

*PEX5 reduction in the dHIP decreased HDAC6 with no effect on HDAC10*

Analysis of western blotting data determined PEX5 downregulation decreased the expression level of HDAC6 ( $P<0.01$ ) in the shPEX5 group (Figures. 3 A and B). However, no significant change in HDAC10 was observed.

*PEX5 down-regulation suppressed SIRT1 without change of SIRT3*

Data represented in Figure 3G shows SIRT1 expresF

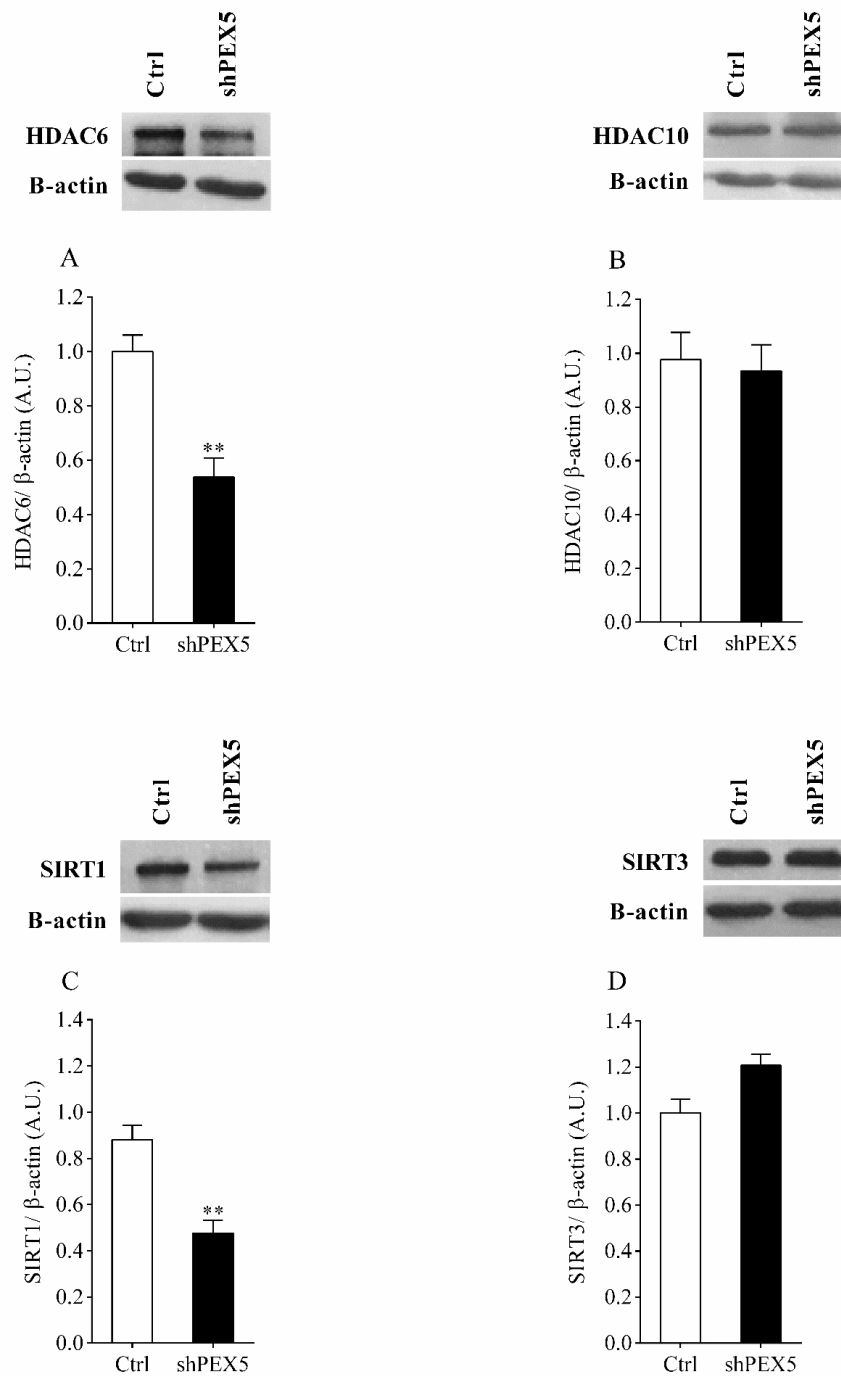
sion in two comparing groups of shPEX5 and control. The analysis revealed a significant reduction of SIRT1 in shPEX5 ( $P<0.01$ ). As it is revealed by Figure 3D, PEX5 down-regulation did not affect SIRT3.

*Down-regulation of PEX5 in the dHIP decreased PGC-1α and TFAM*

Mitochondrial biogenesis factors, PGC-1α and TFAM, were measured by qPCR. Analysis of the data as shown in Figure 4 specifies a significant decrease in mRNA level of PGC-1α ( $P<0.01$ ) and its down-stream transcription factor, TFAM ( $P<0.05$ ) after PEX5 down-regulation in the shPEX5 group.

*PEX5 down-regulation in the dHIP had no significant effect on acquisition and retrieval of long-term spatial memory*

As shown in Figure 5A, a similar decrease in time to find the hidden platform is revealed by repeated-measures two-way ANOVA analysis in three consecutive training days in both groups. The time spent in the target quadrant in the probe test was higher than the opposite one for both groups (Figure 5B,  $P<0.0001$ ). It was disclosed that the shPEX5 group spent lesser time in the target quadrant than the control group, but this parameter was not statistically significant (Figure 5B). Moreover, the number of entries in the target quadrant was similar in both groups (Figure 5C).



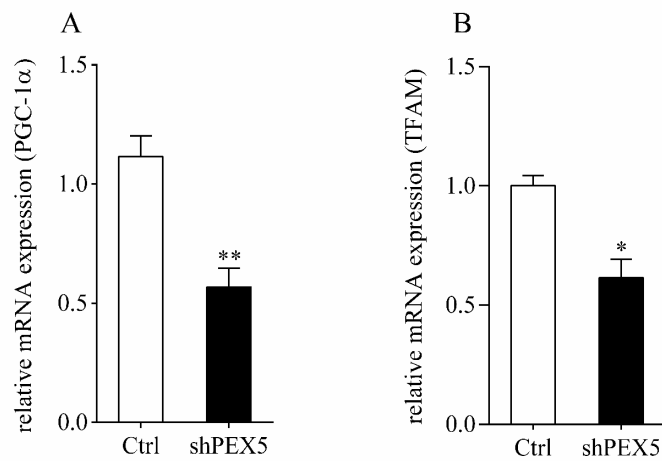
**FIGURE 3.** Impact of PEX5 reduction on HDAC enzymes. Representative and quantification of HDAC6 (A), SIRT1 (B), SIRT3 (C) and HDAC10 (D) protein levels in the dHIP of control and shPEX5 groups. Data are shown as the mean $\pm$ SEM. \*\* $P$ <0.01 versus control group (n=3 per each group).

### Discussion

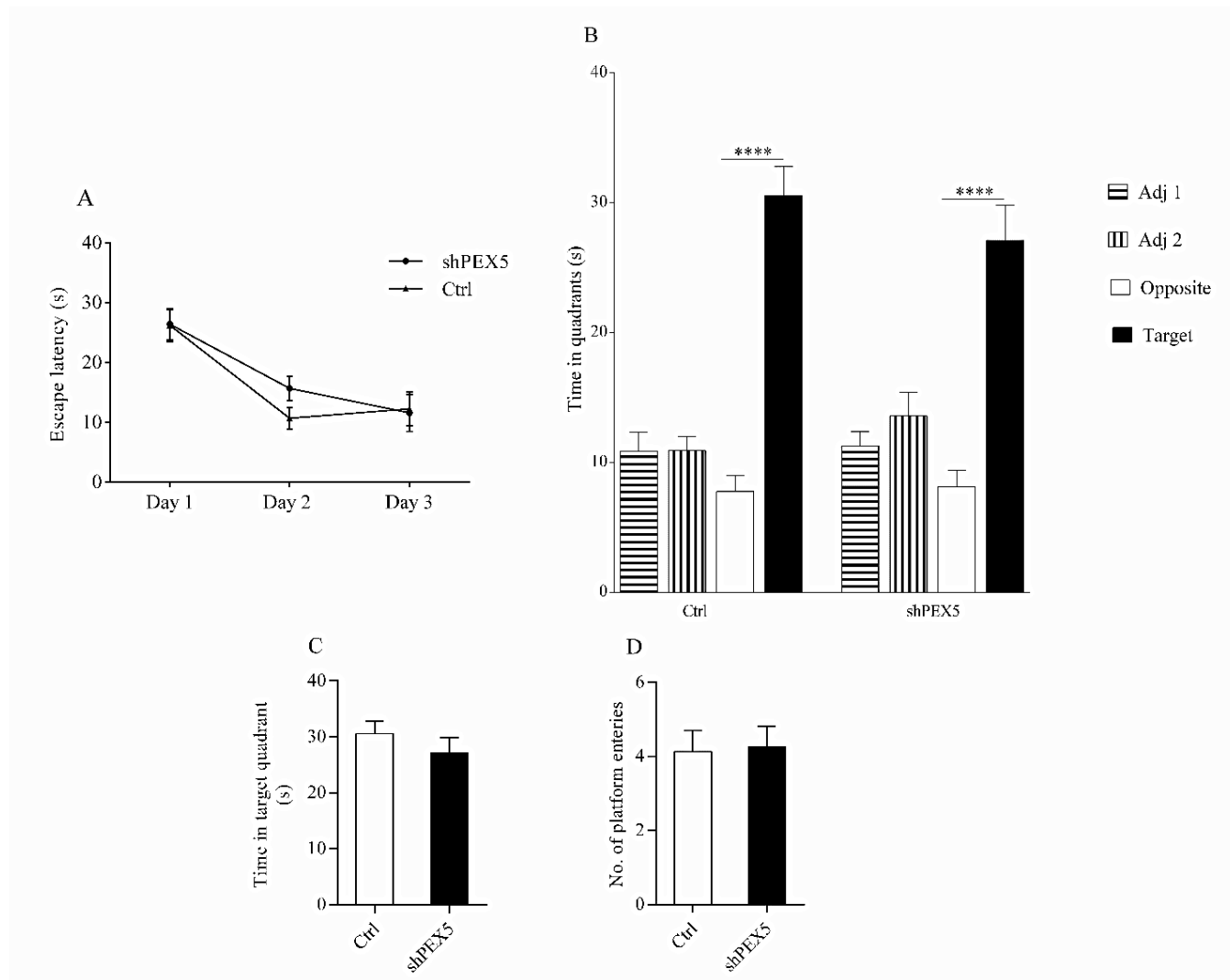
In the present study, we evaluated peroxisome alteration after lentivirus-mediated reduction of PEX5 in dHIP. Consequently, PMP70 expression decreased with enhanced activity of catalase without a change in PEX14. These changes induced significant decrement in the expression of the mitochondrial dynamics and biogenesis factors including, HDAC6, SIRT1, PGC-1 $\alpha$

and TFAM. Nevertheless, the acquisition and retrieval of spatial memory in the MWM task were not affected by these molecular changes.

PMP70 and PEX14, the peroxisome membrane proteins, were considered markers of peroxisome abundance in several experimental studies (Ahlemeyer et al., 2007; Eun et al., 2018; Fanelli et al., 2013; Santos et al., 1994). Moreover, it was revealed that PEX14 is a



**FIGURE 4.** Mitochondrial biogenesis was prohibited by down-regulation of PEX5. Quantification of PGC-1 $\alpha$  (A) and TFAM (B) mRNA levels in the dHIP of control and shPEX5 groups. Data are reported as the mean $\pm$ SEM. \* $P$ <0.05, versus control group (n=3 per each group).



**FIGURE 5.** Spatial learning and memory in Morris water maze were not affected by down-regulation of PEX5. Escape latency for both control and shPEX5 groups for each day of training (with four trials per day pooled) (A). Time spent in each quadrant during the probe trial (B). Number of entries into the platform area by both groups during the probe trial (C). Data are reported as the mean $\pm$ SEM. \*\*\*\* $P$ <0.001, target quadrant compared to other quadrants (n=8 per each group).



feasible marker to detect peroxisome numbers in various developmental stages in several brain regions like the hippocampus (Ahlemeyer et al., 2007). PMP70 is an ABC transporter, imports fatty acids into the peroxisomes. There is no association between increment in the expression of PMP70 and peroxisome proliferation (Gärtner and Valle, 1993; Imanaka et al., 1999). Our results revealed a significant reduction in expression of PMP70 and no change in PEX14. The lack of correlation between these proteins in our study is in harmony with Fanelli and colleagues (2013) findings reported in a previous study. These results can indicate numerically unchanged peroxisomes along with a decrease in PMP70 function.

Catalase activity significantly increased in PEX5-down-regulated rats. In mammalian cells, depending on efficient import into the matrix, catalase is found in high concentrations inside peroxisomes. The target sequence for catalase import is different from the peroxisome target signal, its interaction with PEX5 is less efficient, resulting in retain the enzyme in the cytosol under some conditions such as oxidative stress (Walton et al., 2017). As an explanation, it was implicated by Legakis et al. (2002) that catalase import become ineffective by aging because of aberrant PEX5 trafficking. Furthermore, it was determined that total catalase activity is up-regulated with aging in different brain areas in Wistar rats (Tsay et al., 2000). Therefore, the increase in catalase activity depicted by our results can propose enzyme misplacement from the matrix after PEX5 down-regulation.

Due to the increment of mitochondrial biogenesis, IRT1/PGC-1 $\alpha$  pathway plays a protective role against apoptosis in the intracerebral hemorrhage rat model (Zhou et al., 2018). In addition to that, the neuroprotection effect of SIRT1 is also indicated against ischemia and Alzheimer's disease (Xu et al., 2018). It has revealed by a recent study on the beta-amyloid toxicity model, altered SIRT1/PGC-1 $\alpha$  signaling induces related disturbance of mitochondrial homeostasis (Panes et al., 2020). Thus, restoration of the enzymatic activity of SIRT1 can be considered as a potential strategy for treating axon degeneration in the peroxisome-associated disorders as exemplified by X-ALD (Morató et al., 2015). SIRT1 reduction following PEX5 down-regulation, illustrated by our finding, may be related to peroxisome dysfunction.

We also observed a reduced level of PGC-1 $\alpha$  and its down-stream effector, TFAM, without a significant

change in SIRT3. The PGC-1 $\alpha$  reduction can be attributed to the PEX5 down-regulation, considered a sign of peroxisome biogenesis defects. These results are in concert with previous researches (Eun et al., 2018). On the ground that mitochondrial DNA replication that controls mitochondrial respiratory activity and its gene expression is regulated by TFAM, it is proposed by our experiment that the mitochondrial function might be affected by PEX5 down-regulation as well. It is proved that under both normal physiological and oxidative stress conditions in the brain, PGC-1 $\alpha$  is a principal regulator of ROS metabolism (Lin et al., 2005). Moreover, PGC-1 $\alpha$  down-stream target, SIRT3, is also involved in ROS detoxifying (Kong et al., 2010). Bringing these together, unchanged SIRT3 in our study may indicate the other regulatory pathways distinct from PGC-1 $\alpha$  in controlling SIRT3 expression.

As it is depicted by our results, the level of HDAC6 significantly decreased in PEX5- down-regulated rats besides, no changes in the level of HDAC10. There is a vast body of evidence that dysregulation of HDAC6 expression and function can be considered as the underlying etiology of neurodegenerative diseases. Nonetheless, the contradictory role of HDAC6 in neurodegenerative contexts should be noted. In more detail, while HDAC6 plays a neuroprotective role in Parkinson's disease, its inhibition can be protective in Alzheimer's disease by increasing mitochondrial transport and dynamics (Du et al., 2010; Guedes-Dias et al., 2015; Simões-Pires et al., 2013). In peroxisome-associated disorders, HDAC inhibitors are regarded, as the potential therapeutics (Nagai, 2015; Singh et al., 2011). Nevertheless, it should be mentioned that a specific HDAC in these studies has not been targeted.

Lentivirus-mediated shRNA delivery system stably integrates shRNA sequence into the cell genome, providing a permanent reduction of the target gene (Bot et al., 2005). Therefore, it can be a conventional technique to achieve a long-lasting effect of gene silencing or its manifestation at behavioral levels. We performed behavioral test 21 days after a single virus- injection, inducing a persistent PEX5 reduction in dHIP. Our results detected no significant change between the two compared groups. MWM was selected owing to the involvement of dHIP in this type of memory (Khatami et al., 2018). There is no experimental study considering a direct connection of peroxisome function with learning

and memory. In the adult mammalian brain, SIRT1 is expressed broadly in different brain regions, specifically the hippocampus and hypothalamus (Ramadori et al., 2008). It is proven that SIRT1 plays an essential role in hippocampus-dependent short- and long-term memory formation (Michán et al., 2010). In addition, the potential role of HDAC6 in drosophila memory retention and homeostatic plasticity was disclosed recently (Perry et al., 2017). One implication for the absence of spatial memory impairment in our study might be that the reduction of PEX5 in the dHIP did not affect spatial memory. However, we cannot ignore the fact that the level of PEX5 decrement in this study was not sufficient to elicit spatial memory impairment. As it is depicted in Figure. 5B, the PEX5 down-regulated group tended to spend lesser time in the target quadrant, but not statistically significant, than the control group, which needs further studies.

## Conclusion

In this study, PEX5 down-regulation decreased the peroxisome marker of PMP70 and increased catalase activity. These alterations were followed by the reduction of SIRT1, HDAC6, and mitochondrial biogenesis factors. It could be concluded that peroxisomes play a role upstream to mitochondrial biogenesis, possibly through SIRT1 and HDAC6. They can be considered as potential therapeutic targets to correct mitochondrial disturbances.

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## Conflict of interest

There is no conflict of interest.

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